Neurobehavioral Methods and Effects in Occupational and Environmental Health

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Foreword

Every three years since 1982, the Scientific Committee on Neurotoxicology and Psychophysiology of the International Commission on Occupational Health (ICOH) and the World Health Organization (WHO) have jointly sponsored international symposia on neurobehavioral methods in occupational and environmental health. These symposia have been major events that have contributed substantially to advancement of this new field. The Fourth Symposium in this series was held in Tokyo in 1991. For this event, the International Labour Office (ILO) joined the ICOH and the WHO as a co-organizor. It was a great honor that we could publish the proceedings of this outstanding symposium in *Environmental Research*. We have now compiled the Symposium articles into a hard-cover version for distribution to symposium participants and associates.

The Symposium was aimed at exchanging state-of-the-art information on current studies in the following two areas: (1) development and application of neuropsychobehavioral methods in occupational and environmental health and (2) current advances in the knowledge of the effects on the nervous system and human behavior of occupational and environmental factors. Another crucial objective was to share information in this field with colleagues in developing countries in order to devise preventive strategies compatible with resources in those nations.

At the Symposium, the keynote address, the special lecture, and the Hänninen lecture were delivered by seven distinguished guest professors, including Dr. Hiroshi Nakajima, Director-General of the WHO. Eleven key areas in neuropsychobehavioral methods and effects were presented and reviewed by 31 distinguished speakers in plenary sessions, and were subsequently discussed in detail in the corresponding 11 workshops. Seventy-five oral and 64 poster presentations on subjects relevant to symposium topics were made by active participants. Also, new methods and equipment were demonstrated. A total of 288 colleagues from 34 countries, including Japan, U.S.A., Italy, Sweden, China, Korea, Singapore, Germany, Finland, Australia, South Africa, Brazil, and the Philippines, participated in the Symposium.

In the Symposium proceedings, a total of 106 approved and peer-reviewed papers were accepted and published in successive issues of the journal. All of these papers, together with an additional 44 submissions, were reviewed by an international group of scientific peer reviewers:

U.S.A.	*Europe*	*Japan*
W. Kent Anger	Donatella Camerino	Shunichi Araki
Dean B. Baker	Marie G. Cassitto	Kazuo Hashimoto
Edward L. Baker	Vito Foa	Masayuki Ikeda
Robert G. Feldman	Renato Gilioli	Norito Kawakami
Frederic E. Gerr	Helena Hänninen	Kiyotaro Kondo
Philip J. Landrigan	Jacob Hooisma	Katsuytuki Murata
Richard Letz	Anders Iregren	Akio Ohnishi
Ruth Lilis	Juhani Juntunen	Kazuo Saito
Rob McConnell	Marcello Lotti	Shosuke Suzuki
David A. Otto	Esko J. Matikainen	Yasuhiro Takeuchi
Ellen K. Silbergeld	Antonio Mutti	Eiji Yano
Peter S. Spencer		Kazuhito Yokoyama
Yasunosuke Suzuki		
Richard D. Thomas		
Jose A. Valciukas		

I thank all of our reviewers.

Finally, I express my sincere gratitude to Professor Renato Gilioli, Former Chairman of the ICOH Scientific Committee on Neurotoxicology and Psychophysiology, and to the members of the International Organizing Committee of the Symposium; to Professor Philip Landrigan, Editor-in-Chief of *Environmental Research*, and to his entire staff; to Dr. Kazuhito Yokoyama, Secretary General of the Symposium, and to the members of the Local Organizing Committee; and to all participants in the Symposium, for their valuable contributions to the success of the Symposium and to the publication of the proceedings and hard-cover copy book.

SHUNICHI ARAKI, M.D., D.M.SC., M.SC.
Editor and President of the Symposium

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An International Perspective in Neurobehavioral Toxicology[1,2]

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World Health Organization

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OPENING REMARKS

Since 1982, the World Health Organization (WHO) has supported three international symposia on neurobehavioral methods and effects in occupational and environmental health. As it attempts to advance public health, the Organization has benefited greatly from its association in these symposia with other international organizations. This year I am particularly pleased to note that the fourth symposium in the series is being organized with the joint participation of the International Labour Office (ILO) the International Commission of Occupational Health, and WHO. The Organizing Committee and the Japanese Scientific Programme Committee are to be congratulated on their success in obtaining such cooperation from international groups interested in the protection of human health from neurotoxic agents. I should also like to thank the Organizing Committee for its efforts to support the attendance of scientists from the lesser developed countries of the region. This action greatly assists the work of WHO. For my part, it is a pleasure and an honor to present this lecture.

INTRODUCTION

The Constitution of the World Health Organization, adopted by Member States 45 years ago, defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." When one considers the total environment as including such extragenetic factors as diet; lifestyle choices, such as drugs, alcohol, and tobacco; occupational exposure; and exposure to agents in the ambient environment, agents in the total human environment do play a role in affecting human health.

The full extent of the public health problems arising from environmental factors is still poorly defined. However, in view of its complex structure and functions, and its role in homeostasis, the human nervous system is particularly vulnerable to toxic substances. It should be among the first organs assessed for adverse effects due to environmental factors. As a measure of the integrated functions of all organ systems, including the nervous system, neurobehavioral methodology can play a pivotal role in this area of public health.

At present it is not possible to give a precise estimate of the number of chemicals, and/or physical agents, that have a behavioral or a neurotoxic effect on humans or animals. More than 850 chemicals have been reported to have such an effect, and the neurotoxic effects on humans of such physical factors as noise

[1] Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

[2] This special lecture was delivered at the opening ceremony, on the afternoon of Monday, 8 July 1991. Hiroshi Nakajima is Director-General of the World Health Organization.

vibration and repetitive motion have been well documented. The extent of possible interactive effects of chemical exposure and such physical factors remains to be ascertained.

Although my presentation will focus on chemicals and neurotoxicity, prevention of the neurotoxic effects of physical factors must also continue, in the workplace and, as urbanization increases, in the general environment.

SCOPE AND MAGNITUDE OF THE PROBLEM

The question may be asked whether the public health problem arising from human exposure to man-made and natural neurotoxic chemicals is truly an international problem of sufficient magnitude to warrant the continuing involvement of WHO, ILO, and other international bodies. Certainly, when one examines the wide range of chemicals showing neurotoxicity in humans, it is evident that we are dealing with a major problem (Table 1). Neurotoxins are found in several classes of pesticide, solvents (methanol and ethanol), metals (lead, mercury, and manganese), and plastic intermediates (acetonitrile and acrylamide), and natural toxins are found in the staple foods of several developing countries, producing neurotoxic effects in large segments of the population (for example, from the consumption of chick peas and the root of the cassava). Therefore, in many countries of Africa and the Indian subcontinent there exists a unique problem, that of neurotoxic effects from many classes of chemical introduced for development compounding an already serious health problem arising from the consumption of the types of food made necessary by drought and socioeconomic factors. This is a major international public health problem requiring appropriate action on the part of WHO.

Neurotoxicity, with its insidious effects on the quality of human life, is not confined to either the developed or the developing regions of the world. Nor is it a public health problem of the twentieth century. Incidents of neurotoxicity have occurred since antiquity, in most regions of the world (Table 2). There are many more examples, including a reference by Hippocrates in 370 BC and by Pliny in the first century AD, both to the toxicity of lead. In 1837, evidence of the neuro-

TABLE 1
SOME CHEMICALS AND PLANTS SHOWING NEUROTOXICITY IN HUMANS

	Neurotoxic manifestation
Chemical	
Acetonitrile	Seizure, convulsions
Acrylamide	Abnormal sweating, proprioception
Carbon Monoxide	Apathy/lethargy
Chlordecone	Tremors
Ergot	Hallucination
Ethanol	Incoordination, central nervous system depression
Lead	Hyperactivity, impaired development of cognitive function
Manganese	Dystonia, hallucination
Methanol	Abnormal vision
Organomercurials	Incoordination, psychomotor retardation
Organophosphate pesticides	Fosciculation, anxiety, paralysis
Plant toxin	
Lathyrus sativus (chick pea)	Irreversible spasticity (lathyrism)
Manihot esculenta (cassava)	Spasticity, auditory and visual deficits

TABLE 2
EXAMPLES OF NEUROTOXIC INCIDENTS

Year	Location	Substance	Incidents
1924	United States	Tetraethyl lead	Workers processing additive—5 deaths, over 300 workers with neurological symptoms
1930	United States	Tri-*o*-cresyl phosphate	Adulteration of ginger extract—5000 paralyzed; over 20,000 affected
1950	Morocco	Manganese	Neurobehavioural problems—150 miners suffering severely
1950–1964	Japan	Methyl mercury	Methylmercury poisoning—more than 1200 cases among fish-eating populations; symptoms ranging from paresthesia and ataxia to death
1971–1972	Iraq	Organomercurial fungicide	Treated seed grain used as food—over 450 deaths and 6500 hospital admissions
1981	Spain	Toxic oil	20,000 poisoned, more than 500 deaths; severe neuropathy
1988	India	Tri-*o*-cresyl phosphate	Adulterated rapeseed oil—about 600 cases of polyneuritis

toxicity of manganese was reported from Scotland. Similar neurotoxic incidents can occur at any time. Unless immediate action is taken, the disastrous environmental conditions in central and eastern Europe could provide such opportunities. Incidents shown here do not include neurotoxic outbreaks caused by the consumption of foodstuffs containing excitatory amino acids. It has been reported that there is a 3% incidence of lathyrism in the peasant farmers of Ethiopia, and there is no alternative food source from what is immediately available.

Accurate data on the number of people exposed to neurotoxins are extremely difficult to obtain; however, examples of the large number of people exposed to a few neurotoxins reinforce the need for more international action (Table 3). We see here only the numbers exposed in a few regions to two classes of chemicals—pesticides and organic solvents—and to lead. Many thousands, if not millions,

TABLE 3
DATA SHOWING EXTENT OF HUMAN EXPOSURE TO SOME NEUROTOXIC CHEMICALS

Chemical	Extent of exposure and numbers affected
Pesticides	
Worldwide	At least 15,000 deaths; more than 1 million poison cases
United States	4–5 million workers exposed; about 300,000 poison cases a year
Honduras	32% of 1100 farmers examined in a study had a decrease in cholinesterase levels in excess of 25%
Cuba	17,500 workers exposed
Organic solvents	
Germany (former Federal Republic)	1–2 million workers exposed
United States	9.8 million workers exposed
Venezuela	7000 workers exposed
Lead	
United States	12.5 million children at risk from lead exposure
Venezuela	13,760 workers exposed
China	1.7% prevalence of chronic poisoning in 355,000 workers examined

may be exposed to other known—or as yet unknown—neurotoxic chemicals. In view of the millions exposed to pesticides throughout the world, and the costs to society of the resulting neurotoxicity, more scientific and regulatory activity is essential to prevent further adverse effects. Similarly with lead. Millions of children are at risk, and thousands of workers are still exposed to lead in traditional cosmetics and drugs in developing countries. Solutions must be found in order to prevent further damage to human health.

We still know nothing of the neurotoxic properties of thousands of man-made and natural chemicals, or the number of people exposed to such chemicals. If we fail to prevent further outbreaks of neurotoxic disease from chemical exposure, the effects on human health and the economic costs may be horrendous. Very few economic analyses have been made of the costs of a failure to develop appropriate regulatory strategies for neurotoxic agents. In the United States of America mental disorders and diseases of the nervous system and sense organs accounted in 1980 for an annual personal health care expenditure of about 38 thousand million U.S. dollars. One study has shown that 2–3% of these costs result from neurotoxicity alone, approximately 1 thousand million U.S. dollars annually. These figures, startling as they may be, do not include the cost of lost productivity, or other social costs, such as the rehabilitation of drug and alcohol abusers in working populations.

Economists in the United States have examined the health benefits derived from strategies designed to reduce the neurotoxic effects of lead poisoning in children (Table 4). The figures shown are for the special education facilities needed for children mentally impaired from exposure to lead and the health care costs of the placing of such children in institutions. Savings from reducing the lead exposure can be substantial—approximately 500 million U.S. dollars annually between 1986 and 1988. It is obvious that, apart from substantial monetary benefits from the prevention of neurotoxic disorders, there are great benefits to the health and well-being of society.

There is a group of neurotoxic chemicals of particular relevance at the present time. These are the nerve gases that are highly toxic organophosphorus compounds designed for use in warfare. In Iraq at this moment there are tons of these chemicals in stores, containers, and projectiles, of which many are damaged and leaking. In order to comply with a United Nations Security Council resolution, all chemical warfare agents in Iraq must be disposed of safely. An initial investigation of the situation shows that this will be a formidable technological and time-

TABLE 4
SAVINGS THROUGH REDUCTIONS IN THE NEUROTOXIC EFFECTS OF LEAD POISONING IN CHILDREN

Service area	Savings (in million U.S. dollars)					
	1985	1986	1987	1988	1989	1990
Compensatory education	187	447	408	374	338	309
Medical care	65	155	141	130	117	107
Total	252	602	549	504	455	416

Note. Estimates in 1983 (U.S. dollars).

Source. United States Congress, Office of Technology Assessment; "Neurotoxicity: Identifying and Controlling Poisons of the Nervous System," Washington, DC, April 1990 (OTA-BA-436).

consuming task which must be carried out safely so that human health and the environment are not damaged in any way. The estimated cost of the disposal operation runs into hundreds of millions of U.S. dollars. It should also be borne in mind that other nations too have spent large sums on the disposal of their chemical warfare agents. Thus, an immense amount of financial resources has been, and is being, committed to dealing with chemicals whose only intended use was for warfare and destruction.

PUBLIC HEALTH NEEDS FROM AN INTERNATIONAL PERSPECTIVE

So far I have discussed the magnitude of the problem and its worldwide character. In my opinion it does not present a pleasant picture, rather a potential time bomb waiting to explode, or to be defused by appropriate scientific and regulatory action. The choice is ours. You may ask, what are the requirements for an internationally coordinated effort to address this emerging public health crisis and what action has WHO taken up to the present?

The current situation provides an excellent opportunity for the more developed industrialized countries to work closely with WHO in support of the national programs, for addressing this problem, of all Member States. Countries with scientific expertise in neurotoxicity, epidemiology, and occupational medicine need to continue to work closely with WHO, as it attempts to assemble and coordinate the scientific information which will form the cornerstone of any national regulatory strategy to prevent the neurotoxic effects of chemicals. Through such support WHO will be able to:

(1) facilitate and coordinate the exchange among Member States of scientific information and the results of evaluations;

(2) develop an internationally acceptable testing methodology and epidemiological procedures to facilitate the international acceptance of data;

(3) develop in-country training activities on the safe handling and disposal of chemicals, and the development of national standards.

It is a pleasure to acknowledge that several countries have already played a major role in supporting WHO's activities in the area of neurotoxicity and in the development of behavioral methodology. Some 30 countries and national institutions support the International Programme on Chemical Safety, as it continues to provide to all Member States peer-reviewed evaluations of chemicals, practical guidance on their safe handling and disposal, and internationally accepted methodology in neurobehavioral toxicology. Support from countries has also allowed WHO to develop a neurobehavioral core test battery for use in places of work, permitting the rapid screening of workers for subtle neurotoxic effects.

INTERNATIONAL COLLABORATION IN THE DEVELOPMENT OF NEUROBEHAVIORAL METHODOLOGY

Over the past 6 or 7 years, WHO has been actively developing neurobehavioral methods for use in animal testing for hazard identification and for the assessment of neurobehavioral effects in workers and the risks to children from exposure to lead.

WHO is making efforts to foster and support preventive occupational health programs to deal with the exposure of workers to toxic chemicals, especially in developing countries. As part of these efforts, it has developed, as I have already mentioned, a neurobehavioral core test battery, which can be used for the simple

screening of workers, even in health care programs with limited resources. It consists mainly of paper and pencil tests, it is short and easy to administer to poorly literate subjects, and it is relatively free of cultural bias. The tests included in the battery and the order in which they are administered are shown in Table 5. This battery measures motor steadiness, response speed, perceptual-motor speed, manual dexterity, visual perception, visual memory, auditory memory, and affect. At present it is being evaluated in some eight countries. I am looking forward to hearing the results of this evaluation.

As part of its environmental epidemiology program, the WHO Regional Office for Europe developed a protocol which was used in a multicenter study to assess the risk to children from exposure to lead. The methodology allows epidemiologists to combine for analysis the results from several studies, thus markedly increasing the force of the overall investigation. Each study was relatively small (fewer than 400 subjects); however, the final results were from over 1800 children.

In general, the results of this multicenter study agree with those already published, namely, a strong exposure-related deficit in visual–motor integration and a weak association with psychometric intelligence. However, a positive association between blood lead level and a deficit in IQ was not found across groups. Although the protocol was specifically designed for lead, it has been shown that such a multicentered methodology can be used to assess exposure to other environmental pollutants. This is a definite advantage in cases where large numbers of subjects are needed to ascertain subtle effects at low exposure levels.

FUTURE DIRECTIONS IN NEUROBEHAVIORAL TOXICOLOGY

The neurosciences, in particular the neurobehavioral and neurotoxicological disciplines, have made significant contributions to the protection of human health from neurotoxic agents. If such progress is to continue, there is a pressing need for closer integration of the work of all disciplines within the neurosciences, and work in general toxicology. In particular, neurobehavioral toxicology must be built on a more multidisciplinary foundation, so that a better understanding is developed of the nervous system and its varied responses to chemicals, at the biochemical, functional, and structural, as well as behavioral, levels.

If data collected from animal research are to be useful in assessing human risk from neurotoxic chemicals, they must be related to data collected from human studies. At present, this does not seem to be taking place. A review of current animal screening tests, and the neurobehavioral test batteries used to assess humans, indicates that these batteries do not measure similar functions. A serious

TABLE 5
WHO NEUROBEHAVIORAL CORE TEST BATTERY

Test	Functional domain tested
Aiming (Pursuit Aiming II)	Motor steadiness
Simple Visual Reaction Time	Attention/response speed
Digit Symbol	Perceptual–motor speed
Santa Ana Dexterity Test (Helsinki version)	Manual dexterity
Benton Visual Retention (Recognition form)	Visual perception/memory
Digital Span	Auditory memory
Profile of Mood States	Affect

impediment exists to using animals to assess the neurotoxicity of chemicals in humans. This problem needs to be rectified soon.

The neurobehavioral batteries being proposed for assessment of the effects of chemicals on humans have to be validated. In particular, the neurobehavioral core test battery developed by WHO has to be rapidly validated and made available to all countries. In addition, efforts must be intensified to incorporate other functional tests within this core battery, to make it more sensitive and thus provide greater protection.

As now evaluated, neither the human nor the animal neurobehavioral batteries can identify disease complexes newly suspected of having a chemical etiology. Perhaps there is a need to incorporate other neurological tests in these batteries if they are too sensitive to such complex phenomena as Parkinson's disease, manganism, amyotrophic lateral sclerosis, and neuropsychiatric disorders in workers. To achieve this may pose a challenge to those in basic research. Multidisciplinary research may be needed for the development of a better understanding of the relationship of behavioral test results to these complex disease states.

CONCLUDING REMARKS

Preventing an increase in neurotoxic disease arising from all sources of the human environment is a formidable task. I would ask you to consider how your own discipline can assist, internationally. A multidisciplinary approach is, I repeat, the most cost-effective approach to any public health problem. As we derive more scientific information from testing, and from epidemiological investigations, the development of regulatory strategies on the basis of such information must always be in the context of prevention, not of treatment. Chemicals can play an important role in global sustainable development but they become a severe burden on society when their use results in adverse effects on human health. As part of the global community we must strive for progress in reaching toward better health and well-being.

ENVIRONMENTAL RESEARCH 61, 164–175 (1993)

Assessing the Neurotoxic Potential of Chemicals—A Multidisciplinary Approach[1]

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Since 1981, the development of methodology to assess the neurotoxic potential of chemicals has been a high priority within the International Programme on Chemical Safety (IPCS). Following the completion of an in-depth review of the scientific principles and methods for the assessment of neurotoxicity associated with chemical exposures, IPCS started to develop a multidisciplinary and comprehensive approach for neurotoxicity testing of chemicals. In view of the complexity of the nervous system and the variety of effects caused by chemicals, no single test or approach will be appropriate. Initially, IPCS evaluated neurobehavioral tests as well as *in vitro* procedures as screening tests, and an international collaborative study of neurobehavioral tests appropriate for screening chemicals for neurotoxicity is now in progress. Possible integration of higher level neurobehavioral tests with neurophysiological, biochemical, and pathological procedures in future testing strategies are discussed.

INTRODUCTION

From a public health perspective, the possibility that large groups of humans may be exposed to neurotoxicants is unacceptable, whether these are man-made or natural agents. Protection is essential from neurotoxicants already present in the environment as well as from the potential effects of new chemicals released into commerce. Such protection is best provided by testing chemicals for neurotoxicity in experimental models prior to human exposure, thus allowing a sound and thorough scientific evaluation of neurotoxic potential. Where populations and individuals have already been exposed, the early detection of disease or dysfunction is essential in order to prevent further exposures which might lead to frank neurotoxic disease. While public health programs must integrate the results from studies on both experimental animals and humans, this paper addresses only the methodologies needed to identify and characterize neurotoxic risks using nonhuman models.

A short summary is first presented of the present activities within the International Programme on Chemical Safety (IPCS) related to the development of methodology in neurotoxicology. Based on the scientific methods presently available, some views on the development of integrated multidisciplinary testing strategies

[1] Presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

are then presented. In no way should these proposals be considered definitive or reflecting the official views of any national or international agency. They are presented here to stimulate scientific thought in an attempt to improve the scientific basis of risk assessments carried out within the field of neurotoxicology.

DEVELOPMENT OF INTERNATIONALLY ACCEPTED METHODOLOGY IN NEUROTOXICOLOGY

For more than a decade, the World Health Organization (WHO) and in particular the International Programme on Chemical Safety (IPCS) have been working closely with many institutes worldwide to develop and utilize methods for detecting neurotoxicity to improve public health protection. Some of these activities are shown in Table 1. Other papers in this volume deal with the WHO Neurobehavioral Core Battery described previously by Cassitto *et al.* (1990) and the results of the multicenter study on lead previously summarized by Winneke *et al.* (1990).

The International Programme on Chemical Safety (IPCS)

IPCS is a cooperative program of the International Labour Organisation (ILO), the United Nations Environment Programme (UNEP), and the World Health Organization (WHO), with the overall objective to catalyze and coordinate activities worldwide in relation to chemical safety (Mercier, 1990). It is scientific rather than regulatory in nature.

The scientific activities of IPCS listed in Table 1 were initiated by IPCS in an attempt to attain the objective—"To promote the development, improvement, validation, and use of laboratory testing and ecological and epidemiological studies and other methods suitable for the evaluation of health and environmental hazards and risks from chemicals." Through such activities, IPCS hopes to foster the development of internationally accepted approaches and methods for assessing chemical effects on human health that will result in the generation of data and evaluations accepted worldwide.

TABLE 1
SOME ACTIVITIES WITHIN THE WORLD HEALTH ORGANIZATION (WHO) AND INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY (IPCS) ON THE NEUROTOXIC EFFECTS OF CHEMICALS

(A) Occupational health (WHO)
WHO Neurobehavioural Core Test Battery for use in occupational health
(B) Environmental Health (WHO)
European multicenter study on lead neurotoxicity in children (WHO/EURO)
(C) IPCS (1981–ongoing)
(i) Publication of EHC 60—"Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals"
(ii) Development and promotion of a multidisciplinary, comprehensive strategy for neurotoxicity testing
—Collaborative study on neurotoxicity screening tests
—*In vitro* assays
—Developmental neurotoxicology

Neurotoxicology within IPCS

A group of experts in 1981 recommended that IPCS give high priority to the development of methods for the evaluation of nervous system toxicity resulting from chemical exposures (Mercier, 1990). The preparation of a monograph providing a critical evaluation of the test methods available was given the highest priority, followed by the coordination of international collaborative studies in neurotoxicology.

As shown in Table 1, IPCS, with the assistance of some 40 scientists from 18 countries, published a comprehensive review of the scientific principles and methodologies utilized in neurotoxicology (WHO, 1986). The same group of scientists who developed the monograph on neurotoxicological methods provided a series of important recommendations to IPCS which have guided the Programme since 1986. These recommendations were summarized by Mercier (1990). In essence, these experts supported the concept of a tiered testing strategy in neurotoxicology. It was also recommended that IPCS promote the validation of a generic set of behavioral tests to serve as a primary neurotoxicity screen, consider validation of more complex neurobehavioral assays and other neurotoxicity tests, and develop an international consensus on a multidisciplinary strategy for establishing the neurotoxic potential of chemicals.

The following paragraphs describe the progress made by IPCS in addressing the above recommendations (see Table 1).

COLLABORATIVE STUDY ON NEUROBEHAVIORAL TESTS

The panel of experts convened by IPCS reviewed many of the principles and practices in neurotoxicology research in the 1986 Environmental Health Criteria (Vol. 60) publication. Behavioral tests were divided into those that were primarily useful for the initial evaluation of an agent's neurotoxic potential (i.e., screening tests), and those used to better characterize the nature and mechanism of effects produced by neurotoxic chemicals. Such a stratification is useful for developing tiered approaches for efficiently evaluating the effects of a broad range of new and existing chemicals, many of which may have never been evaluated previously for neurotoxicity.

Screening tests are used to provide preliminary data on an agent's neurotoxic effects. They generally are comprised of a battery of tests used to rapidly evaluate the effects of toxicants on several domains of neurobehavioral function (e.g., sensory–motor reactivity, autonomic). The types of tests include assessments of animals while in the home-cage, while being held, and after being placed in an open arena for examination. A variety of batteries are available that differ in the detail with which behavioral and neurological signs are noted and scored. Almost all batteries, however, include the following types of evaluations: observations of general appearance and autonomic signs, tests of reactivity, coordination, and muscle tone, and a measure of an animal's activity level. Ideally, a battery should be sensitive and reliable, capable of discriminating between neurotoxic and non-neurotoxic compounds, and capable of discriminating between different classes of neurotoxic substances. General principles related to neurotoxicity screening, and

details of many of the screening batteries in use can be found in Gad (1989), Haggerty (1989), Moser (1989), and O'Donoghue (1989).

The IPCS and many other expert organizations have advocated the use of a neurobehavioral battery for hazard identification, that is for providing preliminary evidence of a compound's neurotoxic potential. Many of the tests that make up the battery require semiquantitative or subjective evaluations of the animals. As a consequence, there may be grounds for concern over the reproducibility of results in different laboratories or in the same laboratory over time (e.g., when different technicians make the evaluations). The IPCS recognized this concern and the potential barrier it represented to a broad-based international application of neurotoxicity screening batteries. The IPCS therefore recommended in 1986 that an interlaboratory validation effort be undertaken using a neurobehavioral screening battery. A steering group was established to develop a testing protocol, recruit laboratories to participate in the project, and oversee its progress. Eight laboratories are currently participating in the project. Each laboratory is evaluating in a blind fashion the effects of seven compounds (five neurotoxic and two nonneurotoxic agents) using both acute and repeated-dosing protocols. A standardized battery is being used, with all participating laboratories receiving prior training in its use. In addition, each laboratory was required to provide evidence of their ability to detect the effects of five prototype neurotoxicants. All laboratories have passed this proficiency phase of testing and are now collecting data on the seven unknown compounds. Data collection and analysis are targeted for completion in the spring of 1993. The salient features of the study are given in Table 2.

In Vitro Tests

Over the last few years there has been a marked interest in the use of *in vitro*

TABLE 2
IPCS COLLABORATIVE STUDY ON NEUROBEHAVIOURAL SCREENING TESTS

Test methods
Functional Observational Battery (FOB)
Motor Activity Assessment (MAA)
Laboratories
Eight laboratories from five countries representing academic, industrial, and governmental facilities
Chemicals[a]
Five known neurotoxicants
Two nonneurotoxic substances
Animals
Young adult male rats
Quality assurance
Training[b]—video and laboratory training (completed before formal data collection)
Proficiency—demonstrated ability of each laboratory to detect effects of 5 prototypic neurotoxicants

[a] All chemicals purchased, characterized, and shipped on behalf of IPCS by the National Institute of Environmental Health Sciences/National Toxicology Program.

[b] Training video and in-laboratory training carried out on behalf of IPCS by the U.S. Environmental Protection Agency/Health Effects Research Laboratory.

tests for assessing the neurotoxic potential of chemicals (Walum *et al.*, 1990). IPCS convened a panel of scientists in 1989 to review the status of *in vitro* methods in neurotoxicology. Although many tests were identified, the panel did not believe that any were ready for routine use in the testing of chemicals. However, they did believe that it is a fertile scientific area for international collaboration and urged IPCS to seek support for such work from their network of participating institutions. For the validation of *in vitro* methods for the screening of chemicals for neurotoxicity the panel advocated the use of the same compounds being tested in the IPCS collaborative neurobehavioral study described above.

DEVELOPMENTAL NEUROTOXICOLOGY

IPCS efforts in developmental neurotoxicology (Table 1) are in the final planning stage with work expected to start in late 1991 or early 1992. It will be part of the larger project to update and rewrite the IPCS Monograph "Principles for Evaluating Health Risks to Progeny Associated with Exposure to Chemicals during Pregnancy" (WHO, 1984). In view of the importance of the subject the preparation of a separate monograph on the scientific principles and methods in developmental neurotoxicology is being considered by IPCS. To date, two IPCS Participating Institutions (U.S. Environmental Protection Agency and National Institute of Environmental Health Sciences) have expressed their willingness to support this project. It is planned to invite scientists from a few centers of excellence in Europe and Japan to attend a planning meeting in early 1994 to obtain advice on the scientific issues and to recommend activities which should be undertaken by IPCS.

MULTIDISCIPLINARY APPROACHES FOR NEUROTOXICITY TESTING

In Vitro Approaches

In recent years, efforts to develop *in vitro* tests for assessing neurotoxicity have received increased attention (Shahar and Goldberg, 1987). *In vitro* techniques have played a vital role in increasing our understanding of the molecular, cellular, and biochemical makeup of the nervous system. Numerous *in vitro* techniques have been developed to describe in precise detail the events involved in the development of the nervous system, the requirements for normal neuronal function, and the disruption caused by neurotoxic chemicals. Examples of the latter include studies on the mechanism of action of neurotoxic heavy metals (Cooper and Manalis, 1983) and insecticides (Narahashi, 1984; Abou-Donia and Lapadula, 1990).

A broad range of *in vitro* systems (cell lines, brain slices, organotypic and explant cultures, etc.) are available for studying the neurotoxic impact of chemicals (Davenport *et al.*, 1989). Biochemical and neurophysiological methods can be applied to these systems to provide valuable information on the mechanisms by which neurotoxic substances exert their effects. However, the specificity, reliability, and predictability of *in vitro* tests in screening for neurotoxicity, when little is known about the mechanism of action of a chemical, have not been adequately examined. The panel of experts convened by IPCS in 1989 to review

the status of *in vitro* methods in neurotoxicology recognized that there were many important issues yet to be resolved regarding the broad-scaled use of *in vitro* methods in neurotoxicity screening. Issues unresolved include differentiating specific neurotoxicity from general cytotoxicity and extrapolating *in vitro* effects to effects seen in the whole animal. For example, *in vitro* effects might not be seen in the whole animal because of toxicokinetic and metabolic differences. Also, since *in vitro* systems do not duplicate the complex neural circuitry of the entire animal, a number of toxic endpoints (e.g., sensory, motor, cognitive, perceptual disorders) may be difficult if not impossible to define. The relevance of *in vitro* results for predicting effects of long-term exposures is also uncertain.

Nevertheless, the many advantages of *in vitro* testing, particularly as they relate to the ability to define potential mechanisms of action, present a strong incentive for their increased development and utilization. A program to study the relationship between *in vitro,* whole animal, and human studies for a well-defined set of neurotoxic chemicals is urgently needed to address how *in vitro* systems can predict *in vivo* effects. The continued development and application of *in vitro* approaches in neurotoxicity is urgently needed.

In Vivo Neurotoxicity Approaches

A variety of techniques are available for studying neurotoxicity in the whole organism. These different approaches can provide information over many levels of analysis, ranging from the actions of neurotoxic chemicals on discrete biochemical processes to complex systems that govern behavior. A consideration of neurochemical, neurophysiological, and behavioral approaches will illustrate the power which can be brought to multidisciplinary studies of neurotoxicity.

Biochemical approaches and techniques can be utilized in studies on the neurotoxic potential of chemicals, and many different endpoints can be measured. These include: effects on neurotransmitters (i.e., synthesis, transport, storage, release, re-uptake or degradation of serotonin, norepinephrine, acetylcholine, amino acids), neuromodulators (e.g., opioid peptides, neurohormones), and their receptors or second messengers; lipids, glycolipids, or other constituents of neural membranes; ion channels or membrane-bound enzymes that regulate neuronal activity; and the metabolic machinery necessary to maintain neural functioning (e.g., glycogen and creatine phosphate levels, glucose availability, mitochondrial respiration). Such changes can be wide-ranging if they can occur in localized brain regions, specific cell types, or particular subcellular organelles. These processes can be studied in living animals, in brain slices and homogenates, or in tissue cultures derived from nervous system components. Descriptions of the diversity of biochemical approaches and their limitations can be found in a number of reviews (Damstra and Bondy, 1982; WHO, 1986; Mailman, 1987; O'Callaghan, 1988).

Neurophysiological procedures can provide information regarding neural function on many levels of analysis. These range from events studied best *in vitro* such as the operation of single ion channels assessed with patch and voltage clamp techniques to events studied *in vivo,* such as the operation of large ensembles of cells and complete neural systems. Between these extremes, levels of neurophys-

iological analysis include studies of cell membranes, synaptic function, single cells, nerve fiber bundles, simple neural circuits, the function of sensory, motor, autonomic or limbic system pathways, and mapping of regional activity patterns across the brain. Neurophysiological analysis can be useful in studying the effects of both acute and chronic treatment with neurotoxic compounds. Electrophysiological techniques can also help identify the target sites of chemicals within the central and peripheral nervous systems. Many techniques can be used in humans and laboratory animals alike. The IPCS has previously surveyed neurophysiological measures which are useful in neurotoxicological analysis of the peripherial, autonomic, and central nervous systems (WHO, 1986). In-depth reviews of the literature regarding several neurophysiological techniques used in neurotoxicology are also available (Rebert, 1983; Benignus, 1984; Dyer, 1985, 1987; Lowndes, 1987; Seppalainen, 1988; Mattsson *et al.*, 1990).

Behavioral methods are available for evaluating the effects of chemical exposures in laboratory animals and in humans. The methods used in laboratory animals evaluate to varying degrees the types of effects reported in humans exposed to neurotoxic chemicals, (i.e., deficits in sensory, motor, cognitive, affective, and autonomic function). The types of methods range vary from those commonly used in screening studies, which involve a number of semiquantitative assessments made by an observer while holding the animal, to the highly sophisticated analysis of sensory thresholds made in psychophysical research. Because these methods are noninvasive, animals can be studied repeatedly throughout almost the entire life span, and at key times before, during, and after exposure to toxic substances. Many of the methods can also be incorporated into other ongoing toxicity studies (e.g., acute, subchronic studies, lifetime carcinogen feeding studies). Moreover, in many cases studies can also be carried out using similar approaches in laboratory animals and humans in order to evaluate the predictive power of animal tests of neurotoxicity. A number of excellent reviews of the literature regarding behavioral measurements in neurotoxicology are available (Gad, 1989; Haggerty, 1989; Moser, 1989; O'Donoghue, 1989; Crofton and Sheets, 1989; Miller and Eckerman, 1986; Peele and Vincent, 1989).

Although experiments in neurotoxicology often employ only a single level of analysis, there are now many cases in which multidisciplinary approaches have been employed successfully. These are too numerous to discuss in detail, but a few examples can be mentioned. Miller and O'Callaghan (1984) used biochemical, morphological, and behavioral approaches to study the developmental consequences of exposure of rat pups to trimethyltin (TMT). Time- and dose-dependent lowering of whole-brain, cerebellar, and hippocampal weight, loss of hippocampal CA3-4 pyramidal cells, and reduction of a synaptically localized protein called synapsin 1 were found along with changes in development of locomotor activity, the acquisition and retention of behavior, and in performance in a radial-arm maze. Thus, there was good correspondence between functional, structural, and biochemical measures. Working with TMT in adult treated rats, Bushnell (1990) demonstrated a high correlation between decreases in accuracy in a spatial memory task and increases in hippocampal glial fibrillary acidic protein (GFAP), a glial protein which increases in response to neural injury. Crofton *et al.* (1990) exam-

ined auditory dysfunction in adult rats exposed to TMT using behavioral, electrophysiological, and morphological indices. Both behavioral and electrophysiological procedures demonstrated high-frequency hearing loss resulting from TMT treatment. Cochlear histology demonstrated destroyed hair cells in the basal regions of the cochlea associated with high-frequency hearing. Similar effects have also been reported recently in guinea pigs (Fechter and Carlisle, 1990). Thus, complementary measurements in two species strongly confirm the ototoxicity of trimethyltin. In each of these cases, the multiple measures provided complementary information which together provided a clear understanding of the structural and functional deficits produced by neurotoxicant exposure.

When complementary measurements yield differing results, it is also possible to gain information regarding the toxicity of the compound in question. For example, Boyes *et al.* (1985) examined the hypothesis that the effects of the formamidine insecticide, chlordimeform, on rat visual electrophysiology were due to its actions as an inhibitor of monoamine oxidase, an effect reported previously in other systems. It was found, however, that although monoamine oxidase was equally inhibited in rats treated with either chlordimeform or the drug pargyline, the visual effects were obtained only after treatment with chlordimeform. This discrepancy between neurochemical and electrophysiological findings strongly indicated that the visual effects of chlordimeform could not be attributed to monoamine oxidase inhibition. This was confirmed subsequently when it was demonstrated that the visual effects of chlordimeform were most likely due to an α_2-adrenergic agonist effect (Boyes and Moser, 1988).

One of the pressing needs in neurotoxicology is for more human data. Both behavioral and electrophysiological procedures have already played major roles in identifying and characterizing human neurotoxicity. Anger (1990) has recently reviewed the use of behavioral methods in human occupational neurotoxicology and Seppalainen (1988) has extensively reviewed the literature regarding neurophysiological approaches to neurotoxicity in humans. Given the growing data base in human behavioral and neurophysiological assessments, it is unfortunately rare for behavioral and electrophysiological investigations to be conducted in the same exposed humans. Such a confluence would substantially increase our understanding of the neurobiological basis of the deficits measured, as well as our level of confidence in the effects produced by any given exposure condition.

When neurophysiological, neurochemical, and/or behavioral procedures are used in conjunction, the complementary information available from the different techniques can provide a great deal of information regarding the toxicity of chemicals. Used individually, each type of approach can yield information regarding the functional or structural consequences of exposure to neurotoxic compounds. Combinations of these procedures with neuropathological or neuroanatomical techniques can provide a good understanding of structure–function relationships. In the past, discussions among neurotoxicologists frequently revolved around which type of approach was most appropriate, or most sensitive, for neurotoxicological assessments. These discussions frequently ignored the fact that different approaches provide qualitatively different types of information. The different techniques are best considered to be complementary. Our understanding of neu-

rotoxicological phenomena is enhanced tremendously by multidisciplinary approaches. A multidisciplinary approach is needed to understand of the cellular and molecular basis of behavior, as well as identify the functional consequences of nervous-system lesions. These approaches provide the seldom realized potential to understand the relationships between cellular and molecular actions of chemicals, effects on function of nerve cells, neural circuits and systems, and ultimately, the behavior of the whole organism.

CONCLUSIONS AND FUTURE DIRECTIONS

Tremendous strides have been made in advancing our knowledge in neurotoxicology. These advances include a better understanding of the types of effects produced by neurotoxic chemicals, including sensory, motor, autonomic, and cognitive effects. Our knowledge has also advanced regarding the types of chemicals that may pose neurotoxic risks. These include a wide array of pesticides, solvents and metals including organometals. Advances have also been made regarding the conditions of exposure that can result in neurotoxicity as well as susceptible populations (e.g., the fetus and neonate). Considerable progress has also been made in developing predictive animal models of human neurotoxicity. Without exception, chemicals first shown to produce neurotoxicity in humans have ultimately been shown to produce neurotoxicity in laboratory animals. The availability of animal models has also led to improvements in our understanding of the mechanisms by which neurotoxic chemicals act. As indicated earlier, research has established important links between functional manifestations of neurotoxicity measured at the behavioral and neurophysiological levels, and the cellular and molecular events underlying these functional changes. *In vitro* techniques have also improved to the point where they have contributed substantially to identifying the mechanisms of action of neurotoxicants. Indeed these improvements have led to recent advances in relating the *in vitro* effects of neurotoxicants to their effects produced *in vivo*.

The above advances notwithstanding, further expansion of knowledge in neurotoxicology depends on progress along several lines of research. For instance, we are far from realizing the full range of effects occurring in humans exposed to neurotoxic substances. The use of standardized neurobehavioral test batteries has begun to reveal more subtle forms of impairment than were appreciated previously. At the same time, standardization has significantly enhanced the prospect of identifying impairments that are reproducible in different experimental settings and countries. Better understanding of the dimensions of human neurotoxicity will also lead to improved animal models, which can then be used to provide a comprehensive account of the cascade of events from initial molecular insult to the ultimate behavioral expression of neurotoxicity.

Research is also needed to improve neurotoxicity screening methods. Current toxicity testing practices that rely solely on cage-side observations of clinical signs have been widely recognized as inadequate for identifying neurotoxic substances. Neurotoxicity screening batteries have been developed in many laboratories and are being used by regulatory agencies in the United States and other countries. The Collaborative Study on Neurotoxicity Assessment sponsored by the IPCS

will provide important data on the reproducibility of neurobehavioral screening tests across laboratories. While such tests represent a considerable improvement over existing (i.e., cage-side) practices, concerns have been raised regarding their ability to provide a thorough account of neurotoxic potential. For example, sensory function is evaluated in a relatively crude manner. Moreover, tests of cognitive function are not included in screening batteries. Future research is needed to ensure that neurotoxic agents producing subtle sensory deficits, or deficits in learning and memory, are detected using screening batteries.

It is widely recognized that no screening battery is perfect. At the same time, however, neurotoxicity screening has advanced to the stage where the use of batteries can and should become commonplace. Widespread acceptance of such batteries will, in general, increase in proportion to their compatability with existing toxicological testing practices. Owing to their noninvasive nature, behavioral tests of neurotoxicity offer particular promise in this regard. A consensus needs to be reached, however, regarding whether compatability should be the sole criterion for judging the adequacy—and consequently the future—of a screening battery. As research advances, it is not inconceivable that subtle neurotoxicity alterations may be found, for example, in the electrical and/or biochemical properties of the nervous system, in the absence of behavioral changes.

Current neurotoxicity screening batteries will be indispensible in identifying new neurotoxic substances long before significant human exposures occur. The batteries are also needed to identify the neurotoxic potential of large volume chemicals for which human exposure is significant. At the same time, however, it will be impossible to evaluate more than a small fraction of the chemicals currently in existence. *In vitro* techniques offer the prospect of broad-scale evaluation of considerably greater numbers of chemicals than is otherwise possible using whole-animal methods. Intensive efforts are therefore needed to determine the utility of *in vitro* techniques in screening chemicals for neurotoxic potential.

Research is needed to improve methods for evaluating human neurotoxicity, develop more predictive animal models, and enhance our ability to screen chemicals for neurotoxicity. These three areas represent key elements needed for developing a comprehensive tiered neurotoxicity testing strategy for collecting *de novo* data on new and/or previously untested chemicals, identifying their mechanisms of action, and making accurate predictions regarding human risk. Multidisciplinary investigations will be critical to the ultimate success of this venture. Such a strategy will be vital to the protection of human health and the environment. Its creation, however, will depend heavily on both continued support for research worldwide, and in forging a consensus throughout the scientific community regarding the collection, analysis, and interpretation of neurotoxicity data.

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EURONEST: A Concerted Action of the European Community for the Study of Organic Solvents Neurotoxicity[1]

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EURONEST, a concerted action of the European Community with the participation of most EC and some COST countries, is a project aimed at studying the epidemiological impact of CNS effects from protracted exposure to industrial chemicals in the participating countries. After a review of current knowledge, the paper examines the structure and detailed aims, i.e., the qualitative and quantitative study of exposure to neurotoxic chemicals, the performance of an investigation to contribute to the controversial issue of organic solvents CNS toxicity, the definition of the solvent-induced psychoorganic syndrome as well as its epidemiological relevance, and the influence of exposure on the quality of life, in particular on aging. The primary target population consists of workers with long-term exposure to toluene in the printing industry to establish CNS effects and no-effect level; depending on local priorities mixed solvents exposure can also be investigated. The study design is an ambidirectional cohort and sample size requirements are given. The approach as to the methods is not to choose at once among the available neurobehavioral batteries, but to produce a register of the 20–25 tests more commonly used in the different participating laboratories, in order to examine their validity, sensitivity, and reliability. From this exercise, a new more flexible battery is expected to be more effectively applicable under different conditions. Criteria for clinical diagnosis of the solvent-induced psychoorganic syndrome are given in order to be utilized in the investigation of the prevalence rates of these disorders included in the concerted action's program.

INTRODUCTION

In the framework of the Fourth Medical and Health Research Programme, the Commission of the European Communities has been promoting multicenter studies structured as concerted actions, dealing with major scientific issues of epidemiological research and possibly with the participation of teams from all the 12 member states. In this context, epidemiological investigations in occupational and environmental neurotoxicology were absent until 1990 when a 2-year project was approved as a preparatory activity for a concerted action, a comprehensive program to be developed in several years for the study of the epidemiological relevance of chronic disorders of the central nervous system (CNS) due to exposure to industrial chemicals.

Neurotoxicity from chemical agents is considered a worldwide major public health concern. A search of the international literature from 5 common databases (Juntunen, 1989) shows that, during the last 10 years, 5000–6000 articles were published in this field, ranging from basic neurosciences to social sciences. Most studies regard toxicity to the peripheral nervous system (PNS), while only about 10% concentrate on CNS effects. As to the categories of chemicals, pesticides, metals, and organic solvents were the main focus of research. Data regarding the

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number of workers at risk of developing neurotoxic disorders are scanty; however, estimates were made in the United States where the population exposed to the major categories of neurotoxic chemicals amounts to several millions. Because of the comparable type of socioeconomic development, it is reasonable to hypothesize that figures of the same magnitude may be expected in other industrialized countries. On these grounds, also in the EC countries, many millions of workers may be expected to be at risk.

Another crucial aspect of the current modes of exposure is that, while in industrialized countries there is a general tendency toward a reduction of the airborne concentrations at the workplace, increased levels of toxic chemicals are being found in the general environment, due also to the spreading of pollution outside worksites. This raises the question of the potential involvement of the general population with thus far unknown effects.

The impact on health of these issues is expected to be relevant not only considering the high numbers of people occupationally exposed, but also because it was suggested that irreversible brain damage may occur as a sequel of exposure to solvents and to other chemicals and early disability may ensue (Arlien-Soborg *et al.*, 1979; Juntunen *et al.*, 1980; Mikkelsen, 1980). In fact, there are 60–100,000 chemicals in commerce today and for some 750 chemicals there is evidence of direct or indirect nervous system effects and over 2000 new chemicals of unknown neurotoxic potential are yearly introduced into the market. On these grounds, the U.S. National Institute of Occupational Safety and Health considers neurotoxicity disorders as one of the 10 major occupational problems in the United States (Anger, 1990). Furthermore, because of the potential interaction between the CNS effects of toxic exposure and aging, the possible impact of exposure to toxic chemicals on the cerebral functions is increasingly relevant because of higher life expectancy in the industrialized countries. Specifically, it is estimated that 60 years from now the proportion of the elderly will be nearly twice as much as that of the 1980s with a threefold increase of Alzheimer's disease (Spencer, 1990a) and similar figures are to be expected in the other industrialized areas.

EFFECTS OF LONG-TERM OCCUPATIONAL EXPOSURE ON THE CNS FUNCTIONS

The literature on the solvents effects on the CNS can be divided into two periods. In the 1970–1979 decade, there was a prevalence of experimental studies focusing on the acute effects of various solvents. These effects were shown to be of a narcotic type inducing the reduction of the activation levels and consequently affecting those performances which require quick response such as reaction time, eye–hand coordination, and manual dexterity. Most of these studies pleaded in favor of a quick reversibility of symptomatology when exposure was discontinued (Gamberale and Hultengren, 1972, 1973, 1974, 1975; Gamberale *et al.*, 1975, 1976, 1978; Konietzko *et al.*, 1975; Matsushita *et al.*, 1979; Putz *et al.*, 1979; Salvini *et al.*, 1971a,b; Stewart *et al.*, 1970, 1973; Cherry *et al.*, 1983). In the same period, animal studies confirmed that solvents could induce peripheral neuropathy and mild toxic encephalopathy shown not only by behavioral and neurophysiological alterations but also by irreversible cellular changes in the brain and by an increase of brain protein levels known as indicators of irreversible brain damage (Bignami and Dahl, 1974; Persson *et al.*, 1976; Haglid *et al.*, 1981, 1985; Schaumburg and Spencer, 1976). Subsequently, studies carried out on workers occupationally ex-

posed to organic solvents have progressively increased and presently represent the main bulk of solvents literature. The information issuing from these studies indicates in personality and mood changes and poor performance on psychometric testing the target areas of solvents neurotoxicity (Lindstrom, 1973, 1980; Hanninen *et al.*, 1976, 1978; Axelson *et al.*, 1976b; Cassitto *et al.*, 1978; Elofsson *et al.*, 1980; Flodin *et al.*, 1984; Gregersen *et al.*, 1984; Cherry *et al.*, 1985; Cassitto and Gilioli, 1985; Valciukas *et al.*, 1985; Ekberg *et al.*, 1986). All the above-mentioned studies have shown signs of brain dysfunction, although in different degrees, supported by statistically significant differences between exposed subjects and the referents. However, results are not homogeneous through the different investigators. A certain number of studies, mainly carried out in the Nordic countries, have reported a high occurrence of the so-called "chronic solvents syndrome" in the solvent utilizers possibly evolving toward a dementing disorder (Axelson *et al.*, 1976; Harkonen, 1977; Arlien-Soborg *et al.*, 1979; Mikkelsen, 1980; Olsen and Sabroe, 1980; Juntunen *et al.*, 1980; King *et al.*, 1981; Spencer and Schaumburg, 1985; Mikkelsen *et al.*, 1985; Linz *et al.*, 1986; Gregersen *et al.*, 1987). These data have been confuted by other investigators on methodological and diagnostic grounds (Errebo-Knudsen and Olsen, 1986, 1987; Van Vliet *et al.*, 1987; Triebig *et al.*, 1987, 1988; Gade *et al.*, 1988; Bruhn *et al.*, 1981). Further uncertainty derives from the somewhat different methodological approaches. In fact, although the diagnostic tools used in the different studies investigated the same functions, a direct comparison of the results is questionable.

The most frequently observed signs and symptoms concern the emotional changes, the reduction of concentration, attention, and cognitive abilities as well as memory deficits and impaired visuomotor abilities. All these dysfunctions support the picture of an early aging process in the affected subjects.

Other investigators have focused their efforts on another important aspect of solvents neurotoxicity, namely the reversibility of the observed effects. Data are controversial also in this domain (Lindstrom *et al.*, 1982; WHO, 1985). As a consequence, national and international agencies such as the World Health Organization, the Commission of the European Communities, the International Commission on Occupational Health, and the U.S. National Institute of Occupational Safety and Health have convened meetings and workshops to categorize the solvent-induced CNS disorders, foster the harmonization and standardization of methodologies, and support multicentered efforts aimed at a better insight into possible effects and their reversibility. As a result of these meetings pointing to the numerous existing gaps of knowledge, a certain number of joint international activities have been planned and some are in progress.

According to the WHO core protocol "solvents and the central nervous system" (1989), limitations and weaknesses of the previous studies regard (1) inadequate study designs not taking into account workers removed from exposure, (2) the role played by recent exposure which may account for acute confounding effects, (3) the individual susceptibility, (4) the diversity of methods, and (5) the effects of numerous confounders such as age, sex, primary intellectual abilities, and alcohol consumption. Therefore, it is necessary to verify with well-designed studies the existence of CNS changes, to determine whether they are chronic or reversible, and to establish whether they have any bearing on the brain aging process. As to this last issue, the combined action of natural neuronal deficiency and that induced by neurotoxins was proposed as a putative mechanism of sub-

clinical and overt neurologic diseases. Since this process has been shown to be dose dependent (Calne *et al.*, 1986), it can be hypothesized that occupational exposure to industrial chemicals known as potential neurotoxicants may interact with the brain aging process.

STRUCTURE AND AIMS OF THE CONCERTED ACTION

The structure and the aims of the concerted action were defined at the plenary meeting of the participating teams held in May 1991 in Segovia, Spain. The following EC countries expressed their wish to take part in the final project: France, Germany, Greece, Italy, the Netherlands, Portugal, Spain, and the United Kingdom. Other EC countries, Belgium, Denmark, and Ireland, were present in the previous meetings of the group and will again be invited to join the activities. In addition, Austria, Finland, and Sweden, countries not belonging to the European Community but with formal agreements for scientific research (COST countries) are likely to participate in the concerted action. Dr. Renato Gilioli from the Institute of Occupational Health in Milan, Italy, was appointed project leader and Professor Claes-Goran Westrin, from Uppsala University, Sweden, was appointed liaison officer.

As a general rule of all the concerted actions, the project is funded by the Commission of the European Communities for the coordination costs, while the actual studies are to be supported by individual countries.

The objectives of the concerted action are the following:

1. The qualitative assessment in the participating countries of the different types of exposure to neurotoxic chemicals, with special reference to organic solvents.
2. The assessment of the amount of the populations occupationally exposed to neurotoxic chemicals and in particular to organic solvents in the participating countries.
3. The undertaking of a scientific investigation to be carried out in the participating countries to contribute to the resolution of the controversial issue of the chronic CNS effects due to prolonged exposure to organic solvents, with a particular emphasis on the establishment of the no-effect level. In fact, according to some investigators (Spencer, 1990) this issue is still quite controversial.
4. The definition of the CNS solvent-related clinical entity.
5. The study of the epidemiology of such disorders in the working populations of the participating countries.
6. The study of the impact of the solvent-induced neurobehavioral changes on the quality of life of the individual. In particular there is a suspicion that such changes may interact with the brain aging process by accelerating its development. This is crucial since it is known that only a very slight acceleration of brain aging can cause deleterious consequences on functional mental age (Weiss, 1980).

As specified earlier, the concerted action is being preceded by a 2-year preparatory activity aiming at the finalization of protocols, the performance of common exercises, and of small-scale pilot studies. In this context, a plan for exchange of investigators and materials within the scope on the concerted action was included in the program and was at least partially implemented. The outcome of the preparatory activity will be published with a view to presenting not only the scientific results but also to pointing out the mechanisms activated in international scientific cooperation as well as the main scientific and organizational difficulties encountered.

STUDY PROTOCOL

Types of Exposure

Professor Christer Edling from the Uppsala University, Sweden, chaired the group of experts dealing with exposure and epidemiology issues.

Two options are open depending on the aims which are twofold, i.e., the primary goal being the resolution of the scientific questions previously formulated and the second goal being the educational need for information exchanges among the participating countries.

As to the first issue, the decision was made to carry out an epidemiological investigation aimed at determining chronic CNS effects of toluene exposure in the printing industry. The reasons for this choice were the following:

1. The advantage of single substance exposure which would make results easier to interpret as compared to studies of mixed exposure.
2. The possibility of using both environmental and biological exposure assessment.
3. The availability of animal data which propose a useful model for the mechanisms of toluene's neurotoxicity.

A multicenter study using a core set of methods for measuring both exposure and effects may represent the basis for the determination of no-effect level. This approach implies a high level of professionalism and a high degree of standardization of the methods.

As to the issue more directly related to the need for more extensive exchange of information within the participating countries, it became evident that the choice of the exposed population depends to a large extent on the local possibilities in the different countries. This approach leaves the options of studying single substance exposure or mixed solvent exposure according to local priorities, but reduces the possibility of lumping data from the different countries.

Epidemiological Model

Whether the first or the second approach is chosen, the proposal was to adhere to the suggestions of the previously cited WHO core protocol on solvents and the central nervous system.

The ideal design would be a longitudinal study of a cohort of young persons at the beginning of their working life. However, the follow-up period would be extremely protracted, thus making the study unlikely to succeed. Therefore, the design should consist of a retrospective cohort study including workers with an exposure duration of 10–15 years. The first examination will be cross-sectional. Therefore, the study will examine the prevalence (cross-sectional) of abnormalities as associated with the exposure history (longitudinal) of the subject, thus constituting an ambidirectional cohort.

Sample Size Calculation

In our previous experiences which served as a basis for sample size determination in the WHO core protocol (WHO, 1989), in calculating the sample size needed to achieve a desired level of study power (90%) neuropsychological tests were considered whose background variability was known. For each test the least departure of interest from the reference value was assumed to be 10% (values obtained from 250 male subjects covering the same age intervals of the study

population and living in the same geographical area). Given the direction of the department of interest, a one sided critical point (one-tailed 95th percentile) was used. For calculations, a method suggested by Snedecor and Cochran was used. Results for 11 neuropsychological tests gave samples size requirements ranging from 100 to 244. Similar calculations for critical neurophysiological tests (BEP) yielded results of 216 and 222, respectively. Thus, a sample size between 200 and 250 seemed theoretically advisable. The approach used was rather conservative to ensure ability to detect small departures from the reference mean. Therefore, the size of the cohort should be of no less than 100 persons in each country in the 30–55 age ranges. A reference cohort matched for sex, age, and education must be established and investigated in the same way as the exposed cohort.

Neurobehavioral Battery

Professor Francesco Gamberale from the Swedish National Institute of Occupational Health chaired the group of experts dealing with neurobehavioral methods, while Dr Anders Iregren from the same Institute and Dr Jacob Hooisma from TNO in The Netherlands coordinated the group of psychometrics experts aimed at choosing the battery to be used in the study which should investigate at least memory, reaction time, visuospatial coordination, and verbal competence. These functions are partially covered on some widely employed neurobehavioral batteries and some of these, namely the WHO NCTB, benefits by a wide standardization program and guidelines for its administration and interpretation. However, the decision made by the psychometrics experts of our concerted action was that it is inappropriate, at this stage, to choose one test battery for all the participating groups, since the tests eventually selected should reflect special needs and resources peculiar to the study to be conducted as well as to the study participants. The unwillingness to adhere at once to one of the existing batteries stems from previous experiences according to which the success in the implementation of the neurobehavioral study largely depends on the availability of a substantially homogeneous background regarding the features of both the study and the participants. As an alternative, the following approach was adopted: the psychometrics experts of our concerted action agreed to draw on their experience in using a number of psychological tests, both computer and paper-and-pencil administered, to produce a register of the 25 most used tests. This register would provide information additional to that normally available in published papers. Specifically, it would include descriptive statistics, data on reliability, and validity, as well as a description of the implementation of the tests, the populations involved, and the influence of the effects. It is intended that this register should aid those without prior experience in this field to assess which tests would be useful and practical from their point of view. It would also enable the experts in psychometrics to gain an overview of the usefulness of the various tests in terms of different criteria such as validity, reliability, and ease of administration. The ultimate objective of this exercise is to enable the psychologist to make a final choice of tests that will satisfy the needs of the study as well as of the participants. The consensus of opinion is that this more flexible approach will increase the chances of the participating groups being able to adhere to a common test battery. This process will be carried out within the next few months and the final selection of the tests will be made soon after.

The use in all participating countries of more refined neurophysiological and

neuroradiological techniques is questionable and should be weighed against feasibility and in terms of cost–benefit. However, if adequate resources are available, brain-evoked potentials and neuroimaging techniques could be used.

Diagnostic Criteria

Dr. Juhani Juntunen from Finland chaired the group of experts dealing with diagnostic criteria.

Because one of the aims of the concerted action is the definition of the prevalence rates of the solvent-induced psychoorganic syndrome, the establishment of firm diagnostic criteria is crucial. As a general principle, a distinction has to be made between two problems which are parallel, but which require an entirely different approach:

1. The diagnosis of individual cases in clinical settings; it is obvious that in this case, everything possible for establishing a diagnosis has to be made; in-depth clinical examination with all the available ancillary neurophysiogical (evoked potentials, brain mapping) and neuroradiological (CT, NMI, PET, CBF, etc.) tests must be performed;
2. For field studies settings, simple methods mainly pertaining to the field of neuropsychology should be used as discussed earlier.

CRITERIA FOR THE CLINICAL DIAGNOSIS OF PSYCHOORGANIC SYNDROME AND EFFECTS

The diagnosis of psychoorganic syndrome can only be made when a coherent set of changes in symptoms and objective findings is demonstrated in a subject with verified relevant exposure to solvents.

1. Verified relevant exposure to solvents.

The qualitative and quantitative assessment of the working environment as well as the length of exposure must be taken into account; usually years are considered to be necessary before signs of chronic effects appear; however, it is felt that the establishment of a precise number of years of exposure to develop solvent-induced effects is inappropriate because many factors such as age, individual susceptibility, types of solvent exposure, and working condition, play a role in their appearance.

2. Clinical picture of the nervous system disturbance.

—Subjective symptoms from the subject or from others (family, co-workers, supervisors); the most common are headache, memory disturbance, tiredness, dizziness;

—neuropsychological findings; it is crucial to establish valid norms and cutoff points; generally, abnormal functioning is shown by a decrement of the performance below the lower 5% distribution level for a normal and comparable population;

—changes in sensitive neurophysiogical tests with the same cutoff criteria specified for neuropsychological findings;

—minor signs of impairment of the vestibulocerebellar system.

—the possibility of reasonably excluding other known causes of encephalopathy (brain concussion, history of excessive alcohol intake, other exogenous factors, etc.).

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Epidemiological and Clinical Features of Minamata Disease[1]

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Minamata disease is methyl mercury intoxication from fish contaminated by a chemical factory in Minamata city. Based on the results of our regional survey, cardinal clinical features of the disease were clarified by a multivariant analysis of all symptoms in inhabitants in the polluted area. The clinical features were found to be essentially the same as those of Hunter Russell syndrome; however, some additional symptoms were also found. Those symptoms are influenced by many factors, such as degree of exposure and duration of pollution. The disposition of each inhabitant also plays a role in clinical manifestation. This analysis contributes to a correct individual diagnosis and to the correct estimation of patients in polluted areas. Long-term studies also uncovered a few inhabitants who claimed to have begun to experience some neurological symptoms after pollution ceased. These symptoms were attributed mainly to aging. As many inhabitants with mild neurological complaints were not easily diagnosed, a questionable borderline group should be postulated for social settlement of Minamata disease. The characteristics of Minamata disease are discussed and compared to cases of methyl mercury poisoning in other parts of the world.

INTRODUCTION

Since 1956, methyl mercury pollution has been found near Minamata city in Kumamoto prefecture. This pollution was discharged from a chemical factory in Minamata city. The pollution was so widespread that the number of victims increased to more than 2000 in Kumamoto and Kagoshima prefectures. Since 1974 much effect has been undertaken to elucidate the pathomechanism of the disease and to develop a new treatment under the sponsorship of the Environment Agency of the Japanese government (Environmental Agency, 1975) (Table 1).

QUANTITATIVE DIAGNOSTIC PROCEDURE

The diagnosis of Minamata disease is usually not difficult in typical and severe cases, but it is not always easy to diagnose in mild cases since the health of those affected ranges from severly damaged to healthy. Because of this difficulty, it is important to decide where the line should be drawn in recognizing Minamata disease. This problem should be settled only on a medical basis. In actual situations, an objective, not subjective, diagnosis should be made, so that everyone can agree with the conclusion based on reasonable evidence (Igata, 1974). To diagnose Minamata disease objectively, a quantitative method was proposed by us, using multivariant analysis based on all information obtained in the population survey performed in 1973 and 1974 in the polluted area of Kagoshima prefecture, which neighbors Minamata city with a population of 80,000. Independent of individual diagnosis, by which many cases with Minamata disease were newly found, all data for these inhabitants were analyzed by multivariant analysis. As

[1] Presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

TABLE 1
PATIENTS WITH MINAMATA DISEASE (JUNE 1990)

Prefecture	Diagnosed	Rejected	Pending	Not examined
Kumamoto	1760	8234	844	1859
Kagoshima	474	2780	50	320

the first step in a population survey, patients with any neurological symptoms indicating Minamata disease were identified from the survey answers; the response rates were higher than 97%. After a simple neurological examination as the second step, a third precise neurological examination was undertaken in patients showing any indication of neurological symptoms. This took 2 hr for each patient. Thereafter all information on this third extracted group was analyzed by principal-factor and discriminant analysis. As a result, a group with Minamata disease was found, as shown in Fig. 1, differentiated from nonaffected inhabitants. From this diagram, a reasonable dividing point between those with Minamata disease and the nonaffected group would be 9.13.

If A in Fig. 1 is made the dividing point, all Minamata disease patients will be included with many erroneously diagnosed normal inhabitants. If B is made the dividing point, all patients are definite Minamata cases but some of the left side of this point are erroneously discarded. Therefore, point D (9.13) was settled on as the most reasonable dividing point, to minimize misdiagnosis. Using point D to separate those with Minamata disease from healthy persons, the ratio of erroneous diagnosis is kept to less than 2.5% of individual diagnoses. Using a two-dimensional diagram, each case can be checked as to whether the diagnosis is correct. Seen from diagrams of inhabitants in each area by principal analysis as in Fig. 2, the number of cases suspected of having Minamata disease increases with distance from Minamata city (see also Table 2).

In addition, it became possible to determine which symptoms should be included among those of Minamata disease. The results made clear, that symptoms of cervical spondylosis, arteriosclerosis of the retina, or systemic hypertension

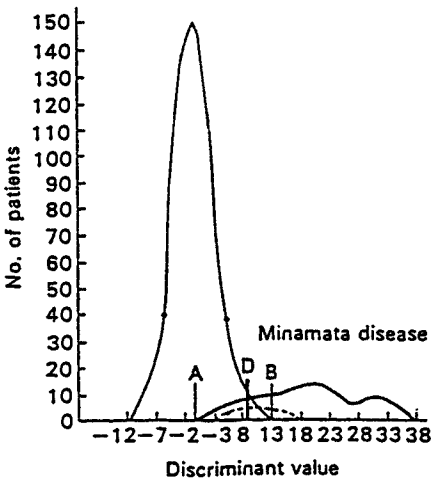

FIG. 1. Histogram of discriminant values.

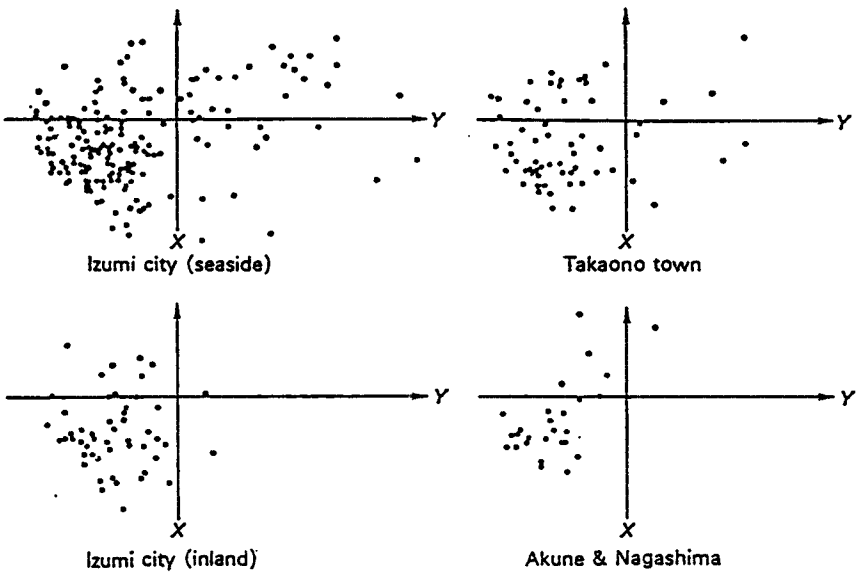

FIG. 2. Distribution of patients in various areas. *X*, factor I; *Y*, factor II (principal analysis). In parallel to the distance from Minamata city, the groups toward the right disappear.

are not related to those of Minamata disease; on the other hand, polyneuritis (Fig. 3), visual field constriction, cerebellar ataxia, and others have a close severity–response relation to Minamata disease. Thus some new symptoms in addition to those of the Hunter–Russell syndrome, for example, anosmia and hyperrefexia, were found.

Thus, this diagnostic method is the most reliable one, if symptoms are correctly identified. Still there are some minor disadvantages in this method; e.g., epidemiological conditions, which are not easy to express quantitatively, are not considered. In diagnosing Minamata disease, there may be errors originating from the erroneous confirmation of each symptom, because the symptoms sometimes fluc-

TABLE 2
MISDIAGNOSIS OF MINAMATA DISEASE

	Minamata disease	Non-Minamata disease	Not decided
		1. Based on 52 items of information	
Minamata disease	37	0	5
Non-Minamata disease	6	228	2
Misdiagnosis: 6/236, 2.5%			
		2. Based on 24 items of information	
Minamata disease	36	4	6
Non-Minamata disease	4	225	1
Misdiagnosis: 8/230, 3.5%			
		3. Based on 12 items of information	
Minamata disease	34	7	8
Non-Minamata disease	7	223	2
Misdiagnosis: 14/232, 6.0%			

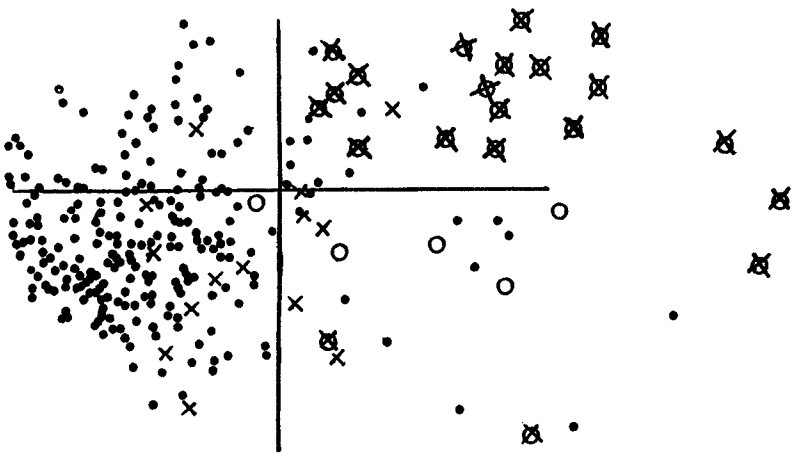

FIG. 3. Sensory polyneuritis and Minamata disease. O, Minamata disease; X, glove and stocking-type polyneuropathy.

tuate from day to day or from morning to night, even within a day to a slight extent. In addition, there are psychological factors in patients, especially those who are eager to be compensated for being kept waiting for a long time for diagnosis. Needless to say, these errors are not caused by this method, but are inevitable in any study of this kind. Concerning the importance of each symptom contributing to the correct diagnosis of Minamata disease, the quantitative scores for each symptom (discriminant values) were calculated on the basis of our own statistical analysis, which is shown in Table 3. These scores can be regarded as the quantitative criteria for Minamata disease, valid for methyl mercury poisoning under one mode of pollution. It is therefore not always applicable to organic mercury intoxication in other areas, where the mode of pollution is different. Through our experience, we postulate that there are borderline inhabitants, who cannot be diagnosed as definitely having Minamata disease, but still have complaints partially compatible with those of Minamata disease. This proposal is now being adopted as an administrative countermeasure. In fact, social settlement is being decided on the basis of the proposal, except for some cases which are now on trial after being rejected. This countermeasure will be suitable for evaluation of any health impairment by pollution.

TABLE 3
DISCRIMINANT VALUES OF MINAMATA DISEASE

Clumsy finger–nose test	5.02
Clumsy movement of tongue	4.13
Perioral dysesthesia	2.68
No coarse nystagmus	2.66
Abnormal ocular movement	2.53
Weakness	2.25
Visual field constriction	2.02
Dysesthesia of glove–stocking type	0.61
Anosmia	0.30
Hearing loss	0.30

EPIDEMIOLOGICAL INFORMATION

Concerning epidemiological information contributing to the correct diagnosis, the amount of fish intake and mercury concentration in blood, hair, and urine are useful in diagnosing acute intoxication for chronic Minamata disease, which persisted after the pollution was stopped, new procedures to indicate past contamination were devised; e.g., the measurement β_2-microglobulin in urine was found to parallel the mercury content in the hair during the period of pollution (Fig. 4), and the grade of past contamination can be estimated from it. However, in the present patients, in whom contamination ceased 20 years ago, such procedures are not useful.

The excretion of mercury after the administration of a chelating agent is also helpful in estimating past contamination (Fig. 5). A history of residency in polluted areas, a career as a fisherman, and food qualities suggest exposure to a high degree of pollution. In congenital Minamata disease, the content of methyl mercury in the umbilical cord, which is usually preserved by Japanese custom, helps to indicate the severity of pollution at the time of delivery.

IDENTIFICATION OF EACH SYMPTOM

There is no available pathognomonic test to indicate Minamata disease such as the TPHA test for syphilis. The diagnosis should be based on a characteristic combination of symptoms. Therefore, it is very important to identify each symptom; in other words, it is very important to confirm each symptom objectively and correctly.

Peripheral Neuropathy

Peripheral neuropathy is one of the cardinal symptoms of Minamata disease. Sensory impairment of the extremities is of the glove–stocking type, sometimes with perioral dysesthesia (Hamada, 1981). There are practically no cases of Minamata disease without peripheral neuropathy, although some rare exceptions have been reported. Evidence of peripheral neuropathy is manifested by morphological changes in the peripheral nerves, both on autopsy and in biopsy. The characteristics of pathological involvement of peripheral nerves in Minamata dis-

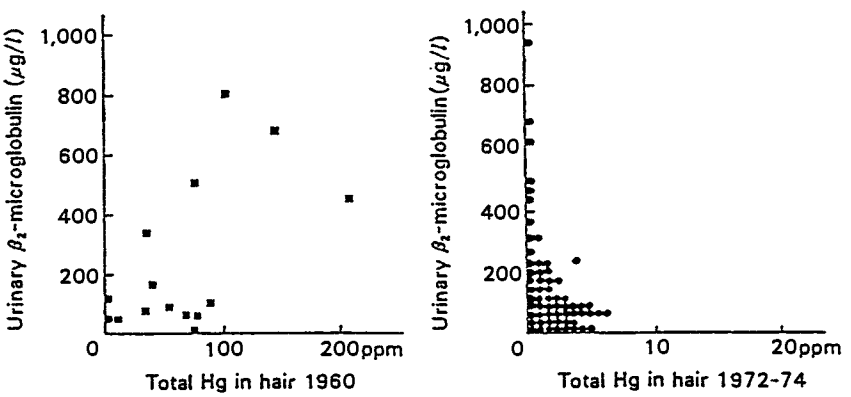

FIG. 4. Relationship between total Hg in hair and urinary β_2-microglobin.

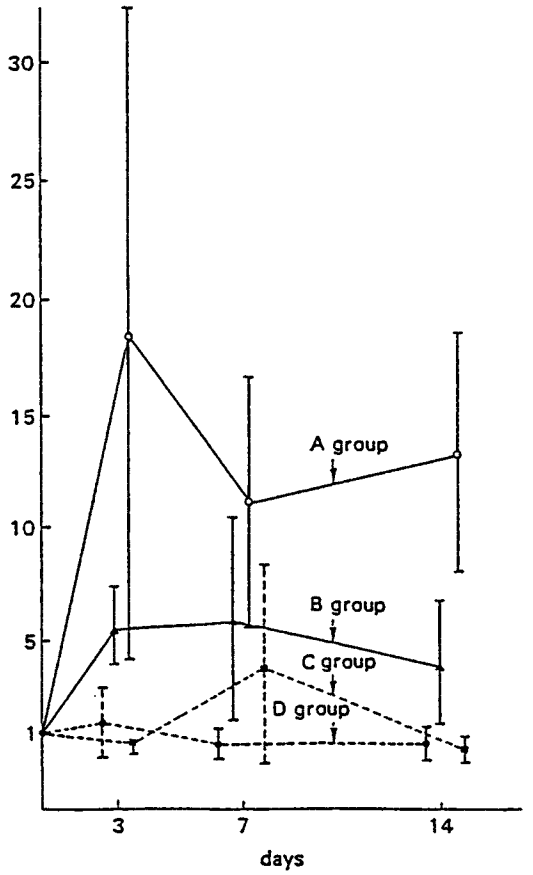

FIG. 5. Excretion of total Hg after administraiton of tiopronin. A, hair content of Hg was higher than 15 ppm in the past; B, hair content of Hg was lower than 15 ppm; C, placebo; and D, without any drug.

ease are the general loss of myelinated fibers with a relative increase in small-sized myelinated fibers which can be regarded as the result of regeneration (Fig. 6).

A typical histogram of myelinated fiber diameters in a patient with Minamata disease is shown in Fig. 7. The disturbance of peripheral autonomic nerves can also be confirmed by loss of unmyelinated fibers and autonomic dysfunctions such as low skin temperature and low sweating especially in distal extremities. In thermography (Kamitsuchihashi, 1985), the general temperatures are low and the recovery from low temperature is slower than that in normal age-matched controls (Fig. 8). Deep tendon reflexes are usually diminished or absent, although exaggerated ones can be confirmed in 40% of the patients. The motor and sensory conduction velocities are usually delayed in accordance with the severity of the disease, the sensory one being more severely affected, although there are no patients in whom conduction velocities are normal even when there is numbness (Fig. 9).

Sensory impairment does not always mean diminished sensation but sometimes the subjective complaints of numbness, a tingling sensation, or hyperethesia. Recently somatosensory-evoked potentials have come to be used to check lesions

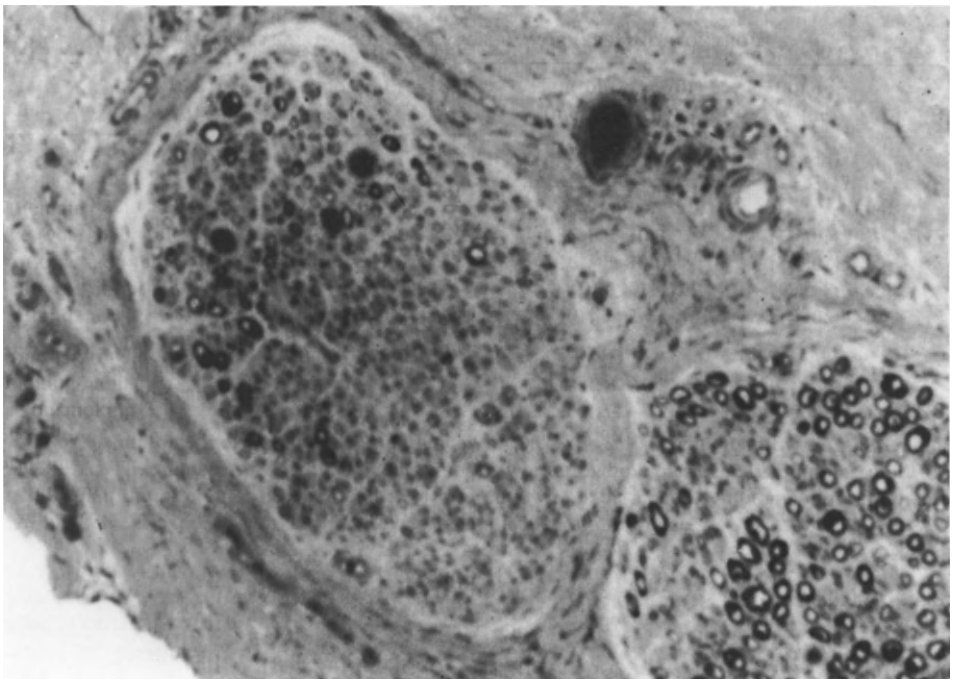

FIG. 6. Typical biopsied sural nerve in a patient: 35-year-old male with Minamata disease.

in the brain (Arimura, 1985). This method assumes that lesions in the central nervous system might be mainly responsible for the sensory impairment seen in Minamata disease.

Cerebellar Ataxia

Although ataxia can be identified by the usual neurological examination, it is sometimes not easy, especially in borderline cases, to identify the ataxia objec-

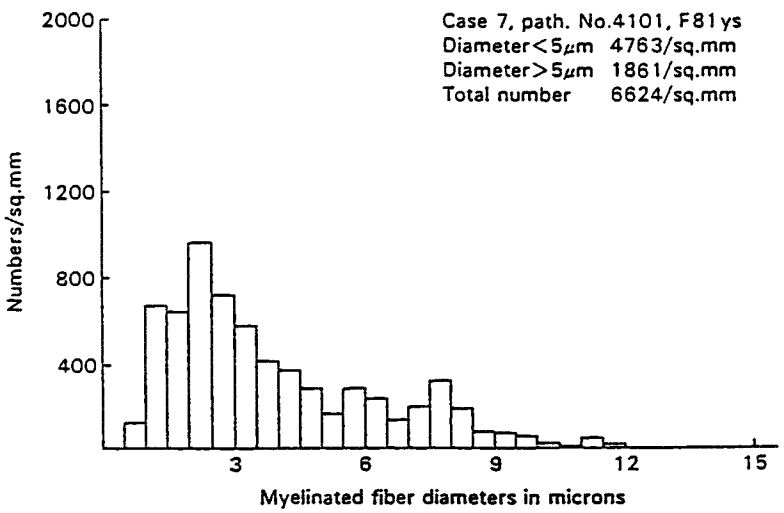

FIG. 7. Histogram of the biopsied sural nerve fibers in a patient with Minamata disease.

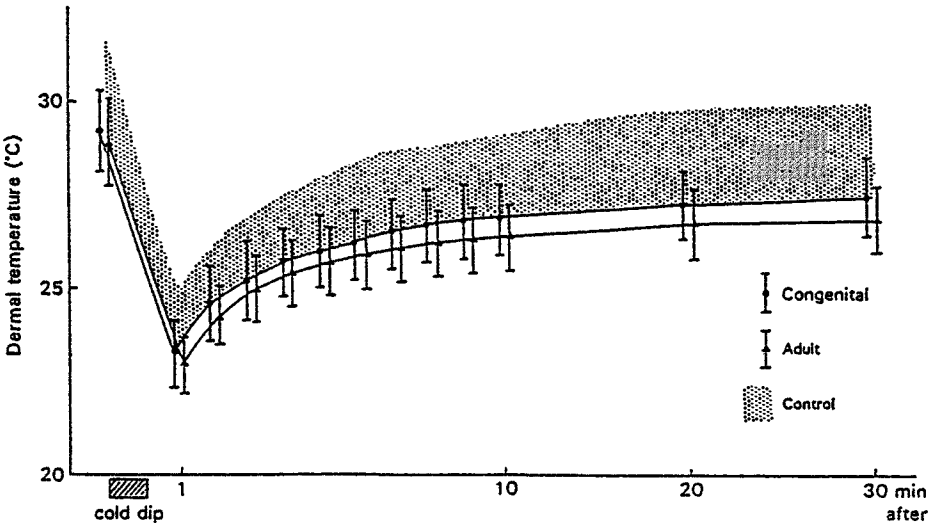

FIG. 8. Cold dip test in Minamata disease.

tively and quantitatively. For the objective evaluation of cerebellar ataxia, many procedures are now being applied, including use of the gravidimeter, objective registration of the finger–nose test, analysis of voice, writing, and gait (Hamada, 1980). In the results, clumsy movement is not directly related to cerebellar ataxia but to concomitant rigidity, tremor, weakness, spasms, etc. A CT scan is also useful in evaluating the morphological changes in the central nervous system, including the occipital lobe, cerebellum, and cerebrum (Fig. 10).

Comparing the age-matched controls, some quantitative scores of Minamata disease based on CT findings are possible, although cerebellar atrophy due to other diseases, cannot be excluded. Using many parameters for the cerebellum, the cerebrum, and the brain stem in the CT scan, the differential diagnosis of

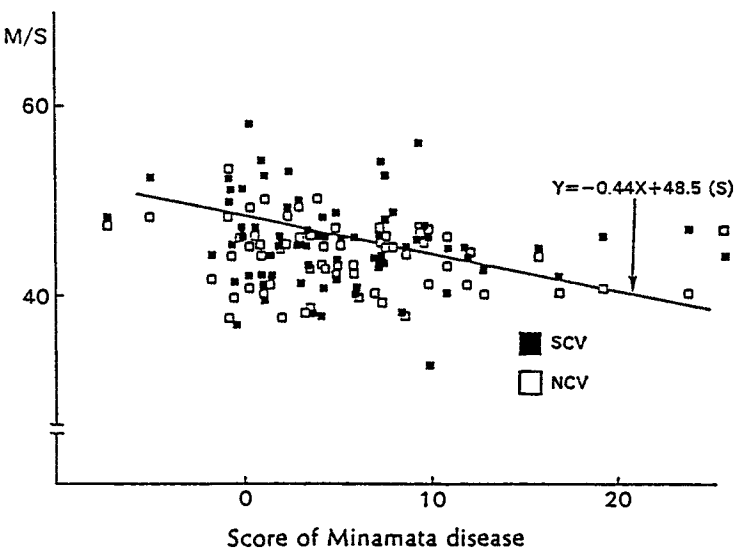

FIG. 9. Index of Minamata disease and NCV.

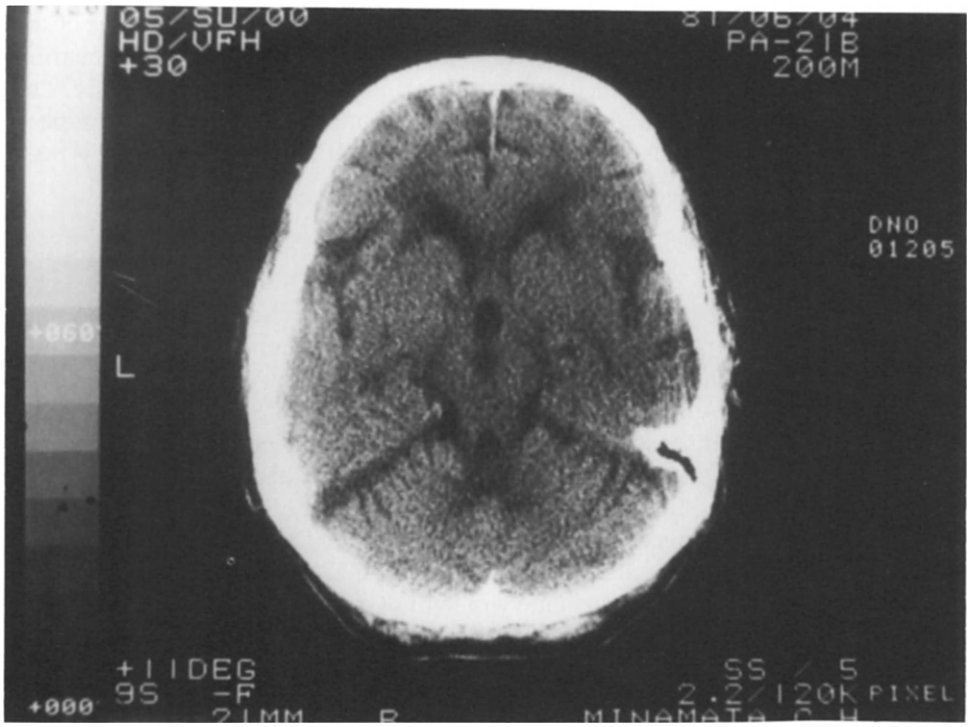

FIG. 10. Cerebella atrophy in a patient with Minamata disease (65-year-old male).

Minamata disease can be made with an accuracy higher than 80%. Akinesia-like slowness of movement can also be observed.

Tremor

Tremor can also be objectively evaluated by computer analysis. The frequency and amplitude of tremor in this disease is rather characteristic, compared to tremors of other diseases such as Parkinson's disease or hyperthyroidism.

Gait

Gait disturbance is also rather common in Minamata disease and can be analyzed with a special precise gait analyzer using a computer. The characteristic gait in Minamata disease, which includes elements of weakness, ataxia, rigidity, and/or apasticity, partially influenced by numbness, cannot by itself be differentiated from other diseases with certainty.

Mental Deterioration

In severe cases of Minamata disease, mental deterioration is inevitable and mild mental symptoms such as erroneous calculation, disturbed memory, and character change can be found in mild cases. However, severe dementia like Alzheimer's disease could not be found in the affected patients.

Ophthalmological and Otological Symptoms

Even in a routine neurological examination, characteristic signs of Minamata disease, such as visual field constriction and disturbed ocular movement, can be

detected. Abnormal visual evoked potential can be an objective parameter of this disease. From the otological point of view, hearing loss or impairment of hearing, discriminative understanding of voice, and hearing fatiguability are specific. Disturbance of equilibrium is also characteristic of Minamata disease. These aspects are discussed in the corresponding sections for each. Abnormal auditory brain stem response is also suggestive of Minamata disease.

CLINICAL COURSE

In any intoxication, the symptoms usually improve gradually after contamination ceases. However, there are some patients with Minamata disease in whom the symptoms worsened after contamination ceased or in whom the symptoms appeared a few years later (Shirakawa, 1979; Igata 1978, 1975). This is called late-onset Minamata disease. Generally speaking, late onset is difficult to understand. Nevertheless, such cases do exist and some of them are verified on autopsy. This late-onset Minamata disease might be accounted for in the following ways: (1) aging may affect latent Minamata disease; (2) long-lasting but slight damage may be due to a minimal amount of organic mercury remaining in the brain, although this is difficult to believe in light of many data on the accumulation and metabolism of ingested mercury; and (3) there may be psychological conditions in people who are eager to be compensated. We have no definite evidence for any of these, but it is an important problem that needs to be solved on a medical and social basis. Roughly speaking, such late onset and late progression are limited to few patients and the peak was reached before 1975, incidence now being rare.

OTHER NEUROLOGICAL SIGNS AND SYMPTOMS

Some other neurological signs and symptoms of Minamata disease have been clarified by a study group sponsored by the Japanese government over the past years (Igata, 1986). These signs include anosmia, loss of taste, abnormal latency in evoked potentials, tonic painful spasm, paroxysmal fainting, and bladder disturbance. Among the inhabitants of the polluted areas, there are some patients with a total loss of superficial sensation (Igata, 1981) and/or total ophthalmoplegia with reflectoric eye jerks (Arimura, 1980). This peculiar symptom might be explained as a psychological reaction. In addition, no abnormal evoked potentials are confirmed in these cases. No relation to the severity or to the high incidence of typical symptoms was found. However, it might be a symptom due to a peculiar effect on the brain due to organic mercury, since there are some in whom no other psychological or hysterical reactions are found.

DIAGNOSTIC CRITERIA OF MINAMATA DISEASE

The committee, sponsored by the Japanese government, had proposed official guidelines for the diagnosis of Minamata disease, compatible with our quantitative diagnostic criteria. Cases with subjective numbness in the extremities only or without any objective signs, such as areflexia, delayed sensory conduction velocity, or abnormal findings in biopsied sural nerves, are excluded from Minamata disease by these criteria.

INVOLVEMENT OF OTHER ORGANS

Although the target organ in organic mercury poisoning is nervous tissue, including the peripheral nerves, other organs, such as kidney and liver, are sometimes also involved, especially in severely affected patients (Igata, 1973). According to our data, methyl mercury has some effect on platelet aggregation *in vitro*. This result might explain the diffuse ischemic change in the acute stage of very severe cases, which are confirmed on autopsy, although general convulsions can also be the cause of them (Shaw, 1979). In the chronic cases now in question, we have no evidence that contamination in the past can be a causative factor in arteriosclerosis or cerebral vascular diseases, although many cases, complicated with cerebrovascular diseases, have been reported probably due to the aging in the polluted areas (Nagashima, 1985). To check the real causal relationship between cerebrovascular diseases and mercury, the severity–response relationship should be confirmed; i.e., the more severe the case, the higher the incidence should be. No data suggesting the possibility of arteriosclerosis directly induced by methyl mercury have been reported. According to the neuropathological studies, arteriosclerosis-like vascular changes can be frequently found in severely damaged and atrophied brain, regardless of its causes. In fact, our experience revealed no relationship between the severity of Minamata disease and the grade of arteriosclerosis or incidence of strokes. The situation is the same in cases complicated by hypertension or diabetes mellitus. Among the patients now being considered, there are some cases complicated by kidney and liver damage. In the autopsied cases, the concentration of total mercury in kidney and liver is slightly higher than that in other organs. Since the concentration of methyl mercury is not always high in the organs, it is possible that such damage is due to transformed inorganic mercury, or at least partly. There is a higher incidence of proteinuria, high β_2-microglobulin, or *n*-acetylhexosaminidase in those with Minamata disease than in nonaffected inhabitants (Igata, 1985; Ohkatsu and Igata, 1978).

CONGENITAL MINAMATA DISEASE

During the period of severe pollution, the incidence of cerebral palsy was found to be much higher than normal (Harada, 1979; Moriyama, 1974). This was the first hint of the existence of congenital Minamata disease born from affected mothers. Altogether about 50 cases have been found in Kumamoto and Kagoshima prefectures. These cases are sometimes quite difficult to differentiate from those children with Minamata disease who became ill in childhood after being born healthy. To diagnose congenital Minamata disease, such epidemiological information as the mother's history of residence in the polluted areas during the severe pollution prior to 1967 is important. This date as the end of pollution was suggested from the data indicating that the methyl mercury content in preserved umbilical cords had normalized by this year. The methyl mercury content in their mothers' hair is also contributory. No late onset or late progression has been seen in congenital cases. Usually the symptoms improve gradually as the child develops. The symptoms of this congenital Minamata disease are essentially similar to those of adult Minamata disease. However, peripheral neuropathy is not manifest as a cardinal symptom, but mental retardation with symmetrical motor disturbance is rather characteristic. If any characteristic symptoms such as cerebellar ataxia, visual congenital constriction, or peripheral neuropathy are confirmed, a diagnosis of Minamata disease is likely to be made. It is also very important that the mother's

symptoms contribute to the diagnosis of congenital Minamata disease, although as a general rule the disease is less severe in mothers than in affected children. Brain damage in congenital Minamata disease is diffuse and symmetrical as seen in the CT scan; so diseases with focal lesions can be excluded as non-Minamata diseases. The evoked potentials including auditory brain stem response on both sides are sometimes abnormal. These findings without any focal lesions sometimes indicate congenital Minamata disease.

DISCUSSION AND CONCLUSION

As society progresses, health hazards due to some industrial products and by-products have been reported. As a result public pollution and hazards have become a very important medical and social problem in our era, of which Minamata disease is a typical example. The outbreak of Minamata disease should serve as a warning to the developed world. This problem should be properly dealt with to avoid its resurgence in any part of the world. In Minamata disease, the pollution was stopped many years ago, but, to our regret, many problems remain unsolved, although a considerable effort has been maintained for many years. As the results of our studies show, the pathomechanism, clinical characteristics, and procedure for prevention and treatment have become clear for typical Minamata disease. However, the diagnosis of mild cases cannot be made in the sense of "all or none." To rationalize diagnosis of mild cases of Minamata disease, quantitative evidence is necessary, so that everyone can agree with the conclusion. In 1971–1972 an outbreak of organic mercury pollution was reported in Iraq (Bakir *et al.*, 1974). An effort was made to compare the clinical aspects of this outbreak with those of Minamata disease (Rustum *et al.*, 1974). Although the symptoms in both outbreaks are quite similar, there are some differences between the two. In Iraq, the pollution was not of long duration, compared with that in Minamata. The loss of visual acuity was reported there but no residual or chronic symptoms have been reported. In addition, in other parts of the world, a lot of new outbreaks of mercury pollution have been reported, including those of natural origin (Clarkson, 1976). Nevertheless, the results of studies on Minamata pollution should be a textbook of new mercury pollution or health hazards or possible ones in the future. In this sense, the clinical studies should be continued to obtain a thorough understanding of mercury poisonings (Table 3, Fig. 11).

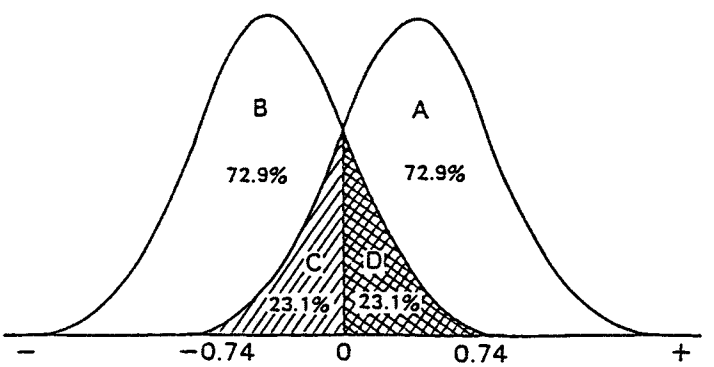

FIG. 11. Discriminant value in CT scan. Diagnosis of Minamata disease using 13 parameters of CT scan. A, minamata disease; B, normal controls; C, Minamata disease, which was diagnosed as non-Minamata disease; D, non-Minamata disease, which was diagnosed as Minamata disease.

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Workplace Strategies for the Control of Work-Related Risks[1]

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The prevention of work-related diseases and disorders, including those of neuropsychobehavioral nature, calls for integrated action to improve both job content and the working environment. Recent international labor standards have highlighted the importance of a good safety and health management system that ensures the assessment of risks and the appropriate control of these risks. Internationally, programs to cope with work-related risks place an increasing emphasis on participatory management of preventive measures, practical methods of risk assessment, and immediate improvements with the support of action-oriented training and information. Psychosocial factors, work performance, and job content need special attention. Workplace strategies must be developed by active participation of employers and workers, with their informed consent. These strategies and linked training activities should build on local practice and achievements rather than administrative models and be geared to the real local needs. In coping with the neurobehavioral effects, international sharing of positive experiences is useful especially with respect to: (i) providing information on potential neurobehavioral effects including labeling, safety datasheets, and precautions; (ii) providing practical advice about locally achieved improvements; (iii) providing action-oriented training; and (iv) creating opportunities for participatory workplace improvements.

INTRODUCTION

The prevention of work-related diseases and disorders calls for integrated action to improve both job content and the working environment. This applies to all occupational hazards and is especially true for health effects of neurobehavioral nature. Neuropsychobehavioral changes are known to occur not only due to poor working environments, but also due to poor job contents and inappropriate work organization. It is thus important to emphasize practical action that takes into account, in an integrated manner, the various workplace factors affecting the physical, mental, and social well-being of workers.

The scientific program of this symposium is very broad and we benefit from sharing experience and scientific knowledge presented by eminent specialists. In doing so, we should not forget that efforts and resources invested in research and studies will become fully meaningful only when they are translated into practical improvements at the workplace level. This paper therefore focuses on strategies for controlling various work-related risks including neurobehavioral effects. Such strategies need to take into account the basic principles of occupational health programs gained internationally.

In many countries, there are encouraging signs that well-organized safety and health programs can result in concrete improvements at the workplace with the reduction of work-related accidents and diseases and the promotion of workers' health (Rantanen, 1990; Kogi, 1991). Positive trends seen throughout the 1980s

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included the gradual extension of services to different working populations and the more active participation of employers and workers in cooperation with safety and health personnel. This development is reflected in recent international standards and guidelines as well as in technical cooperation activities. Collaborative efforts of the WHO, the ILO, and other international organizations and programs stress national action that incorporates such development (International Labour Conference, 1984; WHO Regional Office for Europe, 1990).

An important feature of effective workplace strategies is to adjust activities to varying local needs. This feature is especially important in filling the gaps we have in coping with new risks or in extending services to small workplaces and rural areas. Gaps are larger in many developing countries. The need for local adjustment of safety and health programs is gaining importance in technical cooperation activities, as shown by the experiences within the ILO's International Programme for the Improvement of Working Conditions and Environment (PIACT) (ILO, 1984, 1985; Kogi, 1985, 1991). In particular, good workplace strategies for the control of work-related risks should focus on:

- establishing workplace structures suitable for participatory management of potential risks;
- using practical methods of risk assessment which are easily applicable by those responsible for risk management; and
- using action-oriented training and information activities.

STRUCTURE FOR PARTICIPATORY PROGRAMS

Our common objective in minimizing risks at work is to improve working conditions and environment in all occupations. In achieving this objective, it is necessary to develop means of action adapted to the local situation. Recent international standards on occupational safety and health clearly point out the need to have a workplace structure for management of risks by direct participation of employers and workers. We should note that the role of safety and health services has also changed accordingly.

The Occupational Safety and Health Convention, 1981 (No. 155), and its accompanying Recommendation (No. 164) prescribe the progressive application of comprehensive preventive measures based on a coherent national policy. They establish the responsibility of employers for making work and equipment safe and without risk to health, and duties and rights of workers. The Occupational Health Services Convention, 1985 (No. 161), and its accompanying Recommendation (No. 171) stress that occupational health services are entrusted essentially with preventive functions and responsible for advising employers, workers, and their representatives on maintaining a safe and healthy working environment as well as on the adaptation of work to the capabilities of workers. The emphasis of these Conventions and Recommendations are on roles and cooperation rather than on administrative models. While the administrative structure at the enterprise level can be flexible, it must always ensure that responsibilities for safety and health action are incorporated into daily management at the workplace, from top management to line managers and to the shop floor (Coppée, 1989).

In filling the existing gaps, it is particularly important, as provided in Convention No. 161, to emphasize the advisory role of occupational health services in providing support for the participatory management of risks. The management of workplace risks includes a sequence of activities:

(a) assessing the nature and extent of risks;
(b) planning the improvement action required;
(c) implementing the actions; and
(d) evaluating their effectiveness.

To deal with risks associated with neurobehavioral health effects, these activities should focus on risk assessment based on work surveys, environmental monitoring, and health surveillance; planning and organization of work (including the design of workplaces, the choice and maintenance of machinery and equipment, and the use of chemicals); and organization of adaptive services, counseling, emergency treatment, and rehabilitation. This means that neurobehavioral aspects should be treated as part of various workplace factors and never in isolation from other risks of acute or chronic nature. Many diseases such as those listed in the ILO list of occupational diseases appended to the Employment Injuries Benefit Convention (No. 121) have neurobehavioral aspects, and a variety of work-related diseases and psychosocial factors are also relevant. Careful consideration should be given as to whether these aspects are priority in the local situation and how they are dealt with in the overall effort of improving workplace conditions.

The international exchange of experiences in promoting participatory programs that take account of neurobehavioral aspects in an integrated manner is vital in this regard.

PRACTICAL METHODS OF RISK ASSESSMENT

Risk assessment is not merely a task for safety and health specialists. It should be undertaken by active participation of employers and workers. Practical methods must be made available for this purpose, for example, through scrutiny of potential risks at planning stages, walk-through surveys, and examination of accident data or health-related problems of workers.

In general, the assessment of risks to health may involve

- considering the health status of workers in general;
- identifying potential hazards from materials, processes, and work organization;
- initial and repeated assessment of the extent of risks arising out of these hazards;
- inspection and maintenance of control measures;
- monitoring personal exposures to chemicals;
- health surveillance; and
- identifying the information, training, and supervision required by workers and staff.

Often, the results of environmental monitoring and health surveillance may present effective information for risk assessment. These results, nevertheless, constitute only a part of the activities that can be undertaken. A broader scope is needed to facilitate other activities. For example, depending on the local situation in which detailed surveillance may be difficult, identifying potential hazards from materials and processes and providing information and training about necessary precautions can well be a useful step toward risk assessment.

Comprehensive measures for safety in the use of chemicals at work are presented by the Chemicals Convention, 1990 (No. 170). These measures have special implications for measures as regards neurobehavioral effects of chemicals

used at work (International Labour Conference, 1988). The environmental health criteria documents of the UNEP/ILO/WHO International Programme on Chemical Safety (IPCS) point out the relevance to these neurobehavioral effects of a number of chemicals used at work.

The Chemicals Convention provides that the following are within the responsibilities of employers:

- ensure that hazardous chemicals used at work are labeled according to classification;
- make chemical safety datasheets available to workers and their representatives;
- obtain the relevant information when receiving chemicals which have not been labeled or marked or for which chemical safety datasheets have not been provided;
- relabel hazardous chemicals transferred into other containers;
- assess and monitor the exposure of workers to hazardous chemicals;
- assess the risks and protect workers from such risks by appropriate means of operational control (such as choice of chemicals or technology that minimize the risk, engineering control, improving work practices, hygienic measures, and personal protection);
- provide first aid and arrange for emergencies;
- dispose of residues and empty containers safely;
- inform workers of the hazards; and
- train workers to work safely.

Employers and workers are to cooperate and comply with necessary procedures and practices. Further, workers have

- the right to remove themselves from danger resulting from the use of chemicals when they have reasonable justification to believe there is an imminent and serious risk to safety or health;
- the right to information on the identity and hazardous properties of such chemicals and necessary precautions.

The role of occupational health services in risk assessment is to encourage employers and workers to take the initiative and to provide adequate advice about workplace hazards and control action. The service personnel should know that there are many methods, such as walk-through surveys, assessment through listing potential hazards, occupational hygiene measurements, ergonomic analysis, psychological and toxicological assessment, questionnaire study and interviews. While advice about potential or existing neuropsychobehavioral effects is important, it is equally important to advise about priorities and balanced safety and health management.

As for chemicals which may have neurobehavioral effects, advice should be given concerning the following:

- the extent of such health effects and how information concerning these effects is to be indicated on labels;
- the manner in which information on the effects and precautionary measures is provided to workers and included in chemical safety datasheets;
- practical criteria for monitoring the exposure of workers to these chemicals;
- practical methods for assessing the health effects on workers including both objective and subjective health status (as a group and as individuals);

- procedures to obtain informed consent of workers regarding the use of the chemicals, risk assessment, and health surveillance.

TOWARD PRACTICAL IMPROVEMENTS AND TRAINING

In minimizing work-related risks, different workplaces have different needs. Such differences relate not only to specific hazards at the workplace but also to a variety of situational factors. The health effects of neurobehavioral nature are known to depend greatly on such local, situational factors. Among these local factors, the following seem particularly relevant: worker features including age, sex, and personal adaptability; job content of individual workers; work practices and organization of work; available safety, hygiene, and health services; coping practices; family and social circumstances; and opportunities for participation of employers and workers.

It is interesting that both of the two main types of occupational health services, the undertaking-based services and interundertaking services, have advantages in taking these local factors into account (ILO, 1984; Kogi, 1991). Undertaking-based occupational health services in larger enterprises provide direct internal support to technical aspects of the risk assessment, health surveillance, emergency treatment, and planning of improvements. Risks are relatively better known and the programs to combat these risks can be consistent, while participation of people concerned tends to depend on administrative rules. On the other hand, interundertaking services need to be flexible due to diverse risks and less-consistent programs. Obvious advantages of these services are the relatively easy feedback of local experience and the informal human relations which facilitate participation of all concerned.

In this context, the real needs of employers and workers for occupational health services, irrespective of their type, are:

(i) finding practical ways to reduce risks under local conditions (including the simplest and most cost-effective methods of meeting legal requirements, engineering control, training and health surveillance);

(ii) developing opportunities for participatory implementation of available solutions (while integrating safety and health management into the undertaking's production and quality assurance program).

The basic role of occupational health services should therefore be to provide support for organizing workplace activities aimed at local solutions. This is also the case for neurobehavioral effects. A broad range of action must be planned and managed, including protection against hazards arising from equipment, chemicals, and work processes; improvement of the physical working environment, working time, and rest; maternity protection; protection of young and elderly workers; and the provision of essential welfare facilities and technical services. Particular attention is drawn to work organization and psychosocial factors (ILO, 1986).

Preventive measures relating to neurobehavioral aspects should be taken cautiously. Usually a longer follow-up is necessary (Hänninen, 1991). Increasing concern is being expressed about the social consequences of the screening process and medical decisions. The validity of medical examinations and tests are questionable especially if handling of the results may jeopardize human rights. Utmost care should be taken in this ethical aspect, in particular when the conflicting rights are concerned, such as the right to employment, the right to health,

the right to information, and the right to confidentiality as well as individual rights and collective rights.

Practical improvements should be jointly planned and implemented by employers, workers, and safety and health services. The involvement of safety and health committees and safety representatives is crucial. Direct support must be provided by appropriate training and information activities.

It is useful to learn from successful safety and health programs and recent developments in training for small and medium-sized enterprises. These programs have been successful when they build on local practice, provide positive guidance focusing on locally achievable solutions, pay due attention to the link between working conditions and productivity, and facilitate active participation of employers and workers. As neurobehavioral aspects require particular consideration from ethical points of view, it is extremely important to learn from positive experiences gained in similar local situations.

In coping with neurobehavioral effects, training and information should focus on the following aspects:

(i) ensuring the provision of information on potential neurobehavioral effects (including choice of appropriate work methods, equipment, and work organization; adequate labeling; the use of adequate safety datasheets; and information about hazards associated with work stress and psychosocial factors):

(ii) providing practical advice about workplace improvements (with particular emphasis on those minimizing exposure to agents leading to neurobehavioral effects, which are achieved locally or in a similar situation);

(iii) organizing action-oriented training (so as to enable employers and workers to plan and implement improvements which meet urgent local needs);

(iv) facilitating opportunities for participation in preventive action with fully informed consent and with due respect of human rights (through undertaking a variety of activities such as worksite inspection, feedback of health assessment, improvement planning, campaigns and training activities for work redesign, and other participatory activities).

CONCLUSIONS

The strategies for the control of work-related risks, including those of a neurobehavioral nature, should be based on workplace structure that ensures participatory management of potential risks. As different workplaces have different local needs, it is essential to use practical methods of risk assessment. As for neurobehavioral effects, risk assessment requires presentation of information on hazards, advice about the extent of health effects and precautionary measures, practical criteria for monitoring the exposure, and procedures for obtaining informed consent of workers. Utmost care must be taken to protect human rights in planning and implementing these measures. In coping with neurobehavioral aspects of workplace risks, the occupational health services can play a positive role by providing practical advice, training, and information.

International cooperation is essential in promoting workplace action in this respect. In particular, international sharing of practical experiences is vital in helping organize workplace action aimed at locally practicable solutions.

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REVIEW

Occupational Health Issues in Developing Countries[1]

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Occupational health whether in the developing world or in the industrialized world still remains occupational health. But, the crucial difference lies in the emphasis and priorities in the two situations. To illustrate, the health effects of pesticide usage is a matter of concern to all persons whether from the industrialized world or the developing world. But acute pesticide poisoning is a major health problem in the countries of the developing world requiring urgent attention, while the concerns arising from chronic exposure to pesticides at low dose levels is not a priority issue. The reverse situation obtains in the countries of the industrialized world as they have virtually eliminated the problem of acute pesticide poisoning. Thus, it is important for the countries of the developing world to recognize the priority aspects in an issue in occupational health and address these as a matter of some urgency.

Acute pesticide poisoning is a major concern among the countries of the developing world, whereas it has been well contained in the industrialized world. The developing world, although using only some 20% of the world's agrochemicals suffer almost 99% of the world's deaths due to acute pesticide poisoning. This restated means that the industrialized world using 80% of the world's agrochemicals suffers only 1% of all deaths due to acute pesticide poisoning. The proper understanding of this situation provides several lessons. But the most important and primary lesson is to understand that the problem of acute pesticide poisoning can be controlled as has been done in the countries of the industrialized world. In order to control this problem, it is necessary to have an understanding of the nature and extent of acute pesticide poisoning in the developing world.

A recent article (Jeyaratnam, 1990) reviewed the current status of knowledge regarding the extent of acute pesticide poisoning. It stated that any figures concerning the extent of acute pesticide poisoning on a global scale are largely based, by necessity, on estimates. The first such estimate was made by the World Health Organization (1973) which suggested that 500,000 cases of acute serious pesticide poisoning occurred annually. This estimate included only hospitalized cases of unintentional poisoning. At that time it was considered to be an unacceptably

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large problem, requiring efforts to substantiate this estimate as well as to control the problem. In a national study of hospital cases of acute pesticide poisoning by Jeyaratnam *et al.* (1982) in Sri Lanka, a country with a population of 12 million, approximately 10,000 persons had been admitted to hospitals for acute pesticide poisoning annually, resulting in almost 1000 deaths. The public health importance of this figure was highlighted by the fact that the deaths due to acute pesticide poisoning for that particular year were almost twice the total number of deaths due to malaria, poliomyelitis, whooping cough, diphtheria, and tetanus, the traditional public health problems of developing countries. The equivalent annual figures at a global level were estimated by Jeyaratnam (1985) at approximately 3 million cases hospitalized with approximately 220,000 deaths.

Recently, WHO (1990) reviewed the available estimates and other pesticide poisoning data and summarized the overall public health impact of pesticides, as shown in Fig. 1. WHO (1990) states that "the estimated 3 million cases of acute severe poisonings may be matched by a greater number of unreported, but mild, intoxications and acute conditions such as dermatitis."

It is imperative that every attempt be made to control the extent of what may be described as a controllable problem, that of acute pesticide poisoning.

There is another facet to this problem of acute pesticide poisoning which is of particular relevance to this symposium. The prevalence of acute pesticide poisoning in the developing world also provides an opportunity for the greater understanding of the health effects of exposure to pesticides, which is of relevance

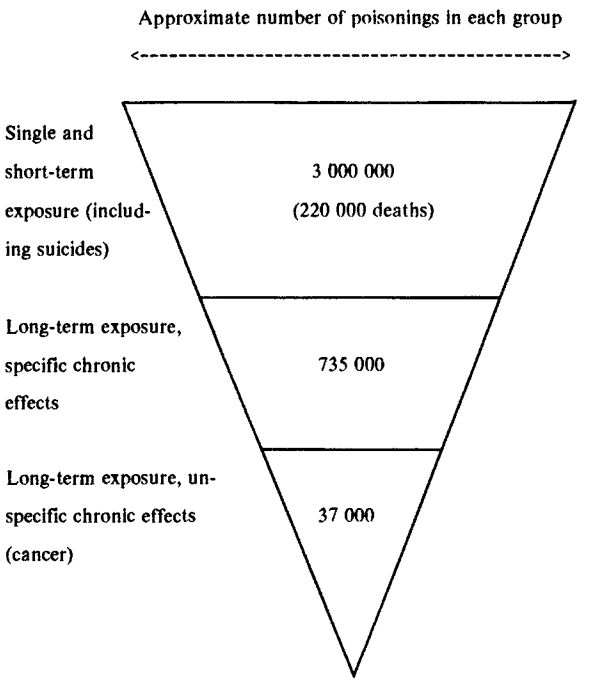

FIG. 1. Estimated overall annual public health impact.

to both the industrialized world as well as to the developing world. A study in Sri Lanka reviewed 23 patients admitted to hospital for acute pesticide poisoning of whom 1 patient died. One year after discharge, 19 out of the 22 surviving patients were traced and examined. The examination consisted of a clinical examination, estimation of blood cholinesterase levels, and recording of electromyograms (EMG) that were done on 18 of the 19 patients. The patients were all clinically normal with normal blood cholinesterase levels.

The results of the electromyographic examination on 18 of the subjects indicated that 7 of them clear evidence of EMG abnormality (Fig. 2). These consisted of a significant reduction in the motor recruitment pattern produced during maximal attempted voluntary muscular contraction and a reduction in the mean voltage and duration of motor unit potentials identified with some evidence suggestive of a fragmentation of motor units. Although some of the subjects gave a history of fasiculations, none were observed during examinations and the EMG showed no evidence of the characteristic giant motor units. There was no detectable evidence of median or ulnar nerve conduction defects. All of the EMG abnormalities were observed among workers exposed to a locally marketed product consisting of mixture of organochlorine and organophosphate pesticide.

This observation of the presence of an abnormal EMG pattern 1 year after an episode of acute pesticide poisoning in the absence of any clinical abnormality was unexpected. Drenth *et al.* (1972) and Roberts and Wilson (1973) too described EMG changes in field sprayers. Their observations were again similar to those observed in the present study.

There seems to be at this stage of the investigation several unusual features in these subjects with abnormal EMG patterns, i.e.,

(a) the presence of normal motor nerve conduction velocities was associated with a significant degree of motor unit damage;

(b) unlike in the typical myopathic lesion there was a gross reduction of the recruitment pattern associated with a reduction of unit amplitude and duration;

(c) muscle power as judged clinically was only marginally diminished in contrast to the severity of EMG abnormality seen.

There is sufficient evidence of EMG abnormality to warrant a much more detailed and quantitated assessment of the problem—muscle biopsy—motor end plate biopsy and more detailed studies to exclude a myasthenic type of lesion are indicated together with an investigation of other subjects who have had significant exposure to the same or similar pesticide.

This study indicates to an extent the interrelationship that exists between the needs of industrialized world and the developing world. Even though the problem of acute pesticide poisoning is seemingly peculiar to the developing world, there are global implications in the related health issues.

FIG. 2. EMG pattern among spray men 1 year after an episode of acute pesticide poisoning.

I wish to examine another issue which is again dependent on the interrelationship between the industrialized world and the developing world, namely the concern over the transfer of hazardous industries (Jeyaratnam, 1990).

The issues associated with the transfer of industries came into focus with the Bhopal incident of 1984. The issue had been previously discussed at various levels globally and nationally, but without much enthusiasm. Associated with and parallel to the problem of transfer of hazardous industries is the issue of export of banned products. The concern is for the hazard to health posed to the nations of the developing world by the dominance of commercial interests. It is basically the commercial factors which exploit the situation of the developing world by exporting hazardous products banned for use on the basis of health consideration in the producer nations.

The problem of transfer of hazardous industries to the developing world is an area of current and growing concern. The factors which lead to such transfer are the conditions prevailing in the industrialized world causing a "push factor" and the conditions in the nations of the developing world leading to "pull factors" (Table 1). The resultant outcome is the movement of hazardous industries largely on the basis of commercial considerations, thereby manifestly increasing the potential health hazards to the recipient nations. Such a situation of divergent needs of the nations of the industrialized world and the developing world often gives rise to the problem of "double standards" in the control of industrial hazards. Double standards arise when one country bans or restricts a product or process and another country does not. The question that arises is whether the standard that is decided on by one country is necessarily appropriate in another. Often decisions on standards are not necessarily always health based but are tempered by economic, social, educational, unemployment, and other considerations. The unacceptability of double standards implies a need for a set of standards which are internationally applicable. In this context the scientific position would be that there must be a uniform set of international standards based on health-based considerations. But, the national decisions on standards could take into consideration other factors in making a decision. This is the crucial issue that has to be

TABLE 1
FACTORS WHICH LEAD TO THE TRANSFER OF HAZARDOUS INDUSTRIES FROM THE INDUSTRIALIZED WORLD TO THE DEVELOPING WORLD

"Push" factors in the industrialized world	"Pull" factors in the developing world
1. Stringent domestic industrial and environmental regulations	1. Lack of poor implementation of labor and industrial regulations
2. Increasing labor costs	2. Cheap labor
3. The "green" movement"	3. Unemployment
	4. Shortage of "hard" currency
	5. National drive toward industrialization

Note. Source: Jeyaratnam (1990).

grappled with in the context of the problem of transfer of hazardous industries, and is discussed in greater detail later.

The solutions to this problem often devolves around international control mechanisms and the responsibility the "exporting" nation to inform the "importing" nation of the health concerns. But the question is whether such an approach is justifiable on the basis of the oneness of mankind and the concept of the global village situation. The health problems associated with hazardous industries is a global concern.

In the past the health concerns of hazardous industries were considered a problem restricted to the immediate vacinity of the hazardous industry. But now it is increasingly realized that it is not so; it is a problem of global concern. For instance, the problem of the chlorofluorocarbon compounds cannot be contained by transferring the process to a developing nation. This is an activity which concerns all of mankind. It is no longer possible to transfer a hazardous industry to a remote corner of the globe and hope that the problem is resolved.

Another aspect of global involvement is related to the issue of reimportation. In this context kepone, a pesticide manufactured in the United States for export only, was sprayed on bananas grown in Guatamala that were intended for consumption in the United States (Hornblower, 1980). Other chemicals such as aldrin, dieldrin, heptachlor, and chlordane, banned in the United States but made available for export, have often been reimported as contaminants of cocoa from Ecuador, coffee from Costa Rica, and sugar and tea from India (Seferovich, 1981).

In the final analysis moral considerations must be an important determinant in human behavior.

General principles of fairness and human decency dictate that we act justly to all of mankind and not take exploitative positions. Seferovich (1981) states "surely there is little or no justification for allowing an uninformed, poverty-stricken country to use a substance that we created but will not use ourselves because we know it is harmful." Often, such a position is circumvented on the basis that if information is provided it is the right of the importing nation to decide independently for itself with regard to its needs. But, the question is whether such nations are in a position to take such decisions, in view of their poverty, poor communications, unemployment, and weak technical and administrative infrastructure with often a background of corruption.

In this paper I have addressed two aspects of the interrelationship between the industrialized world and the developing world in the field of occupational health. It demonstrates a need for both worlds to learn and benefit from each other with the recognition of interdependence.

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Neurobehavioral Toxicology in the 21st Century: A Future or a Failure?[1]

The 1991 Hänninen Lecture

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Toxic substances in the workplace and general environment have been shown to cause adverse health effects in human populations. This has occurred as the result of such factors as industrialization, environmental pollution, and increased reliance on chemicals in agriculture. Effects of toxic substances on the human nervous system have been identified and characterized through use of neurobehavioral methods, essentially over the past 20 years. This paper examines 10 key publications in neurotoxicology and relates them to the future of neurobehavioral toxicology in the next century. The view is expressed that the future of neurobehavioral methods lies in more firmly rooting them in basic mechanisms of neurotoxicity. © 1993 Academic Press, Inc.

INTRODUCTION

The organizers of the Third International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, which was held in Washington, D.C., in 1988, initiated a lecture series in honor of one of the leaders in neurobehavioral toxicology, Dr. Helena Hänninen. Dr. Peter Spencer delivered the first Hänninen Lecture (Spencer, 1990).

It is a great personal honor to contribute to this lecture series. Dr. Helena Hänninen, who pioneered much of what we call neurobehavioral toxicology while at the Finnish Institute of Occupational Health, truly set a remarkable example for others to follow. Her personal research contributions about the effects of solvents, metals, and other toxicants on behavioral parameters are noteworthy. She sowed the seeds from which many of us have reaped a harvest.

I first met Dr. Hänninen at the behavioral toxicology workshop organized by the National Institute for Occupational Safety and Health (NIOSH) in Cincinnati, Ohio, in 1973 (Xintaras *et al.*, 1974). She came to that workshop already a much-published leader in the field. At the workshop she displayed her characteristic patience with new, eager researchers who wished to share their views with her. I was one of them. Over the years I followed her numerous contributions to neurobehavioral toxicology and observed first-hand her assistance to other researchers, especially those from developing countries. She and I were often in Geneva at the same time on consultation for the World Health Organization (WHO). She always had time to share her knowledge and to assist those who needed it. Typical of those contributions was her leadership in helping establish the WHO Neurobehavioral Core Test Battery (NCTB) that was the product of a WHO-convened

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consensus workshop held at NIOSH in 1983. So it is with great humility that I offer, in her honor, some thoughts about neurotoxicology and neurobehavioral methods.

PURPOSES OF THE PAPER

These triennial symposia provide an excellent forum to present timely findings about neurobehavioral methods and their application to occupational and environmental research. As even the casual reader of the scientific literature is aware, the number of scientific publications and specialty journals continues to grow impressively. Keeping up with new findings from neurotoxicologic investigations is now a much more difficult task than it was a decade ago. This explosion in knowledge is laudable—we learn more about what neurotoxicants do to biological systems, for example, and presumably that knowledge leads to improved approaches to preventing human misery caused by contact with toxic substances. This symposium will continue that march of knowledge.

As we come together every 3 years or so, let us commit to understanding where we and the field of neurobehavioral toxicology have come from and where we think we are headed. The Hänninen Lecture provides an opportunity for reflection and, perhaps, redirection and rededication.

As the Japanese philosopher, Nishida Kitaro, noted (Kitaro, 1990)

> In the facts of direct experience there is not the opposition of subject and object; there is not the distinction of spirit and matter; matter equals spirit, spirit equals matter, and there is only one actuality.

The actuality of what faces neurotoxicologists, who develop and use neurobehavioral methods, is that we must not distinguish between the spirit of what we offer to human betterment and the matter of our science. Where has behavioral toxicology, neurobehavioral toxicology, or whatever name you wish to call our science discipline, come from? Although I am neither an historian nor a patriarch of the community of neurobehaviorists, I have had contact with the field for almost 25 years and feel partially qualified to offer some observations about our journey and where we may be headed. [For persons with a history bent, I refer you to articles on the development of behavioral toxicology by Beard (1974), who described the need of regulatory agencies, and Weiss (1990), who discusses behavioral pharmacology.]

TEN SIGNIFICANT NEUROTOXICOLOGIC CONTRIBUTIONS

To address our neurotoxicologic background, I have resorted to the use of a device common in the United States, the compilation of lists of things of presumed importance. I have developed a highly personal list of 10 key contributions in neurotoxicology or neurobehavioral toxicology. The list reflects my view of what constitutes neurotoxicology and how each contribution and its authors have substantially advanced the field. The principal criteria for selecting the 10 publications were (a) significance of the findings in shaping new thinking about a particular neurotoxicant or class of neurotoxicants, (b) generalizability of the findings, and (c) contribution to preventing human health effects of neurotoxicants. I describe in brief what I think makes each paper or report significant and to which area of neurotoxicology the work contributes. In chronological order of publication:

• *Human Laboratory Investigations:* Gamberale, F., and Hultengren, M. (1972). Toluene exposure. II. Psychophysiological functions. *Scand. J. Work Environ. Health* **9**, 131–139.

The effects of specific neurotoxicants on human psychophysiologic functions, as studied under controlled conditions, have been examined by several investigators (Dick and Johnson, 1986). The ability to control exposure levels and conditions of testing represent essential features in advancing our knowledge of the neurotoxicity of specific substances on human health. Gamberale and co-investigators conducted a series of studies on the effects of acute exposure to industrial solvents on human psychophysiologic functions. For example, they exposed human volunteers to various levels of toluene, compared with pure air, and studied the effects on reaction time and perceptual speed. They reported a statistically significant impairment in reaction time at an exposure level of 300 parts per million (ppm) toluene in inspired air. Impairment increased as exposure to toluene increased. Perceptual speed was impaired at 700 ppm and above. This use of human subjects in laboratory investigation appears to be decreasing in frequency. Some researchers are fearful that even acute exposures to commonly found industrial substances at levels permitted in industrial settings may lead later to problems because of latent adverse health effects. Although this is an understandable concern, it is overly cautious. Decreases in these kinds of studies result in considerable loss both for developing laboratory-validated neurobehavioral tests and in improving awareness of the acute effects of low-level exposure to neurotoxicants on human performance.

• *Mechanism of Neurotoxicity:* Spencer, P. S., and Schaumburg, H. H. (1977a). Ultrastructural studies of the dying-back process. III. The evolution of experimental peripheral giant axonal degeneration. *J. Neuropathol. Exp. Neurol.* **36**, 276–299.

Although many substances are known to be neurotoxicants, few have had basic mechanisms of toxicity characterized. An exception is the neurotoxicity of methyl *n*-butyl ketone (MBK), as elaborated by Spencer and colleagues. Workers occupationally exposed to a solvent that contained MBK developed peripheral neuropathy. Through some excellent epidemiology and industrial hygiene work, MBK was identified as the suspect agent. Elegant work by Spencer and Schaumburg (1976, 1977a,b) revealed the mechanism of MBK's neurotoxicity. Their work with experimental animals showed that MBK caused multifocal, giant axonal swellings containing masses of 10-nm neurofilaments, enlarged mitochondria, interdigitated Schwann cell/axon networks, and corrugated myelin sheaths. The investigators' aggressive pursuit of the mechanism of MBK toxicity in humans is a hallmark in neurotoxicology. Public health was enhanced as MBK's use diminished rapidly as occupational standards were revised downward.

• *Identification of Susceptible Population:* Needleman, H., Gunnoe, C., Leviton, A., Peresie, H., Maher, C., and Barret, B. (1979). Deficits in psychologic and classroom performance of children with elevated dentine lead levels. *N. Engl. J. Med.* **300**, 689–695.

The toxic effects of lead have been known since antiquity, but only recently has the true insidious nature of lead's toxicity in children become evident. The work of Needleman and co-workers has been essential in furthering this understanding. This was the first paper to relate a measure of lead exposure to measured deficits in the intelligence and poor classroom performance of children. This work also set

the stage for prospective studies of lead-exposed children that combined this epidemiologic approach with precise measurements of exposure to a major toxicant. Such prospective epidemiology coupled with rigorous exposure assessment and well-established neurobehavioral methods will become the norm, not the exception, as a way to follow the effects of lower exposure levels of neurotoxicants in worker and community populations.

• *Symptoms of Neurotoxicity:* Hänninen, H., Mantere, P., Hernberg, S., Seppäläinen, A., and Kock, B. (1979). Subjective symptoms in low-level exposure to lead. *Neurotoxicology* **1**, 333–348.

There is general acceptance that subjective symptoms of a neurovegetative nature constitute the first response to low-level exposure to neurotoxicants. Mood disturbance, headache, low-grade nausea, fatigue, and other symptoms are often reported by workers and community residents exposed to low concentrations of specific neurotoxicants. Hänninen *et al.* were the first to investigate the dose–response association of subjective symptoms with exposure to a known neurotoxicant. Their work showed an exposure-related pattern for the prevalence of gastrointestinal complaints and for psychologic and neurologic symptoms. In particular, the lead-exposed group showed a higher neuroticism score in the Eysenck Personality Inventory, attributable to an overrepresentation of fatigue, mood disturbances, and difficulties in coping with the environment. As conditions of workplace hygiene improve and environmental remediations continue, human exposures to neurotoxicants will decrease, at least in industrialized countries. Thus, a better understanding of the association between subjective symptoms and exposure to specific neurotoxicants will be needed. This represents an important future research area.

• *Scholarly Codification of Neurotoxicology:* Spencer, P. S., and Schaumburg, H. H. (1980). "Experimental and Clinical Neurotoxicology." Williams & Wilkins, Baltimore.

A body of science exists when the elements of the body are synthesized into a cohesive work. Such a work did not exist prior to Spencer and Schaumburg's reference work. This remarkable book first brought together the various specialty disciplines and toxicologic knowledge that collectively can be called neurotoxicology. Spencer and Schaumburg assembled the neuropathology, neurochemistry, electrophysiology, and behavioral findings on the major neurotoxicants into a codified reference work of lasting significance. This book provided intellectual legitimacy for neurotoxicology when it was most needed and set a standard of scholarship for others to follow.

• *Delayed Toxicity:* Johnson, M. K. (1982). Initiation of organophosphate-induced delayed neuropathy. *Neurobehav. Toxicol. Teratol.* **4**, 759–765.

Many neurotoxicants exert their effects quickly following toxic insult. Other substances induce adverse effects only after a period of time has elapsed following exposure. The first class of compounds identified as causing delayed neurotoxicity was the organophosphates. The work of M. K. Johnson and collaborators provided a central understanding of the delayed neuropathy caused by organophosphates. In particular, the finding that neurotoxic esterase is associated with neuropathy was a major finding in neurotoxicology (Johnson, 1982). Similarly, Spencer extended our knowledge of "silent neurotoxicants" to include a class of natural toxins found in seeds of the cycad plant and identified a putative association with neurodegenerative disease in residents in the Mariana Islands (Spencer, 1990).

• *Neurobehavioral Research in Developing Countries:* Liang, Y., Jing, X., Shen, G., Li, Y., and Li, R. (1983). Health effects of low level CS_2 exposure in viscose rayon workers—An epidemiological study on psychological effects. *Acta Acad. Med. Primae Shanghai* **10**, 15–20.

Recent political changes worldwide evidence the desire for improvements in national economies. As developing countries enlarge their manufacturing and agricultural bases, they run the risk of encountering the same occupational health and environmental contamination problems experienced by industrialized countries. Neurobehavioral toxicologists in developing countries must assume a strong role to protect workers and community residents against adverse health problems caused by neurotoxicants. Indeed, it is in this spirit that the WHO Neurobehavioral Core Test Battery was developed (Johnson *et al.*, 1987). In an excellent example of neurobehavioral methods applied to workers in a developing country, Liang *et al.* (1983) administered a battery of psychological tests involving mood states, intellectual activity, visual perception, short-term memory, and performance speed to workers occupationally exposed to carbon disulfide and to a comparison group from a textile mill. Statistically significant differences in test scores were found between the two groups for most items on the psychological battery. The investigators concluded that 10 mg/m^3 was near the minimum-effect level for carbon disulfide, and they suggested that consideration be given to decreasing the chemical's industrial hygiene action level in China.

• *Failure of Premarketing Testing:* Horan, J., Kurt, T., Landrigan, P., Melius, J., and Singal, M. (1985). Neurologic dysfunction from exposure to 2-t-butylazo-2-hydroxy-5-methylhexane: A new occupational neuropathy. *Am. J. Public Health* **75**, 513–517.

The identification of toxic substances through premarket testing of proposed commercial products is one way to prevent human exposure to new neurotoxicants. This work of Horan *et al.* (1985) is significant because it shows what can happen when premarket testing of new commercial products is not performed adequately. The investigators found that workers using a new kind of epoxy compound in the manufacture of bathroom fixtures exhibited signs of severe neurologic dysfunction. This dysfunction occurred only a few weeks after the manufacturer changed to the new compound because it possessed superior chemical properties that increased the production process. Although the manufacturer had conducted premarket toxicity testing of the reformulated compound, apparently, any signs of toxicity were either not detected or, if detected, were ignored. The bottom line is that although premarket testing of new products is required in the United States, the testing must be ensured as thorough.

• *Risk Assessment of Neurotoxicants:* U.S. Environmental Protection Agency's (EPA) Announcement of Guidelines for Neurotoxicity Testing (1989).

Government agencies are turning increasingly to risk assessment of toxicants as a principal resource to establish regulatory actions (Cohrssen and Covello, 1989). In consideration of this trend, the EPA guidelines for neurotoxicity testing presage the risk assessment of neurotoxicants. The EPA's testing guidelines describe a functional observational battery, motor activity, neuropathology, schedule-controlled operant behavior, and procedures for determining acute, delayed neurotoxicity of organophosphates and provide details of test administration. Incorporating these kinds of tests into testing schemes to assess neurotoxicity of substances will markedly advance our knowledge.

• *Reversibility of Neurotoxicity:* Yokoyama, K., Araki, S., and Aono, H.

(1988). Reversibility of psychological performance in subclinical lead absorption. *Neurotoxicology* 9, 405–410.

It is important to know what substances are neurotoxic and under what conditions. A knowledge of the mechanism of neurotoxicity is equally important. But beyond these important research questions, what happens to the worker or community resident cohort after a substance is found to be exerting neurotoxicity and medical and public health interventions have been taken? Few investigators have pursued this important question (e.g., Baker *et al.*, 1985). One investigation that did follow a cohort for evidence of neurotoxicity reversal is the work of Yokoyama *et al.* (1988), who compared male Japanese workers exposed to lead in a gun-metal foundry with steel foundry workers. Both groups were tested on five scales of the Wechsler Adult Intelligence Scale (WAIS) before and after a local exhaust ventilation system was installed at the facility (a 2-year interval). The investigators found that performance on the picture completion component of the WAIS had returned to normal at the second examination. The improvement in performance was proportional to the decrease in the chelation-mobilized concentration of lead. Yokoyama *et al.* concluded that cognitive performance deficits are reversible if they occur at blood lead levels below 65 μg/dl.

These 10 key publications, and the broad areas of contribution each represents, illustrate the breadth of interest and level of contribution to science and public health that neurotoxicologists have made. Knowledge about the mechanisms of neurotoxicity has advanced for some substances, neurobehavioral methods have been developed and applied for hazard identification, public health has been practiced to identify populations at health risk, and scholarly findings have been compiled and published for the record. That's how far we have come, and the record is a very distinguished one. However, what does the future hold?

FUTURE DIRECTIONS

The discipline of neurotoxicology and the use of neurobehavioral methods have contributed markedly to improved industrial hygiene conditions and environmental regulatory actions. The scholarly contributions cited above are but the tip of a much larger iceberg of science. Do we have any sense of where that iceberg is headed? In a metaphorical sense, is neurobehavioral toxicology adrift, too? What does the future hold?

Does neurobehavioral toxicology, indeed, have a future? What does the 21st century portend for us? A number of trends are already pointing in several directions of importance. Many promising changes are occurring, and although the impressive progress previously described will likely continue, it must be better grounded in more rigorous science.

We must look ahead, assess what we think lies before us, and help lead our discipline toward even more significant developments and applications. The mere passage of time will not guarantee that progress occurs. To borrow a thought from the Rev. Dr. Martin Luther King, Jr. (King, 1990):

> It is the strangely irrational notion that there is something in the very flow of time that will inevitably cure all ills. Actually time is neutral. It can be used either destructively or constructively.

As we approach the future, particularly the 21st century, we can focus on five areas that will develop and lead to even further improvement in the importance of

neurobehavioral toxicology: accelerated research on basic mechanisms of neurotoxicity, increased numbers of neurotoxicants subjected to risk assessment, development and use of personal neurobehavioral test systems allied with health surveillance, an increased number of investigations of community residents exposed to neurotoxicants, and the establishment of international databases on results from neurobehavioral testing of toxicants and populations of concern. Some thoughts about each area follow.

Accelerated Basic Research

Much progress has already advanced the understanding of the toxic mechanisms of specific neurotoxicants, for example, the elegant work of Peter Spencer and colleagues in elucidating the neurotoxic mechanism of MBK. The number of scholarly articles in peer-reviewed publications is impressive. However, we have only just begun this kind of journey. As scientists and health officials, we must too often admit ignorance about which substances will cause harm to the nervous system and how this harm occurs. Lest anyone feels too comfortable about our knowledge, who among us knows just how lead actually causes neurotoxicity?

Notwithstanding our limited knowledge about basic mechanisms of neurotoxicity, accelerated basic research bodes well for the future, but only if we are wise enough to know what to do with the knowledge. As neurotoxicologists, we must ensure that our knowledge of mechanisms of neurotoxicity is not used for destructive purposes. For instance, would our specialty discipline contribute to human welfare if it leads to new and more potent nerve gases? Rather, the accumulating knowledge about how neurotoxic mechanisms work must be used to better our understanding of neurologic disease and its prevention.

National programs of neuroscience research will ultimately lead to a much better understanding of brain function and of human and animal behavior and to an awareness of what we call the mind. Such fundamental knowledge will be vital for a better understanding of abnormal conditions, such as those produced by neurotoxicants. Already, one national program has committed to the study of brain function over this decade (SBBS, 1991). Although not assured, increased funds will be sought in the United States to support studies of the brain and associated functions and, in particular, the study of mechanisms of human neurologic disease.

The study of early- and late-life phenomenona that can be deleteriously affected by exposure to neurotoxic substances is of particular importance. Concerning early life, examples are abundant that *in utero* exposure to toxic substances (e.g., effects of lead on the fetus, ATSDR, 1988) can have grave consequences on the developing fetus and on postnatal development. Regarding late-life phenomena in the populations of several industrial countries, a better understanding is needed of the association between neurotoxic substances and the aging process. As Spencer said in his 1988 Hänninen Lecture, neurodegenerative disease should become a subject of special importance (Spencer, 1990).

Risk Assessment

Risk assessments [defined by the U.S. National Research Council (1983) as hazard identification, exposure assessment, dose–response evaluation, and risk characterization] of individual neurotoxicants have begun. As mentioned earlier, the EPA released neurotoxicity testing guidelines for neurotoxicants and is now

working with other agencies to update them. Government agencies are under increasing pressure from legislative bodies to assess the risk of neurotoxicants (in addition to chemical carcinogens and reproductive toxicants) and to regulate the use of those whose toxicity exceeds accepted risk-level cutoffs (OTA, 1990). These pressures will occur as the number of commercial products that possess potential neurotoxicity increase due simply to economic development. Contemporaneously, as global economic development proceeds, citizens are gaining greater access to information on environmental hazards and risk assessments.

Problems associated with risk assessments of carcinogens [like great differences in risk estimates because of the use of different statistical models (Cohrssen and Covello, 1989)] cast doubt on whether risk assessments of neurotoxicants will fare any better. In any case, we must learn from past mistakes. For example, Silbergeld (1990) has advised that risk assessments of neurotoxicants be grounded in mechanistic information of neurotoxicity. In addition, one area of particular importance is the development of appropriate biologic and analytic methods to measure exposure to neurotoxicants, because exposure assessment is often the area with the least data in the conduct of a risk assessment.

Weiss (1990) has cautioned that behavioral toxicology's emphasis on hazard identification tends to isolate it from new advances in the behavioral and neurosciences. He argues, with justification, that this uncoupling negates much of the early promise of behavioral toxicology. Weiss's concern can be assuaged somewhat if risk assessment of neurotoxicants is oriented toward basic neuroscience principles and mechanisms of toxicity.

Community Investigations

Much of our knowledge about specific neurotoxicants has resulted from concern for the health of workers. Occupational health studies have dominated the literature for the most part. This has resulted from several factors: interest on the part of occupational health agencies, availability of industrial hygiene measurements to characterize exposure conditions, generally higher exposure levels in occupational settings, and evident neurobehavioral problems in specific worker cohorts. Concern for the effects of neurotoxicants on workers led NIOSH to include neurotoxic disorders on its list of 10 leading occupational safety and health concerns. NIOSH notes that more than 750 chemicals have been found to be potentially neurotoxic (NIOSH, 1988), and of the 588 chemicals for which the American Conference of Industrial Hygienists have developed threshold limit values, about one-third affect the nervous system (Anger, 1984).

In the future, additional effort will be made to determine the effects of neurotoxicants on community residents. Already cited was the example of investigations of children exposed to environmental sources of lead (Needleman *et al.*, 1979). Increased attention will be given to children, persons with infirmities, and other potentially susceptible persons exposed to nonoccupational sources of neurotoxicants. For example, the Agency for Toxic Substances and Disease Registry conducted an extensive review of the toxicology of substances released from hazardous waste sites and included neurotoxic disorders on its list of priority health conditions associated with hazardous waste releases. Hazardous waste sites contain discarded industrial materials and many of these substances possess neurotoxic properties; but, the exposure conditions at waste sites differ markedly from those found in occupational settings. Human exposure at waste sites is

principally from ingestion of toxicants; occupational exposures are primarily through inhalation and dermal routes (Johnson, 1992). Investigations of communities exposed to neurotoxic substances will present major new challenges to investigators.

International Databases on Neurotoxicants

Increased knowledge about neurotoxicants will be gained from more risk assessments of neurotoxicants, basic research studies of neurotoxic mechanisms, and occupational and community health studies. The international nature of modern economies will result in increased attention and resources being devoted to developing and maintaining international databases on environmental contamination and toxic substances; these will include neurotoxicity and neurobehavioral effects. Some progress in this area has already been made. The United Nations Environment Program and the World Health Organization are working together to develop such systems. Easier access to such data will be available in the future, using satellite systems and personal computers. For instance in the next century, a farm manager in a remote part of the globe could receive direct transmission of pesticide application data using a hand-held computer linked into national or international data systems.

Personal Neurobehavioral Battery and Surveillance

Perhaps the greatest progress in the next century will be in the development and use of miniature, personal neurobehavioral testing systems. The use of computer neurobehavioral test batteries is already a reality (Letz, 1990). Computer systems will continue to become more prominent in neurobehavioral investigations, but an important new direction will occur. Personal, miniaturized neurobehavioral test systems will appear that will evaluate each person through a workplace or community environmental experience, linked to health surveillance systems. Using each individual as his or her own control, such systems will permit individualized evaluations across long periods of time. This will be important as levels of exposure to neurotoxicants decrease in the next century. These personal neurobehavioral test systems will also be able to appropriately account for each person's subjective adverse health symptoms.

SUMMARY

Returning to the title of this paper, will neurobehavioral toxicology have a future in the next century? Yes, but the future is not assured by any means. A review of major accomplishments in neurobehavioral toxicology indicates that progress *is* being made in identifying specific neurotoxic substances, improving understanding of neurotoxic mechanisms, validating neurobehavioral test methods, and making applications to public health. However, much remains to be done as we approach the next century. In particular, basic research in neuroscience, joined with the development and validation of neurobehavioral methods, will be crucial to continued advances in protecting humans against disease associated with neurotoxic substances. In other words, mechanisms of neurotoxicity must serve as the mortar necessary to build neurobehavioral toxicology into the 21st century.

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Comparison of Performance from Three Continents on the WHO-Recommended Neurobehavioral Core Test Battery[1]

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To address the need for standardized test batteries, an expert group convened by the World Health Organization (WHO) and the U.S. National Institute for Occupational Safety and Health during 1983 proposed the Neurobehavioral Core Test Battery (NCTB) to identify nervous system effects of chemical exposures in human populations worldwide. To determine the feasibility of using the NCTB in varied cultures, a cross-cultural assessment was conducted under WHO auspices. Data were collected in 10 countries of Europe, North and Central America, and Asia from over 2300 males and females who were not exposed to chemicals at work, within five age ranges between 16 and 65. Results suggest that performance on two NCTB tests (Simple Reaction Time, Benton Visual Retention) is very similar in a broad range of countries and that performance on four other NCTB tests (Santa Ana, Digit Symbol, Digit Span, Aiming) is relatively more variable from country to country, in both males and females. However, data collected from very poorly educated males in one country revealed very low performance levels suggesting that the NCTB may not provide an adequate reference group for identifying (behavioral) neurotoxic effects in such populations. More research is thus needed on evaluating neurotoxicity in poorly educated subjects.

INTRODUCTION

Behavioral testing of occupational populations exposed to chemicals began in the 1960s (Hänninen, 1966) using the methods of neuropsychology and experimental psychology. Hänninen in Finland developed the first such behavioral test battery, refining it in a series of studies in the 1970s (Hänninen and Lindström, 1979). Research in this field has generally employed behavioral test batteries to assess the effects of neurotoxic or unknown chemicals, but the number of unique tests used in this field has expanded continuously. Johnson and Anger (1983) identified 60 unique tests that had been used in this research at the outset of the 1980s, and 250 unique tests had been employed in worksite behavioral research by the end of that decade (Anger, 1990).

Calls for standardized tests (e.g., Dews, 1975) were answered by a group con-

[1] Presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

vened in Cincinnati, Ohio, during 1983 by the World Health Organization (WHO) and the U.S. National Institute for Occupational Safety and Health (NIOSH). That group proposed a battery which could identify or screen for nervous system effects of chemical exposures in human populations worldwide. This battery, the Neurobehavioral Core Test Battery (NCTB), is composed of seven behavioral tests: Digit Span, Digit Symbol, Benton Visual Retention, Simple Reaction Time, Santa Ana dexterity, Pursuit Aiming II, and Profile of Mood States (Johnson *et al.*, 1987).

Contemporaneously, Baker *et al.* (1985) developed a battery of 19 tests, the Neurobehavioral Evaluation System (NES), including variants of five of the NCTB tests, using computer implementation. The NES has become the most widely used behavioral test battery to assess the neurotoxicity of chemicals by computer (Letz, 1990; Anger, 1990), and the NCTB is the most widely used battery administered by a human tester (Cassitto *et al.*, 1990; Anger, 1990).

Sensitivity, Reliability and Validity of NCTB Tests

Although the NCTB is of recent origin, one or more of the seven tests in the NCTB (or very similar variants) have been employed widely in behavioral neurotoxicology research in occupational settings. Indeed, while the functional areas assessed by the NCTB were selected to represent a range of important nervous system functions, the main criterion applied in selecting individual tests was sensitivity to changes associated with chemical exposures. Practically, this meant that the tests had successfully discriminated between worker groups exposed to chemicals known to be neurotoxic (viz. lead, mercury, carbon disulfide) and unexposed worker groups (Johnson *et al.*, 1987).

Data from a recent comprehensive review (Anger, 1990) of worksite research published from 1966 through 1989 substantiates the sensitivity of the NCTB tests. The NCTB tests are listed in Table 1, along with the number of studies (column 2) in which each has been employed out of a total of 185 worksite studies identified in the review. The Pursuit Aiming II test has not been used in any such study, but the other six tests have been used in between 2 and 60 studies. Column 3 lists the number of studies in which the author reported that the exposed subjects differed significantly from the control subjects. As noted in column 4 of Table 1, the tests selected for the NCTB have identified statistically significant group differences (between chemical-exposed and unexposed referent groups) in 44–62% of the studies in which they have been employed (the number in column 3 divided by the

TABLE 1
NCTB TESTS, NUMBER OF WORKSITE STUDIES (OF 185) EMPLOYING EACH TEST, AND NUMBER OF THOSE STUDIES WHERE THE TEST IDENTIFIED SIGNIFICANT DIFFERENCES

Test	Studies employing test	Significant results	%
Santa Ana	29	18	62
Simple Reaction Time	52	27	52
Benton Visual Retention	20	10	50
Digit Symbol	60	27	45
Digit Span	39	17	44
Profile of Mood States	2	1	50
Pursuit Aiming Test	0	0	—

number in column 2). This compares favorably with an overall mean of 43% significant differences (calculated in the same way) reported for all 250 unique tests administered in the 185 studies surveyed. Within the functional categories selected (e.g., memory, coordination, speed), the NCTB tests are the most frequently employed in worksite research and among the most sensitive (defined as identifying significant differences) to chemical exposures of the tests employed in this field of research (Anger, 1990).

The two primary psychometric requirements of behavioral tests are reliability and validity. Each NCTB test was developed and extensively used in either neuropsychology or experimental psychology (Johnson *et al.*, 1987). It was thus assumed that they were reliable, although test–retest reliability data for these tests are limited (especially in normal adult populations) or inaccessible (unpublished). Since the NCTB was intended to be a screening instrument to identify chemical-induced health effects, traditional measures of content or construct validity (i.e., the tests assessed the functions intended) are of limited importance. Rather, criterion-related validity is the operative validation principle (Murphy and Davidshofer, 1988). Here, the criterion is the ability of the tests to reflect performance deficits in chemical-exposed groups when compared to a group which is not exposed to the chemicals under scrutiny. Since most worksite research is cross-sectional in nature, only one-time assessments are available to establish the validity criterion in any given population. Thus, replication of findings in different populations exposed to the same chemical using the same tests serves to establish the validity criterion for this area of research. An exclusive analysis of the NCTB tests has not been conducted. However, Anger's 1990 review demonstrates that control vs exposed group differences in populations exposed to lead, mercury, and carbon disulfide have been replicated extensively in independent studies using NCTB tests and, more broadly, evaluations of those functions assessed by NCTB tests. Therefore, the NCTB tests satisfy the validity criterion.

NCTB Cross-Cultural Assessment

What was not clear to the original group that proposed the NCTB as a battery was whether it could be employed in the wide array of cultural settings in which neurotoxic chemicals are employed (e.g., pesticides are used in virtually every country). A specific concern was that the NCTB tests were developed primarily in Western European or Western European-derived populations. Further, both construct validity and the criterion validity or sensitivity of the tests to chemical-exposed groups were also determined primarily in such populations. While the evidence is limited, research on cultural differences in performance testing indicates that some motor (e.g., Bernard, 1989) and cognitive processes are affected by racial or cultural differences, although it is difficult to eliminate educational factors in this research. However, most research employing intercountry or interculture comparisons suggests that cognitive processes are fundamentally similar in diverse cultures (D'Andrade, 1989). Nonetheless, the extent to which the NCTB tests would be reliable and sensitive to chemical-induced health effects in very different cultures is largely untested.

Under the auspices of WHO's Office of Occupational Health, a Cross-Cultural Assessment (CCA) was planned. The CCA's primary goal was to administer the NCTB to working populations unexposed to chemicals in a minimum of eight geographically dispersed countries representing diverse cultures. Specific objec-

tives were to evaluate potential problems that could occur in the translation of instructions or test materials into different languages and in test administration to populations unfamiliar with such tests. This effort was thus aimed at assessing the NCTB's feasibility for testing subjects in diverse international populations. On a more utilitarian level, the CCA sought to develop for the NCTB tests baseline or normative data on diverse cultures in five age ranges between 16 and 65.

MATERIALS AND METHODS

Subjects

Listed in Table 2 are cities and countries in which there are verified reports that Cross-Cultural Assessment data have been collected from volunteer subjects who were selected from occupations, jobs, or companies in which there were no known exposures to neurotoxic chemicals. Subjects from most countries came from large (People's Republic of China, France, Hungary), medium (The Netherlands, United States), or small (Austria, Canada, Poland) urban settings (Italian subjects were from settings of all sizes) and lived in working class or entry-level white collar housing for that country. Nicaraguan subjects were peasants who lived and worked in a rural setting. Also listed in Table 2 are primary subject occupations, incentives given to motivate participation, and an estimate of the years of education of the groups studied. Most subjects in all countries had completed 7–9 years of education, or a few years more. U.S. subjects had the highest number of years of education completed (13–15), while most Nicaraguan subjects had completed 3 or fewer years of school.

It was impossible to provide chemical sampling data in the various countries where this study was conducted. Rather, the principals running the study in each country took the responsibility for seeing that subjects met the criterion of being "unexposed" to chemicals. Subjects in most cities are exposed to some level of pollution. Subjects from the one rural area studied, in Nicaragua, were selected from agricultural farms (coffee, cattle) which specifically did not use pesticides, and each worker's occupational history was taken to verify that there were no significant chemical exposures in the prior 2 years. The principal from Poland indicated concern that the control subjects from Lodz may have been exposed to pollutants in this highly industrialized area, but the lack of a defined comparison basis makes it difficult to offer reliable information on this matter.

Subjects from the United States were paid U.S. $25.00 to take the NCTB and seven NES tests (the latter not reported here) in 2 hours of testing. This amount exceeded slightly the subject's hourly wage in most cases; most testing was conducted outside regular working hours. Subjects from the Netherlands were paid Dfl 25 (approximately U.S. $15 in early 1991), and subjects in Nicaragua were paid the equivalent of 2 days wages (approximately U.S. $4 in early 1991) for taking the tests during working hours. Canadian subjects were paid Canadian $75.00 (approximately U.S. $66 in early 1991) to participate in a 6-hr test session which included four NCTB tests. Subjects from Austria, China, France (in the annual medical examination), Hungary (in preemployment or periodic employee medical examinations), Italy, and Poland were not paid for their performance but were tested during regular work hours, which can be considered "time off work."

The number of male and female subjects in each of the five selected age ranges is listed in Table 3 for each country included in the results. (Submitted data were

TABLE 2

STUDY SITES PROVIDING DATA TO THE CROSS-CULTURAL ASSESSMENT, PRINCIPAL'S RESPONSIBLE, PRINCIPAL OCCUPATION OF GROUP STUDIED, INCENTIVES PROVIDED, AND ESTIMATED YEARS OF EDUCATION

Country	Study site	Sponsor	Principal	Study group occupations	Incentive	Years education[e]
Europe (east)						
Hungary[a]	Budapest	Institute Occupational Health	E. Zsögön A. Jóni	Pesticide factory wkrs[f] (not exposed)	Time off work	13
Poland	Lodz	Institute Occupational Health	B. Dudek B. Bazylewicz-Walczak	Power plant wkrs, cooks, drivers, cleaners	Time off work	9–13
Europe (west)						
Austria	Wiesing	Dept. Social Medicine	J. Hörtnagl	Household, office wkrs	No	8–9
France[d]	Paris	University of Paris	L. Fournier	Auto manufacturing wkrs	Time off work	10
Italy	Chieti	Svcs. Occup. Medicine	C. Fanelli	Cleaners	Time off work	8
	Potenza	Svcs. Occup. Medicine	C. Fanelli	Metallurgical wkrs	Time off work	8
	Poggibonsi	Svcs. Occup. Medicine	C. Fanelli	Food market wkrs	Time off work	8
	Bari	Institute Occup. Health	C. Fanelli	Mechanical plant wkrs	Time off work	8
	Milan	University of Milan	M. Cassitto	Job applicants	Time off work	8
The Netherlands	Hague	TNO	J. Hooisma	City residents	Money	11
Asia						
People's Republic of China	Shanghai	Shanghai Medical University	Y. Liang Z. Chen	Embroidery wkrs, inspectors, clerks	Time off work	11–12
	Beijing[c]	Inst. Occup. Medicine	Z. Zhou	Waiters, teachers	Time off work	10
North America						
United States	Salt Lake City	NIOSH	K. Anger	Postal, hospital wkrs	Money	13–15
	Cincinnati	NIOSH	K. Anger	Hospital, insurance wkrs	Money	13–15
	Portland (OR)	NIOSH	K. Anger	Hospital, government wkrs	Money	13–15
Canada[b]	Beauhamois	University of Québec in Montréal	D. Mergler	Utility wkrs, police, manufacturing wkrs	Money	9–12
Central America						
Nicaragua[d]	Leon Matagalpa	Swedish International Aid Agency	R. Amador M. Keifer	Cattle farm, coffee wkrs	Money	3

[a] Due to equipment costs, the Santa Ana and Simple Reaction Time tests were not used in this country.
[b] Used only four NCTB tests (three reported here) in a study involving other tests; the NCTB tests were administered following prescribed NCTB training procedures.
[c] Data not presented in this article.
[d] Due to concern over translation uncertainties, the POMS was not administered.
[e] Approximated in some cases; some subjects in younger age groups were still in school and are thus not reflected in these estimates.
[f] Wkrs, Workers.

TABLE 3
NUMBER OF NCTB SUBJECTS BY COUNTRY, SEX, AND AGE RANGES FOR DATA REPORTED IN ARTICLE

	Male subjects					Female subjects					
Country	16–25	26–35	36–45	46–55	56–65	16–25	26–35	36–45	46–55	56–65	Totals
Europe (east)											
Hungary		24									24
Poland	19	28	25	17	10		16	14	16		145
Europe (west)											
Austria	30	19	15	12	21	31	18	12	19	25	202
France		24	22								46
Italy		192	11				16	14	16		249
The Netherlands	26	35	41		67	32	13	14		27	255
Asia											
People's Republic of China[a]	27	49	19	40		25	53	33	36		282
North America											
United States	80	73	85	50	29	140	163	162	92	41	915
Canada			70	37							107
Central America											
Nicaragua	31	25	28	12	10						106
Totals	213	469	316	168	137	228	279	249	179	93	2331
			Male	subtotal	1303			Female	subtotal	1028	

[a] Data from Shanghai only.

omitted when the number of subjects was less than 10 in a given age range.) Data exclusions due to equipment malfunctions or test interruptions variably reduced the number of subjects on some tests.

Tests

The NCTB tests are described below in the order the tests were presented. Each test description is brief, since they are thoroughly described in other publications (Johnson *et al.*, 1987; Cassitto *et al.*, 1990). The entire battery of tests was given in all countries except Canada (where only the Santa Ana, Benton, Digit Span, and Profile of Mood States (POMS) tests were administered), Hungary (where the Santa Ana and Simple Reaction Time tests were not given), and Nicaragua (where the POMS was not administered).

Profile of Mood States. This test contains 65 adjectives that describe different moods. Subjects were asked to indicate their mood states during the past week on a five-point scale ranging from "not-at-all" to "extremely."

Simple Reaction Time. The test of reaction time requires the subject to place the index finger of their dominant hand so it is just touching the response button and then to press the button whenever a nearby light is illuminated, in a series of 64 trials of random-length intervals (mean 5.6 sec; range 1.0–11.0 sec).

Digit Span. A series of digits are read to the subject who is asked to recall them immediately, in the order presented, and, subsequently, in the reverse of the order presented. Beginning with three-digit strings forward and two-digit strings backward, two sets of strings at each string length (i.e., number of digits) were presented until the subject missed both strings at a given length, terminating the test.

Santa Ana. This test has a base plate with square depressions and fitted pegs with a cylindrical upper section. The subject takes each peg in succession, lifts it from the depression, turns it 180 degrees, and puts it back as quickly as possible; the subject has 30 sec in which to turn as many pegs as possible.

Digit Symbol. The worksheet on this test contains a list of numbers (1–9) that are associated with nine simple symbols. Below this code list is a string of random digits (1–9) paired with blank squares in which the subject must draw the appropriate symbols (i.e., the symbol paired to their corresponding digit) as fast as possible in 90 sec.

Benton Visual Retention (Recognition form). This test contains 10 test cards, 2 presenting one geometric figure and 8 presenting three horizontally arranged geometric figures. Subjects are shown figure(s) on a card for 10 sec and then asked to recognize the same figure from a set of four similar alternatives shown subsequently on a second card.

Pursuit Aiming II. Subjects are required to use a moderately sharp pencil to place a dot in 2-mm-diameter circles for two 60-sec trials, following a back-and-forth pattern on a single page.

Procedures

Three WHO Collaborating Centres were identified as coordinating centers for the CCA. These were in Helsinki (Institute of Occupational Health) under Dr. H. Hänninen, in Milan (Institute of Occupational Health) under Dr. M. Cassitto, and in Cincinnati (National Institute for Occupational Safety and Health) under Dr. K. Anger. Each coordinating center assembled the NCTB tests into "kits" for distribution (loan), trained test administrators (to assure consistency of test admin-

istration), and collated results. An Operational Guide for the NCTB (WHO, unpublished; see References for availability), specifying detailed instructions and procedures for administering the seven NCTB tests in English, was developed and used as a procedural blueprint for each principal investigator.

Translation of the instructions was the responsibility of the host principal investigator, sometimes with assistance of a coordinating center. The people who administered the NCTB in the CCA were trained by Drs. Anger, Cassitto, or Hänninen (one of four testers in the People's Republic of China, Italy; one of three testers in The Netherlands; one of six testers in Nicaragua, Poland, United States) or they were trained by someone trained by Anger, Cassitto, or Hänninen (Austria; Canada; three of four testers in the People's Republic of China; two of three testers in The Netherlands; five of six testers in Nicaragua). The administrators in France and Hungary were self-taught using the NCTB Operational Guide. Training took between 4 and 80 hr, depending on the expertise (and translation needs) of the persons receiving training and their degree of preparation for the training. The instructions and information in the Operational Guide were the basis for the training. NCTB tests were administered in a variety of settings, including laboratories, offices, conference rooms, and clinics in companies, government buildings, hospitals, schools, apartment buildings, and trailers. Minimization of noise and other extraneous factors, as specified in the Operational Guide, was a responsibility of the principal investigator in each country. The degree of compliance with these guidelines was not monitored.

RESULTS

Results from some countries in the Cross-Cultural Assessment have been presented in tabular form in other publications (Cassitto *et al.*, 1990; Liang *et al.*, 1990). Those and other data are collected here to provide a graphic presentation of the two basic descriptive parameters, mean and standard deviation. Inferential statistics were not applied to the data summarized in this article because the primary goal was to provide a descriptive presentation of findings in several countries for the purposes of data exploration and generation of hypotheses, rather than to demonstrate (significant) differences. Thus, comments on group differences or trends are made purely at the descriptive level and are not necessarily statistically reliable.

Mean and standard deviation data from all locations and age ranges where a minimum of 10 subjects was obtained are presented in Figs. 1–10 for six NCTB tests. (POMS data are not presented due to the expectation of high population specificity and thus large between-country variability due to concerns about translating context-sensitive words.) Data were excluded where testing errors were identified (e.g., Digit Span data from Poland are omitted due to the use of a different scoring method, and Simple Reaction Time data from Nicaragua were omitted because the initial finger location on each trial was different than specified in the Operational Guide). Figures 1–6 include data for each country where at least three age ranges are represented for each NCTB test; data from male subjects are in the top panel and data from female subjects are in the bottom panel. Countries that provided data in only one or two age ranges (viz. France, Italy, Hungary, Canada) are represented only in Figs. 7–10. Figure 7 presents results on all test

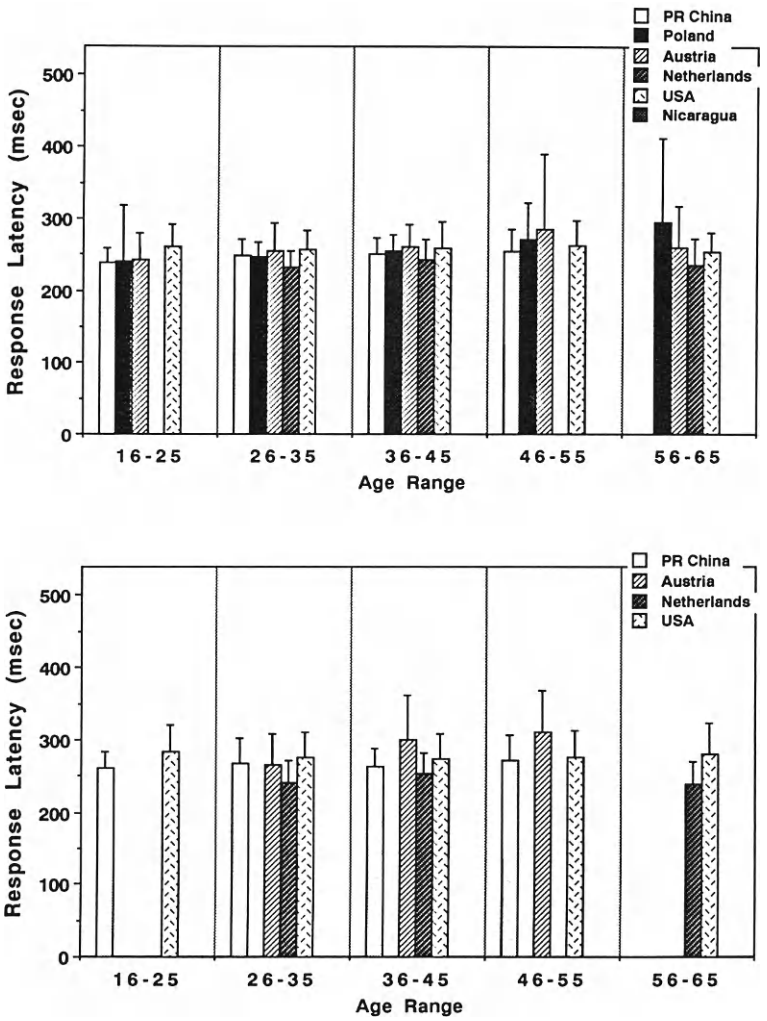

FIG. 1. Mean (±SD) response latency on the Simple Reaction Time test in age ranges between 16 and 65 for males (top) and females (bottom).

data from all countries with female subjects in the 26–35 age range; similarly, data from male subjects in the 26–35, 36–45, and 46–55 age ranges are presented in Figs. 8–10.

From the Simple Reaction Time test, Fig. 1 presents the mean response latency (from onset of light until the button was pressed) in milliseconds. The mean male (top panel) latencies are clustered tightly around 250 msec, and the mean response latencies in females (bottom panel) were between 239 and 311 msec.

Figure 2 presents the mean number of digit strings correctly recalled (sum of strings forward and backward) in the Digit Span test. (For example, if the subject recalled both strings of 3 and 4 digits then failed both of 5 digits forward, recalled one out of two strings of 2 then failed both at 3 backward, the score would be 5.) Male and female subjects remembered between 11 and 19 digit string sequences, with a small reduction in the 56–65 age range in subjects from Austria, The

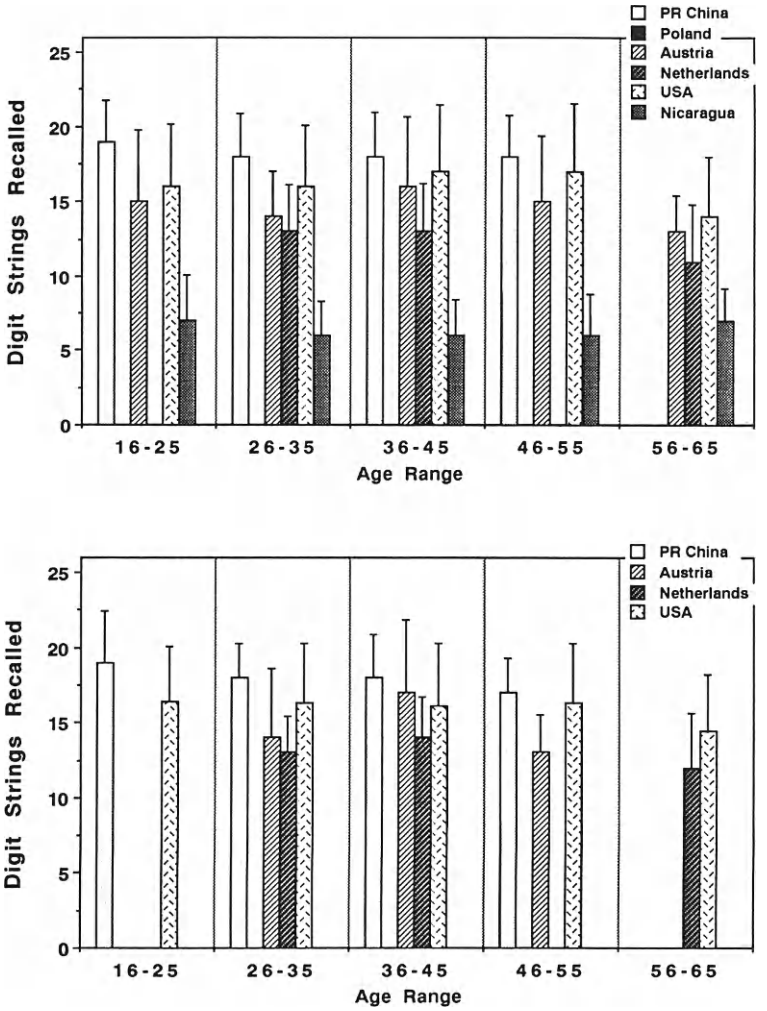

FIG. 2. Mean (±SD) number of digit strings recalled in the Digit Span test in age ranges between 16 and 65 for males (top) and females (bottom).

Netherlands, and the United States (within-country comparisons). Nicaragua (male subjects) is an exception in that the mean number of digit strings recalled was only 6–7 and there was no decline in the older age range. Performance within a given country is relatively consistent (similar) across the four age ranges between 16 and 55 in both male and female subjects.

From the Santa Ana dexterity test, Fig. 3 presents the mean total number of pegs turned by the preferred (dominant) hand on two 30-sec trials. Total pegs turned ranged from 28 to 47 in male and 33 to 45 in female subjects. Within each country, the range was 5–8 across the five age ranges in all cases except Netherlands females who had a range of 11 pegs across the four age ranges they spanned. A country by country comparison suggests a consistent decrement in the number of pegs turned between subjects in the three younger age ranges and subjects in the 56–65 age range. The Santa Ana is the one NCTB test on which performance by subjects from Nicaragua was close (within 1 SD) to that seen in

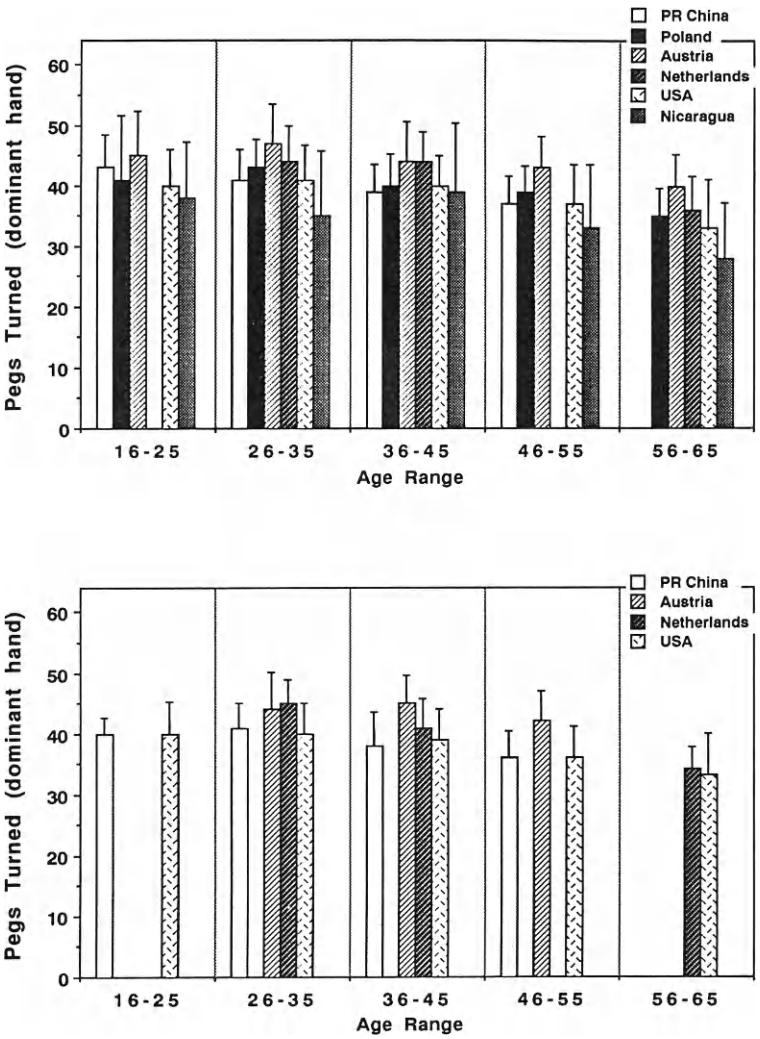

FIG. 3. Mean (±SD) number of pegs turned by the dominant hand in the Santa Ana dexterity test in age ranges between 16 and 65 for males (top) and females (bottom).

other countries, although Nicaraguan subjects turned the smallest number of pegs in every age range except 36–45 where they tied for the smallest number with subjects from China.

Figure 4 presents the mean number of correct symbols drawn in 90 sec in the Digit Symbol test. In Nicaragua (male subjects), the mean number of symbols ranged between 6 and 26. In all countries except Nicaragua, the mean number of symbols drawn by males ranged from 31 to 65; females drew between 44 and 72 symbols. Within-country data follow a consistent decreasing trend from younger to older age ranges and a trend to higher scores in females than in males.

Figure 5 presents the number of correctly recognized geometric figures (of 10 possible) in the Benton Visual Retention test. The mean number of figures remembered was between 7.6 and 9.4 for both male and female subjects. The sole exception is Nicaragua (male subjects) where the range was from just under 4 to

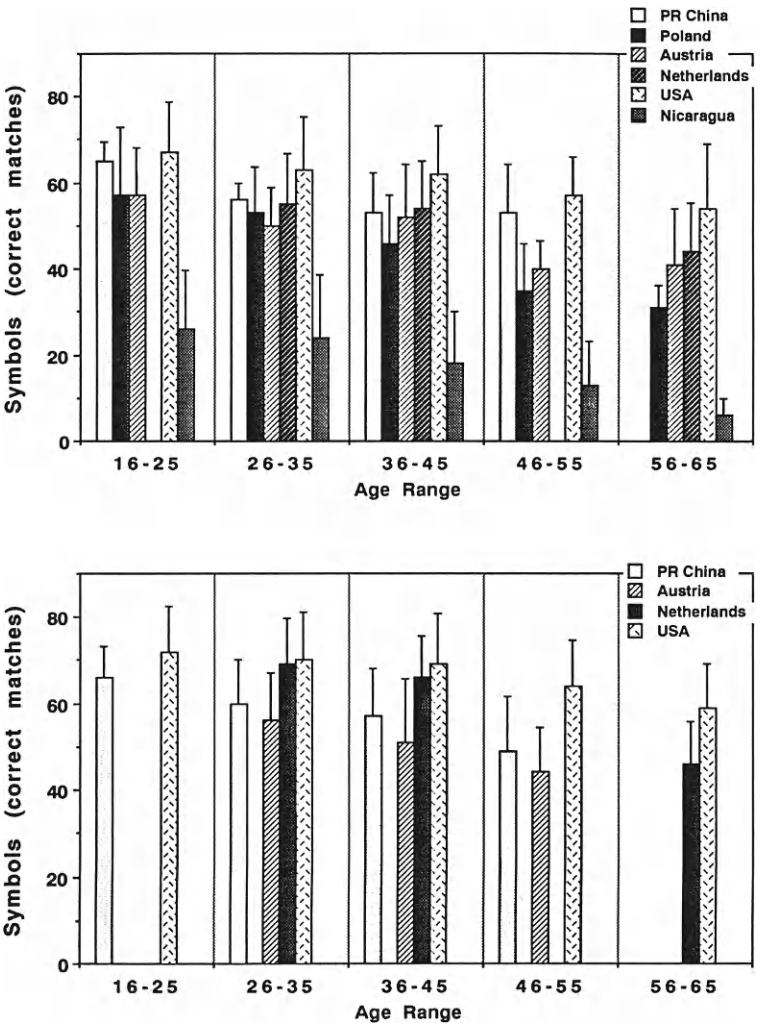

FIG. 4. Mean (±SD) number of symbols (correct matches) drawn in the Digit Symbol test in age ranges between 16 and 65 for males (top) and females (bottom).

just over 4.5. If Nicaragua is excluded, within-country data are highly consistent (similar), as are between-country data.

From Pursuit Aiming II, Fig. 6 records the number of dots placed in circles in two 60-sec trials. Male subjects from all countries except Nicaragua placed a mean number of between 93 and 240 dots, while the range in (male) Nicaraguan subjects was between 18 and 25 dots. Within-country data are relatively more consistent than between-country data, with a tendency to slight declines in the 46–55 and/or 56–65 age group. Females follow the same pattern (range from 108 to 256).

The larger number of countries represented in the 26–35, 36–45, and 46–55 age ranges, shown in figures 7–10, reinforces the evidence of between-country consistency or similarity seen in Figs. 1–6. Between-country data are highly consistent (similar) for the Simple Reaction Time test and, excepting Nicaragua, the

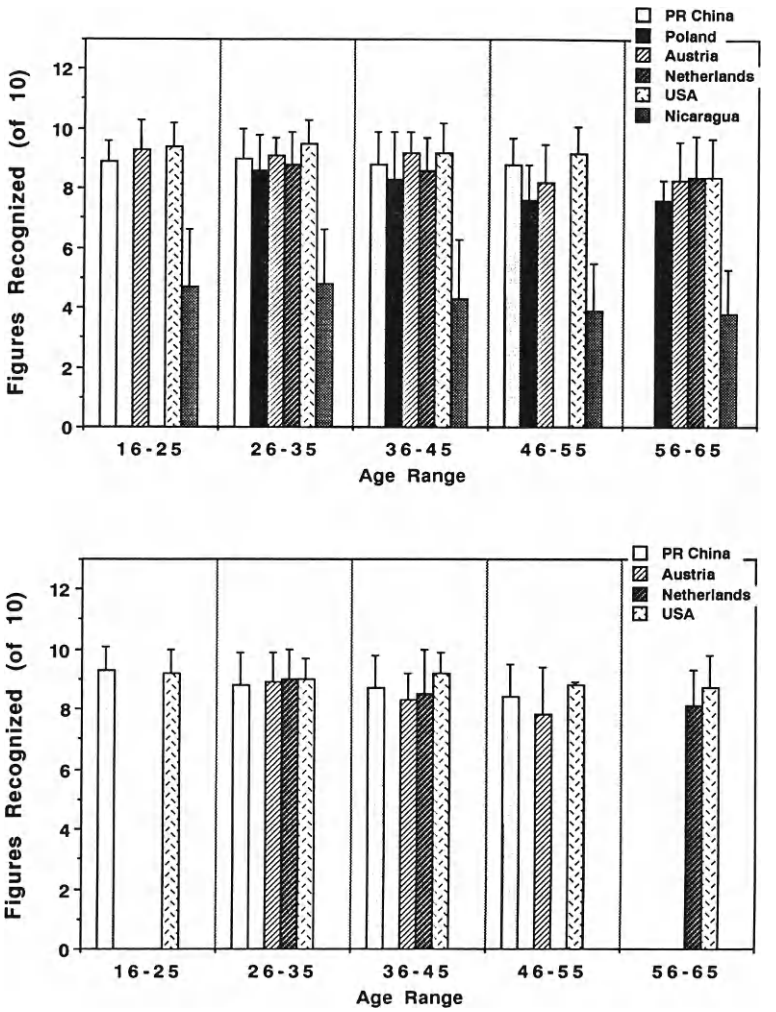

FIG. 5. Mean (±SD) number of figures recognized (of 10) in the Benton Visual Retention test in age ranges between 16 and 65 for males (top) and females (bottom).

Benton test, whereas the data are less consistent for, in declining order, the Santa Ana, Digit Symbol, Digit Span, and Aiming tests.

DISCUSSION

The Cross-Cultural Assessment of the NCTB exceeded by two the goal of collecting data in eight countries, although the distribution did not achieve the degree of cultural diversity originally sought. Data presented here represent performance of over 2300 participants (1303 males and 1028 females) from European (involving, at a minimum, slavic, grecolatin, and germanic cultural groups), North American (predominantly Western European), Central American, and Asian populations. Collection of NCTB data from other populations (e.g., Venezuela and India) also appears achievable in the near future.

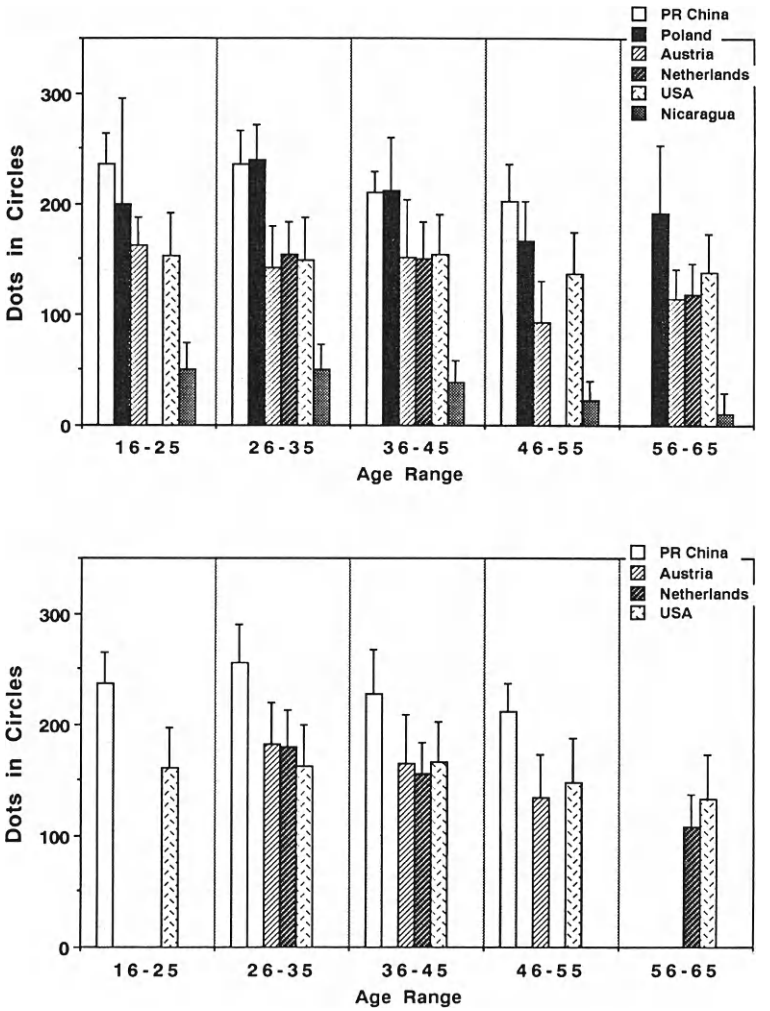

FIG. 6. Mean (±SD) number of dots placed in circles in the Pursuit Aiming II test in age ranges between 16 and 65 for males (top) and females (bottom).

Between-Country Consistency

The data collected on the Simple Reaction Time test demonstrate consistency or similarity in absolute scores across all countries studied. With the exception of Nicaragua, data from the Benton test also demonstrate consistency between countries. Santa Ana, Digit Span, Digit Symbol, and Aiming data demonstrate more diversity between countries, although data on these tests appear relatively consistent within each country when viewed across age ranges (Figs. 1–6).

The data from Nicaragua stand in stark contrast to results of the NCTB in other countries. The explanation for this performance difference does not appear to relate to procedural problems. The test administrators in Nicaragua were trained by Dr. R. Amador, who was trained in NCTB administration by Dr. H. Hänninen. Dr. Amador conducted the study and served as one of the six Nicaraguan test administrators. In addition, Dr. M. Keifer, who received NCTB training from Dr. K. Anger, collaborated on project testing. Drs. Amador and Keifer judged the test

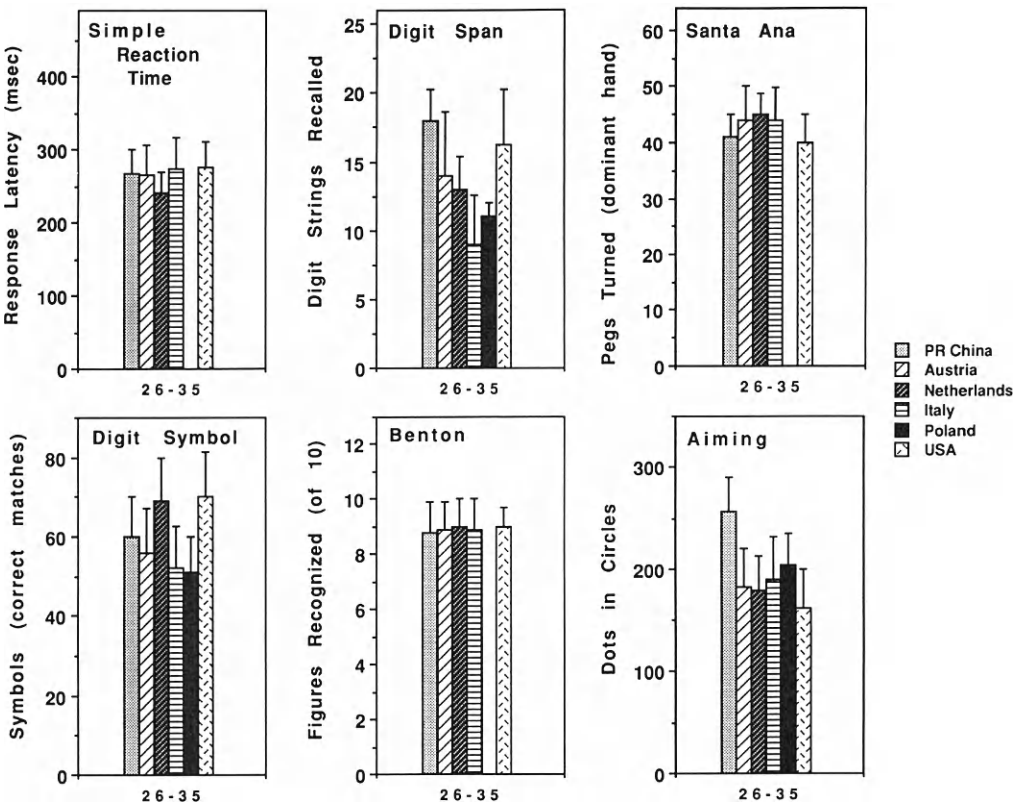

FIG. 7. Mean (±SD) on NCTB tests for female subjects ages 26–35 (including countries which only reported results in limited age ranges).

administrators to be well-trained and motivated. Most subjects had some difficulty with all tests, but test administrators and observers (Amador, Keifer) were convinced that subjects understood the instructions, responded positively to the testers and test setting, were motivated to perform the tests (many appeared competitive, inquiring how they did compared to others), and supported the study's goals (both unions and management supported the study in open meetings in which potential subjects asked many questions about who would benefit and who was running the study). Mean performance by Nicaraguan subjects on the Santa Ana, though slightly lower than that in other countries, was within one standard deviation of mean scores in other countries (Fig. 3). This suggests that the Nicaraguan subjects had a reasonable level of motor competence, motivation, understanding of instructions, and comfort with the test conditions.

Evidence to support this conclusion was sought from the findings of the Simple Reaction Time test which, as noted above, were excluded from the results because the Operational Guide instructions had failed in Nicaragua to produce the appropriate response behavior and were thus altered. The principals changed the procedure to require the subjects to place their fingers to the side of the response button and, when they saw the light, to "press the trigger." While the Nicaraguan subjects had little education, many have had recent military experience. Their mean response latency varied between 385 and 450 msec, or roughly twice that of subjects in the other countries (Fig. 1) who had a far shorter distance to respond

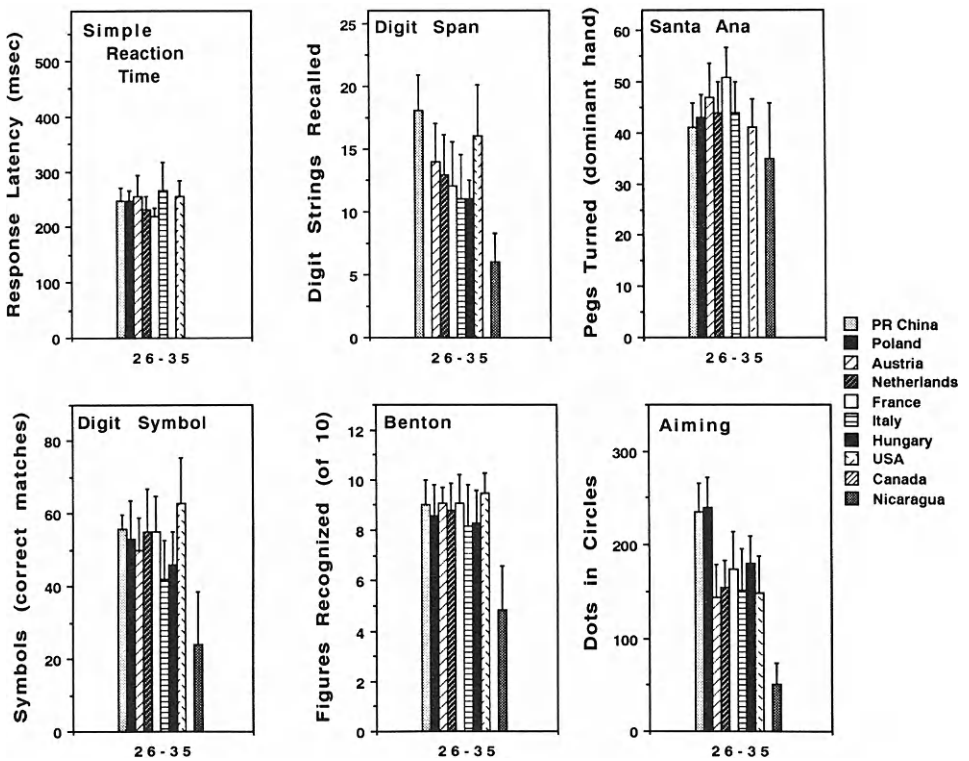

FIG. 8. Mean (±SD) on NCTB tests for male subjects ages 26–35 (including countries which only reported results in limited age ranges).

(viz. 0 mm, as they were touching the button). To further investigate this finding, 32 faculty members (mean 17 years education) of the University of Nicaragua in Leon were given this test using the correct instructions; their mean reaction time latency was 255 msec for subjects aged 16–25 and 258 msec for subjects 26–35; this is in the range found in all other countries (Fig. 1). When administered to the same 32 faculty members using the modified (incorrect) instructions, the mean reaction time latency was 355 and 334 msec for the 16–25 and 26–35 age ranges, respectively. Finally, 45 of the original control subjects were retested on this the Reaction Time test using the correct instructions; the mean time latency was 330–402 msec in the five age ranges, suggesting that performance was slower in Nicaragua than in other countries but within 1–2 SD of performance in other countries. The performance of the faculty demonstrates that a highly educated group of Nicaraguans can perform this test very well. This suggests a hypothesis related to education, explored below.

Aside from the results on the Santa Ana, mean scores by Nicaraguan subjects on the remaining NCTB performance tests are roughly half the level achieved in other countries. The most straightforward explanation for this finding is in factors inherent to the subjects tested. Nicaragua is the one country in the CCA where a predominantly rural population with almost no formal education (approximately 3 years) was tested. Specifically, 32% of the Nicaraguan subjects had no formal education, 42% had 1–3 years, and 26% had 4–19 years of education. Most Nicaraguan subjects responded to a structured question set that they did not write or

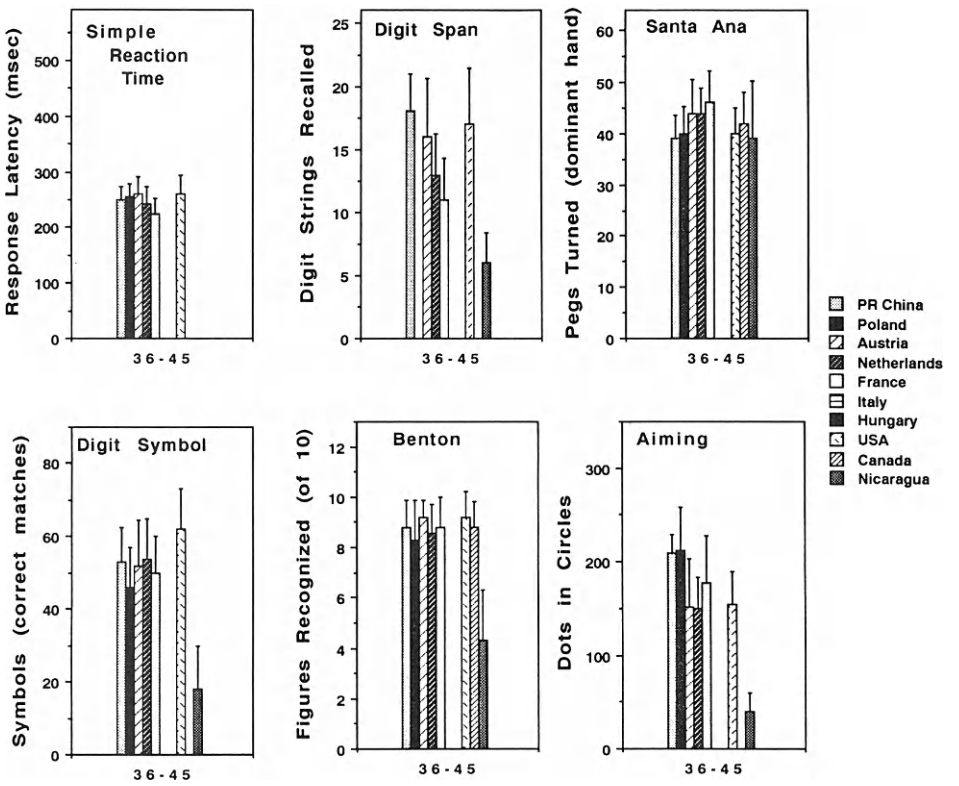

FIG. 9. Mean (±SD) on NCTB tests for male subjects ages 36–45 (including countries which only reported results in limited age ranges).

read in their daily lives. Thus, at least 74% of these subjects can only be characterized as marginally literate or illiterate.

Drs. Amador and Keifer provide enlightening comments on Nicaraguan subject performance relating to the lack of formal education and an associated unfamiliarity with performance tests such as those in the NCTB. On the Benton, the lack of experience with geometric figures is hypothesized as the key problem. Inexperience with writing was a significant handicap in the Aiming and Digit Symbol tests; test administrators noted that about one-third of the subjects appeared unused to having pencils in their hands. The Aiming test may also have been affected by uncorrected vision problems, especially in older subjects. The Digit Symbol test amounted to a symbol–symbol test (and perhaps was further affected by uncorrected vision deficits) in these marginally literate subjects, and the Digit Span may also have been affected by inexperience with numbers.

To assess the possibility that education was a major factor in the Nicaraguan results, Pearson r and Kendall's τ correlations were run between years of education and scores on each test. The nonparametric Kendall's τ correlations were included because the large number of subjects with 0–3 years education produced nonnormal distributions. The results for each test were: Digit Symbol ($r = 0.69$; $\tau = 0.64$); Digit Span ($r = 0.44$; $\tau = 0.52$), Benton ($r = 0.33$; $\tau = 0.42$); Aiming ($r = 0.58$; $\tau = 0.58$). As would be expected, the lowest correlation was on the Santa Ana ($r = 0.29$; $\tau = 0.31$) where performance was close to that seen in other countries and the Simple Reaction Time test ($r = -0.12$; $\tau = 0.11$).

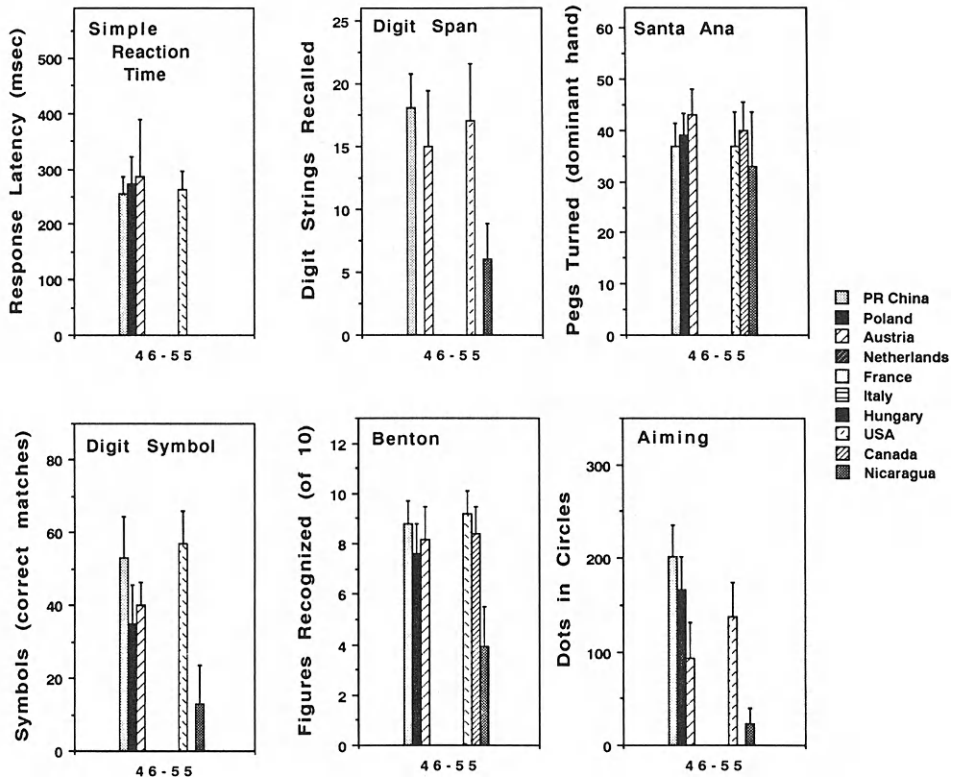

FIG. 10. Mean (±SD) on NCTB tests for male subjects in ages 46–55 (including countries which only reported results in limited age ranges).

These correlations are consistent with other reports. Neuropsychological test performance is positively associated with education, including tasks (e.g., memory) independent of academic performance (Lezak, 1983). There is a 0.44 and 0.42 correlation between years of education and performance on the Digit Symbol and Digit Span, Wechsler Adult Intelligence Scale (WAIS) subtests, respectively. Scores on WAIS "performance" tests, including the Digit Symbol test, are roughly 10–20% lower in subjects with a range of 0–11 years of education than in subjects with 12–15 years of education (Albert and Heaton, 1988). Similar though smaller performance differences related to education have been reported for cognitive tests such as those in the Halstead-Reitan (Leckliter and Matarazzo, 1989) and the WAIS Digit Span (Heaton *et al.*, 1987) and simple motor tests such as tapping (Warner *et al.*, 1987) and grooved pegboard tests (Bornstein, 1985).

While there are large differences in education between Nicaraguan and other CCA subjects, the Nicaraguan population also represents a unique cultural group (American Indian–Spanish) in this study. Cultural differences provide a reasonable competing hypothesis (to education) as the cause of the performance differences between Nicaragua and the other countries. However, the evidence on the educational factor in cultural differences in cognitive performance (D'Andrade, 1989), evidence in many populations of a correlation between performance and education on some NCTB tests (noted above), and the high correlation between years of education and NCTB performance reported in this study reinforce the

proposal that educational factors provide the more supportable hypothesis to explain the poorer performance of Nicaraguan subjects.

The huge performance differences between subjects from Nicaragua and those from other CCA countries raises doubts about the NCTB's hoped-for universal utility. The high correlation between years of education and several NCTB tests indicates that this variable must be taken into account (through subject selection criteria or data analysis) in the analysis of NCTB data in any study. However, more research is clearly needed on education as a factor in NCTB performance. A different battery may be needed to provide an adequate baseline (i.e., sufficiently high performance and a sufficiently small standard deviation relative to the mean) to detect behavioral effects (performance decrements) of neurotoxic chemicals in poorly educated or poorly literate populations. *Data from other Latin American countries and from sparsely educated subjects in other countries and in different socioeconomic strata are needed to address these findings.*

Other Subject Factors (Age, Sex)

Data on two NCTB tests demonstrate slight (Santa Ana, Digit Span) or marked (Digit Symbol) age-related performance declines, particularly when viewed in within-country comparisons. This is consistent with other research. WAIS verbal tests remain stable into later decades, while performance tests show a characteristic age-related decline (Nolan *et al.*, 1986). The one WAIS verbal test in the NCTB is the Digit Span and the only performance test in the NCTB is the Digit Symbol (Murphy and Davidshofer, 1991). CCA data are thus consistent with these findings; declines in the 56–65 age range are more apparent in the Digit Symbol (Fig. 4) than in the Digit Span (Fig. 2). Further, age-related memory losses are found on free recall (e.g., Digit Span) but not on recognition tests (e.g., Benton) (Shimamura, 1990; Botwinick, 1981). This is consistent with CCA data in that performance on the Benton is virtually the same in all age ranges (Fig. 5), while there appears to be a slight decline in the 56–65 age range on the Digit Span (Fig. 2). With regard to motor tests, mean scores on the Santa Ana are lowest in the 56–65 age range, when compared to subjects of younger ages within each country, but there are no such differences on the Simple Reaction Time test.

Age-related declines in performance are reported on tests of memory (Nolan *et al.*, 1986), a variety of visual discrimination tasks (Hochanadel and Kaplan, 1984), and motor tests, including Simple Reaction Time (Botwinick, 1967, 1984; Salthouse, 1985; Nolan *et al.*, 1986) and grooved pegboard (Bornstein, 1985). While age-related performance declines do occur in the fifth decade, they are more typical of the sixth decade or beyond (Albert and Heaton, 1988; Nolan *et al.*, 1986), and age-correlated differences are exaggerated by cross-sectional research such as the present study, due presumably to improvements in early health care, nutrition, and education (Albert, 1988). Since the age-related declines seen in this study are minimal, the NCTB would appear relatively resistant to group differences in age, especially below age 55.

There are performance differences between men and women on many behavioral tests. For example, women perform significantly better on the Digit Symbol test than do men (Albert and Heaton, 1988), and the reverse is the case for the grooved pegboard test (Bornstein, 1985). Similar trends are seen in the NCTB's Digit Symbol data in some countries (e.g., United States) and in the Santa Ana (pegboard) test, but the differences are very small and not completely consistent

in the latter case. Generally, however, CCA test results of women subjects are remarkably similar to test results of men subjects on most NCTB tests when comparing data country by country (Figs. 1–6).

In summary, trends in the CCA data are generally consistent with findings in the literature with regard to the variables of education, age, and sex. Sex appears to have a smaller impact on NCTB performance, and the changes in NCTB performance over age ranges of 16–65 (noted within each country) are also relatively slight. This suggests that, in worksite research, small differences between the mean ages of referent and exposed groups with only relatively equal distributions of men and women would not seriously jeopardize conclusions from data on most NCTB tests. Rather, concern in a given study should be focused on education and perhaps other potential variables if sex and age differences between groups are not large, and especially when subject ages are below 55 years.

Technical Factors

For those tests where there are substantial differences between countries (e.g., Digit Span, Aiming), the differences could result from a variety of factors, including cultural, educational, nutritional, prenatal, health status, genetic, motivational, or socioeconomic. Performance variability on all tests could also result from a variety of technical factors such as instructions, test administration consistency, and test grading. Relevant technical factors that could have produced between-country variability and within-country consistency include the following: (a) the orally presented Digit Span test is particularly susceptible to vagaries of administration; (b) the relation between subject height/arm length and table height in the Santa Ana dexterity test may have been variably optimal across countries; (c) the Pursuit Aiming test is susceptible to substantial grading criterion differences such that all tests in a given study should be graded by one person; (d) substitute equipment in the Simple Reaction Time test was used after the original equipment became prohibitively expensive; button travel distance, button resistance, and other factors could have differed, despite heroic efforts to assure that alternative devices retained the same timing characteristics as the original source; and, (e) the POMS test is so highly dependent on the existence of precisely equivalent terminology in diverse languages that translation limitations must be expected to be unresolvable (e.g., D'Andrade, 1989).

CONCLUSIONS

The data presented here serve an illustrative purpose. The initial goal of the Cross-Cultural Assessment was to determine if a wide variety of populations could perform in a roughly similar manner on the NCTB tests, thus satisfying a loosely defined "feasibility" criterion. While there are clearly differences in mean performance between countries on many tests (e.g., Aiming, Digit Symbol, Digit Span), within-country performance across age ranges is remarkably consistent in the six countries in which such data have been obtained by the CCA. Feasibility is thus clearly demonstrated in a range of cultural groups, as long as within-country comparisons are planned. The NCTB can therefore be used widely, perhaps universally, in male and female working age populations with 8–12 years of formal education, as long as the education variable is equated among groups or included in the data analysis. However, the degree to which subjects with less than 8 years education can provide baseline performance on most NCTB tests

which will allow detection of neurotoxic effects in exposed populations remains an open question.

It was recognized by the original expert group that data from this Assessment in unexposed workers would *not substitute for control or reference groups in future studies* because factors such as living conditions, health status, education, socioeconomic status, genetics, motivation, and culture (e.g., deemphasis on individual achievement) can be dominant variables. The baseline data presented here tend to support that contention with regard to education and perhaps correlated socioeconomic background (Nicaraguan subjects were described as rural peasants while all other subject groups came from towns or cities and were typically not on the bottom socioeconomic rung). However, the data in this article can serve as a *guide to the range within which control data would be expected to fall.* More useful is the relative lack of performance changes across age ranges which suggests that conclusions based on most NCTB tests can be drawn in cross-sectional studies when group ages are relatively similar (especially between the ages of 16 and 45). Similarly, minor differences in group gender composition in cross-sectional studies with the NCTB would not be expected to affect conclusions on most tests.

The Cross-Cultural Assessment was designed to develop baseline data across the working age span in a minimum of eight countries by testing working populations unexposed to chemical substances where they work. The minimum number of countries was met, although the cultural and socioeconomic dispersion is insufficient at present. Further, the feasibility of using the NCTB in poorly educated populations remains an unresolved question that urgently needs an answer before the NCTB can be recommended for use in such populations. Thus, additional data are being sought to further expand the CCA in breadth (across countries) and depth (multiple sites within countries) in order to increase the generality of the baseline data and to answer questions related to feasibility of use in poorly educated populations. Continuation of this effort has been undertaken directly by Drs. Anger and Cassitto and their host institutions (Oregon Health Sciences University and Milan's Institute of Occupational Health, respectively), with tacit but not administrative support of WHO.

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Individual-Administered Human Behavioral Test Batteries to Identify Neurotoxic Chemicals[1]

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Most research demonstrating behavioral effects of occupational chemical exposures is produced in established laboratories using a consistent set or battery of tests. Exemplifying this tradition are batteries developed at Finland's Institute of Occupational Health, Milan's Institute of Occupational Health, Sweden's National Institute of Occupational Health, Australia's National Institute of Occupational Safety and Health, and at universities in the United States and other countries. In 1983, under the World Health Organization (WHO) aegis, experienced human occupational researchers recommended the Neurobehavioral Core Test Battery (NCTB) as a screening instrument to be administered by an individual to subjects exposed to chemicals believed to be neurotoxic. Health professionals from 50 cities in 27 countries distributed on every large continent have been trained to administer the NCTB according to its Operational Guide. Six issues need to be addressed regarding human-administered test batteries: (a) The critical role of individual-administered batteries to screen chemically exposed populations in a field increasingly dominated by computer-administered batteries; (b) selection criteria for tests to assess known and unknown chemicals; (c) utility of baseline data for study analysis and interpretation; (d) test battery validation; (e) availability and cost of inexpensive test batteries; and (f) equivalence of computer- and human-administered variants of the same tests.

INTRODUCTION

Research assessing occupationally exposed persons with behavioral tests began in the 1960s (Hänninen, 1966) and has been consistently productive since the 1970s (Anger, 1990). Various laboratories and agencies developed their own test batteries early in this area of research (e.g., Hänninen and Lindström, 1979), a process that has continued through the 1980s and into 1990 (e.g., Gamberale *et al.*, 1990; Williamson *et al.*, 1982; Williamson, 1990; Almirall-Hernández, *et al.*, 1987; Hogstedt *et al.*, 1980; Valciukas and Lilis, 1980; Hänninen and Lindström, 1979; Hänninen, 1990). Research in various countries where laboratories use a consistent set or battery of tests produces the majority of findings demonstrating behavioral effects of occupational chemical exposures (Anger, 1990).

Behavioral test batteries that have been employed in neurotoxicology research or that were developed by members of the behavioral neurotoxicology community are listed in Table 1. The table is divided into batteries administered by a person (top) and those administered by a computer (bottom). The use of computer-

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TABLE 1
HUMAN TEST BATTERIES IN BEHAVIORAL NEUROTOXICOLOGY

Battery name	Country of origin	Source
	Individual-administered batteries	
—[Information Theory Battery]	Australia	Williamson *et al.*, 1982; Williamson, 1990
London School of Hygiene Battery	Britain	Cherry *et al.*, 1984
Finland Institute of Occupational Health (FIOH) Battery	Finland	Hänninen and Lindström, 1979, 1989
Neuropsychological Screening Battery (NPS)[a]	Finland	Hänninen, 1990
Test Battery for Investigating Functional Disorders (TUFF)	Sweden	Hogstedt *et al.*, 1980; Ekberg and Hane, 1984
—[Mt. Sinai 1970s–1980s battery]	United States	Valciukas and Lilis, 1980
Neurobehavioral Core Test Battery (NCTB)	World Health Organization	Johnson *et al.*, 1987
Pittsburgh Occupational Exposures Test Battery (POET)[a]	United States	Ryan *et al.*, 1987a
CNS-B[a]	United States	Bowler *et al.*, 1986
IMT	Cuba	Almirall-Hernández *et al.*, 1987
—[Milan IOH Battery]	Italy	Angotzi *et al.*, 1980
	Computer-administered batteries	
Neurobehavioral Evaluation System (NES)	United States	Letz and Baker, 1986; Letz, 1990
Armed Forces Cooperative Performance Assessment Battery (UTC-PAB)	United States	Englund *et al.*, 1987
Microtox Test System (MTS)	United States	Eckerman *et al.*, 1985
Milan Automated Neurobehavioral System (MANS)	Italy	Cassitto *et al.*, 1989
Swedish Performance Evaluation System (SPES)	Sweden	Gamberale *et al.*, 1990
Cognitive Function Scanner	Denmark	Laursen, 1990
Automated Performance Test System (APTS)	United States	Kennedy *et al.*, 1987

[a] Neuropsychological battery.

administered test batteries (described by Letz in this volume) has grown with tremendous rapidity and has tended to dominate the field in recent years. This domination is due in part to the influence of publication in journals from industrialized countries where this interest is centered and where microprocessors are widely available and inexpensive.

Test batteries administered by a human do not achieve the level of efficiency or

presentation consistency as do computer-administered batteries. However, aided by precision equipment, human- or individual-administered batteries can be equivalent, or in some cases, superior to their computer-implemented clones. Reasons include the capability of the individual-administered batteries to employ a wider range of tests, the capacity of a human tester to elicit cooperation and responding from nervous, reluctant, or poorly literate subjects, and the greater potential for detecting subtle subject responses leading to important follow-up testing recommendations. Obviously, a combination of human administration and use of precision instruments (e.g., as employed by Williamson *et al.*, 1990, and Gamberale *et al.*, 1990) is the most effective approach. This suggests that the frequently mentioned distinction between human- and computer-administered batteries has a degree of artificiality and is unnecessarily limiting. Improved technology should be used to test subjects whenever it is appropriate; human judgment is needed to evaluate results and is often desirable for test administration.

Individual-Administered Human Behavioral Batteries

Hänninen developed the first behavioral test battery used in worksite research in her early studies of carbon disulfide-exposed workers (Hänninen, 1966, 1971, 1974). A handbook describing the battery and its implementation is available from the Finnish Institute of Occupational Health (FIOH) (Hänninen and Lindström, 1989) where it has been modified and refined over the years. Following the development of Hänninen's FIOH battery, there was a period of aggressive development of test batteries by scientists at other institutions, as reflected in Table 1. The batteries listed in Table 1 can be divided into neuropsychological test batteries, batteries that are no longer used actively, batteries that have been replaced by other (computer-implemented) batteries, and batteries undergoing active development and use. Supplementing the batteries in Table 1 are new batteries appearing in the literature in recent years. Each of these batteries is described below.

Neuropsychological batteries. Hänninen (1990) has developed the Neuropsychological Screening Battery (NPS), a more focused screening test battery, as distinct from a research tool (the FIOH battery). She has initiated a series of longitudinal studies in Finland, installing the battery in factories where it is given by in-plant occupational health professionals. The NPS is comprised of seven neuropsychological tests (Finger Tapping, Flanagan Coordination, Mira test, Block Design, Memory for Design, Digit Span, and Associative Learning) and its sensitivity to neurotoxic chemicals has been demonstrated in solvent-exposed workers at five manufacturing plants in Finland. The battery is now used routinely in select Finnish industries where its administration is triggered by worker complaints or evidence of high-concentration exposures.

Two other neuropsychological test batteries (Bowler *et al.*, 1986; Ryan *et al.*, 1987a) have been recommended for assessing neurotoxicity in working populations (Table 1). Both rely heavily on the Wechsler Memory Scale (WMS) and Wechsler Adult Intelligence Scale (WAIS) subtests, plus other cognitive and visual/motor (e.g., pegboard) tests. These batteries have been employed recently in worksite research (Ryan *et al.*, 1988; Bowler *et al.*, 1991) and have been used in methodological assessments (e.g., Law *et al.*, 1990). The use of neuropsycholog-

ical batteries is increasing in worksite research, due particularly to the need to evaluate individual subjects for worker compensation decisions and to identify individuals in need of follow-up care. Such judgmental decisions cannot be accomplished by screening batteries at present, rather clinical neuropsychologists select tests in order to provide such individual diagnosis and assessment.

Batteries not used in recent years. Two of the individual-administered batteries in Table 1 appear to have fallen into disuse in recent years as their developers turned to other interests. The London School of Hygiene Battery (Cherry *et al.*, 1984) employs a combination of neuropsychological and experimental psychology tests that have been used in Britain to study solvents. This battery includes perceptual/motor (grooved pegboard, simple reaction time, dotting) and cognitive function tests (visual search, Digit Symbol, Buschke Memory, Block Design, British National Reading test). The battery used in a series of studies by Valciukas and Lilis at Mt. Sinai in the 1970s and 1980s included only two WAIS tests (Digit Symbol and Block Design) and the Embedded Figures test (drawings of simple figures with distracting lines through them), which they adapted from neuropsychological testing. Research with these batteries has not been published in several years.

Batteries replaced. The test battery for Investigating Functional Disorders (TUFF) was developed in Sweden in an aggressive campaign to assess industrial exposures. The TUFF developed the most widely used symptoms questionnaire (Anger, 1990), and it employs a broad array of 15 motor ([manipulation of] bolts, cylinders, pins), perceptual/motor (Dots/cancellation), and cognitive tests (Figure Classification, Block Design, unfolding, visual gestalt, Digit Symbol, Same Number, Benton Visual Retention, Auditory Perception/Retention, Synonyms, opposites) (Hogstedt *et al.*, 1980). However, the computer-implemented Swedish Performance Evaluation System (SPES) (Gamberale *et al.*, 1990) has taken the major role in recent worksite evaluations in Sweden (e.g., Iregren, 1990). A similar fate has befallen the Milan IOH battery developed in the 1980s (Angotzi *et al.*, 1980) which is now used primarily for individual neuropsychological testing. For population assessments, it has been replaced by the computer-implemented Milan Automated Neurobehavioral System and the Neurobehavioral Core Test Battery, both described below.

Batteries in active development. The IMT was developed in Cuba (Almirall-Hernández, *et al.*, 1987). It includes motor and cognitive tests adapted from familiar English-language precursors. Testifying to the fact that the battery has been extensively evaluated in Cuba, the test manual includes baseline data for IMT tests (Almirall-Hernández, *et al.*, 1987). There is interest in Latin America in using this Spanish-language battery for neurotoxicity evaluations. Williamson and co-workers (1982, 1990) developed a battery based on information processing theory that is the most firmly grounded in experimental psychology of the batteries now in use. Tests are critical flicker fusion, vigilance, hand steadiness (stylus in hole), Simple Reaction Time, visual pursuit, sensory store memory, Sternberg memory test, and Paired Associates (short- and long-term memory). Key factors have been carefully assessed. Test–retest reliability exceeds 0.8 in most tests, and the effects of potential study confounders age, sex, education, job type, and

length of residence have been evaluated using multivariate linear regression analysis (7–26% of the variance was explained by these factors on the various tests). Finally, test sensitivity was evaluated in working populations exposed to mercury and lead. The tests show specificity for these chemicals and show promise for the development of hypotheses about the brain function or area where damage is producing the performance deficits.

Recently described batteries. New batteries appear poised to supplement the more established individual-administered batteries in Table 1, particularly in Germany (Triebig, 1989; Seeber *et al.*, 1990). These batteries employ standard neuropsychological tests (e.g., Digit Symbol), established experimental psychology tests (e.g., simple and choice reaction time), modifications of widely used vigilance tests (e.g., d2) (Seeber *et al.*, 1990), and unique tests based on cognitive theory (e.g., WES, KAI) (e.g., Triebig, 1989).

Strategies of Test Selection

More important than the method of presentation is the strategy employed for selecting tests into a battery. Information about health effects of the chemical and symptoms of the chemically exposed group under study likely serve as a guide to test selection in most studies (e.g., Anger, 1985), although the rationale for test selection in any given study is rarely addressed in published reports. Some behavioral test batteries provide a menu of choices (e.g., the computer-implemented Neurobehavioral Evaluation System by Letz and Baker, 1986) with only limited guidance on test selection. Others have addressed the strategy for test selection. Eckerman *et al.* (1985) selected cognitive tests based on a factor analysis of a large sample of cognitive test results, identifying eight cognitive factors (Eckerman and Gullion, 1986). Williamson and co-workers (1982, 1990) took a theoretical approach, as noted above, selecting tests that could be interpreted within the constructs of information processing theory. Recommendations of expert gatherings also serve as an important test selection guide for many researchers.

International Recommendations

Although appropriate concern has been voiced over limiting development of new methods (e.g., Letz and Singer, 1985), the use of standardized test instruments has been encouraged by many scientists at many international meetings (Laties, 1973; Dews, 1975), including the series of triennial meetings termed International Symposia on Neurobehavioral Methods (and Effects) in Environmental and Occupational Health (Johnson *et al.*, 1985; Eckerman, 1990). While behavioral test batteries to assess neurotoxic effects are used primarily in the country in which they are developed, events in the 1980s have begun to concentrate international interest on two test batteries (Anger, 1990; Letz, 1990). One, the Neurobehavioral Core Test battery, has been recommended by an international group of experts and has now been used in 10 countries in an evaluation of its feasibility for international use (Cassitto *et al.*, 1990; Liang *et al.*, 1990; Anger *et al.*, 1993). The other is the Neurobehavioral Evaluation System which is the most extensively used worksite research behavioral test battery in the world (Letz,

1990). The unifying element was the 1983 meeting at the National Institute for Occupational Safety and Health (NIOSH) in Cincinnati of experienced human occupational researchers convened from around the world under the aegis of the World Health Organization (WHO) (Johnson, 1983).

At the 1983 meeting in Cincinnati, scientists who had conducted behavioral research in working populations proposed a "core" test battery which could be used as a screening instrument to detect (and characterize in a limited way) a wide range of neurotoxic effects in human populations. The tests selected had been among the most useful in discriminating between groups exposed to the neurotoxic chemicals lead, mercury, and carbon disulfide in past research, and the tests could further be administered in even primitive settings by trained technicians. The proposed test battery was named the World Health Organization (WHO)-recommended Neurobehavioral Core Test Battery (NCTB) and consisted of seven tests: Digit Span, Digit Symbol, Simple Reaction Time, Pursuit Aiming II, Santa Ana dexterity, Benton Visual Retention, and Profile of Mood States (Johnson *et al.*, 1987). Since the NCTB was developed by many prominent researchers in the field of neurotoxicology, its tests were used individually in studies long before formal publication of the Battery's reference monograph in 1987.

WHO NCTB Cross-Cultural Assessment

A small group (Xintaras, Cassitto, Hänninen, Anger, Johnson, and Lindström at various stages) involved in recommending the NCTB subsequently met in Geneva to develop an Operational Guide for the NCTB. The Guide described the administration procedures, pitfalls, and general analysis of results. Members of this group also developed a plan to institute a two-phase Cross-Cultural Assessment (CCA) of the NCTB to address their primary concerns regarding the feasibility of using the battery worldwide. Since the NCTB tests were developed and validated in Western European and derivative populations, the CCA was focused on evaluating the NCTB's feasibility in a broad range of culturally diverse subject populations. Phase 1 was aimed at developing baseline or normative data in populations unexposed to chemicals at work, and phase 2 (which has not been implemented) was intended to demonstrate the sensitivity of the battery in people exposed at their workplace to established neurotoxic chemicals, both in a broad range of cultures (WHO, 1986a).

WHO's Office of Occupational Health under Drs. M. El-Batawi and C. Xintaras, and subsequently Dr. T. Ng, provided administrative support for the initial stages of phase I. The CCA proposal was distributed to all WHO Collaborating Centres and an article was published to further broaden contacts in Africa (Cassitto *et al.*, 1987). WHO's Office of Occupational Health collected the applications to participate in the CCA and distributed them to CCA coordinators. Applications to participate in the Cross-Cultural Assessment were received from 16 countries (Table 2, left two columns). Contacts with several applicant countries were successful and ultimately led to conducting the CCA in the countries listed in the right-hand columns of Table 2.

Training classes in administration of the NCTB were conducted by CCA coordinators Drs. Cassitto, Hänninen, or Anger to provide a measure of consistency in test application. A report (unpublished) describing a full-featured training pro-

TABLE 2
INITIAL APPLICANTS AND FINAL PARTICIPANTS IN WHO-SPONSORED CROSS-CULTURAL ASSESSMENT (CCA) OF THE NCTB

Initial applicants		Final participants	
Country	Proposed site	Country	Final study site
Africa			
Egypt	Cairo		
Kenya	Nairobi		
America (Central)			
Cuba	Havana		
		Nicaragua	Leon
			Maracay
America (North)			
		United States	Salt Lake City
			Cincinnati
			Portland, OR
		Canada	Montréal
America (South)			
Brazil	Sao Paolo		
Asia			
India	Tiruchiripalli		
People's Republic of China	Beijing	People's Republic of China	Beijing
	Shanghai		Shanghai
Thailand	Bangkok		
Europe (East)			
Poland	Lodz	Poland	Lodz
		Hungary	Budapest
Europe (West)			
		Austria	Innsbruck
		France	Paris
		Italy	Bari
			Chieti
			Milan
			Poggibonsi
			Potenza
		The Netherlands	The Hague
Germany	Berlin		
Oceania			
Australia	Sydney		

gram was prepared by Drs. R. Gilioli and M. Cassitto (1986) at Milan's Institute of Occupational Health. At least 75 people have been trained to administer the NCTB by one of the three CCA coordinaters in the manner prescribed in the NCTB Operational Guide (WHO, 1986b). Countries from which personnel were trained are listed in Table 3. There is now a large coterie of trained NCTB administrators distributed in all permanently inhabited continents, except Oceania.

Extensions of WHO NCTB Implementation

The WHO-recommended NCTB has had its greatest impact in the People's Republic of China. Dr. Chen Zi-qiang of Shanghai Medical University learned to

TABLE 3
TRAINEES TAUGHT BY DRS. CASSITTO, ANGER, OR HÄNNINEN FOR THE NCTB CROSS-CULTURAL ASSESSMENT

- **Africa**
  - Kenya
    - Nairobi
  - Tanzania
    - Dar Es Salaam
  - South Africa
    - Cape Town
- **Asia**
  - China
    - Beijing (2)
    - Shanghai
    - Guangzhou
  - India
    - Lucknow
  - Japan
    - Tokyo
  - Korea
    - Seoul
- **Europe**
  - Austria
    - Innsbruck
  - Bulgaria
    - Sofia
  - Czechoslovakia
    - Prague
  - Greece
    - Athens
  - Hungary
    - Budapest
  - Italy
    - Bergamo
    - Bologna
    - Desio
    - Grosseto
    - Milan
    - Naples
    - Lecco
    - Lodi
    - Novara
    - Rome
    - Siena
    - Sondrio
    - Volterra
  - Poland
    - Lodz
  - Portugal
    - Anodia
    - Coimbra
- **North America**
  - Canada
    - Toronto (2)
  - United States
    - Atlanta, GA (>10)
    - Baldwin, MD
    - Blacksburg, VA
    - Cincinnati, OH (6)
    - Los Angeles, CA
    - Madison, WI
    - Portland, OR (4)
    - Salt Lake, UT (6)
    - Seattle, WA
    - Washington, DC
- **South America**
  - Argentina
    - Buenos Aires (2)
  - Brazil
    - Sao Paolo
  - Chile
    - Santiago
  - Columbia
    - Bogota
  - Venezuela
    - Maracay
- **Central America**
  - Cuba
    - Havana
  - Dominican Republic
    - San Domingo
  - Mexico
    - Iztocala
  - Nicaragua
    - Leon
- **Middle East**
  - Israel
    - Jerusalem (2)
- **Oceania**

Note. Numbers in parentheses indicate number of people from city receiving training.

administer the NCTB at NIOSH in Cincinnati. Returning to Shanghai with an NCTB kit, he and Dr. Liang You-xin, with colleagues, undertook phase 1 of the Cross-Cultural Assessment to evaluate the NCTB's feasibility in unexposed workers. They quickly pressed on to evaluate the battery's sensitivity to lead

exposures (Liang *et al.*, 1990), the proposed phase 2 of the Cross-Cultural Assessment. Dr. Kuang Shou-ren of the Institute of Occupational Medicine in Guangzhou was also trained at NIOSH to administer the NCTB and began studying manganese-exposed workers.

In Beijing, Professor He Fengsheng of the Institute of Occupational Medicine arranged for local construction of the most expensive instrument in the NCTB, the Simple Reaction Time device, to minimize costs. She also conducted, along with members of her Institute and from Shanghai Medical University, NCTB training classes for health professionals in China. Cities represented at the training class by at least one person are listed in Table 4. Availability of an economical test battery and the support of scientists through publications and presentations from widely respected institutions in China built interest in the battery. A large cadre of trained NCTB test administrators are now distributed among more cities than in any other country, suggesting the likelihood of widespread use in the People's Republic of China.

Test Batteries Based on the NCTB

Two prominent members in the 1983 WHO-sponsored meeting in Cincinnati, Drs. E. Baker and R. Letz, then of Harvard School of Public Health, were at the time selecting behavioral tests to assess neurotoxicity in human populations. They implemented their tests in a computer format for reasons of efficiency and application consistency (Baker *et al.*, 1985). This battery of 19 tests named the Neurobehavioral Evaluation System (NES) includes computer-implemented variants of five of the seven NCTB tests. The NES, and its recent successor, the NES II (22 tests), has now been translated into several languages and has been employed in diverse countries and cultures. It has become the most widely used human behavioral neurotoxicology test battery in the world (Letz and Baker, 1986; Letz, 1990), as publications will soon demonstrate. A users group has been developed

TABLE 4
CITIES OF RESIDENCE (BY PROVINCE) OF TRAINEES AT BEIJING'S INSTITUTE OF OCCUPATIONAL MEDICINE-SPONSORED NCTB TRAINING CLASS

Province	City	Province	City
Liaoning	Shenyang (4)	Fujian	Fuzhou
Liaoning	FuShun	Fujian	Nanping
Liaoning	Jinxi	Qinghai	Xining
Jilin	Jilin (2)	Shanxi	Xian (5)
Jilin	Qiqihar	Hubei	Wuhan
Heilongjiang	Harbin (4)	Hubei	Yichang (2)
Jiangsu	Nanjing (3)	Hainan	Hainan (2)
Shanxi	Datong	Shandong	Jinan
Sichuan	Chengdu (2)	Shandong	Qingdao
Sichuan	Panzhihua	Hebei	Qinhuangdao
Henan	Zhengzhou (2)	Hunan	Changsha (2)
Henan	Xinxiang (2)	Anhui	Hefei
Henan	Houyang	Yunan	Kunming
Henan	Luoyang		Beijing (2)

and centralized distribution of the software assures control over this battery's development. However, since the developers do not recommend use of a particular subset of NES tests in all investigations, the tests used in any given study may include a wide variety of tests from the NES menu. Ongoing research is directed at assessing whether the computer-implemented (NES) variants produce the same results as the same test given by an individual (e.g., in the NCTB) (e.g., Hooisma *et al.*, 1990). Of course, this was the form in which the tests were originally developed and validated.

Drs. M. Cassitto and R. Gilioli at the Institute of Occupational Health in Milan focused exclusively on the WHO-recommended NCTB. They developed a computer-implemented presentation of the NCTB cognitive tests which they named the Milan Automated Neurobehavioral System (MANS). This battery has been used in seven projects undertaken by its developers, and it is also being translated for use in Greece (Cassitto *et al.*, 1989). The MANS has been used in a multicenter study conducted by scientists in the Federal Republic of Germany, Italy, the United Kingdom, and the United States to study exposures to solvent mixtures due to growing concern over this problem (Triebig *et al.*, 1990).

DISCUSSION

Test batteries have been employed in human behavioral neurotoxicology since 1966 (Hänninen, 1966). The development of standardized test instruments has been encouraged by many scientists, particularly in the series of triennial meetings termed International Symposia on Neurobehavioral Methods (and Effects) in Environmental and Occupational Health (Laties, 1973; Dews, 1975). This was strongly reaffirmed in 1988, at the third such meeting: "After vigorous discussion, the [clinical and field-testing batteries workshop] group recommended inclusion of the World Health Organization (WHO) Neurobehavioral Core Test Battery (NCTB) in all clinical and field studies of neurobehavioral function. This recommendation was offered without dissent." The primary reasons, closely paraphrased, given for this recommendation were (a) provision of a reference point for interstudy comparisons, (b) inclusion of tests of demonstrated sensitivity in all studies, and (c) the potential development of normative data for selected tests (Eckerman, 1990).

The development of technology has had a significant impact on the field of behavioral neurotoxicology, including the largest segment of research, field, or worksite epidemiological assessments. Computer-administered batteries will likely dominate the field for many reasons, particularly ease, consistency, and efficiency of administration, recording, and analysis. However, this reliance on computer-administered batteries will be constrained by high initial costs, the frequent need for competent computer service now widely available only in highly industrialized countries and the need to assess motor and sensory functions not easily elicited or measured by current computer hardware. This trend to computer-implemented tests should not limit research in locations where such hardware is unavailable because many of the same tests administered by a computer can be administered with equal competence and greater subject acceptability by a human administrator.

There is a clear need for administration of behavioral tests by both computers and humans (e.g., Williamson, 1990). Several test batteries administered by a human can be employed to study neurotoxic exposures to working populations. The WHO-recommended Neurobehavioral Core Test Battery (NCTB) is the most widely distributed individual-administered test battery and employs field-proven tests for research where humans are the test administrator of choice or where there are constraints on computer administration.

While test batteries administered by an individual have a significant niche in the field, at least six issues need to be addressed regarding such test batteries:

(a) The critical role of human-administered test batteries for screening working populations exposed to chemical agents in a field increasingly dominated by computer-administered test batteries;

(b) Selection criteria for tests to study the effects of known and unknown chemicals in humans;

(c) Utility of baseline or normative data for analysis and interpretation of future studies;

(d) The need for international validation of test batteries such as the NCTB (proposed as Phase II of the Cross-Cultural Assessment) and as a comparison for findings with unknown chemicals;

(e) Availability and cost of inexpensive test batteries for developing countries or underfunded researchers;

(f) Equivalence of computer- and human-administered variants of the same tests.

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Covariates of Computerized Neurobehavioral Test Performance in Epidemiologic Investigations[1]

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Computerized neurobehavioral tests are being used increasingly in occupational and environmental health to measure potential effects of exposure to neurotoxicants. Many factors affect performance on these tests. Data sets from application of a computerized neurobehavioral evaluation system in epidemiologic investigations of two occupational groups, printing pressmen and construction painters, were analyzed. Age and education were the major covariates of performance in these groups. The reliability of these computerized tests was also reviewed. Computerized neurobehavioral tests are similar to conventional tests in terms of reliability and relationships to known covariates.

INTRODUCTION

Computerized neurobehavioral testing of humans has become an integral part of neurotoxicologic assessment in occupational and environmental health (Anger, 1989). Several computerized systems for assessing neurobehavioral performance in humans have been developed for use in occupational and environmental health (Baker *et al.*, 1985; Eckerman *et al.*, 1985; Cassitto *et al.*, 1989; Gamberale *et al.*, 1989; Laursen, 1990; Williamson, 1990).

Performance on computerized neurobehavioral tests is affected largely by the same factors that can affect performance on traditional neurobehavioral tests. A large number of factors can contribute to variance in neurobehavioral test outcomes. Some of these are between-subjects factors and some are within-subjects factors.

The most important between-subject factors affecting neuropsychological test performance are age and education (Franzen, 1989). Other between-subjects factors reported to affect neurobehavioral performance are sex and history of head injury. Chronic alcohol intake is also commonly presumed to affect neurobehavioral test performance. Experience with computers or video games is an additional between-subjects variable that may specifically affect performance on computerized neurobehavioral tests.

Within-subjects factors are ones that vary within an individual over time. Time of day, day of week, acute alcohol and drug (including caffeine and nicotine) intoxication, fatigue, and level of motivation have been reported to affect neurobehavioral performance.

Error in measurement of neurobehavioral outcomes can be controlled in a

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number of ways. In laboratory investigations the effects of between-subjects variation are often avoided by employing within-subjects, or crossover, designs ("the subject is his own control"). In addition, within-subject variation is minimized by selection of highly motivated subjects or use of monetary payment, extensive training of subjects on the behavioral tasks, and strict control of factors such as drug intake and time of day. However, in most epidemiologic investigations assessment of outcome is performed only once, and usually there is little time available for training. Reduced precision may be compensated for by matching subjects on age and education, testing larger numbers of subjects, and, to the extent possible, removing in the data analysis systematic variance attributable to covariates. The more variance that is accounted for by known covariates, the less remains as residual (error) variance, thus allowing more powerful statistical tests of potential effects of exposure to neurotoxicants. However, inclusion of presumed covariates that do not in fact account for any variance may weaken the statistical tests or even bias the estimates of exposure effects.

The purpose of the present paper is to provide a preliminary empirical basis for discussing the factors that contribute most to systematic variance in computerized neurobehavioral test outcomes in epidemiologic investigations.

METHODS

Subjects

Data were available from two relatively large epidemiologic studies employing a computerized neurobehavioral testing system codeveloped by the author (Baker *et al.*, 1985). The two studies were (1) a study of printing pressmen at a large newspaper in the northeastern United States (the neurobehavioral results are unpublished, but other aspects of the study are described by Hashimoto *et al.*, 1991) and (2) a study of construction painters (Baker *et al.*, 1988).

The group studied at the newspaper consisted of 215 printing pressmen and 32 compositors located at two facilities. Neurobehavioral testing was performed in conjunction with other tests (pulmonary function, urinary sedimentation, and dermatologic examinations) as part of health screening resulting from worker concerns about solvent exposure. Industrial hygiene sampling indicated that solvent exposures were low except in a room used for cleaning and storing cleaning-up equipment where only a few participants worked on occasion. After a priori exclusion criteria (history of head trauma, alcoholism, etc.) were applied to the group, 217 male printing pressmen and compositors remained.

Data were also available from a study of the neurobehavioral effects of mixed solvent exposure among construction painters (Baker *et al.*, 1988). Application of the same exclusion criteria mentioned above to members of the painters' union in two cities in the southern United States yielded a total of 182 male painters. Solvent exposure was characterized by a solvent exposure intensity index derived from questionnaire data (Fidler *et al.*, 1987).

Variables available for examination as potential covariates of neurobehavioral performance were age, number of years of formal education, experience with video games and computers (none, some, a lot), self-assessment of effort ex-

pended in performing the neurobehavioral tests, usual number of alcoholic drinks consumed per week, day of the week that testing was performed, and time of day that testing was performed. The effort variable was measured on a 4-point scale in the pressmen study (0 = not at all, 1 = somewhat hard, 2 = very hard, 3 = as hard as I could) and on a 5-point scale in the painters study (0 = not at all, 1 = somewhat hard, 2 = moderately hard, 3 = very hard, 4 = as hard as I could).

Categorical data were inspected, and minor adjustments were made to assure adequate numbers of observations in all the categories to be used in the multiple regression analyses: only 2 pressmen were tested on Sunday, so they were dropped from the analyses; only 8 painters were tested on Tuesday, so they were recorded as Wednesday. These changes left at least 34 pressmen in each of the test-day groups for Monday, Tuesday, Wednesday, Thursday, and Saturday, and at least 20 painters in groups representing every weekday except Tuesday. For the pressmen, time of day was coded as morning (0800–1100, n = 78) or night (2100–0100, n = 137), and for the painters time of day was coded as morning (0800–1200, n = 30), afternoon (1300–1700, n = 111), and evening (1800–2100, n = 41).

Summary statistics for interval or continuous variables for the two study groups are presented in Table 1. The pressmen were slightly older and more educated than the painters, but both groups included a broad range on these variables. The pressmen also reported greater familiarity with computers and video games than the painters.

Although the vocabulary variable is an outcome from an NES test (see below), it was included among the covariates, as it is considered a surrogate for general intellectual ability and a measure that is relatively resistant to the effects of toxic insult, i.e., a "hold" test. As measured by vocabulary, the pressmen had slightly higher intellectual abilities than the painters.

In addition, since performance on some neurobehavioral tests was associated with exposure in the painters study, an exposure variable, an index of solvent exposure intensity (Fidler *et al.*, 1987), was also included as a covariate in the analyses of painters' data.

Neurobehavioral Tests

Eight tests from the Neurobehavioral Evaluation System 2 (NES2) were em-

TABLE 1
MEANS AND STANDARD DEVIATIONS OF COVARIATES FOR THE TWO STUDY GROUPS

	Pressmen (n = 215)		Painters (n = 186)	
Variable	Mean	Std. Dev.	Mean	Std. Dev.
Age (years)	44.7	13.14	38.7	12.17
Education (years)	12.7	1.90	11.3	2.10
Video games	0.75	0.59	0.37	0.57
Effort[a]	2.54	0.78	3.49	0.77
Alcohol (drinks/week)	9.1	10.55	7.5	15.85
Vocabulary	17.6	4.83	16.0	4.65

[a] Different scales in the two groups, see text.

ployed in the study of printing pressmen: (1) symbol–digit substitution, a visual scanning and coding task; (2) continuous performance test, a visual reaction time, sustained attention task; (3) hand–eye coordination; (4) pattern comparison, a visual perceptual task; (5) pattern memory, a short-term visual memory task; (6) serial digit learning, a visually presented learning and memory task; (7) finger tapping with the dominant hand; and (8) a multiple-choice vocabulary test. All tests were administered according to the NES2 User's Manual (available from the author). A single summary measure from each neurobehavioral test was selected for analysis in this paper.

Seven tests from the original Neurobehavioral Evaluation System (Baker *et al.*, 1985) employed in the painters' study that corresponded to the tests given to the pressmen were analyzed. These were the same as for the pressmen with the following exceptions: Finger tapping was not administered; visual digit span was given instead of serial digit learning. The two digit span outcomes (digits forward and backward) appeared very similar in their relationships to the covariates of interest, so only results for the Backward measure are included here. All tests were administered according to the NES Users' Manual, Version 3 (also available from the author).

Statistical methods

Multiple regression models were fitted using the Statistical Analysis System (SAS, 1985). A single summary measure from each neurobehavioral test was regressed upon the potential covariates using Proc GLM. Separate models were fitted for pressmen's data and painters' data. The estimated sum of squares for each covariate was divided by the total sum of squares corrected for the mean to yield an estimate of the proportion of variance accounted for by each covariate.

RESULTS

Pearson product moment correlations between the covariates are presented in Table 2 to assist in interpretation of the results that follow. With these sample sizes, a correlation of approximately 0.15 is statistically significant at the $P < 0.05$ level. The highest correlation ($r = 0.48$) was between age and education among the painters. Experience with computers and video games was negatively correlated with age in both samples. None of the correlations was considered of sufficient magnitude to cause difficulty with collinearity in the regression analyses.

The results of the multiple regression analyses are summarized in Table 3. In the pressmen analyses all effects that accounted for more than 1.2% of the variance, except weekday, which had more than one degree of freedom, would be considered statistically significant at the $P < 0.05$ level. In the painters analyses all effects that accounted for more than 1.8% of the variance, except for weekday and time-of-day, would be considered statistically significant at the $P < 0.05$ level.

The most striking result shown in Table 3 is the, at best, modest total proportion of variance in neurobehavioral tests outcomes that was accounted for by all the covariates in the regression model. Clearly, age and general intellectual ability, as measured by vocabulary, accounted for most of the systematic variance in these test scores.

TABLE 2
CORRELATIONS AMONG POTENTIAL COVARIATES IN THE TWO STUDY GROUPS

	A. Pressmen (n = 215)				
	Age	Vocabulary	Education	Video	Effort
Vocabulary	0.144				
Education	−0.264	0.191			
Video games	−0.394	0.058	0.200		
Effort	0.141	0.107	−0.028	−0.036	
Alcohol	0.018	−0.005	−0.104	−0.185	0.014

	B. Painters (n = 182)					
	Age	Vocabulary	Education	Video	Effort	Alcohol
Vocabulary	0.084					
Education	−0.484	0.379				
Video games	−0.368	0.134	0.183			
Effort	0.075	0.159	−0.081	0.055		
Alcohol	0.068	−0.093	−0.097	−0.026	−0.054	
Exposure	−0.180	−0.074	0.101	0.063	−0.014	0.200

In these analyses, years of education completed did not account for much of the variance in test scores. When other regression models were fitted without Vocabulary as a predictor (not tabulated), years of education picked up some of the variance in test scores on some of the tests. However, as a surrogate for general intellectual ability, vocabulary appears to be a better choice than years of education.

Other effects in the regression models accounted for, at most, a small percentage of the total variance in NES test performance. Reported experience with computers and video games accounted for 1 to 3% of the test variance among the pressmen and for virtually none of the variance among the painters. Self-report of effort in performing the NES tests accounted for a small percentage of the variance on some NES tests, but the tests affected were not the same for pressmen and painters.

Self-report of current alcohol intake accounted for none of the variance in NES test performance among pressmen, and the two small effects noted among the painters were in the direction of better performance with increased drinking. Solvent exposure was associated with poorer performance on two NES tests among the painters. The magnitude of this effect was near the limit of detection with a sample size of 186.

Time of day, coded as morning/night for the pressmen and morning, afternoon, or evening for the painters, accounted for virtually none of the variance in scores on these tests. Day of week appeared to account for some variance in neurobehavioral performance, although these apparent effects were not statistically significant since this factor contained several degrees of freedom. Also, the pattern of apparent differences, i.e., which day's performance was best or worst, was not consistent among the different neurobehavioral tests.

DISCUSSION

In these two occupational groups with a substantial range of ages and some

TABLE 3
PERCENTAGE OF VARIANCE OF NES TEST PERFORMANCE ACCOUNTED FOR BY POTENTIAL COVARIATES AMONG TWO GROUPS OF WORKERS

Covariate	NES Test						
	SyD	CPT	HE	PC	PM	SDL	Tap
	A. Printing Pressmen (n = 215)						
Age	30	—	9	17	4	5	8
Vocabulary	3	—	—	3	6	10	—
Education	2	—	—	—	—	—	—
Video games	3	1	3	2	2	—	1
Effort	1	1	—	—	—	—	2
Alcohol	—	—	—	—	—	—	—
Day of week[b]	2	5	—	—	1	—	2
Time of day[b]	—	1	—	—	—	2	—
Total	41	9	14	23	13	18	14
	B. Painters (n = 186)						
Age	21	—	3	10	3	5	
Vocabulary	12	7	7	9	2	11	
Education	—	—	—	—	1	—	
Video games	—	—	—	1	—	—	
Effort	—	—	—	2	1	3	
Alcohol	—	—	1[a]	—	—	3[a]	
Day of week[b]	7	5	3	5	3	2	
Time of day[b]	—	1	—	2	—	—	
Exposure	3	—	—	2	—	—	
Total	44	15	16	33	11	26	

Note. SyD, Symbol-digit; CPT, continuous performance test; HE, hand–eye coordination; PC, pattern comparison; PM, pattern memory; SDL, serial digit learning; Tap, finger tapping (dominant hand); DSp, digit span backward. —, indicates that <1% of variance was accounted for.

[a] Greater reported drinking was associated with better performance.

[b] Coded differently for pressmen and painters; see text.

variation in education, age and vocabulary were the most important covariates. When vocabulary was not in one of the models, education accounted for some of the test variance. Similar results indicating the predominant effects of age and education on neurobehavioral test performance have been reported for tests in the Halstead–Reitan battery and the Wechsler Adult Intelligence Scale (Heaton *et al.*, 1986; Franzen, 1989).

Greater experience with computers and video games was associated with better performance on NES tests among the pressmen but not among the painters. These results might be due to few painters having any experience with computers and video games (see Table 1). Additional effort to quantify this important variable, as well as the motivation of the subject performing the tests, is required.

Self-reported current alcohol intake accounted for virtually none of the variance in neurobehavioral test performance, and when it did account for a small percentage of the variance, the estimated effect was in the direction of better performance with increased drinking. The absence of an effect of reported drinking has been

reported in unexposed workers by investigators standardizing a manually administered test battery (Ryan *et al.*, 1987) and a computerized test battery (Laursen, 1990). Although these results may be due, in part, to the potential lack of reliability of self-reported drinking as a predictor variable, effects of chronic alcohol intake in the absence of alcoholism do not appear to be readily observable.

The total proportion of variance in neurobehavioral test outcomes that was accounted for by all the covariates in the regression models ranged from 9 to 44%. These r^2, although not high, are entirely consistent with others reported in the literature (e.g., Williamson, 1990).

Although there is a certain amount of intrinsic error in measurement of neurobehavioral outcomes, it is much less than the 60–90% of total variance that these regression models may seem to imply. Measurement error can be estimated from data collected on the same individuals tested under similar conditions on two or more occasions. A substantial amount of information is available on the reliability of NES tests (Letz, 1990; Arcia and Otto, 1992). The data available come from a number of laboratories, and testing was conducted under a variety of conditions. Most of the test–retest correlations have been in the range of 0.7–0.9. These test–retest correlations yield crude estimates of measurement error of 10 to 25% of the total variance, implying that the maximum variance that can be explained by potential covariates is 75–90%.

The difference between the variance accounted for in the regression models (10–40%) and the estimated maximum capable of being explained (75–90%) suggests that a substantial proportion of the variance in neurobehavioral outcomes currently remains unexplained. The results presented above suggest that factors such as time of day and day of week account for only a small percentage of the total variance in neurobehavioral test outcomes in between-subjects study designs. The results presented do not imply that these and other factors are not important determinants of neurobehavioral performance, only that they probably do not act in a systematic way across individuals. Thus, it is likely that the considerable variance left unexplained by the factors in the regression models may result from an *interaction* between these factors and subjects, or stated another way, that these factors are expressed differentially across subjects.

CONCLUSIONS AND RECOMMENDATIONS

One implication of the substantial variability in neurobehavioral performance between people that is not readily attributable to known covariates is that large sample sizes are necessary to observe reliably even modest-sized exposure effects. Furthermore, this limitation is reduced only a moderate amount by matching of subjects on age and education; as such matching will reduce between-subject variance by a small percentage to at most 30%.

Another implication of the large variability among people in neurobehavioral performance is that within-subjects (i.e., prospective) designs should be used whenever possible. In potential exposure situations it would be beneficial to obtain preplacement neurobehavioral baselines in workers (Letz, 1988).

Finally, in testing situations in which individuals are tested only once, it is

highly desirable to include a test (or tests) of general intellectual ability for use as a covariate in data analyses.

Computerized neurobehavioral testing is likely to play an increasing role in assessment of the effects of exposure to neurotoxicants. The major contributions of the computerized tests presently available may be increased standardization and efficiency of data collection rather than enhanced sensitivity over conventional methods.

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Prevalence of Abnormal Neurobehavioral Scores in Populations Exposed to Different Industrial Chemicals[1]

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The aim of the study is to establish the prevalence of neurobehavioral scores of occupationally exposed subjects below the 10th percentile rank of normalized curves obtained on a referent population. The Milan Automated Neurobehavioral System (MANS) was administered to 400 drivers from public and private firms; their data were distributed on the basis of age and years of school attendance and were normalized by determining percentile rank equivalence. The exposed population is made up of 20 lead- and zinc-exposed subjects, 18 welders exposed to aluminum for less than 1 year, 150 exposed to different metals in the ferromanganese production, 73 lithographic operators exposed to gasoline and petroleum, 197 exposed to solvents mixtures in the paint manufacture, and 23 dropouts of the same firm. The percentages of scores below 10th percentile rank were calculated in the different exposure groups and in the different age–school attendance ranges. The prevalence of results below the 10th percentile rank was found to be related to the intensity of the exposures and to the low levels of school attendance. In the 20–29 and the 30–39 age ranges, there was a prevalence of POMS scale scores below 10th percentile rank, in the 50–59 age range, the percentages were high for the Digit Symbol, the mean value of Simple Reaction Time, Serial Digit Learning, and Benton Visual Recognition.

INTRODUCTION

Over recent years, together with an increasing interest for neurobehavioral studies in occupational toxicology, the disappointment in the methods applied has become common. The production of software for automated neurobehavioral test administration has fed the dissatisfaction because it seemed that the efforts and creativity had not been used to resolve the toxicological questions still unanswered because of the inadequacy of the traditional methods. It was not fully realized that these software have bridged the passage from the traditional methods to an, albeit unrealized innovative approach and were, in practice, a productive effort in detecting problems and possible solutions. In a recent article (Beaumont, 1990) the author was concerned by the diffusion of these software even though they were distributed only in order to be evaluated and validated and not as a final product. Another concern of some experts (Beaumont, 1990, Gamberale *et al.*, 1990) is that these software are composed by many tests originating from the WHO–NCTB. This choice reflects the current prevailing aim of providing uniform and standardized methods. The fact that we are still pondering over and studying these computer methods is not aimed at sponsoring the one or the other of these

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batteries, but at gathering together efforts and results, without excluding different choices in the future (Williamson, 1990).

In this direction this paper presents a study carried out to establish the prevalence of neurobehavioral scores of occupationally exposed subjects below the 10th and the 30th percentile ranks of normalized curves obtained in a referent population using one of those computerized batteries, i.e., the Milan Automated Neurobehavioral System (MANS).

MATERIAL AND METHODS

MANS is a software written in Pascal for an IBM compatible personal computer including six tests: Profile of Mood State (POMS), Simple Visual Reaction Time, Digit Span, Serial Digit Learning, Digit Symbol, Visual Recognition, and Aiming Pursuit II. Their characteristics were described in previous articles (Cassitto *et al.*, 1989, Gilioli and Cassitto, 1990). Of the 7 POMS scales (Tension, Anger, Depression, Vigor, Fatigue, Confusion, and Sociability) Tension and Confusion scales scores were not included in the present analysis because of mistakes in the first preparation of the software. Aiming percentages were also excluded because this test had not been used in all studies.

The computerized tests (MANS) were administered to 400 nonexposed subjects, mainly bus drivers or company drivers. The data were grouped in terms of age and educational levels and their distribution was normalized by percentile rank equivalence. MANS was also applied in the health monitoring of subjects occupationally exposed to different neurotoxic agents. The exposed population is made up of 20 lead- and zinc-exposed subjects, 18 welders exposed to aluminum for less than 1 year, 150 exposed to different metals in the ferromanganese production, 73 lithographic operators exposed to gasoline and petroleum, 197 exposed to solvents mixtures in the paint manufacture, and 23 dropouts of the same firm.

The percentages of scores below the 10th percentile rank were calculated in the different exposure groups and in the different age–school attendance ranges.

The characteristics of the groups examined are illustrated in Table 1.

RESULTS AND DISCUSSION

Comparison among Exposure Groups

Table 2 illustrates the percentage of subjects with scores below the 10th and the 30th percentile ranks of the reference values. The subjects with very short exposure to aluminum fumes and dust had the lowest percentage of results below the 10th percentile rank. The subjects exposed to organic solvent mixtures in a paint industry and exposed in the last 10 years to levels much below the TLV had a percentage of values below the 10 and the 30th percentile ranks practically coincident with the percentage of the reference population. Moreover, no significant correlations were found between these results and past exposure indexes.

Dropouts of the same paint industry that had been removed from the hazardous tasks showed a high percentage of values below the 30th percentile rank (POMS: Anger, Depression, Fatigue, Sociability) and the 10th percentile rank (Vigor,

TABLE 1
GROUPS OF SUBJECTS OCCUPATIONALLY EXPOSED TO NEUROTOXIC AGENTS TESTED WITH THE MILAN AUTOMATED NEUROBEHAVIORAL SYSTEM (MANS)

N	Age (years)	Substances	Length of exposure (years)	Environmental–biological monitoring
20	Range: 20–61 $X \pm SD$: 38.8 ± 12.0	Lead, zinc	Range: 1–40 $X \pm SD$: 10.2 ± 10.6	Range: PbB 39–72.4 mg/100 ml Range: FEP 47–255 mg/100 ml
18	Range: 21–55 $X \pm SD$: 40.0 ± 10.3	Aluminum	Range: <1 $X \pm SD$: 4.9 ± 3.6	Range: 1.6–3.5 mg/m^3 Personal sampler × 4 hr
150	Range: 18–51 $X \pm SD$: 36.9 ± 8.2	Chromium Manganese Lead, zinc Iron	Range: 0–32 $X \pm SD$: 11.3 ± 7.9	Range (μg/m^3) Iron 41–390 Chromium 2–817 Mn 6–1628 Pb 3–456 Zinc 4.7–367 Personal sampler × 8 hr
73	Range: 18–52 $X \pm SD$: 32.8 ± 7.2	Gasoline Petroleum	Range: 1–27 $X \pm SD$: 8.4 ± 5.7	Range in ambient air gasoline: 6800–10,100 mg/m^3 Range in ambient air petroleum: 307–1860 mg/m^3
23	Range: 25–63 $X \pm SD$: 46.8 ± 9.6	Organic solvents dropouts	Range: 6–29 $X \pm SD$: 18.3 ± 6.5	
197	Range: 23–63 $X \pm SD$: 42.7 ± 9.2	Organic solvents	Range: 2–33 $X \pm SD$: 17.6 ± 7.5	<TLV ACGIH mixtures Mainly TWA 8 hr personal sampler

Sociability, Digit Symbol, Reaction Time mean and standard deviation values, Serial Digit Learning). However, since past exposure data of these subjects were lacking, it was impossible to establish a relationship between the observed effects and past working conditions; in addition, the reasons causing their removal might have been different from exposure itself.

Although 73 offset operators of a graphic industry participated in the study with adequate motivation and effort, they showed a high prevalence of values below the 30th percentile rank in POMS indexes and focused attention scores (Reaction Time standard deviation values, Digit Span (*f*), Digit Learning). Unfortunately, also in this study, it was impossible to obtain and to plot environmental and biochemical data with behavioral measurements.

The subjects of the ferromanganese industry had slightly higher scores only for POMS, specifically Anger and Sociability scales. In this group, manganese and other metals concentrations were found to be below TLV values while alcohol consumption was extremely high. Comparison of these subjects with referents having similar drinking habits did not show significant differences between the two groups.

TABLE 2
COMPARISON AMONG EXPOSURE GROUPS

	Anger	Depression	Vigor	Fatigue	Sociability
	% Subjects with values below the 30th P.R.[a]				
Referents	23	21	21	20	20
Lead	25	25	13	25	31
Aluminum	17	11	6	22	22
Petroleum—gasoline	38	29	23	30	30
Solvents (dropouts)	39	26	13	35	39
Solvent mixtures	25	16	17	26	25
Ferromanganese	27.8	20.9	19.5	24.1	23.4
	% Subjects with values below the 10th P.R.				
Referents	9	9	9	10	11
Lead	19	13	50	25	50
Aluminum	11	0	6	0	6
Petroleum—gasoline	14	14	20	14	18
Solvents (dropouts)	13	9	26	4	34
Solvent mixtures	13	12	5	9	6
Ferromanganese	21.5	11.4	11.4	13.3	19.6

	Digit symbol	Reaction time (*X*)	Reaction time (SD)	Digit span forward
	% Subjects with values below the 30th P.R.			
Referents	19	20	20	20
Lead	25	38	38	13
Aluminum	17	17	11	17
Petroleum—gasoline	21	18	29	30
Solvents (dropouts)	17	35	22	30
Solvent mixtures	20	18	21	13
Ferromanganese	18.4	18.4	27.8	23.4
	% Subjects with values below the 10th P.R.			
Referents	10	9	9	9
Lead	31	13	13	25
Aluminum	6	0	6	6
Petroleum—gasoline	16	5	14	4
Solvents (dropouts)	22	30	17	4
Solvent mixtures	15	13	9	4
Ferromanganese	4.4	10.8	9.5	9.5

	Digit span		Serial digit learning	Visual recognition
	Backward	Total		
	% Subjects with values below the 30th P.R.			
Referents	22	19	21	18
Lead	13	25	44	31
Aluminum	11	6	28	11
Petroleum—gasoline	23	27	33	23
Solvents (dropouts)	22	22	22	13
Solvent mixtures	16	12	11	18
Ferromanganese	25.9	29.1	17.7	20.3

TABLE 2—*Continued*

	Digit span		Serial digit learning	Visual recognition
	Backward	Total		
	% Subjects with values below the 10th P.R.			
Referents	8	10	10	8
Lead	13	13	25	25
Aluminum	0	6	11	0
Petroleum—gasoline	5	4	11	3
Solvents (dropouts)	13	13	26	9
Solvent mixtures	8	4	12	16
Ferromanganese	5.7	10.8	14.6	5.7

[a] P.R., percentile rank.

Subjects exposed to lead and zinc had high scores even below the 10th percentile rank (Vigor, Sociability, Digit Symbol, Digit Span (*f*), Digit Learning, and Benton Visual Recognition). POMS scales were found to be related to blood lead levels or free erythroporphyrins.

As a whole, these results seemed to reflect the working conditions but it can also be hypothesized that other concurrent factors might have played a role; for instance, the heavier the job and higher its risks, the more the subjects involved were low educated, more unsatisfied, and with increased need for smoking and alcohol consumption.

Comparison among Subgroups per Age and School Ranges

Subdividing the total number of exposed subjects in age and educational levels subgroups (Table 3), a high prevalence of scores below the 10th percentile rank was observed in the subjects of 20–29 and 30–39 age ranges, mostly with 5 years of school attendance. Mood indexes were markedly altered, as were the performance scales to a lesser degree.

In the subjects aged 50–60 years and with lower educational level, performance scores were more abnormal, namely in Digit Symbol, Serial Digit Learning, and Benton Visual Recognition.

Scores of mood indexes below the 10th percentile rank in the younger and less educated subjects cannot be simply referred to adjustment problems in the work environment and to increasing responsibilities since these factors were shared also by the control group. However, exposure might interfere with the compensation mechanisms, thus reducing the subjective resources to cope with other environmental stressors. This might explain the general feeling of discomfort and determine poor performances in those tests which require concentration and mental effort.

CONCLUSIONS

This preliminary and crude analysis indicates an increase of scores below the 10th percentile rank in keeping with the data regarding the intensity of the exposure to neurotoxic agents and with the educational level of the subjects.

TABLE 3
COMPARISON AMONG AGE–SCHOOL RANGES: PERCENTAGE OF SUBJECTS WITH VALUES BELOW THE 10th P.R.

	Age			
	20–29	30–39	40–49	50–59
	5 Years of school attendance			
Anger	0	43.24	9.47	5.88
Depression	0	32.43	6.31	7.84
Vigor	33.33	18.91	8.42	11.76
Fatigue	0	29.72	6.31	7.84
Sociability	33.33	40.54	12.63	13.72
	8 Years of school attendance			
Anger	27.77	6.45	17.77	25
Depression	22.22	12.90	2.22	0
Vigor	11.11	9.67	11.11	0
Fatigue	18.51	9.67	11.11	12.5
Sociability	18.61	22.58	8.88	0
	5 Years of school attendance			
Digit Symbol	0	16.21	11.57	29.41
Reaction Time *X*	0	8.10	10.52	13.72
Reaction Time SD	0	10.81	3.15	11.76
Digit Span forward	33.33	18.91	7.36	5.88
Digit Span backward	0	10.81	5.26	9.80
Digit Span total	0	21.62	5.26	9.80
Serial Digit Learning	0	13.51	11.57	29.41
Visual Recognition	0	16.21	14.73	17.64
	8 Years of school attendance			
Digit Symbol	16.16	19.35	6.66	12.50
Reaction Time *X*	11.11	11.29	2.22	25.00
Reaction Time SD	22.22	14.51	4.44	6.25
Digit Span forward	11.11	4.83	8.88	0
Digit Span backward	12.96	1.61	4.44	12.50
Digit Span total	5.55	3.22	6.66	0
Serial Digit Learning	5.55	9.67	2.22	6.25
Visual Recognition	7.40	4.83	2.22	12.50

Therefore, these findings seem to justify further studies on the sensitivity of these tests and on the relationship among sensitivity, validity, and reliability (Vorhees, 1987).

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Psychological Effects of Low Exposure to Mercury Vapor: Application of a Computer-Administered Neurobehavioral Evaluation System[1]

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A computer-administered neurobehavioral evaluation system in a Chinese language version (NES-C) and a mood inventory of the profile of mood states (POMS) were applied to assess the psychological effects of low-level exposure to mercury vapor in a group of 88 workers (19 males and 69 females, with mean age of 34.2 years) exposed to mercury vapor (average duration of exposure 10.4 years). The well-matched group of 97 nonexposed workers was treated as the control. The intensity of current mercury vapor was relatively mild as reflected by the average level of mercury in the air of the workplace (0.033 mg/m^3) and in urine (0.025 mg/liter). The results indicated that the profile of mood states posed was moving to the negative side in Hg-exposed group and most of the NES-C performances, in particular, the mental arithmetic, two-digit search, switching attention, visual choice reaction time, and finger tapping, were also significantly affected compared with those obtained from controls ($P < 0.05$–0.01). The present study and the previous study on the validation of the system suggest that the NES-C we developed is valid for the neurotoxicity screening among the working population exposed to neurotoxic agents.

INTRODUCTION

Neurobehavioral tests have been developed primarily to assess the functional changes in the central nervous system following human exposure to neurotoxic agents and other environmental or occupational hazards. The growing interest in the measurement of neurobehavioral tests is most likely due to the sensitivity shown by these methods in unveiling the subtle health effects in the exposed population that would usually not be detected by other measurements, and the accessibility was facilitated by providing a noninvasive, affordable, and transportable even portable technique for the purpose of occupational epidemiological studies.

A variety of test methods and batteries have been derived from certain types of traditional psychological scales since the most pioneer test battery for occupational epidemiology was created and used in Finland during the mid-1960s (Hanninen and Linstrom, 1979). Although somewhat similar techniques have been introduced, significant variability in testing procedures between studies is created which promotes confusion and hinders cross-study comparison.

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To further achieve the standardization of test protocols, the computer-administered behavioral testing techniques have been developed since the mid-1980s (Baker *et al.*, 1985a, 1985b; Maizlish *et al.*, 1985; Iregren *et al.*, 1985). There are many reasons for developing computerized methods in neurobehavioral evaluation. This technique offers certain advantages which include the standardization of test procedures, improvement of the reproducibility of tests, ease of data handling, scoring, and immediate reporting. All of these progresses have not only perfected the test and evaluation system but also have improved the level of motivation and thereby encouraged the participation (Letz, 1988).

Being initiated by the international efforts, in particular, the pioneer work contributed by Baker and Letz, a computer-administered neurobehavioral evaluation system programmed in Chinese version (NES-C) has jointly been developed by Shanghai Medical University and Shanghai University of Technology, the People's Republic of China (Letz, 1990; Sun and Liang, 1990). The present paper attempts to assess the psychological effects of low exposure to mercury vapor using the NES-C and to validate the appropriateness of the system for neurotoxicity screening among a working population exposed to neurotoxic agents.

SUBJECTS AND METHODS

Study Population

In the study a total of 158 workers consisting of 115 males and 43 females was examined. The group of 88 (69 males and 19 females) exposed to mercury vapor was recruited from a fluorescent lamp factory and the 70 well-matched controls were recruited from an embroidery factory. The exposed group had been uninterruptedly exposed to mercury vapor for at least 2 years prior to the study. The exposure level of mercury vapor in the air of workplace was found to be 0.033 mg/m^3 on average, slightly higher than the maximum allowable concentration (MAC) of 0.01 mg/m^3 currently adopted in China. The control workers had never been occupationally exposed to mercury. The neurologic or neuropsychiatric affectations could not be found in the current medical history of both Hg-exposed and control groups. Other covariables, such as smoking, alcohol drinking, and computer using experience, etc., were checked by pretest interview. No important difference was found between both groups as shown in Table 1.

Exposure

Exposure was evaluated by determining the current air mercury in the working environment and by measuring urine mercury concentrations.

Air mercury was surveyed by samples fixed in various worksites for 4–8 hr depending on the work process. Atomic absorption with the cold vapor technique was used to analyze the samples (Ma *et al.*, 1984).

Urine mercury was used as the indicator of biological monitoring for all workers investigated. All workers collected 24-hr urine samples and an atomic absorption spectrophotometric method was used for the analysis.

Psychological Tests

To standardize as much as possible with respect to test schedule, environment,

TABLE 1
CHARACTERISTICS OF Hg-EXPOSED WORKERS AND CONTROLS

	Hg-exposed (n = 88) (means ± SD)	Control (n = 70) (means ± SD)	t or χ^2 value	P
Sex				
Male	69	46	$\chi^2 = 3.17$	NS[a]
Female	19	24		
Age (years)	34.2 ± 6.9	35.1 ± 10.3	$t = 0.46$	NS
Working age (years)	15.8 ± 7.7	16.7 ± 10.0	$t = 0.45$	NS
Years of school attendance (years)	10.4 ± 2.2	10.4 ± 2.2	$t = 0.04$	NS
Urine Hg (mg/liter)	0.024 ± 0.058	ND[b]		
Smoking habit				
Nonsmoking	49	34	$\chi^2 = 0.79$	NS
Smoking	39	36		
Drinking				
Frequent	21	8		
Seldom	45	42	$\chi^2 = 4.03$	NS
Never	22	20		
Drank within 24 hr				
Yes	45	33	$\chi^2 = 0.25$	NS
No	43	37		
Computer experience				
Yes	3	1	$t = 0.63$	NS
No	85	69		

[a] NS, not significant.
[b] ND, not detected.

and procedure, the examinations were separately carried out by a study team of two to three well-trained researchers in quiet rooms affiliated with the worker clinics of the two factories. Ten to twelve subjects were asked to participate in the examination between 0900 and 1600 for a workshift day. Each subject was interviewed by a pretest questionnaire and followed by answering a mood inventory sheet of the profile of mood states (POMS), and finally by performing 10 tasks of NES-C subtests on the keyboard of an IBM-PC/XT computer.

1. Mood test. The POMS inventory contains 65 adjectives, which were translated into Chinese strictly based on the real meaning as it is in the English language origin, describing different moods: Tension, Depression, Anger, Vigor, Fatigue, and Confusion. Subjects are asked to indicate their mood states during the past week (including the test day) on a five-point scale ranging from "not-at-all" to "extremely" by marking one of the points from the five-point scale which is most likely to describe his/her present mood state. The overlay stencil was used as "scoring key" to calculate the raw scores for different moods mentioned above (WHO, 1986).

2. Computerized neurobehavioral test. A computer-administered neurobehavioral evaluation system in the Chinese version (NES-C) was used for the study. The system consists of 20 subtests which have adapted testing for intelligence, memory, visual perception, and psychomotor functions in an IBM-PC/XT com-

puter (Table 2). Of these, 10 subtests were chosen for the study. Subjects were individually asked to complete the 10 subtasks according to the instructions displayed on the screen and the inspection of the investigator in a quiet computer room.

Statistical Analysis

The data were analyzed by the IBM-PC/XT computer using dBASE III and SPSSPC statistical package for health sciences. The significant level was set at 0.05.

RESULTS

1. Exposure

As shown in Table 3, the ambient mercury concentration ranged from 0.008 to 0.085 mg/m^3 in various worksites in which the processes of lamp vacuumizing and mercury pumping seemed to be two of the most polluted sites of the fluorescent lamp factory in the study. The air mercury levels of these two reached 0.085 and 0.078 mg/m^3, respectively, on average, which were eight to nine times of the current MAC (0.01 mg/m^3) adopted in China and nearly three times higher than those of the average level from worksites investigated. The remainder showed a slight excess compared with the current MAC (Table 3).

TABLE 2
CONSTITUTES OF COMPUTER-ADMINISTERED NEUROBEHAVIORAL EVALUATION SYSTEM IN CHINESE VERSION (NES-C)

Function domain	Test	Source
Intelligence	Mental arithmetics*	Gon, 1981
	Serial add/subtract	Baker *et al.*, 1985b
Memory	Visual retention*	Baker *et al.*, 1985a
	Memory scanning	Maizlish *et al.*, 1985
	Visual memory span	Maizlish *et al.*, 1985
	Pattern memory	Baker *et al.*, 1985a
	Paired-associate learning*	Xi *et al.*, 1984
	Visual digit span	Baker *et al.*, 1985b
	Auditory digit span	Sun and Liang, 1990
	Continuous recognition memory	Maizlish *et al.*, 1985
Visual perception	Symbol-digit substitution*	Baker *et al.*, 1985a
	Pattern comparison*	Letz and Baker, 1988
	Two-digit search*	Thorne *et al.*, 1985
	Length discrimination	Baker *et al.*, 1985a
	Continuous performance	Maizlish *et al.*, 1985
Psychomotor	Visual simple reaction time*	Baker *et al.*, 1985a
	Visual choice reaction time*	Thorne *et al.*, 1985
	Cursor tracing	Baker *et al.*, 1985a
	Switching attention*	Letz and Baker, 1988
	Finger tapping*	Anger, 1985

Note. Asterisks denote the tests which were chosen in this study.

TABLE 3
AIR MERCURY CONCENTRATIONS AT DIFFERENT WORKPLACES

Sampling sites	No. of sample	Means ± SD (mg/m^3)
Lamp vacuumizing	4	0.085 ± 0.075
Mercury pumping into lamp	4	0.078 ± 0.042
Lamp baking	2	0.019 ± 0.007
Maintaining	4	0.018 ± 0.006
Aging	2	0.017 ± 0.004
Tube shaping	2	0.015 ± 0.013
Tube rolling	4	0.011 ± 0.002
Quality inspecting	6	0.008 ± 0.003

2. *POMS Questionnairing*

No significant difference was found in terms of overall scoring between the Hg-exposed and the control groups. However, scores from two of the negative mood states categorized as fatigue and confusion showed significant higher in the Hg-exposed group compared with the controls as shown in Table 4.

3. *Performance of Neurobehavioral Tests*

Results from most of the neurobehavioral tests introduced showed that the numerical ability and cognitive performance in Hg-exposed workers was significantly poorer than those in the controls. It was particularly demonstrated by the lowered scores of mental arithmetic and two-digit search tests, prolonged latency and decreased correct response in switching attention test, and retarded finger tapping test as shown in Table 5.

To assess the possible long-term effects from the past Hg exposure, analysis of variance was conducted based on the stratified working age. It was found that the significant differences did exist among the different working age groups ($P < 0.05$).

In view of the fact that the working age is positively proportional to the chronological age, the analysis of covariance was conducted to exclude the confounding effect of age. After the confounding factor of chronological age being controlled, the effects from working age, in particular, on the neurobehavioral tests of mental

TABLE 4
COMPARISON OF THE MOOD STATES BETWEEN TWO GROUPS (SCORED IN MEANS ± SD)

Mood states	Hg-exposed (n = 88)	Control (n = 70)	t value
Tension	11.40 ± 6.58	10.07 ± 5.55	1.79
Depression	14.49 ± 11.44	12.23 ± 9.81	1.71
Anger	14.38 ± 9.30	13.29 ± 8.36	0.58
Vigor	14.07 ± 6.43	15.24 ± 5.57	1.44
Fatigue	10.55 ± 5.53	8.04 ± 5.00	8.62**
Confusion	9.65 ± 5.29	6.93 ± 3.68	13.21**

** $P < 0.01$.

TABLE 5
RESULTS OF NEUROBEHAVIORAL TESTS IN Hg-EXPOSED AND CONTROL GROUPS (MEANS ± SD)[a]

Subtest	Hg-exposed (n = 88)	Control (n = 70)	t value
Mental arithmetic	17.57 ± 8.46	23.77 ± 7.27	23.72**
Visual retention	5.53 ± 1.98	5.59 ± 1.87	0.03
Paired-associate learning	16.01 ± 6.74	17.01 ± 8.00	0.92
Two-digit search			
No. correct reaction	11.74 ± 3.92	12.90 ± 1.82	5.25*
Max. time consumed (sec)	9.67 ± 7.01	7.57 ± 3.63	6.24*
Min. time consumed (sec)	1.82 ± 0.91	1.98 ± 0.86	1.33
Symbol-digit substitu'n	3.65 ± 2.98	2.98 ± 0.88	3.30
Switching attention			
No. correction reaction	51.96 ± 9.26	55.91 ± 4.26	10.96*
Mean reaction time (sec)	1.07 ± 0.87	0.78 ± 0.22	7.06*
Visual SRT			
Mean reaction time (sec)	0.32 ± 0.49	0.29 ± 0.29	0.22
Visual CRT			
Maximum (sec)	1.07 ± 0.65	1.05 ± 0.05	9.36*
Minimum (sec)	0.59 ± 0.15	0.63 ± 0.14	4.21*
Mean (sec)	0.85 ± 0.07	0.86 ± 0.07	0.16
Finger tapping	62.73 ± 13.21	67.91 ± 15.92	4.98*
Pattern comparison (sec)	3.81 ± 1.44	3.85 ± 0.89	0.03

[a] Expressed as scores except for items with mark of (sec) which indicates the reaction time in second.

* $P < 0.05$.

** $P < 0.01$.

arithmetic, paired-associate learning, two-digit search, switching attention, and reaction time as well as pattern comparison still existed (Table 6).

DISCUSSION

The effects of low-level exposure to mercury vapor, as found at present workplaces of the fluorescent lamp manufacturing factory, appear to be a more subtle symptomatology that is characterized by neuroticism and introversion as well as minor changes in psychological performance (Soleo *et al.*, 1990). In that case, the neurobehavioral test battery with sensitive measures seems to be the only tool with which to monitor the insidious effects of the exposed workers at the subclinical stage. It is, therefore, important to demonstrate that performance on the tests is sensitive to the subtle effects of known neurotoxicants under occupational exposure conditions.

The subjects in this study had a moderate exposure to inorganic mercury vapor at an average air level of 0.033 mg/m^3 (ranging 0.005–0.190 mg/m^3) with urinary mercury excretion of 25 μg/liter (25 ± 59 μg/liter) during the study period which is about three times of the maximum allowable concentration, 0.01 mg/m^3, of the elemental mercury vapor at workplace and the similar level of the biological exposure index of urinary mercury, 0.05 mg/liter, currently adopted in China. The results indicated that changes both in the pattern of the POMS questionnairing and some performance tests from the NES-C were found in the Hg-exposed group. In

TABLE 6
RESULTS (MEANS ± SD) OF ANALYSIS OF COVARIANCE BETWEEN NEUROBEHAVIORAL PERFORMANCE AND WORKING AGE[a]

Subtests	Working age (years)			F
	<10	10–19	>20	
M. arithmetic	28.66 ± 11.83	21.64 ± 8.51	18.33 ± 8.67	4.10*
V. retention	6.52 ± 1.91	5.67 ± 1.76	5.08 ± 1.89	2.72
PA. learning.	23.75 ± 7.48	16.64 ± 6.24	14.50 ± 7.15	3.85*
TD. search (sec)	1.58 ± 1.55	3.68 ± 1.75	4.26 ± 1.67	7.03**
SD substitut'n (sec)	2.43 ± 1.88	3.32 ± 2.42	3.47 ± 1.04	2.05
S. attention (sec)	0.58 ± 0.66	0.92 ± 0.57	0.92 ± 0.51	2.09
V. SRT (sec)	0.28 ± 0.34	0.35 ± 0.47	0.32 ± 0.25	0.69
V. CRT (sec)	0.51 ± 0.14	0.62 ± 0.14	0.63 ± 0.16	3.44*
F. Tapping	154.10 ± 42.88	162.00 ± 52.30	152.70 ± 79.10	0.65
P. Comparison (sec)	2.46 ± 1.60	4.00 ± 1.50	3.87 ± 1.11	8.98*

[a] Chronological age was treated as the covariable.
* $P < 0.05$.
** $P < 0.01$.

comparison with controls, the mood states were posed moving to the negative side characterized by having higher scores of the mood scale of Fatigue and Confusion. One might supposedly link these mood states with the early sign of "introversion." Moreover, the alteration of neurobehavioral pattern was most likely to be connected to the impairment of cognitive function appearing as the poorer performance particularly in mental arithmetic, switching attention, and two-digit search tests.

Furthermore, as shown in Table 6, subjects with longer working age exerted a poorer neurobehavioral performance despite the fact that the confounder of age was controlled. It seemed to imply that the cumulative effects on psychological function might play an important role in long-term exposure to a low level of mercury.

In conclusion, it is evident that the computer-administered evaluation system in the Chinese version is basically valid for the purpose of neurotoxicity screening tests among the working population exposed to neurotoxic agents. Fluorescent lamp manufacturing workers exposed to moderate mercury vapor for 10.4 years on average were found to be affected in mood states as well as neurobehavioral performance by the evaluation system. On the basis of our findings that changes in psychological tests may be observed with an average air exposure level of 0.03 (ranging from 0.005 to 0.19) mg/m^3 and the urinary mercury level as low as 0.025 mg/liter. However, further study is needed to provide more information with respect to the reference data and essential baseline data for neurobehavioral health surveillance using NES-C for workers at risk.

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Subjective Symptoms and Neurobehavioral Performances of Ex-Mercury Miners at an Average of 18 Years after the Cessation of Chronic Exposure to Mercury Vapor[1]

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In order to determine if there are any residual effects of long-term exposure to mercury vapor, neurobehavioral tests were given to ex-mercury miners about 18 years after the cessation of mercury exposure. Seventy-six male ex-mercury miners who had been exposed to relatively high concentrations of mercury vapor (over 1.0 mg/m^3) and with a past history of mercury intoxication were compared to age (±3 years)-, sex-, and years of education-matched controls. Although the extent of the workers' symptoms caused by mercury poisoning, termed erethismus merculialis, markedly decreased after the cessation of exposure, the prevalence of neurological symptoms (such as hand tremors, headaches, and slurred speech) and symptoms of senility (such as low-back pain, loss of sexual desire) in the exminers was significantly higher than those in the controls. Matched-pair analysis showed that performances of motor coordination, Simple reaction time, and Short-term memory in the exminers were significantly deteriorated compared to those of controls. There are slight but persistent effects on neurobehavioral function, especially on motor coordination function, among mercury miners more than 10 years after the cessation of exposure.

INTRODUCTION

Chronic inorganic mercury poisoning in man is characterized by the development of tremors, psychic disturbances, and altered behavior; the latter two are known as erethismus mercurialis (Bidstrup, 1964). According to the literature, the neurobehavioral effects of mercury vapor appear as a wide spectrum of functional and subjective disturbances, categorized into the following four groups: (1) disturbances of the motor system, such as fine tremors of the extremities and poor psychomotor performance, (Miller *et al.*, 1975; Langolf *et al.*, 1978; Fawer *et al.*, 1983), (2) deterioration of intellectual capacities, such as memory disturbances and poor verbal intelligence (Forzi *et al.*, 1976; Williamson *et al.*, 1982; Smith *et al.*, 1983; Piikivi *et al.*, 1984; Piikivi and Hanninen, 1989; Soleo *et al.*, 1990), (3) alterations of the emotional state and such symptoms as depressive moods, irritability, and listlessness (Forzi *et al.*, 1976), and (4) peripheral neurotoxicity, i.e., polyneuropathy with prolonged motor and sensory nerve conduction (Gilioli *et al.*, 1976; Levine *et al.*, 1982; Albers *et al.*, 1982; Singer *et al.*, 1987). However, only a limited number of reports about whether the effects of mercury vapor

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exposure on neurobehavioral function after cessation of exposure persist is available (Albers *et al.*, 1988). We have surveyed subjective symptoms and neurobehavioral performances of ex-mercury miners, about 18 years after the cessation of mercury exposure, in order to determine if there were any adverse residual effects of long-term exposure to mercury vapor.

METHODS

Subjects

The subjects were exworkers of a mercury mine in Hokkaido, Japan, which started mining in 1939 and closed in 1970. About 1000 men were engaged in mining at the plant before 1945 (the end of World War II), while 517 men and 143 women worked there in 1947. Then the number of employees gradually decreased because of declines in both the amount and the quality of the mercury; in 1969, 361 men and 64 women worked in this mine.

In 1987, 18 years after the closure of the mine, 149 exworkers' addresses were obtained from lists of employees and the worker's union, but 15 of them had already died. An introductory letter concerning the epidemiological survey was sent to these exworkers. Finally, we were successfully able to survey the health status of 117 male workers, including exminers and workers in other job categories from the same mine. Then subgroups were formed according to the history of mercury intoxication, which was supposed to be related to long-term and actual exposure. For the evaluation of neurobehavioral performance tests, a subgroup of 76 ex-mercury miners who had a history of suffering from mercury poisoning during their employment in the mercury mine was compared with matched referents on the bases of age (±3 years), sex, and years of education.

As the referent group, 76 referents were selected following random sampling strategy from among 154 participants who visited seven local municipal health centers in the neighborhood of this mercury mine for the annual physical health check up for the screening of the chronic diseases. This health-check system for persons above 40 years of age has been carried out by the Ministry of Welfare in Japan since 1983. This selection used individual matching to the mercury-exposed groups within 3-year birth intervals, as well as on the bases of sex, residency, and educational attainment.

Collection of Data and Test Procedures

The examination of the study group included both mailed questionnaires and personal interviews for data collection concerning the workers' occupational and health histories and subjective symptoms, and a health survey using neurobehavioral tests, clinical and neurological examinations, and biochemical laboratory tests for blood, liver, and kidney functions. Mercury levels in blood, urine, and hair were also measured. (The results of the clinical examinations will be reported elsewhere.)

Occupational history. The subjects were asked about the duration of their employment and job categories in the mercury mines, the types and duration of occupations and industries that they had been employed in throughout their lives, and their current job status or reasons for unemployment.

Medical/lifestyle questionnaire. A standardized questionnaire was sent to each subject. Specific questions were asked concerning (1) the history of mercury

poisoning, i.e., the frequency of (a) acute mercury poisoning, (b) hospital admissions for inpatient treatment of mercury poisoning, and (c) transfer from inside-pit labor to labor outside the mine in order to decrease the mercury exposure; (2) medical history of diseases, including hypertension, diabetes, ischemic heart disease, stroke, pneumoconiosis, cancer, and other chronic diseases, and use of medications; (3) lifestyle habits, including smoking, alcohol consumption, and food intake frequency, such as the extent of fish consumption; and (4) exposure to other chemicals at the mercury mine, before employment at the mercury mine, and after its closing.

Subjective Symptoms

The questionnaire used in the symptom survey was divided into two parts: one part concerned acute symptoms ($N = 20$) experienced during the mercury poisoning and the other concerned chronic symptoms ($N = 79$). Acute symptoms were examined with questions about the acute effects of mercury vapor, such as tremors, gingivitis, and the central nervous system syndrome termed erethism merculialis. The questions dealing with chronic symptoms were formulated so that they covered almost all symptoms revealed in earlier clinical and epidemiological reports on mercury poisoning. The main symptoms were nervous system disorders. Sensory disorders such as eye and nose symptoms were included. Psychiatric symptoms such as irritability, sleeping difficulties, impaired memory, and concentration difficulties were also included. In addition, general symptoms, including those related to the autonomic nervous system, skin, and digestive system, as well as the cardiac respiratory system were added. We also considered the symptoms caused by mining work (such as respiratory dysfunction, low-back pain, hearing loss, and vibration syndrome) and the symptoms of senility (such as presbyopia, joint or low-back pain, loss of sexual desire), because the mean age of the ex-mercury miners was over 60 years old. The questions concerning chronic symptoms were asked so that responses were separated, i.e., present symptoms experienced within the past year and symptoms experienced during the employment at the mercury mine (or about 20 years ago for the referents). A three-step rating scale (never, sometimes, often) was used in the questionnaire, which was constructed in language that was simple and unambiguous in order to facilitate replies.

Neurobehavioral Evaluation

The objective of the testing program was to determine if there were in fact measurable residual effects of mercury exposure among exworkers using the characteristics that have been hypothesized as critical in toxic causation based on previous studies. In selecting tests the following criteria were emphasized:

1. The test should cover many functions, including motor coordination, cognitive and psychomotor function.
2. It should be easy to conduct under field conditions.
3. It should be easy to conduct in elderly persons.
4. Administration should be economical in terms of both time and equipment.

The neurobehavioral examination consisted of a short interview and a brief test battery, which included (1) Sensorimotor performance [Grip strength, Equilibrium duration test, and a writing test for the screening of tremors (Bender Gestalt test)]. (2) Psychomotor function (Simple reaction time, Tapping and Pegboard),

(3) Hand–eye coordination (Aiming and Tracing), (4) Color card reading test, (5) Verbal/Nonverbal cognition (Hasegawa's scale of dementia and Kohs' Block design test), and (6) Short-term memory (Digit span).

Each subject was required to complete the following procedures for Sensorimotor performance:

Grip strength. Grip strength for both hands was determined using a hand dynamometer. Subjects squeezed the handle as hard as they could for 5 sec. The maximum force for three 5-sec trials was recorded.

Equilibrium duration test. Subjects were asked to stand erect with one leg bent at the knee, both eyes shut, and arms outstretched. A stopwatch was used to record the duration of the period that the subjects were able to maintain the stance before returning the second foot to the ground. The test was performed three times and the mean endurance time was calculated.

Writing test for the screening of hand tremors (Bender Gestalt test). A modified Bender Gestalt test for hand-tremor screening was used. Since we did not intend to apply the Bender Gestalt test for the usual purposes, we only employed Figs. 4 and 7 to detect tremors in hands. This was because these two figures and Figs. 2, 6, and 8 are used to check the presence of tremors in the usual scoring. We excluded Figs. 2, 6, and 8, since they seemed to be relatively insensitive for detecting tremors. We exhibited a set of figures to an examinee, instructing him to reproduce the same figures on a blank piece of paper. The tremor status of the reproduced figures was read blindly by one of us unaware of the neurological status of the subjects. The sensitivity and specificity of this method were studies by Fukuda (Fukuda *et al.*, 1975).

For Psychomotor function, subjects completed the following:

Simple reaction time. The subject was required to grasp a falling steel pole as quickly as possible following a visual cue from the pole (Ohnishi, 1974). The task was repeated seven times. Each subject's score was the average reaction time for five of the seven trials, excluding maximum and minimum reaction times.

Tapping. Finger tapping is a motor-speed task performed with a simple counter. The subject taps it with his index finger as fast as possible. The whole task consists of 60-sec trials with both hands.

Pegboard and Finger-dexterity board: The subjects' task in this test consisted of three different kinds of Figure dexterity tests. The first test (replacement) was to replace as many sets of metal pegs in small round holes upside down as he could in 30 seconds. The task was performed with the preferred hand. In the second test of assemblage using a Finger-dexterity board, the subject was instructed to assemble rivets and washers using both hands and to use them to fill in as many holes as possible within the allotted time (90 sec). In the third test (disjointing), the subject was asked to place the rivets on one side of a board and washers on a pole on the opposite side, separating as many of the sets as possible within 60 sec. These tests are standardized tests used by the Japan Ministry of Labor for vocational aptitude (1969).

For Hand–eye coordination, the subjects were tested on the following:

Aiming and Tracing. Aiming (a motor-coordination test published by the Tokyo University Department of Rehabilitation; Ueda, 1971) and Tracing (MacQuarrie, 1953) both are paper–pencil tests which require a seated subject to aim a center point or trace a (spiral) pathway with a pencil. The standardized instruction for the administration of the test was followed (Ueda, 1971). Fifty seconds were allowed

for the completion of each of Aiming and Tracing tests. The test score (error) was a function of the distance plotted from a center and the number of times the edges of a concentric circle were touched with the pencil used for the aiming.

For the Color card reading test, subjects performed the following procedure.

Subject performance on the Color card reading test (Takei Instrument Co.) was assessed in terms of word reading speed and reading fluency (Karino, 1975). The color card had five color strips (red, blue, yellow, black, and white), printed on a gray board and set in a 10 × 10 matrix, each color strip appearing 20 times. The instructions stressed the need for both speed and accuracy. The experimenter had a coded copy of each card with the correct response and recorded any errors.

For Verbal/Nonverbal cognition, the following tests were conducted:

Kohs' Block design test. Kohs' Block design is a test for visual ability, i.e., the ability to see and reproduce spatial relations. The subject was given 4 or 9 two-colored blocks. The test includes 10 items, and it becomes more difficult from item to item (Kohs, 1920).

Hasegawa's dementia screening scale. A test for the screening of dementia was given to both the exworkers and the referents. The 11 elements included (a) 5 primitive questions about date, name of the place, age, birth place, and time; (b) 2 intelligence questions about the year of the end of World War II and the name of the prime minister; (c) 1 series involving calculations, i.e., subtract 7 from 100, then 7 from 93; (d) Digit span, i.e., name numbers in reverse, e.g., 6–8–2, 3–5–2–9; (e) immediate visual memory, i.e., identify five items (coin, toothbrush, watch, comb, and spoon) and recall them. There are scoring norms for normal, borderline, predementia, and dementia (Hasegawa, 1984).

To determine Short-term memory, subjects performed the following:

Digit span test (Wechsler Adult Intelligence scale; WAIS). Lists of successively increasing numbers of randomly generated digits were orally presented to subjects, e.g., 3–6–2, 4–8–5–1. The task was to repeat the numbers presented, first in a digits-forward and then in a digits-backward manner. The test score was the total number of digits correctly repeated forward and backward (Wechsler, 1955).

Statistical Methods

First the sign test (Siegel, 1956) was used to compare the subjective symptoms of the exminers during employment at the mercury mine with those at present; then it was utilized to compare the symptoms of exminers with those of nonexposed matched referents symptom by symptom. Neuropsychological performance levels of the exposed workers and the nonexposed matched referents were represented as means and standard deviations. The significance of the differences between the two groups was tested by one-tailed t tests for unpaired data.

RESULTS

Description of Work Process and Exposure

The mine, work process, and working conditions have been described by an industrial physician (Hashiba, 1954). The workers were exposed to relatively high concentrations of mercury vapor from native mercury. The atmospheric mercury concentration was usually over 1.0 mg/m^3, e.g., 1.9–3.3 mg at the pit face, and 1.5 mg at the overchute. The mean working time of these workers was 378 min/day. Although these workers' exact medical records were unfortunately lost, it was

reported that their exposure had been monitored by regular health examinations and urine mercury analyses once a month by an industrial physician and other health personnel in the mercury mine (Hashiba, 1954). Many of the miners had a history of mercury intoxication. Suwa and Takahata (1969) reported that the mercury concentrations of urine at the time of diagnosis in the clinic were quite high: 72.4% of cases (60 cases among 83 patients) in a survey showed urine mercury levels of 500–2000 μg/liter, 22.8% (19 cases) showed over 2000 μg/liter, while 4.8% (4 cases) showed under 500 μg/liter.

Characteristics of Subjects

The 117 exworkers' mean age was 60.3 ± 7.3 years (range, 46–78). The mean age of 76 exworkers with a history of mercury intoxication was 60.6 ± 7.0 years (range, 47–78). The mean duration of the employment of exworkers at the mercury mine was 14.9 + 9.0 years (range, 1–42) and 15.5 ± 8.7 (range, 1–42), for the total 117 exworkers and the 76 subgroup members with a history of mercury poisoning, respectively. The mean durations from the cessation of mercury exposure to the present survey were 19.5 ± 6.3 years (range, 2–44) and 17.9 ± 5.1 years (range, 2–29 years), for the 117 exworkers and the 76 workers of the subgroup, respectively. After the closure of this mine, all of the exworkers had alternate jobs. Some of them (7 miners) had transferred to another mercury mine and 22 miners had been engaged in other metal mines in the same prefecture (11 in coal mines, 9 in a copper mine, and 2 in a zinc mine), although those mines were also closed within several years after the mercury mine was closed.

Among 76 exworkers with a history of mercury intoxication, workers who engaged in mining, refining, and transporting jobs showed higher percentages of having a past history of mercury poisoning, while workers who had engaged maintenance jobs showed lower percentages of mercury intoxication history. Since we matched exposed cases with referents by sex, age, residence, and educational years, there were no differences of mean age and education among the exworkers and the referents. The answers to the questions concerning frequency of alcohol consumption, amount of alcohol used, and smoking status were almost the same in the exposed and nonexposed groups. There were differences in current working status among exmercury miners and referents. A higher percentage of referents were currently working than exmercury mine workers, mainly because many of the referents over 60 years old were farmers. There were some differences in distribution of the job/industry categories, although there was no difference in the ratio of jobs involving supervisory, clerical, and engineering between exminers and referents (Table 1).

Past History of Mercury Intoxication and Subjective Symptoms

Table 2 shows the number of hospital admissions for inpatient care among the 76 exworkers (subgroup) who had a history of mercury poisoning. More than 70% of the workers had experienced inpatient care in the hospital. The distribution of the number of workers' job transfers from inside-pit labor to jobs outside the mine is also shown in Table 2. These job transfers were recommended for the workers (patients) in order to decrease mercury-exposure levels when they suffered acute mercury poisoning.

The most common subjective symptom which exminers with a past history of mercury intoxication reported was gingivitis (73.7%), followed by hand or foot

TABLE 1
CURRENT (OR MOST RECENT) JOB OR INDUSTRY CLASSIFICATION OF THE EXMINERS WITH A HISTORY OF MERCURY POISONING AND REFERENTS

Job category	Exposed (%)	Referents (%)
1. Supervisory	3 (4.0)	4 (5.3)
2. Clerical and engineering	17 (22.4)	17 (22.4)
3. Sales	5 (6.6)	8 (10.5)
4. Services	5 (6.6)	9 (11.8)
5. Manufacturing (skilled)	16 (21.1)	8 (10.5)
6. Construction (semi-skilled, unskilled)	14 (18.4)	5 (6.6)
7. Agriculture (farmer)	3 (3.9)	23 (30.3)
8. Mining (miner)	7 (9.2)	1 (1.3)
9. Transport	6 (7.9)	1 (1.3)
Total	76 (100.0)	76 (100.0)

tremors (72.4%), tiredness (69.7%), insomnia (59.2%), fatigue (59.2%), and irritability (56.6%) (Table 3). This distribution of subjective syndromes was almost the same as those already published by Hashiba (1954) and Suwa and Takahata (1969) while the mine was still operating.

Results of pairwise comparison of the symptoms of the 76 exminers with a history of mercury intoxication are shown in Table 4. The symptoms experienced more frequently during employment at the mine rather than at present were mainly tremors of hands, feet, and fingers; painful swelling of gums; and central nervous system symptoms such as irritability and depression, which were termed erethism. In contrast, the symptoms more frequently experienced now rather than during employment at the mercury mine were symptoms of senility (such as presbyopia, decline in sexual desire, pain in the joints, back or hips, frequent sputum and coughing) (Table 5).

Although the extent of the exworkers' symptoms caused by mercury poisoning markedly decreased after the cessation of exposure, comparison between the exposed workers and the referents showed that the prevalence of neurological symptoms (such as hand tremors, headache, and slurred speech), some symptoms caused by mining work (such as symptoms related to pneumoconiosis, low-back

TABLE 2
NUMBER OF HOSPITAL ADMISSIONS FOR INPATIENT CARE AND NUMBER OF JOB TRANSFERS FROM INSIDE-PIT LABOR TO OUTSIDE THE MINE AMONG THE EXWORKERS WHO HAD A HISTORY OF MERCURY POISONING

	No. of hospital admissions	No. of job transfers
More than 10 times	8 (10.5%)	10 (13.2%)
10 times	2 (2.6%)	9 (11.8%)
5–9 times	9 (11.1%)	5 (6.6%)
3–4 times	16 (21.1%)	13 (17.1%)
1–2 times	18 (23.7%)	11 (14.5%)
None	22 (28.9%)	23 (30.2%)
Unknown	1 (1.3%)	5 (6.6%)
Total	76 (100.0%)	76 (100.0%)

TABLE 3
MAIN SUBJECTIVE SYMPTOMS OF EXMINERS WITH A PAST HISTORY OF MERCURY INTOXICATION DURING TOXICOSIS

Symptom	Case	(%)
1. Gingivitis	56	(73.7%)
2. Hand or foot tremor	55	(72.4%)
3. Tiredness	53	(69.7%)
4. Insomnia	45	(59.2%)
5. Fatigue	45	(59.2%)
6. Irritability	43	(56.6%)
7. Powerless of arm and legs	42	(55.3%)
8. Toothache	42	(55.3%)
9. Bleeding gums	41	(53.9%)
10. Difficulty writing	41	(53.9%)
11. Heaviness in the head	40	(52.6%)
12. Slow moving	35	(46.1%)
13. Unsteadiness in walking	33	(43.4%)
14. Forgetfulness	30	(39.5%)
15. Dropping chopsticks during meal	23	(30.3%)
16. Inarticulate speech	21	(27.3%)
17. Diarrhea	20	(17.1%)
18. Hearing loss	12	(15.8%)
19. Blurred vision	7	(9.2%)
20. Others	4	(5.3%)

TABLE 4
SYMPTOMS EXPERIENCED MORE FREQUENTLY DURING EMPLOYMENT AT THE MERCURY MINE THAN AT PRESENT (76 EXMINERS)

Symptom	Cases		*P* value*
	A	B	
Tremors in fingers	45	3	0.001
Hands or feet tremble	44	5	0.001
Often drop chopsticks during meals	35	9	0.001
Tooth decay	34	9	0.001
Easily irritated without reason	33	9	0.001
Large involuntary weight loss	30	9	0.001
Paleness	27	7	0.001
Easily depressed or melancholy without reason	26	4	0.001
Difficulty in staying asleep	31	14	0.01
Dizziness and unsteadiness	32	8	0.01
Trouble writing due to tremors	25	9	0.01
Loss of appetite	24	8	0.01
Loose tooth (or teeth)	23	8	0.01
Headache	26	10	0.02
Worry about everything	26	11	0.02
Upset by all sorts of little things	16	5	0.02
Difficulty in falling asleep	30	16	0.03
Difficulty in concentrating	20	9	0.05
Listlessness	29	15	0.05

Note. Case A, number of exworkers who experienced symptoms more frequently during their employment at the mercury mine than at present. Case B, number of exworkers who experience symptoms more frequently now than during their employment at the mercury mine.

* Level of significance of difference.

TABLE 5
SYMPTOMS EXPERIENCED MORE FREQUENTLY NOW THAN DURING EMPLOYMENT AT THE MERCURY MINE (76 EXMINERS)

Symptom	Cases		*P* value*
	A	B	
Longsightedness	1	54	0.001
Sight grown dim recently	1	51	0.001
Decline in sexual desire	4	46	0.001
Easily intoxicated	1	41	0.001
Back or hip pain	9	36	0.001
Difficulty in hearing	2	34	0.001
Stiffness in shoulders or neck	7	33	0.001
Often out of breath when just sitting still	5	29	0.001
Frequent sputum	6	28	0.001
Frequent urination during the day	6	28	0.001
Footsore	3	26	0.001
Often ill	8	29	0.001
Coldness of hands and legs	3	24	0.001
Heavy perspiration even in cool weather	5	23	0.001
Cough up sputum more than 4 days per week	5	21	0.002
Tinnitus, a ringing in the ears	8	24	0.005
Thumping of the heart	7	24	0.005
Easily forget things	11	29	0.005
Cough more than 4 days per week	6	21	0.005
Frequent coughing	8	23	0.01
Joint pain	10	26	0.01
Excessive fatigue after the workday	12	26	0.02
Trouble breathing	4	14	0.02
Difficulty in swallowing	4	14	0.02
Arms and legs feel powerless	11	24	0.05
Easily catch cold	10	22	0.05

Note. Case A, number of exworkers who experienced symptoms more frequently during their employment at the mine than now. Case B, number of exworkers who experience symptoms more frequently now than during their employment at the mine.

* Level of significance of difference.

pain), and symptoms of senility (such as longsightedness, loss of sexual desire) were significantly higher than those of controls (Table 6).

Neurobehavioral Comparison between the Exposed Workers and the Referents

Matched-pair analysis showed that Grip strength, Simple reaction time, the performance of Tapping, Finger dexterity, Hand–eye coordination, speed of Color Card Reading, performance on the Block design test, and Short-term memory (Digit span) in exminers had significantly deteriorated compared to those of referents; i.e., the mean number of errors in Aiming was 11.0 ± 11.0 (mean ± SD) and 5.4 ± 6.5 (exmercury miners and referents, respectively, $p < 0.001$). The mean number of counts performed in Tracing were 38.2 + 14.8 (mean ± SD) and 48.3 ± 18.5, respectively. The mean digits of the Digit span (forward) test were 4.9 vs 5.5. The mean of Simple reaction times were 228.6 ± 24.7 msec (mean ± SD) and 216.7 ± 31.8 msec ($P < 0.05$) (Table 7).

DISCUSSION

It is a well-recognized fact that chronic exposure to mercury can result in

TABLE 6
RESULTS OF A PAIRWISE COMPARISON OF THE SUBJECTIVE SYMPTOMS OF 76 MATCHED PAIRS OF EXPOSED AND REFERENT SUBJECTS—SYMPTOMS WITHIN THE PAST YEAR

Symptom	Cases		*P* value*
	A	B	
1. Headaches	33	5	0.000
2. Heaviness in the head	34	6	0.000
3. Easily irritated without reason	30	9	0.001
4. Easily depressed or melancholy without reason	18	5	0.01
5. Worry about everything	30	8	0.001
6. Upset by all sorts of little things	24	8	0.005
7. Difficulty in concentrating	25	10	0.01
8. Need plenty of time to resolve something	29	16	0.05
9. Character said to have changed	21	5	0.005
10. Easily forget things	34	13	0.005
11. Difficulty in falling asleep	29	14	0.05
12. Difficulty in staying asleep	25	14	(N.S.)
13. Nightmares	27	7	0.001
14. Fall asleep in the daytime	23	11	0.05
15. Convulsions	11	5	(N.S.)
16. Arms and legs feel powerless	32	8	0.001
17. Dizziness and unsteadiness	34	6	0.000
18. Cannot walk straight	16	3	0.000
19. Hands or feet tremble	32	3	0.000
20. Tremors in fingers	26	1	0.000
21. Numbness or tingling in fingers or soles of feet	31	5	0.000
22. Often drop chopsticks during meals	15	1	0.001
23. Feel slow moving	30	19	(N.S.)
24. Walking unsteadily	28	9	0.01
25. Feel unsteady when bumped by someone	26	6	0.001
26. Speak with a lisp	28	6	0.001
27. Trouble speaking clearly or rapidly	26	9	0.005
28. Trouble writing due to tremors	35	7	0.000
29. Trouble writing	25	15	(N.S.)
30. Listlessness	31	11	0.005
31. Excessive fatigue after the workday	34	12	0.001
32. Loss of appetite	19	10	(N.S.)
33. Frequent vomiting	20	4	0.001
34. Stomachache	18	3	0.001
35. Frequent diarrhea	18	7	(N.S.)
36. Usually constipated	24	10	0.05
37. Frequent salivation	10	1	0.01
38. Tooth decay	24	16	(N.S.)
39. Painful swelling in the gums	25	18	(N.S.)
40. Loose tooth (or teeth)	21	16	(N.S.)
41. False teeth	10	29	0.01
43. Frequent coughing	37	10	0.000
44. Frequent sputum	37	12	0.001
45. Cough more than 4 days per week	27	6	0.001
46. Cough up sputum more than 4 days per week	28	5	0.000
47. Easily catch cold	31	12	0.005
48. Difficulty in breathing because of wheezing	21	6	0.005
49. Trouble breathing	22	6	0.005
50. Thumping of the heart	36	10	0.001
51. Often out of breath when just sitting still	34	12	0.001
53. Difficulty in hearing	36	8	0.001

TABLE 6—*Continued*

54. Tinnitus, a ringing in the ears	33	7	0.001
55. Sight grown dim recently	41	5	0.000
56. Longsightedness	35	14	0.005
57. Joint pain	35	10	0.001
58. Back or hip pain	42	10	0.000
59. Coldness of hands and legs	32	9	0.001
60. Stiffness in shoulders and legs	39	15	0.001
61. Footsore	28	9	0.005
62. Easily intoxicated	31	15	0.05
63. Frequent urination during the day	29	10	0.005
64. Decline in sexual desire	36	12	0.005
65. Difficulty in swallowing	15	3	0.005
67. Swollen feet and hands	25	3	0.000
68. Large involuntary weight loss	19	18	(N.S.)
69. Paleness	17	3	0.005
70. Cheeks often flushed	16	4	0.01
71. Heavy perspiration even in cool weather	28	6	0.001
72. Often ill	30	12	0.005

Note. Case A, the ex-mercury miners experienced symptoms more frequently than the referents. Case B, referents experienced symptoms more frequently than the ex-mercury miners.

* Level of significance of difference.

complex alterations of a person's physiological state with the primary effects being in the central nervous system. Tremors and erethism are especially emphasized as toxic symptoms (Kark, 1979). Many exminers in the present study with toxicosis during employment in the mercury mine suffered severe mercury poisoning and complained mostly of such kinds of subjective symptoms. Deficiencies in short-term memory and those detected by a verbal intelligence test (similarities) have been also detected objectively among workers exposed to low levels of mercury such as chlorine-alkali workers (Piikivi and Honninen, 1989) and workers at a fluorescent lamp factory (Soleo *et al.*, 1990), while patients suffering from advanced mercury intoxication have had other neural problems, such as constricted visual fields and disorders of the auditory nerve (Kark, 1979).

It is not so easy, however, to determine if there were any residual effects of long-term exposure to mercury vapor from the present results, because exposure-related neurological dysfunction caused by mercury vapor is often reversible (Kishi *et al.*, 1978) and only rarely is the toxic exposure so massive or the neuronal target so vulnerable that irreversible neuronal changes ensue. The exworkers in this study had been working under conditions in which the mercury levels of the atmosphere were extraordinarily higher than that of the current threshold limit value. Levels were almost more than 100 times higher than the current TLV in Japan. Many of the exminers mentioned that they suffered from mercury poisoning so frequently that they could not remember exactly how many times they took inpatient care treatment. Takahata *et al.* (1970) studied two autopsy cases who had worked for about 10 years in the same mercury mine as those subjects in present study and also worked as pit workers in other types of mines, including coal, gold, sulfur, and asbestos. Both cases showed residual effects of mercury exposure, e.g., rough tremors and ataxia even 10 years after the cessation of mercury vapor. The mercury concentration in the brains of the two cases was high, especially in the occipital cortex (33.56 and 14.80 ppm, respectively), pari-

TABLE 7
MEAN PERFORMANCE ON NEUROPSYCHOLOGICAL TESTS BY EXWORKERS WITH HISTORIES OF MERCURY TOXICOSIS AND REFERENTS

Performance	Exworkers	Referents	*P* value
1. Height	162.5 (4.2)	160.4 (5.5)	**
2. Weight	60.5 (6.6)	60.3 (10.0)	ns
3. Grip strength			
Right	37.6 (7.3)	41.8 (6.7)	**
Left	37.6 (7.1)	39.8 (6.3)	*
4. Romberg	1.2 (0.5)	1.1 (0.3)	ns
5. Equilibrium duration	14.0 (1.3)	12.4 (13.7)	ns
6. Tremor			
(Bender Gestalt)	2.5 (1.3)	2.3 (0.7)	ns
7. Simple reaction time	228.6 (24.7)	216.7 (31.8)	**
8. Tapping			
30 sec	147.1 (27.6)	159.0 (31.6)	**
60 sec	287.9 (50.8)	308.4 (55.6)	*
9. Pegboard and Finger dexterity board			
Replacement	19.2 (4.2)	21.2 (4.5)	**
Assemblage	22.4 (5.0)	23.4 (4.4)	ns
Disjointing	17.7 (4.2)	19.4 (4.2)	**
10. Hand–eye coordination			
Aiming			
Error, right	3.6 (4.3)	3.0 (6.7)	ns
Error, left	11.0 (11.0)	5.4 (6.5)	***
Tracing	38.2 (14.8)	48.3 (18.5)	***
11. Color card reading			
Time	79.6 (22.1)	72.3 (18.0)	*
Error	0.5 (0.9)	0.4 (1.0)	ns
12. Block design	37.0 (15.5)	43.9 (18.2)	**
13. Digit span			
Forward	4.9 (1.1)	5.5 (1.1)	***
Backward	3.5 (0.8)	3.6 (0.9)	ns
14. Dementia scale	28.9 (3.0)	29.7 (2.9)	ns

* $P < 0.05$ (one-tailed).
** $P < 0.01$.
*** $P < 0.001$.

etal cortex (6.21 and 13.80 ppm, respectively), and the substantia nigra (23.05 and 18.00 ppm, respectively), although histopathologically no specific changes were observed except electron-dense granules in the cytoplasm of nerve cells, in particular in Purkinije cells. Albers *et al.* (1988) recently examined 247 exworkers, who had occupational elemental mercury exposures 20 to 35 years previously, to identify potential exposure-related neurological abnormalities. They found that subjects with urine mercury peak levels above 0.6 mg/liter demonstrated significantly decreased strength, decreased coordination, increased tremors, decreased sensation, and increased prevalence of Babinski and snout reflexes when compared with the remaining subjects.

From the epidemiological viewpoint, it is rather difficult to find proper referents for these exworkers, because they are somewhat old and they have had compulsory transfers to several jobs after the closure of the mine. Although we tried to find matched controls with the same educational attainment in individual cases in

addition to age, sex, and residency, the occupational histories of the referents were not exactly in the same category as those of the exminers. From the present data we may say that there are slight but persistent effects on neurobehavioral function, especially on motor coordination function, among mercury miners even more than 15 years after the cessation of exposure. However, further studies following up the neurobehavioral functional assessment of ex-mercury workers are needed to confirm whether the residual effects of chronic mercurialism after the cessation of exposure can be correlated to the exposure indicators, e.g., duration of exposure, period after the cessation of exposure, and job categories.

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Neurobehavioral Effects in Occupational Chemical Exposure[1]

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Neurobehavioral effects in 30 female workers (aged 18–41, mean 25.6) exposed to an average of 341 mg/m^3 (SD 100) toluene for an average of 5.7 years (SD 3.3) compared with 30 matched controls (aged 18–48, mean 25.1), 24 male workers (aged 18–32, mean 24.7) exposed to 268 mg/m^3 (SD 185) toluene equivalent of mixed solvent (82.2% toluene, 12.3% ethyl acetate, and 5.5% methyl ethyl ketone) for 2.3 years (SD 3.0) compared with 24 matched controls (aged 17–31, mean 24.3), and 94 dentists (aged 24–49, mean 31.7) exposed to 0.017 mg/m^3 (SD 0.009) of elemental mercury for 7.4 years (SD 5.3) compared with 54 referents (aged 23–50, mean 33.6) were studied. The Z score (made up of Digit Span, Symbols Digit, and Grooved Peg Board) for the workers exposed to toluene was 0.79, for workers exposed to mixed solvents was 0.38, and for the dentists exposed to mercury was 0.42. The Z score for each group of exposed subjects was statistically poorer than that for its controls. Neurobehavioral performance was statistically related to exposure intensity for the toluene-exposed workers and to years of exposure or dose (exposure intensity × years of exposure) for mixed solvent- and mercury-exposed subjects. The type of chemical species and pattern of exposure appear to influence whether the adverse effects will be cumulative.

INTRODUCTION

Neurobehavioral effects of toluene have been widely reported (Iregren, 1982; Hanninen, 1984; Cherry *et al.*, 1985; Juntunen *et al.*, 1985; Hanninen *et al.*, 1987; Foo *et al.*, 1990). Previous studies indicated that neurobehavioral effects of toluene were most likely related to exposure intensity rather than exposure duration (Juntunen *et al.*, 1985; Iregren, 1982; Cherry *et al.*, 1985). The absence of a statistical relationship between effect and exposure duration suggests that either the neurobehavioral effects of toluene is highly reversible or the cumulative effect was very small under the occupational exposure conditions studied and the study design was not able to observe a small difference between exposed and control groups.

The accumulation of toxic substances depends on the type and the exposure pattern. The present study assessed the neurobehavioral effects of three substances, toluene, mixed solvents, and mercury, under three exposure situations.

MATERIAL AND METHODS

Study Population

Model 1. A total of 30 female electronic factory workers exposed to toluene

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were studied with 30 controls matched for age, sex, and ethnicity. The controls were selected from another section of the same factory where toluene or toluene-containing substances were not used. These workers worked from 8 AM to 5 PM daily, Monday to Friday.

Model 2. A total of 24 male workers exposed to solvent mixture in a factory printing plastic packaging materials were studied with 24 hospital attendants as controls, matched for age, sex, and educational level. These workers were on a 6-day work week (Sunday off), averaged 10 hr daily (including overtime), and worked on a weekly rotating three-shift system.

Model 3. A total of 98 dentists (38 females and 60 males) exposed to metallic mercury in their work were studied with 54 (27 females and 27 males) controls selected from staff at the National University of Singapore. They were matched for educational level. The dentists worked 9 AM–5 PM and 7 PM–9 PM Monday to Friday, 9 AM–12 PM on Saturday, with Sunday off.

The exposed and control groups were screened with a questionnaire to eliminate subjects with (1) diagnosed medical causes of neuropathy, (2) history of central or peripheral nervous system illness or psychiatric disorders, (3) history of upper and or lower limb surgery or injuries, and (4) history of head and or spinal cord surgery or injuries.

Exposure Evaluation

Model 1. Exposure levels for toluene were monitored for the whole shift (8 hr) using personal passive diffusive tubes containing Tenax GC (DCA, Dosimeter Corp, USA). The collection tubes were thermally desorbed and the contents analyzed with gas chromatography. The details of analysis has been described by Foo *et al.* (1988).

Model 2. Exposure levels for mixed solvents were monitored for the whole shift with personal passive diffusive badges containing activated carbon (3500 Organic Vapor Monitor, 3M, USA). The samples were desorbed with carbon disulfide and the contents analyzed with gas chromatography. The details of chemical analysis have been given by Ismail (1990). The toluene equivalent levels (TEq) for mixed solvent exposure were calculated by

$$\text{TEq}(\text{mg/m}^3) = 375\, I_T/375 + I_{EA}/1400 + I_{MEK}/590), \quad (1)$$

where I_T = exposure intensity of toluene in mg/m^3, I_{EA} = exposure intensity of ethyl acetate in mg/m^3, I_{MEK} = exposure intensity of methyl ethyl ketone in mg/m^3.

Model 3. Exposure to metal mercury was monitored for the whole working period with personal passive diffusive mercury badges (Catalog Nos. 520-02 and 520-03, SKC, Inc., USA). Mercury samples were analyzed with cold vapor atomic absorption spectrophotometry. The details of the analysis is given by Ngim (1989).

Neurobehavioral Tests

Three neurobehavioral performance tests were conducted. Digit Span (DSp)

was used to measure short-term memory. Symbols Digit (DSy) test was used to measure the visual motor speed. Grooved Peg Board (GPB) was used to measure the manual dexterity. The details of these tests have been given by Foo *et al.* (1990). Neurobehavioral tests were conducted on Wednesday and Thursday, 9 AM–11 AM before the workers started work.

Statistical Analysis

A neurobehavioral performance Z score was defined as

$$\begin{aligned} Z \text{ score} = [&-(\text{DSp} - \text{mean DSp})/(\text{SD DSp}) \\ &-(\text{DSy} - \text{mean DSy})/(\text{SD DSy}) \\ &+(\text{GPB} - \text{mean GPB})/(\text{SD GPB})]/3. \end{aligned} \quad (2)$$

The means and SD of the respective controls were used to calculate the Z score. The resulting Z score for the controls has a mean of zero and a standard deviation of unity.

The General Linear Model procedure (PROC GLM of SAS statistical analysis software, SAS Inc., USA) was used in analysis of covariance. Covariance analysis was performed for the following models:

$$Z = b_0 + b_1 I + b_2 D + b_3(\text{dose}) + (\text{confounder terms}) \quad (3)$$

$$Z = b_0 + b_1 I + b_2 D + (\text{confounder terms}) \quad (4)$$

$$Z = b_0 + b_3(\text{dose}) + (\text{confounder terms}). \quad (5)$$

The potential confounders included in the regression analysis were age and years of education, for the toluene and mixed solvent group, as well as sex, and consumption of Chinese medical products known to contain mercury for dentists.

Three separate sets of covariance analysis were performed, one for each type of exposure.

RESULTS

The population characteristics are shown in Table 1. The age of the exposed and controls groups were adequately matched. The average age was 25.6 years for the subjects exposed to toluene, 24.7 years for mixed solvent group, and 31.7 years for dentists. The average years of work was 5.7 for toluene-exposed group, 2.3 for mixed solvent group, and 7.4 for dentists. The average years of education was 5.9 for the toluene group, 9.3 for the mixed solvent group, and 16.2 for the dentists.

The TWA (time-weighted average) exposure intensity for the toluene group was 341 mg/m^3 (SD 100) toluene, 268 mg/m^3 (SD 185) toluene equivalent for the mixed solvent group (toluene averaged 59.8 ± 40.8 ppm, ethyl acetate 35.7 ± 25.5 ppm, and methyl ethyl ketone 7.8 ± 5.6 ppm), and 0.017 mg/m^3 (SD 0.009) mercury for dentists.

The major solvent that the plastic printing workers were exposed to was toluene, 82.2% of the solvent exposure based on the ratio of concentration to the

TABLE 1
CHARACTERISTICS OF STUDY POPULATION

Population	Mean age (y)	Years exposed	Mean years of education
Toluene			
Exposed	25.6 (5.6)*	5.7 (3.3)	5.9 (2.8)
Controls	25.1 (6.1)	0.0 (0.0)	8.0 (2.5)
Mixed solvent			
Exposed	24.7 (3.9)	2.3 (3.0)	9.3 (2.0)
Controls	24.3 (4.0)	0.0 (0.0)	9.4 (1.6)
Mercury			
Exposed	31.7 (6.7)	7.4 (5.4)	16.2 (0.6)
Controls	33.6 (7.2)	0.0 (0.0)	17.1 (2.0)

* Values in parentheses are standard deviations.

threshold limit value (ACGIH, 1990), 12.3% of ethyl acetate, and 5.5% of methyl ethyl ketone.

Analysis of covariance showed that the neurobehavioral test performance was poorer in the exposed groups as compared to their respective controls. The difference was statistically significant ($P < 0.05$). The least-square mean of the Z score was 0.79 for the toluene group, 0.38 for mixed solvent group, and 0.42 for dentists. The details are given in Table 2.

The potential confounders included in the regression analysis were age and years of education for the toluene and mixed solvent group as well as sex, and consumption of Chinese medical products known to contain mercury in the dentists.

The statistically significant exposure variable for Model 1, was I in the toluene group and D in the dentists. For Model 2, I was the significant variable in the toluene group, and D in the mixed solvent group and dentists. And for Model 3, the dose variable was statistically significant only in the mixed solvent group and the dentists. The P values are listed in Table 3.

TABLE 2
NEUROBEHAVIORAL PERFORMANCE Z SCORE FOR THE THREE EXPOSED GROUPS AND THEIR CONTROLS

Population	Least-square mean (SE)	P value
Toluene		
Exposed	0.79 (0.14)	0.0005*
Controls	0.04 (0.13)	
Mixed solvent		
Exposed	0.38 (0.11)	0.0212
Controls	0.00 (0.11)	
Mercury		
Exposed	0.42 (0.06)	0.0001
Controls	−0.08 (0.09)	

* P value for exposed differed from that of control.

TABLE 3
REGRESSION MODELS

Model	I	D	Dose
Toluene			
$Z = b_0 + b_1(I) + b_2(D) + b_3(\text{dose})$	0.0113*	0.7437	0.3988
$Z = b_0 + b_1(I) + b_2(D)$	0.0014	0.2042	NA
$Z = b_0 + b_3(\text{dose})$	NA	NA	0.1020
Mixed solvent			
$Z = b_0 + b_1(I) + b_2(D) + b_3(\text{dose})$	0.8481	0.3666	0.2136
$Z = b_0 + b_1(I) + b_2(D)$	0.2022	0.0170	NA
$Z = b_0 + b_3(\text{dose})$	NA	NA	0.0049
Mercury			
$Z = b_0 + b_1(I) + b_2(D) + b_3(\text{dose})$	0.8268	0.0131	0.2598
$Z = b_0 + b_1(I) + b_2(D)$	0.3724	0.0001	NA
$Z = b_0 + b_3(\text{dose})$	NA	NA	0.0001

Note. NA, not applicable.
* P value.

The dentists were further divided into four exposure groups: two exposure levels (0.0007–0.015 and >0.015 mg/m^3) and two exposure durations (up to 75 months and >75 months). The Z scores for the four exposure combinations are shown in Fig. 1.

DISCUSSION

In the present study, the results show that the neurobehavioral effects of mixed solvent and mercury exposure were related to dose according to the Haber's rule. The duration of exposure (years worked) was the major contributor to these

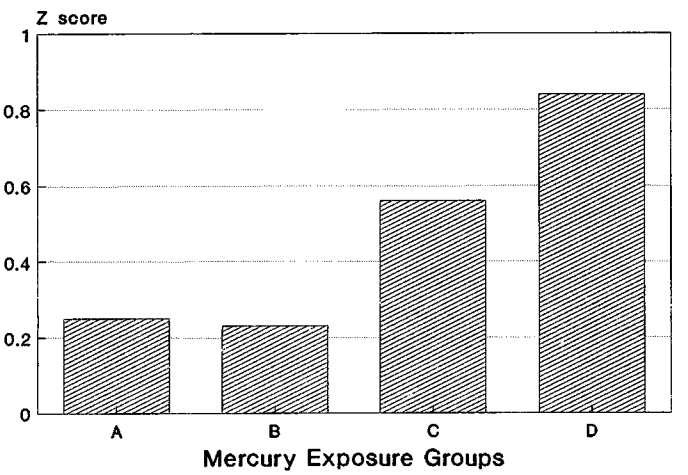

FIG. 1. Z score for exposure intensity–duration groups in dentists exposed to mercury. (A) <75 months, <0.015 mg/m^3; (B) <75 months, >0.015 mg/m^3; (C) >75 months, <0.015 mg/m^3; (D) >75 months, >0.015 mg/m^3. Covariance analysis results, after adjusting for age, sex, education, and consumption of Chinese medical products are (A vs B) $P > 0.05$, (A vs D) $P < 0.05$, (B vs C) $P < 0.05$, (C vs D) $P < 0.05$.

effects. In the case of exposure to toluene, the effect was related mainly to the exposure intensity. These observations indicate that the neurobehavioral effects of mixed solvent and mercury are likely to be cumulative whereas those of toluene are probably highly reversible. These observations are in line with data reported by Iregren (1982), Cherry *et al.* (1985), and Juntunen *et al.* (1985), suggesting that neurobehavioral performance was less affected by the duration of exposure to toluene.

The observed cumulative neurobehavioral effects of mercury are consistent with studies on the absorption and retention of mercury in humans, indicating that excretion and elimination of absorbed and stored mercury is a very slow process (Stein *et al.*, 1974; Gerstner and Huff, 1977; Enwonwu, 1987; Bernard and Purdue, 1984). It is reported to be chemically persistent in biological systems, especially the central and peripheral nervous systems (Enwonwu, 1987). This had been attributed to the "trapping" effect of mercury in the brain by the blood–brain barrier after being transformed in the tissues to the divalent ionic form (Enwonwu, 1987; Cherian *et al.*, 1978; Stein *et al.*, 1974).

Our results indicate that neurobehavioral performance is worse for workers exposed to a single solvent in comparison with those exposed to mixed solvents. The increase in Z score per mg/m^3 toluene was 0.0025 for workers exposed to toluene as compared to 0.0014 for workers exposed to mixed solvents. This observation was different from that of Iregren (1982), who suggested that the risk for adverse effects on behavioral performance are greater in workers exposed to solvent mixture than in workers exposed to single solvent. Further, the observed adverse effects in workers exposed to mixed solvent were related to the exposure duration, whereas, it was only related to exposure intensity in workers exposed to toluene.

A possible explanation for these observed differences could be the different patterns of exposure. In the present study, the exposure patterns were 8 AM–5 PM, Monday to Friday, for the workers exposed to toluene and to 10 hr a day, Monday to Saturday, in rotating shift for workers exposed to mixed solvent. For the toluene-exposed workers, there was more time for recovery from the adverse effects between workdays and during weekends. However, the toluene-exposed workers had worked for a longer period (5.7 years vs 2.3 years for mixed solvent workers) so the residual adverse effects in these workers might have reached their respective dynamic equilibrium levels according to their daily exposure intensity, giving higher adverse effects that were independent of the length of exposure. In the case of the workers exposed to mixed solvents, dynamic equilibrium might not have been reached as the exposure period was shorter. Shift work in the mixed solvent-exposed workers may also affect the time needed for reaching the dynamic equilibrium levels. Furthermore, the adverse effects from some of the solvent components may be less reversible as compared to those of toluene. Hence, the adverse effects for the workers exposed to mixed solvents were related to the length of exposure.

CONCLUSIONS

Adverse neurobehavioral performance was detected in workers exposed to

toluene alone, solvent mixture, or metallic mercury vapor. The adverse effects appear cumulative for workers exposed to solvent mixture or mercury; for workers exposed to toluene alone, the adverse effects were related to exposure intensity. The types of chemical species and patterns of exposure appear to influence whether the adverse effects will be cumulative.

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Neuropsychological Assessment of Organic Solvent Effects in South Africa: Test Selection, Adaptation, Scoring, and Validation Issues[1]

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The neurobehavioral effects of organic solvent exposure were assessed in 68 South African workers in a paint manufacturing plant in which the maximum current combined solvent level in workplace air was 0.72 of the Threshold Limit Value. A 17-test battery was assembled from the WHO-NCTB, NES 2, and the University of South Africa Neuropsychological Assessment Procedure. Extensive modifications were made to some tests in the battery, using a guided learning rationale derived from the cross-cultural literature. In light of test correlations with demographic variables (age, education, and alcohol consumption), the construct and likely predictive validity of the tests in the battery are reviewed, and promising procedures identified. Finally, it is noted that in developing countries, the results of neuropsychological tests that have been well standardized in the West may be misleading unless the underlying validity issues that arise when a test developed in one culture is applied to another have been addressed.

INTRODUCTION

The utility of neuropsychological assessment in the early detection of central nervous system (CNS) pathology caused by neurotoxins is widely accepted in the developed countries. Such assessment is increasingly used in developing countries with non-Western and culturally different populations, for example in China (He *et al.*, 1990; Liang *et al.*, 1990), Chile (Espinosa *et al.*, 1990), Mexico (Ramirez *et al.*, 1990), and India (Saxena, 1990). However, unless validity issues arising from cross-cultural variation are meticulously addressed, the results of standard neuropsychological tests, whether paper-and-pencil or computer administered, may be nondiagnostic or misleading. This arises for two reasons.

In the first place, performance differences may reflect cultural differences rather than differing levels of ability. Accordingly, no cross-national or intercultural comparisons of individual scores or group means can be made until it has been demonstrated not only that the populations are equivalent with regard to age, language, and education, but also that there is cultural equivalence. Such equivalence may be absent not only in developing countries that have "imported" Western tests, but even when comparing ostensibly similar populations in the developed world. Thus, Cassitto *et al.* (1990) note that an analysis of the differences in mean test score performances between five European countries "indicates a lack of similarities between groups" (p. 210), arising in part from cultural

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differences. These cultural factors are important also within the developed countries, in which the absolute performance levels of migrants from other societies and culturally different enclaves within the dominant society (e.g., Valciukas *et al.*, 1986) may raise equivalency problems.

Second, when cultures differ, the truism that "the test as given is not the test as received" becomes operative, and no assumptions of construct validity can be made for even well-standardized Western tests in developing countries or other "exotic" settings. Test instructions acquire unintended meanings, and performance levels may be affected by unexpected variables, so that a neuropsychological test performance gradient, even within a group that is culturally and educationally homogenous, may not reflect CNS integrity.

Cross-cultural psychologists have for years wrestled with these issues of comparability and validity in the assessment of intellectual function in non-Western settings (Dasen, 1984; Deregowski, 1983; Gilbert, 1989; Reuning, 1988). The underlying issue for cognitive psychology (and thus for neuropsychology) is whether human thought proceeds in universal patterns (Gellatly, 1989; Gellner, 1981; Wertsch, 1985) or whether these patterns are fundamentally modified by the social and material conditions of different cultures. Luria's neuropsychological work began by addressing these questions (Luria, 1965, 1971). Taking full account of these and related issues will require developing country neuropsychologists to pay meticulous attention to the profound problems of test validation, rather than making easy assumptions about cognitive universals.

In South Africa, most of the industrial labor force is functionally illiterate and has had no prior experience with computer displays. Though great caution must be used before attributing cognitive difference to one group of people in comparison with another, there is a large body of evidence (reviewed in Berry, 1984) that in sub-Saharan Africa, quickness of thought and execution are less valued than in Western society. Folk understandings of the nature of intelligent behaviour in Zimbabwe and Uganda emphasise caution, prudence, slowness and carefulness rather than the quickness prized in the west and literally rewarded by "bonus points" in neuropsychometric tests. Sternberg (1984) argues that "fast is not smart, although the assumption that it is underlies the overwhelming majority of tests used in identification of the gifted" (p. 282). Thus Verster (1983) showed that black male South Africans traded off speed for greater accuracy to a larger extent than white groups. However, as years of formal education increase, so does the value placed on quickness (Kendall *et al.*, 1988). The current state of psychological research in Africa (reviewed in Irvine and Berry, 1988) indicates that the mental constructs underlying speeded test performances in African SS may be different to those in Western SS.

The objective of the present study was to select a relatively brief battery of neuropsychological questionnaire and test materials that on rational and empirical grounds seemed likely to be sensitive to neuropsychological dysfunction in an illiterate or semi-literate population of Black South African workers.

METHOD

Access was secured to two large South African paint manufacturing plants, one in Johannesburg in which 88 workers were assessed, on which this paper is based, and one in Durban (302 assessments); a second paper (Nell, Myers, Colvin, and Rees, unpublished) reviews and compares results from each factory separately.

The combined total solvent exposure was below the Threshold Limit Value (TLV), reaching a maximum of 0.72 of TLV in workplace air. The substances and their levels are specified in Colvin, Myers, Nell, Rees, and Cronje (1991).

The study uses a cross-sectional design, with a single culturally homogenous group in which the degree of CNS insult is expected to vary from none through to moderate, allowing determination of the response of neuropsychological measures to this gradient of CNS compromise.

SUBJECTS

All subjects were African males with at least 5 years continuous service. Of the 88 who met this criterion, 1 died prior to neuropsychological testing, 5 were excluded because of a history of epilepsy or psychiatric disorder, and a further 15 were excluded because of previous head injuries, even if they reported unconsciousness had been for as little as 5 min. After exclusions, a net total of 67 workers was available for analysis.

The mean *age* of this group was 45.7 years (SD 9.8); 11 were aged 55 or more, and 10 were 34 or less. With regard to *education,* the group mean for the last year of schooling successfully completed was 6.0 (SD 3.1), which is 1 year before the end of South African primary school education. Thirty-two subjects had not progressed beyond primary school, and of these, 7 had no schooling at all; only 2 had graduated from high school. Since most of these workers would have completed primary school some 3 decades earlier, many formal education skills acquired during these early years of schooling would have decayed.

Exposed workers (n = 43) were those with an average lifetime exposure in excess of 0.3 times the TLV; *unexposed* workers (n = 25), with exposure below this value, had in effect nil or very low exposure (Colvin *et al.,* 1991).

MATERIALS

Test selection rationale was derived from three complementary criteria, namely, that the test procedures be sensitive to neurotoxic effects, be appropriate for a non-Western and little-educated subject population, and have international comparability.

Two sources are well known: The World Health Organization's Neurobehavioral Core Test Battery (WHO-NCTB; 1986) and the computer-administered Neurobehavioral Evaluation System (NES 2; Letz and Baker, 1986, 1988).

Three tests were drawn from the University of South Africa's Neuropsychological Assessment Procedure (UNAP; Nell, 1990), comprising 41 procedures in 11 functional domains. Since the first version of this procedure was published (Nell, 1985), norms have been accumulated for black South African adults on selected procedures, and their utility demonstrated for neuropsychodiagnosis after diffuse high-velocity brain injury (Brown *et al.,* 1991a; Jansen, 1988; Moselenyane, 1990), in detecting the effects of surgical and vascular insult (Makunga, 1988), in the detection of manganism (Brown *et al.,* 1991b), and in the early detection of AIDS dementia (Brown, 1991).

WHO-NCTB. All seven core procedures in this battery were used, substituting the NES 2 measure of Simple Reaction Time.

NES 2. The use of computerized neuropsychological assessment has been extensively examined (Division 40, 1981; Law *et al.,* 1990). The issues in developing

countries are more complex in that non-Western populations, especially their older cohorts, have had little experience with computer screen displays and none in the use of keyboards or joysticks. Use of the NES 2 has to date been restricted to the United States, Western Europe, and Japan (Letz, 1990); to our knowledge, this is its first use with an African population.

On a priori grounds, those NES 2 procedures requiring reading, writing, or typing skills were eliminated (Symbol Digit Substitution, Visual Digit Span, Serial Digit Learning, Associate Learning, Associate Recall, Vocabulary, Horizontal Addition, and Grammatical Reasoning).

Using five black laborers comparable in age and education to the factory workers in the target group, informal *pilot testing* was carried out on the remaining NES 2 tests. Finger Tapping and Simple Reaction Time were readily understood and executed by the subjects. Once the changes described below had been made to the tests of Continuous Performance, Pattern Completion, Pattern Recognition, and Switching Attention, the pilot study subjects were able to perform at an acceptable level.

Hand–Eye Coordination was eliminated because the pilot subjects, unfamiliar with the video game arcades now freely accessible in South African cities and popular with their own children, were flustered by this seemingly simple joystick manipulation task and turned in performances that resulted in automatic test termination as a result of too many errors.

UNAP. Three tests from this battery that had demonstrated their utility in previous validation studies were adopted (Four Words, Paragraph Memory, and Geometric Design Reproduction).

The tests included in the final battery are set out by functional domain and source in Table 1. Note that three of these are paper-and-pencil tests; however, they require drawing rather than writing skills.

TRANSLATION AND TESTER TRAINING

Factory personnel records indicated that if test materials were available in two African languages, Zulu and South Sotho, all subjects could be tested in their language of choice. Back-translated texts were produced and bound as test manuals.

Two graduate psychologists and an occupational medicine technician were trained to adopt a warm and facilitative approach during the guided learning phase of the practice trials (see below), but to avoid cuing subjects during testing proper.

PROCEDURE

Two subjects were tested each workday morning and one in the afternoon. Test sessions took between 60 and 90 min per subject, including administration of the four questionnaires. These were a Health Questionnaire (Appendix B of NES 2) which included a set of questions on head injury, a Pretest Questionnaire (Appendix A of NES 2) which established whether the present state of the testee would adversely influence test performance by inquiring about sleep and substance consumption in the previous 24 hr, a Work History Questionnaire (loosely based on Annexe 2 of the WHO-NCTB), and a Subjective Symptoms Questionnaire (Annexe 3 of the WHO-NCTB).

The questionnaires were administered first, followed by the paper-and-pencil

TABLE 1
TEST BATTERY BY FUNCTIONAL DOMAIN

Domain and test	Source battery
Motor Speed	
Simple Reaction Time	NES 2
Finger Tapping Speed	NES 2
Dexterity	
Santa Ana Peg Board	WHO-NCTB
Pursuit Aiming Test	WHO-NCTB
Clerical Speed	
Digit Symbol Substitution	WHO-NCTB
Attention	
Digit Span	WHO-NCTB
Vigilance	
Continuous Performance Test	NES 2
Visuospatial Function	
Benton Visual Retention Test (Recognition Form)	WHO-NCTB
Pattern Comparison	NES 2
Geometric Design Reproduction	UNAP
Verbal Memory	
Four Word Test	UNAP
Paragraph Memory	UNAP
Visual Memory	
Pattern Memory	NES 2
Geometric Design Recall	UNAP
Cognitive Flexibility	
Switching Attention	NES 2
Mood	
Profile of Mood States	WHO-NCTB

tests, and finally the computer-based procedures. Test sequence within each group was in general from easier to more difficult items.

Substantial modifications were made to the standard instructions of several procedures in the battery. These changes and the rationale on which they are based are described in the Appendix.

UNAP Tests

Since these are unique, administration and scoring are briefly described; full instructions appear in the manual (Nell, 1990).

Four Word Test

The examiner asks the subject to repeat in sequence four words (house, cloud, tree, flying), repeating these until criterion is reached up to a maximum of six trials. Delayed trials are given at 5 min, 10 min, and at the end of the session.

Scoring. Only Trial 1 and the three delayed trials are scored for a maximum *recall* score of 16 and a *sequence* score that gives 1 point for each word that precedes those later in the sequence for a maximum of 24.

Paragraph Memory

The examiner reads the subject a 22-item story about a train derailment, loosely based on the shipwreck item from the Logical Memory Subtest of the Wechsler

Memory Scale. A delayed recall is requested at 30 min. Credit is given for each correctly reproduced idea.

Geometric Design Reproduction

This test comprises the five shapes illustrated in Fig. 1. The subject is instructed to copy these designs, making them "about the same size and the same shape," but without lifting the pencil from the paper. A delayed recall is requested at 30 min.

Scoring. A score of 5 is given for accurate shape and size, decreasing to 1 for severely distorted shapes. If the instruction not to lift the pencil is followed, a score of 4 is given, down to 1 if the person ignores the instruction or the examiner's request to return to the lift point.

RESULTS

Mean scores (and standard deviations) of the 68 subjects on the test battery are set out in Table 2 together with the scores of a group of Dutch subjects of comparable age (Cassitto *et al.*, 1990). Before considering correlations between the independent and dependent variables, it is first necessary to consider the intercorrelations of the independent variables (Table 3). There is a striking negative correlation between age and education; this large education-by-generation interaction indicates that the older subjects, in keeping with their generation's opportunities and value system, had less formal education than the younger subjects.

As would be expected in the light of the formulae by which cumulative exposure and average lifetime exposure were computed (Colvin *et al.*, 1991), age was significantly correlated ($r = 0.35$) with the former, and rather less correlated ($r = 0.25$) with the latter.

Alcohol consumption as measured by the number of drinks the subject reports he usually has at a sitting is independent of the other variables, with no correlation exceeding 0.1.

Table 4 sets out the correlation coefficients between each of the independent variables—age, education, alcohol use, average lifetime exposure (Colvin *et al.*, 1991), and reported subjective symptoms—and each of the neuropsychological

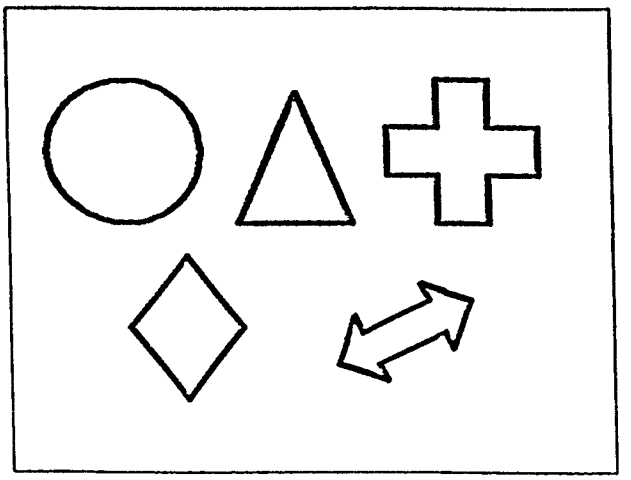

FIG. 1. Geometric Design Reproduction Test (Nell, 1990).

TABLE 2
NEUROPSYCHOLOGICAL TEST SCORE MEANS OF SOUTH AFRICAN PAINT FACTORY WORKERS AND DUTCH WORKERS (CASSITTO *ET AL.*, 1990)

	This study	Netherlands
N	54	41
Age	45.7 (9.8)	36–45
Years education	6.0 (3.1)	—
Digits Forward	5.2 (1.0)	6.4 (1.8)
Digits Backward	3.3 (1.0)	6.1 (1.7)
Four Word		
Recall	13.8 (2.3)	
Paragraph Memory		
Immediate	9.7 (3.0)	
Delayed	8.6 (3.4)	
Digit Symbol	22.1 (10.1)	53.8 (10.9)
Simple Reaction Time	286.3 (56.2)	241.6 (30.1)
Benton Visual Retention Test	7.0 (1.6)	8.6 (1.1)
Pursuit Aiming		
Correct	81.6 (18.3)	149.5 (33.8)
Attempted	163.4 (19.2)	
Santa Ana		
Dominant hand	34.4 (3.7)	43.8 (4.9)
Nondominant hand	30.4 (3.4)	42.7 (4.6)

test scores. Noting that the direction of scoring is reversed for the latency measures, the signs are all positive for education, but negative for age, number of drinks at a sitting, and average lifetime exposure. The neuropsychological measures included in the test battery are thus shown to have substantial construct validity in the target population—as Letz (1990, p. 195) has argued for age-related declines in NES scores.

Education

As predicted by the cross-cultural literature (Irvine and Berry, 1988, p. 29;

TABLE 3
INTERCORRELATIONS (SPEARMAN *r*) OF THE DEMOGRAPHIC VARIABLES

	Age	Highest class passed	Number drinks at a sitting	Average lifetime exposure	Cumulative exposure
Age	1.00	−0.35**	0.10	0.25*	0.35**
Highest class passed		1.00	−0.18	−0.29**	−0.32**
Number of drinks at a sitting			1.00	0.09	0.08
Average lifetime exposure				1.00	0.92***
Cumulative exposure					1.00

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

TABLE 4
CORRELATIONS (SPEARMAN *r*) BETWEEN DEMOGRAPHIC VARIABLES AND NEUROPSYCHOLOGICAL TEST SCORES (DECIMALS OMITTED)

	Age	Highest class passed	Number drinks at a sitting	Average lifetime exposure	Total score on subjective symptoms
WHO-NCTB					
Digit Span					
Forward score		38**			
Backward score		52***			
Santa Ana					
Dominant hand					
Trial 1	−30*	39**	−29*		
Trial 2		37**			
Nondominant hand					
Trial 1					
Trial 2		30*	−28*		
Digit Symbol					
Substitution	−54***	69***			
Benton Visual					
Recognition		42***			
Fleischman					
Pursuit Aiming					
Trial 1 correct	−47***	34**			
Trial 2 correct	−52***	32**			
Trial 1 total attempts			−27*		
Trial 2 total attempts		27**	−29*		29*
NES 2					
Tapping					
Simple Reaction Time	−37**		−24*	28*	−41**
Continuous Performance					
Pattern Recognition					
Number correct	−27*	33**			
Latency					
Pattern Memory					
Number correct					
Switching Attention					
Side	−27*			39**	−30*
Direction					
Switching side					
Switching direction					
UNAP					
Four Word Recall					
Sequence					
Total					
Paragraph Memory					
Immediate					31*
Delayed					
Geometric Design					
Reproduction					
Copy					
Circle		30*	−40**		
Triangle	−34**	51***	−32**		29*
Greek cross	−48***	36**			30*
Diamond	−27*	44***			

TABLE 4—*Continued*

	Age	Highest class passed	Number drinks at a sitting	Average lifetime exposure	Total score on subjective symptoms
Double arrow	−31*	52***			
Recall					
Circle					
Triangle					
Greek cross		42***			
Diamond					
Double arrow					

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Kendall *et al.*, 1988, p. 310), the single most important predictor of test performance in this group is years of formal education.

Age

Age also contributes substantially to the matrix. However, the large negative correlation between age and latency on the reaction time task is counterintuitive, especially in view of the positive relations between reaction time, alcohol consumption, and exposure; this result requires that the other age effects reported in the table, as well as correlations between reaction time and other independent variables, must be treated with caution until replications with other South African worker groups have cast further light on this finding.

Alcohol Consumption

The number of drinks typically consumed at a sitting generated a number of significant correlation coefficients with the neuropsychological variables. However, it is surprising that alcohol consumption did not covary with age, education, or exposure.

Average Lifetime Exposure

Only two relatively weak associations of exposure with the neuropsychological variables emerged, one with Simple Reaction Time, and the other with the NES 2 Switching Attention Test, but only for latency on the "Side" condition, the simplest of the four tasks in this test, and conceptually an analogue of the Simple Reaction Time task. The more complex "Switching" condition, which does not appear to have been well understood by these subjects, generated no significant correlations with any other demographic or symptom variable.

A series of *t* tests between the scores on the neuropsychological variables for the 19 least-exposed and 21 most-exposed workers (Colvin *et al.*, 1991) was noncontributory. It is once again of interest that the only significant difference ($P < 0.05$) between the groups was on the NES 2 Switching Attention task.

Subjective Symptoms

Cumulative scores on the Subjective Symptoms Questionnaire generated six relatively low coefficients with the neuropsychological tests, of which five are in the wrong direction.

Profile of Mood States (POMS)

Two difficulties arose. In the first place, subjects interpreted the adjectives on this scale in a literal and concrete way, responding to a question such as, "Are you feeling tired today?" (itself an elaborated translation of the POMS item "bushed"), with questions such as "Do you mean when I got up? I was feeling very tired. But on the way to work I felt better. Now I am tired again," obviously a reference to the fact that it was then 3 PM. "So what do you mean when you ask if I am tired?"

Second, the test administrators complained that despite the best efforts of the translators there were an inadequate number of equivalents in the African languages for the many synonyms used in the POMS on a particular dimension. For example, fatigue is described by the terms worn out, listless, sluggish, weary, bushed; anger is described by the terms peeved, grouchy, annoyed, ready to fight, and bad-tempered. Subjects therefore responded with irritation to some of these questions with the retort, "I have already given you the answer. Why are you asking me again?"

Such difficulties were the rule rather than the exception, leading to abandonment of this measure after the first 10 subjects.

DISCUSSION

Studies with black South African subjects that give available *normative* and *validation* data for the tests used in the present battery are reviewed below.

Normative Data

Two small normative studies (Moselenyane, 1990; Sesel, 1990) and one using a larger norm group (Makunga, 1988) showed that Digit Span and Digit Symbol Substitution from the WHO-NCTB and the Four Word Test, Paragraph Memory, and Geometric Design Reproduction from UNAP were all readily understood by black subjects across a wide educational and age range and that the mean scores of these groups were comparable with Western norms, though always lower. These findings are set out in Table 5.

Very marked education effects are evident. Sesel's (1990) results show that the combined effects of low education and socioeconomic deprivation among the 15 black laborers are profound. Similarly, the Digit Symbol Substitution scores of the present sample in comparison with those of Avenant's (1988) two groups of prison warders, with their rather better education, show a marked slowing on this task that requires school-type writing and fine dexterity. These are also the skills drawn on by Pursuit Aiming, and when the scores of the present sample are contrasted with those of the Dutch subjects (Table 2), there are 48 and 58% discrepancies in favor of the latter on these two tests. These differences are only 16 and 22% on the purely psychomotor procedures, Simple Reaction Time and Santa Ana.

TABLE 5
SCORES FOR A VARIETY OF SUBJECT GROUPS ON SELECTED NEUROPSYCHOLOGICAL TESTS; MEANS (SD)

	Avenant (1988)	Sesel (1990)		Adonisi	Makunga (1988)	Brown *et al.* (1990)	Jansen (1989)
	Prison warders[a]	Laborers[a]	Scientists	(1988)[a]	students[a]	Laborers[a]	Mixed[b]
N	140	15	20	20	100	20	34
Age	24.8 (3.3)	(45)[c]	(35)[c]	25.3 (7.2)	23.7 (4.2)	40.5 (8.2)	29.6 (11.9)
Years education	9–12	6.1 (1.7)	17.6 (2.7)	10.4 (1.8)	±14	4.9 (8.2)	11.3 (3.6)
Digits Forward	7.4 (2.3)	4.3 (1.7)	7.4 (1.2)		6.2 (0.9)	4.5 (0.9)	Score 10.9 (2.1)[d]
Digits Backward	5.6 (2.2)	2.7 (1.1)	5.6 (1.2)		5.0 (0.9)	2.8 (1.4)	
Four Words Recall		15.0 (19.4)	32.3 (8.5)				
Paragraph Memory							
Immediate				13.4 (4.2)	12.1 (3.5)	8.1 (3.2)	13.3 (4.4)
Delayed				14.2 (4.9)	11.6 (3.8)	7.4 (3.2)	12.4 (4.9)
Digit Symbol	43.5 (10.0)			38.2 (4.9)	15.4 (9.2)		Score 11.9 (2.1)[e]

[a] Black South African subjects.
[b] White South Africans assessed 6 months after mild closed head injury (PTA Between 5 min and 6 hr).
[c] Estimated average age.
[d] Equivalent to raw scores of approximately 6 forward and 5 backward.
[e] Equivalent to a raw score of approximately 47.

Validation Studies

Moselenyane (1990) compared the scores of Adonisi's group (Table 2) with the scores of an older (37.5 years, SD 11.9) and less-educated (6.0 years, SD 3.9) group of 48 brain-damaged individuals. Although some differences in favor of Adonisi's healthy subjects would be anticipated on the grounds of age and education alone, she found that on the 22-item Paragraph Memory Test, the immediate free recall mean scores of the two groups were 13.4(SD 4.2) items as opposed to 6.3(3.4) items; after the 30 min delay, these scores were, respectively, 14.2(4.9) and 6.0(3.8) ($P < 0.001$ in both cases). On Geometric Design Reproduction, all designs except the Greek cross differentiated significantly between the groups; at 30 min delay, scores on the circle, diamond, and double arrow were significantly different.

Makunga (1988) compared the scores of 20 hospitalized controls with no neurological history or abnormality and a mean age of 25.6(SD 4.7) with 20 hospitalized subjects matched for age (25.3 (5.5)) and education with a history of neurological disease or substance abuse. On Paragraph Memory, the mean scores for immediate recall were 8.4 items (SD 4.2) and 4.8 (3.5) ($P < 0.01$) for controls and neurological subjects, respectively; after the 30 min delay, these scores were 7.5 (3.6) and 4.8 (3.5) ($P < 0.05$). For Digit Span, the difference between the groups (standard scores of 8.4 (1.5) and 6.7 (2.1)) was not significant.

Brown *et al.* (1991b) compared low- and high-exposure groups of manganese refinery workers (LE and HE, respectively). The 20 LE workers (Table 2) did not differ significantly from the 19 HE workers on age or education, which for these two groups were, respectively, 40.5 (SD 8.2) and 41.4 (10.3) years of age and, for education, 4.9 (8.2) and 4.2 (3.4) years. Neither Digit Symbol Substitution nor Digit Span (whether for digits forward, backward, or the standard score) differentiated between the LE and HE groups. However, the Four Word Test sequence and total scores, and the copy task on the double-headed arrow in the Geometric Design Reproduction Test, did significantly distinguish between LE and HE groups.

Mood

Moselenyane (1990) applied a back-translated North Sotho version of the POMS to three groups of persons who had suffered mild ($N = 29$), moderate (9), and severe (10) traumatic brain injury. The anger–hostility factor significantly differentiated between the three groups, but, counterintuitively, no significant differences were found for the other five factors.

Brown *et al.* (1991a) applied back-translated Zulu, Pedi, and Afrikaans versions of the POMS to the LE and HE groups: none of the factors differed significantly between groups.

Such equivocal results with the POMS are not unusual. Liang *et al.* (1990) report that in China "the POMS seemed to be too complicated. Some subjects might be puzzled by the equivocal adjectives used, particularly in the Chinese language version" (p. 231).

Validity Issues in the Present Battery

Subjective Symptoms Questionnaire. Assessment of subjective symptoms is a necessary aspect of a neurobehavioral evaluation, since impairments of neuro-

psychological function—unless these are subclinical—are accompanied by a subjective sense of unreliable memory, reduced concentration and motivation, and increased fatigue. In this moderately exposed group, it is to be anticipated that the cummulative Subjective Symptoms score would, if impairments were absent or subclinical, fail to correlate with the neuropsychological scores, or, if impairments were more marked, generate some negative correlation coefficients with these scores. In fact, the obtained correlations indicate that the *presence* of many symptoms leads to *better* neuropsychological test scores. The validity of this questionnaire for the target population is thus in question; future validation studies of subjective symptom expression and elicitation in black working populations in South Africa will have to address this problem.

Profile of Mood States. The difficulties that arose in the present study suggest that with ill-educated worker populations, unaccustomed to introspection and to drawing subtle distinctions between variants of a particular mood state, the POMS may not be an appropriate mood measure. Future validation studies should fundamentally reexamine this method of determining mood states in such populations and, if necessary, devise alternatives.

Switching Attention. Our experience places a question mark against the validity of such higher level procedures to assess "stimulus pull" in illiterate subjects for whom a written stimulus cannot be used.

CONCLUSIONS

There are no short cuts to neuropsychological assessment in developing countries. Easy assumptions of human intellectual universality that have guided too many of the attempts at neuropsychological assessment made thus far in South Africa and other developing countries obscure the cultural differences that modify test responses and test performance levels.

The weakness of the present study is that it set out to detect neurotoxic effects in a workplace without first undertaking a full-scale validation study that applied a wide range of neuropsychological measures of likely utility to comparable groups of impaired and unimpaired black workers. Nonetheless, despite its limitations, inspection of Table 4 suggests that a number of tests in the present battery are sensitive to neuropsychological dysfunction in this population, namely Pursuit Aiming, Geometric Design Reproduction, Santa Ana, Simple Reaction Time, Digit Span, Benton Visual Retention, and Pattern Recognition.

APPENDIX: THE RATIONALE FOR TEST ADAPTATION AND MODIFIED TEST INSTRUCTIONS

Rationale

Test performance as a learning curve. In Western populations, individuals are exposed from an early age to test-type materials, whether in the form of video games requiring hand–eye coordination or rapid problem solution, pattern reproduction and object assembly tasks of the jigsaw puzzle type, party and quiz games based on the rapid learning of new information, or repeated exposure in the classroom situation to individual and group tests of global intelligence or specific scholastic abilities. Children growing up in this environment become "test wise" adults, and although they will undoubtedly improve their scores with repeated exposure to a given test, such improvements tend to be strikingly small. Lezak

(1983) remarks that test–retest differences on standard measures of intelligence seldom amount to more than two or three scale points on global IQ scores.

Among non-Western subjects unfamiliar with intellectual tests the situation is markedly different. In 1976, Verster demonstrated that multiple test exposure to the Classification Test Battery (widely used in the South African mining industry in order to categorize black workers according to their level of "intellectual ability") produced a strong learning curve reaching asymptote at about the fourth retest; the effects of retesting were shown to make a substantially larger contribution to these increased scores than other factors, such as years of service in the technologically rich mine environment. Reanalyzing her unpublished data, Verster and Muller (1975) found that test procedure and environmental stimulation made minor contributions to this learning curve; on the other hand, exposure to the underlying mental operations to the test items, and to their format, made greater contributions. In addition, individuals with a very low or very high level of education made greater gains than those at an intermediate educational level. Reflecting on these findings, Kendall *et al.* (1988) ask a crucial question, namely, whether in any one test exposure, one is measuring at a point that reflects the subject's best performance, or rather "at a point on the acquisition curve for the ability being measured" (p. 308).

Guided learning. These findings have been embodied in UNAP and the present test battery by requiring the examiner to treat the instruction phase of the test procedure as a guided learning experience within what Vygotsky (1988) termed the "zone of proximal development." To facilitate this approach, which is fundamentally different to the cool and arm's-length examiner–testee relationship encouraged by psychometric test manuals, additional learning trials are provided in UNAP, and examiners are encouraged to elaborate on these until the subject has fully understood what is required and has been shown some problem solution strategies. Though this brief guided learning experience cannot be expected to compensate for educational deprivation in an overcrowded and ineffective school system, or for cultural differences, it will at least have the effect of measuring a performance point higher on the learning curve than would otherwise be the case.

In terms of these guidelines, the modifications described below were made to the Benton Visual Retention, Simple Reaction Time, Continuous Performance, Pattern Comparison, Pattern Memory, and Switching Attention Tests.

Modified Test Instructions

Benton Visual Retention Test (Recognition Form). The Operational Guide instructions were modified to incorporate an additional teaching loop: On the first three items, the subject's initial response is recorded for scoring purposes, but followed by an inquiry phase in which the examiner says, "That's right, but I want you to explain to me why you chose that one and not one of the others," or, if the response had been wrong, "No, that's not the right one, let's work out together why that was wrong and which one is right." Detailed guided learning then ensued to the point where the subject was able to verbalize the reasons for a correct choice by specifying the matching characteristics between the stimulus figure and the correct choice and how these did not match up for the remaining three alternatives. Examiners were encouraged to take as much time as needed on cards 1, 2, and 3 to ensure that subjects fully understood test demands; card 3 is a two-unit figure, introducing additional complexity into the teaching phase of the test.

Simple Reaction Time. Since this is the first of the highly speeded tests in the NES 2 series, the instructions were substantially modified and the test was presented as a Wild West situation. The examiner says, "That square on the screen is your enemy. It wants to get you. You have to kill it as soon as you see it. Here is the trigger [showing the blue button]. Keep your finger lightly touching the trigger of this gun so you don't have to waste time moving your finger through the air when you see the square. You shoot the square dead as soon as it comes up. You will see that as soon as you fire your shot, the square disappears." Examiners congratulated subjects enthusiastically every time a square was "killed" for the first six trials. Thereafter, in order to allow the vigilance variable adequate play, the examiner said, "I am not allowed to say any more now. You just remember to keep going and to shoot that square dead as soon as you see it until the end of the test."

Subjects responded well to these instructions.

Pattern Comparison. On the practice item that precedes the test proper, an inquiry procedure as on the Benton was followed. The subject was asked to pick the nonmatching pattern and then to verbalize the reason for this choice. The examiner reinforces correct responses by repeating the correct points of difference; with wrong responses, the examiner raises objections until the subject chooses the correct item and verbalizes the reasons. On the first two items of the test proper, the subject's choice is recorded for scoring purposes, but the same inquiry loop is instituted to provide a total of three guided learning experiences.

A further modification to the instructions stressed the need for considered thoughtfulness. When the subject pointed to the chosen pattern or called out its number, the examiner pressed the appropriate key and was then instructed to ask, without any particular emphasis, "Are you sure? If you want to change your mind, you can." Only when the subject had confirmed that this was indeed his choice, the number was entered by depressing the "+" key.

In this way, the test atmosphere on both Pattern Comparison and Pattern Memory was made more reflective and less rushed—a significant change from the great emphasis that had been laid on speeded responses for the previous test. However, this variation changes a fundamental characteristic of the NES 2, which Law *et al.* (1990) characterize as requiring a visual presentation and a manual response to all items. Whether substitution of a verbal for a manual response affects the psychometric properties of these two tests and their validity is a question only partly answered by the results of the present study. However, the effect of the modification is that because all responses were keyed in by the examiner, variation arising from subjects' differing levels of literacy was eliminated, with the better-educated subjects calling out the number of their choice, while others pointed to the chosen array.

Pattern Memory Test. In light of pilot test experience, an additional guided learning aid was introduced as illustrated in Fig. 2.

This illustration (a hard copy of the screen hand-shaded to simulate the visual presentation) is a composite of the stimulus figure for the first practice trial, together with the two distractors and the target array that then appear on the screen. The illustration is presented to the subject for about 5 sec with the bottom figures covered; the target stimulus is then covered and the subject asked to pick the matching array from the three at the foot of the page, after which, through dialogue, and with both target and distractor figures exposed, the reasons for this

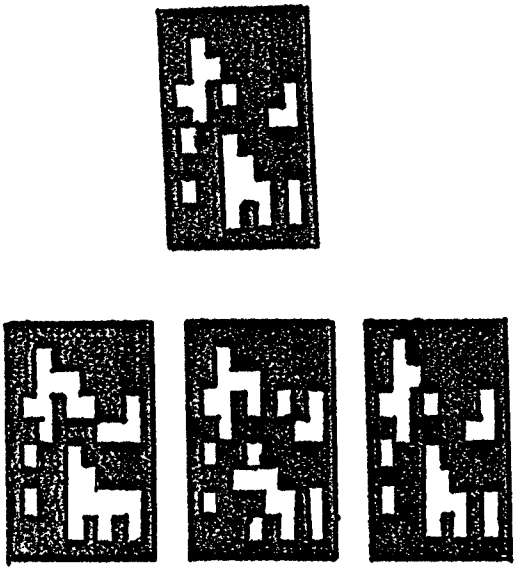

FIG. 2. Guided learning aid for the Pattern Memory Test.

choice and its appropriateness are discussed. The other two practice trials provided by the program were similarly prepared as illustrations of the screen material, and once the subject had worked his way through these, the practice trials on the screen were called up, again introducing the loop described for Pattern Comparison, asking "Are you sure?" before the examiner entered the chosen answer.

Continuous Performance Test. Subjects were reminded of the Wild West situation and urged to respond instantaneously. Despite the pilot test findings, unexpected difficulties arose during the factory administration since many subjects confused the target "S" with other letters of the alphabet, and as rapidity of response presentation increased in the later blocks of trials, errors multiplied. A hard copy print of a specimen letter S was made and kept at the keyboard as a memory aid, but confusion persisted.

Switching Attention Test. For the first two phases, "Side" and "Direction," standard procedure was followed. For the switching phase, the examiner would call out the Zulu or South Sotho word for "side" or "direction" as the English word appeared on the screen, in this way reducing literacy and reading speed differences between subjects.

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A Cross-Sectional Survey of Neurobehavioral Effects of Chronic Solvent Exposure on Workers in a Paint Manufacturing Plant[1]

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Neurobehavioral impairments in 84 workers with long-term exposure to organic solvents in a paint manufacturing plant were examined cross sectionally. The World Health Organization (WHO) Neurobehavioral Core Test Battery, the NES-2 computerized battery, and four additional South African tests were used. Exposure to solvents was determined by using company industrial hygiene data as well as from an industrial hygiene survey of current total solvent levels in air. Indexes for cumulative exposure and average lifetime intensity of exposure were calculated for groups of homogeneously exposed workers in each department. Exposure levels were below the ACGIH threshold limit values. Multiple linear regression revealed that education level, age, and alcohol consumption were strong predictors for several neurobehavioral test scores. After adjusting for potential confounding from this source, average lifetime intensity of solvent exposure was the most significant predictor of the NES-2 Continuous performance test (measuring sustained visual attention) score of the WHO Digit span backward test score (measuring attention span and double tracking). Pursuit aiming (measuring fine visuomotor tracking speed) was significantly associated with the cumulative exposure index, possibly indicating early neurotoxic effects.

INTRODUCTION

The chronic effects on the nervous system of long-term exposure to solvents are not clearly established and are still the subject of considerable debate. Whereas one author claims that "excessive exposure to organic solvents and subsequent development of chronic encephalopathy has been recognized for nearly 100 years" (Linz *et al.*, 1986) another author states categorically that solvent-induced encephalopathy does not exist (O'Flynn 1988).

The main reason for this lack of agreement among researchers is the inconsistency of published research findings. The literature on the effects of heavy metals such as mercury and lead or of particular solvents such as carbon disulfide is much less equivocal than studies on the effects of mixtures of solvents (Anger, 1990). This is partly due to the fact that the effects of solvents or solvent mixtures are subtle and hence need well-designed studies with adequate sample sizes in order to demonstrate such effects.

This is made clear by the World Health Organization (WHO) (1986), "Previous studies on the possible relation between occupational solvent exposure and chronic neurobehavioral impairment show quite different results. This is most

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likely due to differences in design, material and the methods used to demonstrate any adverse effects."

However, as Gamberale (1985) points out, the fact that in every study at least some aspect of psychological functioning is affected means this is unlikely to be due to chance alone. Neither is it probable that there would have been some systematic error in the choice of reference groups in each of these independent studies.

But if severe and irreversible effects of solvents on the central nervous system are disputed, the occurrence of subclinical neurobehavioral effects is more widely accepted, at least on populations in the industralized countries. The bulk of research on this topic has been done in Scandinavia and, to a lesser degree, in other developed nations such as the United States and European countries. In the developing countries little research on this topic has been done and none, to our knowledge, has been undertaken on the African continent.

Being situated in South Africa the authors were therefore faced with two issues. First, the neurobehavioural test batteries that have been used in published studies have been largely untried in African working populations. This meant that it first had to be shown that the tests that were to be used were appropriate for use by South African workers. This issue is addressed elsewhere in a related study by Nell *et al.* (1993). The second aim was to determine whether there was a relationship between exposure to solvents and neuropsychological effects. The focus of this study is on the latter issue.

It is important to note that worker populations in South Africa and other developing countries may differ from their counterparts in the industrialized nations in their performance levels on neuropsychological test batteries. Issues such as level of education, cultural variations, socioeconomic differences, and endemic parasitic infestations all may potentially give rise to significant differences between the two populations and this discussion is covered elsewhere (Nell *et al.*, 1991).

METHODS

This study was a cross-sectional investigation of neuropsychological test responses and subjective symptoms in groups with different exposures to organic solvents. Exposures were assessed from current and historical industrial hygiene records and from detailed work histories from each subject.

The population comprised black monthly paid workers from a paint manufacturing plant. The most important reason for this choice was that these subjects were the most easily accessible solvent-exposed workers with exposure history data. In addition the population was homogeneous in terms of race and socioeconomic background. This homogeneity also cut across exposure categories as the skill or intelligence requirements for employees did not differ between exposed jobs and unexposed ones. This was a great advantage as there were no preexposure neuropsychological data that could be used to rule out selection bias.

A major weakness in many previously published studies has been that the diversity of methods used has made comparability between studies difficult. To overcome this difficulty, the authors adopted the World Health Organization Neurobehavioral Core Test Battery (WHO, 1988), supplementing this procedure, as the operational manual for the battery suggests, with a range of additional items. The selection rationale is fully detailed in the article by Nell *et al.* (1993).

Subjects

The study population comprised all waged, black males who had been employed at the paint factory for at least 5 years. Of the work force of 213, 81 had more than 5 years service.

The following criteria were used to exclude workers from the study: (1) Clinical evidence of encephalopathy of known origin, (2) open or closed head injury resulting in 24 hr or more of unconsciousness, (3) current or previous long-term administration of psychotropic medication, (4) history of inpatient treatment for alcohol/drug dependence, (5) history of proven epilepsy, or (6) previously diagnosed and treated mental illness.

A number of workers in the factory who met the inclusion criteria were not exposed, or only slightly exposed, to solvents. This is because they worked in areas such as the water-based paint section, dry stores, labeling, or elsewhere where exposure is nonexistent or minimal. For the purposes of this study these workers were considered unexposed.

There were 7 nonresponders from the 83 potential subjects, a response rate of 91%. One worker died during the survey, 2 were on prolonged sick leave, and 4 refused to participate for unknown reasons. The exposures of these workers are unknown. Of the remaining 74 workers 4 were excluded because of unconsciousness of longer than 24 hr, another 2 because of a history of epilepsy, and 3 because of a history of mental illness. This left a final sample of 67.

Exposure

Assessment of exposure in the paint manufacturing industry is complicated by a number of factors. The process is essentially a batch operation and because such a variety of formulas are used for different paints the constituents of these products, particularly the solvents, differ considerably in both type and relative concentration. This means that workers are exposed to a variety of solvents at different relative exposure levels.

Another implication of the batch operation is that often one worker will do a number of different tasks throughout the day. For example a machine operator may go and fetch the solvents in his container, mix them with his particular machine, and then clean the equipment himself afterward.

Yet another factor which causes substantial fluctuations of solvent vapor levels is the weather. Most of the workplaces rely on natural ventilation from open windows and the extent of this ventilation obviously depends on wind speed and direction.

The result of this varied exposure is that it becomes difficult to quantify what a worker's past cumulative exposure has been. Even personal monitoring of solvent vapor usually only gives what the levels are on that particular day.

Despite these limitations it is still possible to calculate exposure indexes (EI) for the workers and, although there will be a degree of error in the absolute figures, we believed it to be independent of outcome and therefore a nondifferential exposure misclassification.

The EI was calculated by taking the following considerations into account.

(a) A walk-through inspection was done in order to establish "homogeneous exposure zones." In such a zone all workers would have a similar exposure to solvents. This homogeneous zone might be an entire department or only a

particular operation such as a tin filling point. Measurements were made of solvent levels on a sample of workers from each zone and the measurements were then averaged for that area.

(b) A detailed work history was taken from each worker to obtain information on what jobs they had and in which departments since being employed in the factory. In addition information was asked on exposure to neurotoxins from jobs they may have had before being employed at the paint factory. From this data length of service in exposed and unexposed areas could be obtained.

(c) Industrial hygiene measurements of solvent in air levels that had been done in the past as well as measurements done for the purposes of this study were both taken into account in trying to assess exposure for each of the homogeneous zones. Ten potential solvents were tested for in the current study, namely, MEK, benzene, trichloroethylene, MIBK, toluene, butyl acetate, xylene, cellosolve acetate, isophorone, and white spirits. Mean concentration for each of the solvents was assessed for each of 15 different exposure zones. A measure called the "hygienic effect" was calculated and is defined as the ratio between the actual amount of the compound (C) and its threshold limit value (TLV) (ACGIH). A "total hygienic effect" figure for exposure to all the solvents was calculated in the following way:

$$\sum_{i=1}^{n} \frac{C_i}{\mathrm{TLV}_i}.$$

Therefore, in order for the cumulative time-weighted average (TWA) not to exceed the total hygienic threshold limit value, the value of the above equation must not exceed 1. In this study the total hygienic effect ranged from 0 to 0.72.

Two ways of measuring chronic exposure were used. One was the "total cumulative exposure" (CE) to solvents which occurred over the years that the worker spent at the factory. This was assessed by multiplying the number of years spent in a particular job (t) by the TWA level of mixed solvent exposure for that job (the total hygienic effect figure) and then adding the products of each job that the worker had in the factory.

$$\mathrm{CE} = \sum_{i=1}^{n} (t_i \times \mathrm{TWA}_i).$$

The other measure of chronic exposure was assessed by taking the result of the cumulative exposure and dividing it by the number of years worked in the factory. This gave a result which we called the "average lifetime exposure" (ALEXP). In this study two exposure groups were constructed. One group (N = 43) had an average lifetime exposure (ALEXP) of greater than 0.30 which meant that they had been exposed to an average of at least 0.3 times the hygienic effect level for mixed solvents during their working lifetime at the plant. The other group (N = 24) had an average lifetime exposure of less than 0.3 times the hygienic effect, i.e., nil or very low exposures.

Neuropsychological Test Battery

All procedures in the WHO Neurobehavioral Core Test Battery were applied

with the exception of the Profile of Mood States. This battery was supplemented by six computer-administered procedures drawn from the Neurobehavioral Evaluation System (Letz and Baker, 1988) and three tests from the UNISA Neuropsychological Assessment Procedure (Nell, 1985). The complete battery by functional domain and battery of origin was thus as follows:

WHO tests.
The Santa Ana pegboard
Pursuit aiming
Benton visual retention test
Digit span forward and backward
Digit symbol substitution.
NES 2 computer-administered tests.
Reaction time
Finger tapping
Continuous performance test
Switching attention
Pattern recognition test
Pattern memory.
UNISA tests.
Four word memory test
Paragraph memory—immediate and delayed
Geometric shape drawing.

Test selection, appropriateness testing along with full descriptions of the test procedures are discussed in an accompanying study by Nell *et al.* (1993).

The Testing Procedure

Workers were seen individually during working hours. First, a work and personal history interview was done followed by a brief neurological examination by a doctor who was blind to the worker's exposure. The purpose of the neurological examination was solely to exclude persons who had clinical signs of neurological impairment due to a known cause and information from this examination was not used in the analysis.

Several days later the worker was recalled to the clinic to undergo the neuropsychological tests followed by the administration of a health questionnaire and another questionnaire on subjective symptoms. A quiet, isolated office was used for this part of the study which was done by two trained psychologists who were blind to the workers' exposure.

Because our subjects came from different ethnic groupings with different home languages the "Subjective Symptoms" and "Health" questionnaires were translated into two languages, namely, South Sotho and Zulu. In addition all the instructions that the examiner had to use were also translated into these two languages. To ensure that translations were accurate the technique of back-translation was used (Sundberg, 1981). As a final precaution to limit communication problems, the psychologists that administered the test batteries and questionnaires were fluent in English, South Sotho, and Zulu.

All manually administered tests were scored by one psychologist to ensure consistency and all tests were done at least 16 hr after the subject's last exposure to solvents to minimize acute effects (Hanninen, 1976).

Statistical Methods

All analysis was done using the SAS System (SAS, 1988) for personal computers. A simple division into exposed and unexposed categories was made in order to obtain an idea of the numerical difference in test scores in a simple bivariate analysis. An idea of the strength of the crude association between exposure and test scores was obtained by using Student's *t* test. Bonferroni's correction was not used (Poole, 1991). Multiple linear regression analysis was then performed on all test scores using a model which included solvent exposure, potential confounding, and effect modifying variables.

RESULTS

Table 1 shows that the unexposed group is comparable with the exposed group with respect to age, education level, and alcohol consumption although the exposed workers tended to be slightly older and had a higher alcohol consumption. None of these differences were statistically significant.

Performance on the neuropsychological tests and subjective symptom scores was poorer in the exposed grouping for 27 of 33 test results. However, these poorer results only reached significance for 2 tests: the latency times on 2 of the switching attention tests (Table 1).

There were no differences in reporting of subjective symptoms between the groups when questions were either analyzed separately or grouped into symptom categories.

A multiple linear regression model was used, forcing in the potential confounders age, schooling, and alcohol consumption, to examine the effects of cumulative exposure and average lifetime exposure to solvents on test scores. Average lifetime exposure to solvents was a significant predictor for four tests: Continuous performance latency time, Switching attention latency time, Mean reaction time, and Pattern memory (Table 2).

DISCUSSION

This study has shown that the test battery used was a sensitive indicator of neuropsychological impairment. This was demonstrated by the high correlation between age, alcohol consumption and schooling with poorer performance levels (Nell *et al.*, 1993). However, the effects of solvent exposure were relatively mild and were all subclinical (asymptomatic).

The measure of ALEXP was a better predictor of poor performance on the above four tests then the measure of CE. Another recent cross-sectional study of paint manufacturing workers (Bleecker *et al.*, 1991) also demonstrated a comparable finding with the strongest correlation occurring with "life-time weighted average" as opposed to cumulative exposure.

Our findings are similar to those of other studies on workers occupationally exposed to solvents in that differences are relatively mild and cover a variety of mental functions. A comparative analysis of cross-sectional studies done over the past 2 decades shows no consistent effect of chronic exposure on any particular aspect of mental functioning (Table 3). At the same time only a minority of these studies show no effects from long-term solvent exposure.

The exposed group did perform less well on 27 of 33 tests and this may be due to differences in exposure between the two groups. The reason that most tests did

TABLE 1
MEANS AND STANDARD DEVIATIONS FOR DEMOGRAPHIC VARIABLES AND NEUROBEHAVIORAL TEST RESULTS FOR THE TWO EXPOSURE GROUPS

	Unexposed (24)		Exposed (43)		Worse in exposed?
	Mean	SD	Mean	SD	
Age	43.52	10.04	48.00	9.26	
Alcohol	0.87	1.10	1.26	1.11	
Education	6.43	3.87	6.30	3.36	
Santa Ana 1	17.08	3.81	15.33	4.77	Yes
Santa Ana 2	19.38	5.52	17.95	4.84	Yes
Santa Ana 3	15.25	4.35	14.65	3.81	Yes
Santa Ana 4	16.79	7.41	16.30	3.91	Yes
Aim correct 1	41.21	19.89	38.51	17.04	Yes
Aim correct 2	42.25	19.23	39.26	17.89	Yes
Aim total 1	82.67	22.37	75.84	19.63	Yes
Aim total 2	88.63	18.36	81.16	19.27	Yes
Four word test					
Recall	13.96	2.64	14.02	1.99	No
Sequence	18.28	6.25	17.81	5.07	Yes
Total	32.08	9.03	31.12	7.58	Yes
Digit span forward	5.28	1.06	5.09	0.89	Yes
Digit span backward	3.32	1.07	3.32	0.97	=
Digit score forward	5.72	2.48	5.18	1.50	Yes
Digit score backward	4.04	1.65	4.02	1.81	Yes
Digit/symbol	23.79	13.11	21.51	9.40	Yes
Paragraph memory					
Immediate	9.24	3.15	10.28	2.75	No
Delay	8.24	3.21	9.26	3.32	No
Benton	7.13	1.71	6.95	1.41	Yes
Mean reaction time	272.2	68.08	292.7	49.0	Yes
Pattern recognition					
Number correct	19.28	1.06	19.02	1.20	Yes
Latency time	8.84	2.51	9.48	3.43	Yes
Continuous performance					
Latency time	394.4	41.8	415.8	47.8	Yes
Pattern memory					
Number correct	11.84	2.03	11.14	2.14	Yes
Latency time	7.57	1.66	7.35	2.31	No
Finger tapping	186.8	39.5	175.6	43.7	Yes
Switching direction					
"Side"	316.3	69.0	378.3	84.3	Yes
"Direction"	543.6	93.8	594.0	117.7	Yes
"Switching side"	885.6	148	996.6	167	Yes
"Switching direction"	973.5	149	1076.6	186	Yes
Subjective symptoms					
Night problems	1.00	1.00	1.42	1.07	Yes
Confusion	1.26	1.05	1.07	1.03	No
Activity	5.26	2.45	4.19	2.86	Yes

Note. Numbers in parentheses indicate number of subjects in group.

not attain statistical significance may be because of the small size of the sample and because the effects of solvents are typically slight. For this reason the WHO (1988) recommend a minimal sample size of at least 200 index and 200 reference subjects for a cross-sectional study.

TABLE 2
LINEAR REGRESSION ANALYSES OF OUTCOME VARIABLES THAT DEMONSTRATED A SIGNIFICANT DOSE–EFFECT CORRELATING WITH SOLVENT EXPOSURE

	Regression coefficient	SE	*P* value
Mean reaction time			
INTERCEP	320.769204	42.78997212	0.0001
AGE	−1.828490	0.76462923	0.0201
SCHOOL	2.913319	2.06593589	0.1638
ALCOHOL	15.105863	6.29949030	0.0197
ALEXP	94.355078	43.33918220	0.0336
Continuous performance test: Latency			
INTERCEP	409.989972	35.78065127	0.0001
AGE	−0.267624	0.63176745	0.6734
SCHOOL	−2.142464	1.73690826	0.2223
ALCOHOL	6.303250	5.05867747	0.2177
ALEXP	101.763492	36.43366636	0.0070
Pattern memory—Number correct			
INTERCEP	9.934849	1.68670719	0.0001
AGE	0.028957	0.02978165	0.3349
SCHOOL	0.127211	0.08187821	0.1256
ALCOHOL	−0.109644	0.23846709	0.6474
ALEXP	−3.445444	1.71749045	0.0494
Switching attention			
INTERCEP	451.485293	64.85943278	0.0001
AGE	−2.859228	1.14057302	0.0152
SCHOOL	−1.606736	3.04717857	0.6002
ALCOHOL	18.350236	9.16390737	0.0503
ALEXP	182.393157	64.90672351	0.0069
Pursuit aiming—Total attempted, trial 1			
INTERCEP	101.720910	14.62175809	0.0001
AGE	−0.114456	0.26171181	0.6635
SCHOOL	−0.580192	0.71948645	0.4233
ALCOHOL	−7.825454	2.02589718	0.0003
CE	−2.255045	0.80344727	0.0068

In addition, the exposure levels to solvents for almost all workers in this study was below the TLV-TWA. Previous studies have shown that the effects of solvents at these levels is usually weak.

This issue may be clarified when the results of an identical study to this one in another larger factory become available. In a study such as this there is the danger that the "healthy worker" effect may dilute the results of the study. This would occur if workers affected neurologically by solvents either left the factory or were transferred to low-exposure jobs within the workplace. However, the healthy worker effect probably had a minor impact on this study as there is no evidence that significant numbers of people left or changed their jobs for neurological or psychological reasons.

The calculated exposure indexes were fairly rough estimates of actual exposures. This may have given rise to nondifferential misclassification of exposure which again would have diluted the effects of solvent exposure.

The absence of a relationship existing between subjective symptoms and solvent exposure needs explanation. There is the possibility that the questionnaire on subjective symptoms was an inadequate measurement tool. Unfortunately this

TABLE 3
SUMMARY OF EPIDEMIOLOGICAL STUDIES ON EFFECT OF CHRONIC SOLVENT EXPOSURE (ORIGINAL TABLE IN GAMBERALE, 1985, WITH AUTHOR'S ADDITIONS)

Author	Exposure	Study type	Functions measured	
			Affected	Not affected
Hanninen *et al.*, 1976	Mixed solvents	Retrospective cohort, 100 cases, 101 controls	M, I, PM P	RT
Hane *et al.*, 1977	Mixed solvents	Cross-sectional, 52 cases, 52 controls	I, PM, M	RT
Knave *et al.*, 1978	Jet fuel	Cross-sectional, 30 cases, 30 controls	RT, P Neurasthenia Depression	M, PM
Elofsson *et al.*, 1980	Car and industrial spray painters	Cross-sectional, 80 cases, 80 controls	M, P, PM, RT	I
Gregersen *et al.*, 1984	Mixed solvents	Cross-sectional, 65 cases, 33 controls	I, S Sym Concentration	M, RT Retrospective
Lindstrom *et al.*, 1984	Mixed solvents	Case-referant, 374 matched pairs	Neuroses RR 5.5	Alcoholism, other neuro-psychological disorders
Mutti *et al.*, 1984	Styrene	Cross-sectional, 50 cases, 50 controls	M, PM, RT	Digit symbol
Maizlish *et al.*, 1985	Mixed solvents (isopropanol + hexane)	Cross-sectional, 240 subjects, printers and spray painters	Nil	PM, M
Orbaek *et al.*, 1985	Mixed solvents	Cross-sectional, 50 exposed workers, 50 controls	Sustained attention	PM, M, IQ, RT, Dexterity pns
Valciukas *et al.*, 1985	Mixed solvents	cross-sectional, 55 shipyard painters, 55 controls	Block design Embedded Figs	Digit symbol
Fidler *et al.*, 1987	Mixed solvents	Cross-sectional, 101 cases, 31 controls	S Sym	M, I, PM
Baker *et al.*, 1988	Painters	Cross-sectional, 254 cases	S Sym, PM	I
Triebig *et al.*, 1988	Mixed solvents	Cross-sectional, 105 house painters, 53 controls	Personal change in short-term memory	IQ, EEG, NCV, CAT

Note. RT, reaction time; P, perceptual functions; M, memory functions; PM, other psychomotor functions; IQ, intellectual functions; S Sym, subjective symptoms.

questionnaire was not subjected to a validation study and subjects may have interpreted the questions incorrectly.

Another possible explanation is that the mild neuropsychological impairments that were demonstrated in two tests in this study are the earliest signs of solvent-induced effects that occur before subjective symptoms become apparent. No subject was severely impaired and any impairment was very much subclinical.

This study does not answer the question of whether the poorer test performances are chronic, permanent effects or whether they are reversible subacute reactions. An important area for future research will be to determine the permanence of these effects and how they alter the integrity of the nervous system. The practical significance of the poorer performances by exposed workers is difficult to assess.

A further important aspect that needs investigation is the possible effect of these performance impairments on the quality of lives of those exposed and their families. Hanninen (1985) reports that her studies "suggest a decreased coping ability with extensive negative consequences in everyday life".

In conclusion, this study only demonstrated slight effects of solvents on the nervous systems of exposed paint manufacturing workers. The findings may be clarified when the results of an identical study ($N = 185$) done at another factory are available as there will then be a larger sample size.

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Neurobehavioral Effects of Chronic Occupational Exposure to Organic Solvents among Japanese Industrial Painters[1]

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To investigate the relationship between chronic exposure to organic solvents and changes in central nervous system function, industrial painters were compared with an age- and education-matched referent group of nonexposed workers. Eighty-one male painters completed a symptom questionnaire. Twenty painters underwent both questionnaire and neuropsychological examinations. From the results of pairwise comparisons of the symptoms, dry and scaly skin, being easily depressed without reason, coldness of hands and legs, being easily irritated without reason, loss of appetite, dizziness, and unsteadiness occurred statistically significantly more often among the exposed subjects than among the referents. Performances on the Digit symbol test and vocabulary test scores (synonyms) in exposed subjects were significantly lower than those of controls. In multiple regression models, controlling for age, education, and alcohol intake, a significant relation was found between the duration of the solvent exposure and poor performance in both the Block design and Digit span tests. The relation between toluene exposure and poor performance in both the Santa Ana coordination test and the Benton visual retension test was also significant. The results suggest that a symptom inquiry and some behavioral tests are helpful for detecting the possible effects of exposure to low levels of organic solvents. However, no consistent pattern was observed in regard to the effects of organic solvent exposure on neurobehavioral function, which is coincident with the type I toxic central nervous system disorder as classified by the World Health Organization.

INTRODUCTION

Neurotoxicity of organic solvents is one of the most important issues emerging in the field of occupational health. Psychological testing has proven useful not only in clinical diagnosis but also in experimental and epidemiological studies (WHO, 1985). Although various psychobehavioral performance test batteries have been applied in the study of neurotoxicity of organic solvents during these past 2 decades in European countries and the United States (Johnson, 1987), only a few such studies have been carried out in Japan (Kishi and Miyake, 1990).

Some previous studies have shown widespread neurobehavioral dysfunctions in workers exposed to solvent mixtures with toulene (Hanninen *et al.*, 1976; Hane *et al.*, 1977; Elofsson *et al.*, 1980; Linz *et al.*, 1986) or white spirits (Lindstrom and Wickstrom, 1983) as one of the main components, whereas other studies have reported more limited dysfunction (Maizlich, 1985; Cherry *et al.*, 1985; Fidler *et al.*, 1987) in workers exposed to mixtures of organic solvents.

The purpose of the present study was to determine whether an association could be demonstrated between chronic low-level exposure to mixed solvents and an increased prevalence of symptoms characteristic of painter's syndrome or a

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decreased level of neurobehavioral performance in a group of industrial paint workers in Japan.

METHODS

Subjects

The examination of the study group included an interview questionnaire for data collection about the workers' occupational and health histories and subjective symptoms as well as a health survey on neurobehavioral tests. A questionnaire survey was made of 81 painters of the repair section of the Japan Railway Co. (JR) and two painting shops. The subjective symptoms and health status of these painters were compared to age (±3 years)-, sex-, and education-matched referents who were randomly selected from among 154 nonexposed blue collar workers who took the annual physical health check for the screening of chronic diseases at the same work sites. Referent workers were mainly from JR, since there were only a small number of nonexposed workers in the other two plants.

In addition to the questionnaire survey, a neurobehavioral test battery was given to 20 maintenance painters from JR and to 20 matched referents, who were working in the same factory but were not exposed to any solvents, in order to evaluate the neurobehavioral effects of long-term exposure to mixed organic solvents.

Symptom Survey

The questionnaire used in the symptom survey was divided into two parts, one part concerned acute symptoms during the work shift ($N = 17$) and the other concerned chronic symptoms ($N = 61$), with a total of 78 questions. The questions dealing with chronic symptoms were formulated so that they covered almost all symptoms revealed in earlier clinical reports on organic solvents. The main symptoms were nervous system disorders, i.e., headaches, dizziness, unsteadiness in walking, and symptoms of peripheral neuropathy such as paresthesia. Sensory disorders such as eye and nose symptoms were included. Psychiatric symptoms such as sleeping difficulties, impaired memory, and concentration difficulties were also included. In addition, general symptoms, including those involving the autonomic nervous system, skin, the digestive system, and the cardiac respiratory system were added.

The questions concerning chronic symptoms experienced within the last 6 months were asked separately from questions concerning symptoms experienced farther in the past (6 or more months ago), since such organic-solvent workers usually received medical examinations every 6 months in Japan. A three-step rating scale ("never," "sometimes," "often") was used in the questionnaire, which was constructed using language that was simple and unambiguous in order to facilitate replies.

In the symptom survey, background variables which could have influenced subjective symptoms as potential confounders were also inquired about, i.e., use of alcohol and medicines, smoking habits, present and past medical history of diseases, and years of education.

Neurobehavioral Evaluation

The objective of the testing program was to determine if there were in fact measurable chronic effects of solvent exposure among Japanese painters. In se-

lecting the behavioral test battery the following principles were emphasized; first, the test should cover many functions including motor coordination, as well as cognitive and psychomotor functions and, second, it should exhibit ease of testing under field conditions. The neurobehavioral examination consisted of a short interview and a brief test battery, which included (1) Psychomotor function (Simple reaction time, Digit symbol, and Santa Ana motor coordination), (2) Visual cognitive function (Benton retention test), (3) a Vocabulary test (synonyms of the Tanaka A test), (4) Visual spatial cognition (Block design, WAIS) (5) Short-term memory (Digit span, WAIS), and (6) Mood tests.

The Block design test (WAIS), Digit symbol, Digit span, and Santa Ana motor coordination tests were from the batteries of the WHO-recommended neurobehavioral core test (WHO, 1986), while the Reaction time, Hand-eye motor coordination, and Benton visual retention tests were from the NES computerized test battery (Baker *et al.*, 1985) and the Vocabulary test (synonyms from the Tanaka A test; Tanaka, 1958) was used to evaluate verbal ability. In the vocabulary test, 25 words were presented and subjects were asked to select a synonym for each from sets of 4 words. Neurobehavioral examination for each worker was done on Monday morning before the working shift to avoid daily acute effects of organic solvents. Mood (the profile of mood status, POMS) was determined by the Japanese version of the POMS (Yokoyama *et al.*, 1990).

Working Conditions and Exposure Measurement

The subjects were asked about their occupational history, i.e., the kind and duration of employment/job category, and the extent of exposure to organic solvents and other chemicals at their work sites. Air concentrations of the organic solvents at the work sites of these workers were measured twice a year by industrial hygienists. Under new regulations for biological monitoring of workers exposed to organic solvents, urine hippuric acid levels of the workers were measured individually. Urine of workers was collected after the work shift (from 16:30 to 17:30). The hippuric acid concentrations in urine samples were measured by high-performance liquid chromatography (Ogata *et al.*, 1977).

Statistical Methods

For symptoms, the answers of the organic-solvent workers and their referents were coded by the alternatives 1 (“never”), 2 (“sometimes”), and 3 (“often”). The analysis of pairwise differences was applied in order to diminish the confounding effects of the presumably different age trends of the symptoms. The sign test was used to compare the subjective symptoms of the exposed and nonexposed groups symptom by symptom (Siegel, 1956). The relationship between subjective symptoms and variables of the solvent exposure among 81 painters was investigated by multivariate analysis using stepwise backward regression analysis (Kleinbaum *et al.*, 1987) in which the duration and frequency of solvent exposure, kinds of solvent exposure, age, education, smoking, and alcohol intake were candidate variables.

Neurobehavioral performance levels of the exposed workers and the nonexposed matched referents were described using means and standard deviations. The significance of the differences between the two groups was tested by one-tailed t tests for paired data. The relationships between neurobehavioral perfor-

mance and variables of the solvent exposure among workers were also investigated with stepwise, backward regression models.

RESULTS

Exposure Levels of the Workers

The workers were exposed to moderate levels of mixed organic solvents. Among 81 painters having completed the questionnaire, 56 workers (69.1%) reported that they were exposed to toluene. Toluene, xylene, and mineral spirits were the major constituents of the paints used (Table 1). At some work sites with high exposure levels, such as a section using spray paint, the toluene and xylene levels were usually higher than the current Japanese threshold level (100 ppm). Air concentration of the solvents was especially high 10 years ago (Table 2). Unfortunately there were no detailed records on the ambient exposure levels of other solvents such as acetone, methanol, and gasoline.

Mean duration of exposure to organic solvents was 14.2 ± 11.9 years (range, 1–43). Half of these workers were exposed to organic solvents for more than 5 hr/day and for more than 21 days/month. We could not analyze the relationship between each work site and both symptoms, and neurobehavioral performance of these workers, because the workers were usually shifted to one of these work sites every few months and no detailed personal records of job histories were available.

One month before the neurobehavioral examination, the mean concentration of urine hippuric acids of 20 workers was 18.9 ± 10.4 g/liter (range, 3.2–54.0). We did not analyze the relationship between urine hippuric acid level and neurobehavioral performance, since urinary hippuric acid concentration is an index of acute exposure, being well-correlated with the most recent air concentration of toluene in the work site.

Comparability of the Groups

The ages of the 81 workers ranged from 18 to 65 years (mean ±SD, 31.5 ± 7.3) with a duration of exposure of 11.4 ± 6.4 years (mean), while the mean age of the

TABLE 1
LIST OF PAINT SOLVENTS/THINNERS TO WHICH WORKERS WERE EXPOSED

Solvents	No. of workers exposed	(%)
Toluene	56	(69.1%)
Xylene	30	(37.0%)
Mineral spirits	25	(30.9%)
Methanol	24	(29.6%)
Lacquer	24	(29.6%)
Gasoline	21	(25.9%)
Acetone	13	(16.0%)
MEK	5	(6.2%)
Methyl acetate	5	(6.2%)
Ethyl acetate	4	(4.9%)
N-Hexane	3	(3.7%)
Trichloroethylene	1	(1.2%)
Unknown	1	(1.2%)
Note. No. of workers	81	(100.0%)

TABLE 2
SUMMARY OF THE AIR CONCENTRATION (ppm) OF COMPOUNDS MEASURED IN THE WORKSITES FOR 15 YEARS

		1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	Mean ± SD
Brush paint site no. 1	Toluene	120	40	60	30	0	0	18	15	6	8	17	6	9	3	23	± 23.6
	Xylene	80	—	50	70	1	2	9	9	1	3	11	35	7	48	4	± 30.2 23.5
	Mineral spirits	40	40	20	20	12	30	80	46	—	—	75	97	—	—	—	± 26.5 45.9
Brush paint site no. 2	Toluene	210	200	100	—	22	3	—	155	—	—	249	—	—	—	—	± 27.3 134.2
	Xylene	210	900	450	50	224	23	—	21	—	—	586	—	—	—	—	± 88.3 308.0
	Mineral spirits	10	30	30	30	379	68	45	14	—	—	—	—	—	—	—	± 294.7 75.8
Spray paint site no. 1	Toluene	550	500	60	290	2	2	115	—	—	—	112	130	—	161	74	± 116.0 181.4
	Xylene	1000	1000	350	450	316	29	10	—	—	—	27	8	—	20	4	± 178.9 292.3
	Mineral spirits	80	30	10	40	976	182	10	—	—	—	—		—	—	—	± 367.7 189.8
Spray paint site no. 2	Toluene	600	200	150	500	—	—	86	—	55	—	40	—	23	173	254	± 325.7 173.8
	Xylene	50	700	250	35	25	17	12	—	6	—	8	—	5	7	12	± 186.4 93.9
	Mineral spirits	50	20	20	10	132	113	—	—	—	—	—	—	—	—	—	± 194.1 57.6
Mixing room	Toluene	—	—	10	—	4	—	—	6	4	—	—	—	5	4	2	± 48.0 5.0
	Xylene	20	—	—	—	4	—	—	1	1	—	—	—	6	2	2	± 2.3 5.3
	Mineral spirits	10	—	—	—	22	—	—	—	—	—	—	—	—	—	—	± 6.3 16.2
																	± 6.2

20 workers who were examined for neurobehavioral performance was 39.5 ± 11.6 years (range, 25–60). Since we matched exposed cases with referents by sex, age, and years of education, there were no differences in mean age and education among the solvent-exposed workers and referents. The answers to the questions concerning the frequency of alcohol intake and the amount of alcohol consumption were different between both groups. Thirteen (65%) exposed workers consumed alcohol daily, while only 6 (30%) of the nonexposed workers consumed alcohol every day. The mean alcohol consumption in the exposed group was 41.4 ± 30.7 ml/day, while that of nonexposed referents was 28.1 ± 34.8 ml/day. There were no significant differences either in smoking habits nor in medicines used regularly between the organic-solvent-exposed subjects and the referents. The percentage of current smokers was similar in the two groups, i.e., 75.0% and 65.0%, in the exposed and in nonexposed groups, respectively.

Subjective Symptoms

Table 3 shows the response rates for acute symptoms during the work shift, and Tables 4 and 5 show statistically significant acute symptoms and chronic symptoms of the exposed group (81 workers) in comparison with the symptoms of the unexposed groups by sign test. In this comparison the exposed group was characterized by symptoms of dry and scaly skin, being easily depressed without reason, coldness of hands and legs, being easily irritated without reason, loss of appetite, dizziness, and unsteadiness.

TABLE 3
SYMPTOMS DURING THE WORKSHIFT (ACUTE SYMPTOMS)

Symptoms	Never	Sometimes	Often
1. Do you have headaches?	55 (67.9%)	23 (28.4%)	3 (3.7%)
2. Do you have a drunken feeling?	57 (70.4%)	24 (29.6%)	0 (0.0%)
3. Do you feel nauseated?	76 (93.8%)	5 (6.2%)	0 (0.0%)
4. Do you have spells of dizziness?	47 (58.0%)	34 (42.0%)	0 (0.0%)
5. Do your legs feel heavy?	66 (81.5%)	13 (16.0%)	2 (2.5%)
6. Do you feel your walking is unsteady?	72 (88.9%)	9 (11.1%)	0 (0.0%)
7. Do you feel you cannot walk straight?	78 (76.3%)	3 (3.7%)	0 (0.0%)
8. Do you have numbness in your hands or feet?	73 (90.1%)	7 (8.6%)	1 (1.2%)
9. Do you feel strange feelings in any part of your body?	74 (91.4%)	6 (7.4%)	1 (1.2%)
10. Do you have trouble with a runny nose and nasal irritation?	57 (70.4%)	24 (29.6%)	0 (0.0%)
11. Do you often feel you smell of organic solvents?	68 (84.0%)	9 (11.1%)	4 (4.9%)
12. Are you bothered by itching hands?	68 (84.0%)	11 (13.6%)	2 (2.5%)
13. Do you have trouble with burning or blisters on your hands?	70 (86.4%)	10 (12.3%)	1 (1.2%)
14. Do you feel heaviness in your stomach or upper abdomen?	66 (81.5%)	12 (14.8%)	3 (3.7%)
15. Do you get chills and shiver?	77 (95.1%)	4 (4.9%)	0 (0.0%)
16. Is your eyesight blurred even when you wear glasses?	70 (86.4%)	9 (11.1%)	2 (2.5%)
17. Do you see everything double?	76 (93.8%)	4 (4.9%)	1 (1.2%)

Multiple linear regression analysis of exposure variables with the scores of subjective symptoms demonstrated some positive correlations between increases in the number of symptoms and variables related to organic-solvent exposure (Table 6). The frequency of exposure, i.e., hours of solvent exposure per day or days of exposure per month, had a significant positive relationship to increases of all acute and some chronic symptoms. The exposures to acetone and gasoline had significant relations to increases in subjective symptoms. On the other hand, other variables such as the duration of exposure (years of exposure) and age had no positive relation with any of the current symptoms.

TABLE 4
RESULTS OF A PAIRWISE COMPARISON OF THE ACUTE SYMPTOMS OF 81 MATCHED PAIRS OF EXPOSED AND REFERENT SUBJECTS

	Cases		
Symptoms[a]	A	B	*P* value
1. Drunken feeling	21	5	0.001
2. Dizziness	27	10	0.004
3. Smell of organic solvent	13	0	0.000
4. Itching hands	12	4	0.038

[a] Acute symptoms which were more frequently experienced by exposed subjects than by referents.

Note. A; The number of organic solvent-exposed workers who experienced symptoms more frequently than the referents. B; The number of referents who experienced symptoms more frequently than the solvent-exposed workers. *P;* Level of significance of difference.

TABLE 5
RESULTS OF A PAIRWISE COMPARISON OF THE CHRONIC SYMPTOMS OF 81 MATCHED PAIRS OF EXPOSED AND REFERENT SUBJECTS

Symptoms[a]	Cases		*P* value
	A	B	
1. Dizziness	19	9	0.04
2. Unsteadiness occurred	17	7	0.03
3. Difficulties in hearing	16	7	0.05
4. Easily irritated without reason	23	11	0.03
5. Easily depressed without reason	18	7	0.02
6. Coldness of hands and legs	16	6	0.03
7. Loss of appetite	20	9	0.03
8. Dry and scaly skin	27	12	0.01

[a] Those symptoms more frequently experienced by the exposed subjects than by the referents.

Note. A; The number of organic solvent-exposed workers who experienced symptoms more frequently than the referents. B; The number of referents who experienced symptoms more frequently than the solvent-exposed workers. *P;* Level of significance of difference.

Comparison of Neurobehavioral Performances between the Exposed Workers and the Referents

Neurobehavioral performance levels of the exposed workers and the nonexposed matched referents are shown in Table 7. Matched analysis showed that Vocabulary levels and Digit symbol performance in exposed workers were significantly inferior to those of controls, i.e., the mean numbers of words in Vocabulary tests were 12.0 ± 5.6 (mean ± SD) and 15.8 ± 3.6 (for the exposed workers and referents, respectively, $P < 0.05$), and the mean counts of Digit symbol were 60.0 vs 69.4 (for the exposed workers and referents, respectively, $P < 0.05$). There were no statistically significant differences in the answers to the Mood scales of the exposed and the nonexposed referent groups.

Relationship among Neurobehavioral Tests, Exposure, and Other Background Variables

Multiple linear regression analysis of exposure variables with neurological examination measures demonstrated some positive correlations between poorer neurological performance and variables related to solvent-exposure history (Tables 8 and 9). Organic-solvent exposure had a statistically significant relation with poorer performance for reaction time and "confusion," one of the subcategories of POMS. Duration of exposure had a significant relationship with poorer performance in Vocabulary and Block design tests and Digit span. Toluene exposure also had a significant negative relation to Santa Ana motor coordination, the Benton visual retention test, and the "fatigue" of POMS. However, in another multiple regression model, controlling Vocabulary as an independent variable in addition to age, education, and alcohol intake, a significant relation was found only between solvent exposure and poorer performance in the Santa Ana coordination test, with a higher value for one of the scales of POMS (confusion).

DISCUSSION

The main subjective symptoms characterizing the organic-solvent worker group in general were dizziness, drunken feelings, unsteadiness, being easily depressed

TABLE 6
SUMMARY OF STEPWISE BACKWARD LINEAR REGRESSION ANALYSIS OF ACUTE AND CHRONIC SYMPTOMS

		Chronic symptoms						
	Acute symptoms	Total	Central nervous system	Peripheral nervous system	Autonomic nervous system	Psychiatric	Skin	Digestive
1. Frequency of exposure (days/month)								(+)[a] 0.04
2. Frequency of exposure (hours/day)	(+)* 0.01		(+) 0.00	(+) 0.02	(+) 0.02	(+) 0.02	(+) 0.01	
3. Toluene exposure (0, 1)			(−) 0.05					
4. Xylene exposure (0, 1)				(−) 0.01				
5. Acetone exposure (0, 1)	(+) 0.03	(+) 0.05						
6. Gasoline exposure (0, 1)		(+) 0.04				(+) 0.03		
7. Methanol exposure (0, 1)		(−) 0.01						
8. Age (years)				(−) 0.01				
9. Smoking								(+) 0.03
10. Alcohol intake (frequency)								(+) 0.03
R^2	0.32	0.13	0.18	0.15	0.06	0.14	0.09	0.15

Note. Candidate variables included age, duration of exposure (years), frequency of exposure (months/year, days/month, hours/day), kinds of solvents exposed to, education, smoking, and alcohol intake. No significant relationship was observed by variables of duration of exposure, frequency of exposure (months/year), and education.

[a] (+), Association between higher value or degree of variable and the score of symptoms; (−), association between lower value or degree of variable and the score of symptoms.

* P value of regression coefficient listed only if <0.05.

or irritated without reason, dry and scaly skin, itching hands, and loss of appetite. These results on subjective symptoms are in good agreement with previous reports (Elofsson *et al.*, 1980; Husman, 1980; Cherry *et al.*, 1985; van Vilet *et al.*, 1987). In contrast the significant positive association with exposure was only found with neurobehavioral tests of Digit symbol and synonym in the analyses by pairwise comparison. This may be mainly because subjects were exposed to relatively low levels of organic solvents in past ten years and partly because of limited statistical power due to the small sample size.

The comparability of exposed and reference groups is usually a difficult problem in epidemiological studies. Both the organic-solvent workers and the referent workers were studied in cooperation with an industrial physician. Interviewers and testers collected data from both groups in the same standardized manner. Therefore, bias caused by different attitudes toward the study seems unlikely. The groups of solvent workers and referents did not differ with respect to history of illness, use of medicines, or smoking habits. Because no essential chemical exposure was found in a study of the referent workers' working conditions, the group differences found can be interpreted as having arisen from exposure to organic solvents.

It was not so easy, however, to determine from the present results if there were

TABLE 7
MEAN PERFORMANCE ON NEUROBEHAVIORAL TEST AND SCORE OF PROFILES OF MOOD STATUS

	Exposed (mean ± SD)	Nonexposed (mean ± SD)	*P* value
Neurobehavioral performance			
Synonym	12.0 ± 5.6	15.8 ± 3.6	(*)
Block design	19.6 ± 4.4	21.4 ± 4.0	ns
Santa Ana (right)	40.3 ± 5.9	39.4 ± 6.9	ns
Santa Ana (left)	35.8 ± 5.7	38.8 ± 5.7	ns
Digit span	9.7 ± 2.4	10.9 ± 2.9	ns
Digit symbol	60.0 ± 14.1	69.4 ± 13.6	(*)
Hand–eye coordination	187.8 ± 97.0	193.1 ± 66.3	ns
Reaction time (right)	298.2 ± 58.2	313.8 ± 84.7	ns
Reaction time (left)	312.2 ± 85.8	324.0 ± 86.2	ns
Benton	10.5 ± 1.3	10.4 ± 1.9	ns
POMS subcategory			
Tension–anxiety	10.9 ± 4.2	12.9 ± 6.8	ns
Anger–hostility	7.8 ± 6.0	11.0 ± 7.8	ns
Fatigue	8.5 ± 5.0	9.0 ± 6.5	ns
Depression	10.3 ± 7.0	10.4 ± 9.4	ns
Vigor	9.3 ± 4.2	11.1 ± 5.0	ns
Confusion	8.8 ± 3.4	7.3 ± 4.5	ns

* $P < 0.05$.

any chronic adverse effects of long-term exposure to organic solvents, because exposure-related neurological dysfunction of solvent workers is often reversible (Olson *et al.*, 1981; Olson, 1982) and there are many confounding variables, e.g., age, sex, education, and alcohol intake, which are critical when groups of unexposed or slightly exposed workers are contrasted with an exposed group. In addition, the intelligence of the worker is also an important variable. Unfortunately, in many cross-sectional studies like the present one the workers' intelligence levels before exposure are usually unknown.

TABLE 8
SUMMARY OF STEPWISE BACKWARD LINEAR REGRESSION OF NEUROBEHAVIORAL TESTS

Variables[a]	Synonym	Block design	Santa Ana (left)	Digit span	Digit symbol	HE (right)	RT (right)	Benton
1. Duration of exposure (years)	(−)0.00*	(−)0.04		(−)0.01	(−)0.01	(−)0.00		
2. Mixed-solvents exposure (0, 1)							(−)0.02	
3. Toluene exposure (0, 1)			(−)0.04				(+)0.00	(−)0.00
4. Age (years)	(−)0.03		(−)0.00	(−)0.00				
5. Education period (years)							(−)0.00	
6. Alcohol intake (0, 1)				(−)0.01	(+)0.01			
R^2	0.55	0.11	0.47	0.47	0.69	0.21	0.25	0.22

Note. Abbreviations: HE, hand–eye coordination; RT; simple reaction time. (+), Association between higher value or degree of variable and better performance of the test; (−), association between higher value or degree of variable and poorer performance of the test.

[a] Candidate variables included age, duration of exposure (years), frequency of exposure (months/year, days/month, hours/day), kinds of solvents exposed to, education , smoking, and alcohol intake.

* P value of regression coefficient listed only if <0.05.

TABLE 9
SUMMARY OF STEPWISE BACKWARD LINEAR REGRESSION OF SCORE OF PROFILES OF MOOD STATUS

Variables[a]	Profile of mood status					
	Tension-anxiety	Anger-hostility	Fatigue	Depression	Vigor	Confusion
1. Mixed solvent exposure (0, 1)						(−)0.00
2. Toluene exposure (0, 1)			(−)0.03			
3. Education period (years)	(−)0.04					(−)0.04
R^2	0.08		0.08			0.23

Note. (+), Association between higher value or degree of variable and better performance on the test; (−), association between higher value or degree of variable and poorer performance on the test.
[a] Candidate variables included age, duration of exposure (years), frequency of exposure (months/year, days/month, hours/day), kinds of solvents exposed to, education, smoking, and alcohol intake.
* *P* value of regression coefficient listed only if <0.05.

We used multiple regression analysis to investigate the relation between solvent exposure and both the subjective symptoms and the results on neurobehavioral function while controlling for potential confounders, especially, age, education, and alcohol. Solvent exposure had a significant association with poorer performance in the Vocabulary test, Block design tests, Digit span, Santa Ana motor coordination test, and two subcategories of POMS. However, in another multiple regression model, controlling Vocabulary as a surrogate independent variable of the general level of intelligence in addition to age, education, and alcohol intake, a significant relation was found only between solvent exposure and poor performance in the Santa Ana coordination test, or a higher value for one of the scales of POMS (confusion).

Our results are congruent with the reports of Cherry *et al.* (1985) and Fidler *et al.* (1987). For example, when Cherry *et al.* compared two groups of men exposed to paint solvents or to toluene with age-matched, nonexposed workers, substantial deficits were found in a number of neuropsychological tests. When the exposed workers were then rematched with a second comparison group based on intelligence using a reading test, no differences were found between the groups.

However, the correlations between the duration of exposure (years) and the score on the synonym test were statistically significant. It was interesting because the exposure level at the work sites of the workers more than 10 years ago was considerably higher than that in recent years. Hanninen *et al.* also reported that the painters' verbal intelligence (similarity in WAIS) had deteriorated even when adjusted by age and initial intelligence (Hanninen *et al.*, 1976).

It is always difficult to draw causality conclusions in a prevalence study like the present one. Our results suggest that a symptom inquiry is helpful for detecting the possible effects of exposure to low levels of organic solvents. However, no consistent pattern was observed in regard to the effects of organic-solvent exposure on neurobehavioral function, which is coincident with the type I toxic central nervous system disorder as classified by WHO (1985).

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Human Experimental MIBK Exposure: Effects on Heart Rate, Performance, and Symptoms[1]

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Heart rate, performance, and symptoms were studied in six female and six male volunteers, aged 19 to 47 years, during experimental 2-hr exposures to 10 and to 200 mg/m^3 of methyl isobutyl ketone (MIBK). No effects from exposure on performance of a reaction time task or an arithmetic test could be demonstrated, and no consistent effects on heart rate were found. Subjects reported significantly more symptoms from the central nervous system, e.g., fatigue, due to the exposure. There was also an indication of an increase in ratings of irritation to the airways. A reduction of the threshold limit value (TLV) of 205 mg/m^3 for MIBK exposure presently indicated by the American Conference of Governmental Industrial Hygienists, is therefore recommended.

INTRODUCTION

Neurotoxic effects from exposure to some organic solvents are well known. One group of commonly used solvents, comprising e.g., acetone, methyl ethyl ketone (MEK), and methyl *n*-buthyl ketone (MNBK), is labeled ketones. The neurotoxic effects of MNBK and the mechanisms of action on the nervous system following chronic exposures to this ketone have recently been reviewed by, e.g., Bos and colleagues (1991) and Spencer and Schaumburg (1985). Acetone has been shown in experimental studies (Dick *et al.*, 1989) to have a negative effect on the functioning of the human central nervous system (CNS) following short-term exposures to levels around present threshold limit values (TLVs), while no such effects have been indicated for MEK (Dick *et al.*, 1989).

Another ketone, methyl isobutyl ketone (MIBK) is commonly used in industrial products such as glues and paints. MIBK vapor is irritating to the mucous membranes of the eyes and the airways at exposure levels exceeding 330 mg/m^3 (Silverman *et al.*, 1946; Linari *et al.*, 1964). Effects from MIBK on the functioning of the CNS have rarely been investigated in humans, and in the two early studies reported in the literature the actual solvent concentrations are inadequately described (Linari *et al.*, 1964; Armeli *et al.*, 1968).

Experimental studies on animals exposed to MIBK concentrations below or at the TLV of 205 mg/m^3 recommended by the American Conference of Governmental Industrial Hygienists have indicated that MIBK exposure at this level may impair CNS functioning. Thus, behavior in rats was affected at 100 mg/m^3 (Geller *et al.*, 1978), and in studies of delayed match-to-sample discrimination performance in baboons, response latencies were prolonged after 1 week of exposure to 200 mg/m^3 of MIBK (Geller *et al.*, 1979).

In a study on human subjects experimentally exposed during light physical

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exercise (50 W) for 2 hr to 200 mg/m^3 of MIBK performed at our laboratory indications of a negative effect on performance of a simple reaction time (SRT) task were found (Wigaeus-Hjelm *et al.*, 1990). In this study an increase in the SRT variability following exposure, possibly indicating fatigue, was found for 50% of the subjects. However, since these findings were only indicative, we decided to repeat the study using a modified design.

The primary purpose of the study mentioned above (Wigaeus-Hjelm *et al.*, 1990) was to investigate the toxicokinetics of MIBK. To fulfill this objective, frequent samples of expired air, blood, and urine were taken. The potentially annoying sampling procedures may well have had an alerting effect, counteracting a possible narcotic influence on the CNS from the MIBK exposure. Therefore, it was decided to maximize the sensitivity of this study to narcotic effects on the CNS by excluding biological sampling from the present study.

This study was thus designed to investigate the possible narcotic impact on CNS functioning from exposure to MIBK at the Swedish Permissable Exposure Level (PEL). Heart rate, performance tests, and rating scales for local irritation, CNS symptoms, and mood were chosen as effect indicators.

MATERIALS AND METHODS

Six female and six male employees at the National Institute of Occupational Health, aged 19 to 47 years, volunteered to participate. They were all considered healthy according to a general medical examination preceding the first exposure and gave their consent to participate after being informed of the experimental procedure in general. They were, however, neither aware of the exact MIBK concentrations given nor of the order of administration of the conditions. The study design was approved by the Regional Ethical Committee at the Karolinska Institute (2.2 AI 273/89).

Exposures were performed in a 12-m^3 exposure chamber at 22 to 23°C, and the exposure levels were 10 mg/m^3 under the control condition and 200 mg/m^3 during the exposure condition. This higher exposure was the Swedish PEL at the time of the planning of this study, and it was also close to the TLV recommended by the ACGIH (205 mg/m^3). During the performance of this study the Swedish PELs for some 25 solvents were reduced, following a governmental initiative. The PEL for MIBK was thus halved in 1990 (Lundberg *et al.*, 1991), while the recommendation from the ACGIH is still unchanged (ACGIH, 1991).

The solvent was injected in the influent air stream, and the air was changed 11 times/hr. MIBK concentrations were continuously monitored by an infrared spectrophotometer, and the average solvent concentrations from samples taken every 5 min during exposures ($N > 500$ per exposure condition) were 201 mg/m^3 (SD = 3.0) at the higher exposure level and 11.9 mg/m^3 (SD = 1.44) under the control condition.

Subjects were exposed one at a time for 2 hr, at the same time of day, with a 1-week interval between exposure sessions. Exposure started with a 90-min period of light physical exercise (50 W) on a bicycle ergometer, and during the last 30 min of exposure subjects were relaxing on a bed.

Heart rate (HR) was registered using a portable tape recorder (Medilog Instruments, Cambridge, UK). Output from the analyses of the tapes was HR in beats per minute (bpm) for each minute of the registration.

Two performance tests from the Swedish Performance Evaluation System

(SPES), developed specifically for the measurement of effects from exposure to unfavorable work environmental conditions (Gamberale *et al.*, 1990), were used. Performance on a test of SRT and a test of simple arithmetic (RTadds) was measured immediately before entry to the chamber, as well as directly upon cessation of exposure.

The SRT test measures reaction time to an easily discriminable but temporally uncertain stimulus during 6 min, using a signal density of 16 signals/min. Performance is evaluated with respect to level and variability of latencies to 80 stimuli. Each item of the RTadds test consists of three digits, displayed horizontally for 1 sec. The task is to add the digits as fast as possible and to enter the sum on the keyboard. Performance is evaluated as the mean response latency and variability of the latencies to 40 items.

Mood changes during exposure were measured by a two-dimensional scale from the SPES, evaluating stress and activity. The scale consists of 12 adjectives describing stress and activity states, and ratings are made on a six-point category scale. Mood ratings were performed on five occasions during each session (one before, three during, and one after exposure).

The occurrence of symptoms of irritation and CNS symptoms was evaluated using a questionnaire from the SPES. The scale contains 17 items regarding symptoms from the CNS as well as of local irritation to the eyes and airways. The symptoms scale was used on seven occasions during each session (one before, four during, and two after exposure).

The SRT test has documented its sensitivity to the effects from exposure to organic solvents, as well as other derousing exposures, in more than 20 studies performed at our laboratory (Gamberale *et al.*, 1989). The RTadds test has been applied in some 10 investigations of the neurotoxic effects from occupational exposures and has proved its sensitivity in several of these studies (Gamberale *et al.*, 1989). The Mood scale has been especially developed at our laboratory to evaluate effects from environmental exposures, and it has proved to be sensitive to short-term exposures to heat (Razmjou and Kjellberg, 1992) and noise (Kjellberg *et al.*, 1991) and to long-term exposure to manganese (Iregren, 1990). The Symptom scale has proved to be sensitive in studies of acute toluene (Iregren *et al.*, 1986) and MIBK exposure (Wigaeux-Hjelm *et al.* 1990), as well as to chronic exposure to manganese (Wennberg *et al.* 1991).

Statistical evaluations were performed with the SPSS package for Macintosh computers (Norusis, 1990). For the different performance measures, an ANOVA model for repeated measurements was applied, using exposure level (low and high) and measurement occasion (before and after exposure) as the two sources of variance. While using such a model for the analyses of the present design, it is important to note that an exposure effect would be seen statistically as a significant interaction between the two factors exposure level and measurement occasion.

Effects on HR were evaluated separately for the two subgroups exposed in different orders, using an analysis of variance (ANOVA) model for repeated measures. Exposure level and exposure occasion were introduced to the model as the sources of variance.

Mood and symptom ratings were evaluated as differences from baseline ratings, and the two-way ANOVA was performed with exposure level and measurement occasion (two to five, and two to seven occasions, respectively) as the two

sources of variation. Input to the ANOVA model was thus differences between each of the ratings two to five and the rating before the start of exposure for mood data, and the differences between the ratings two to seven and the preexposure rating for the symptoms data.

RESULTS

Performance data for the tests of SRT and RTadds tests are presented in Table 1. Performance on the SRT over test time is presented in Fig. 1. There were no differences in performance attributable to exposure, since no interactions were found between exposure and measurement occasion in the analyses of any of the performance parameters. For SRT there was, however, a prolonged reaction time from the measurement before exposure to the measurement after exposure ($F_{1,11} = 4.1$; $P < 0.05$). The variability of the response latencies to the RTadds test was reduced from the measurement before to the measurement after exposure ($F_{1,11} = 7.7$; $P < 0.05$). No interactions were found for any parameter.

Heart rate varied between 71 and 135 bpm for the different subjects during exercise and between 44 and 85 bpm during rest. The increase in heart rate due to the 50 W work load varied between 24 and 53 bpm in this group of subjects. During the high-exposure condition HR was lower for seven subjects, unchanged for one subject, and higher for four subjects, as compared to HR during the low-exposure condition. Thus, no consistent effect from exposure on heart rate was found.

Mood ratings of activity, presented in Fig. 2, and stress varied during sessions ($F_{3,33} = 21.4$; $P < 0.001$ and $F_{3,33} = 9.7$; $P < 0.001$, respectively), but did not differ between exposure conditions. There was no interaction between exposure and measurement occasion.

Symptoms of irritation, which are presented in Fig. 3, were not significantly different between the two exposure levels. They varied however over measurement occasions ($F_{5,55} = 8.5$; $P < 0.001$). No interactions were found.

The occurrence and/or the intensity of CNS symptoms, which are presented in Fig. 4, increased with exposure ($F_{1,11} = 5.2$; $P < 0.05$) and with repeated measurements ($F_{5,55} = 4.9$; $P < 0.001$). There was no interaction.

DISCUSSION

No consistent effects from MIBK exposure were found on HR, performance, or mood ratings. However, there was a clear increase in reported CNS symptoms

TABLE 1
PERFORMANCE ON THE TESTS OF SRT AND RTADDS OVER EXPOSURE CONDITIONS AND MEASUREMENT OCCASIONS

	Exposure			
	Low		High	
Test/variable	Before	After	Before	After
SRT				
Mean (msec)	209	215	212	215
SD (msec)	27	36	34	39
RTadds				
Mean (sec)	2.5	2.4	2.5	2.3
SD (sec)	0.7	0.5	0.7	0.6

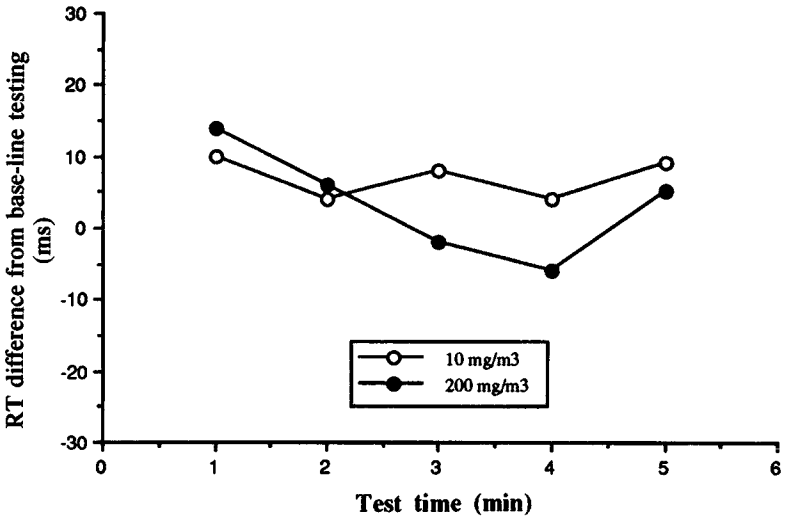

FIG. 1. Performance on the simple reaction time test over the 5-min test time for the two exposure conditions. Reaction time is expressed as the difference from the preexposure measurement.

due to the MIBK exposure, and a trend to the same effect was observed for symptoms of irritation.

Performance on the two tests used, the SRT and the RTadds, was not affected by MIBK exposure. For both the tests there was, however, an effect from the repeated testing. Reaction time on the SRT increased, and the variability of the response latencies on the RTadds decreased, from the measurement before exposure to the one following exposure. The increase in SRT may well be due to fatigue following the physical work. The decrease in variability on the RTadds task is probably due to training effects and increased familiarity with the task. Similar effects were found in our previous study with MIBK exposure (Wigaeus-Hjelm *et al.*, 1990). The latencies on the RTadds test were reduced following

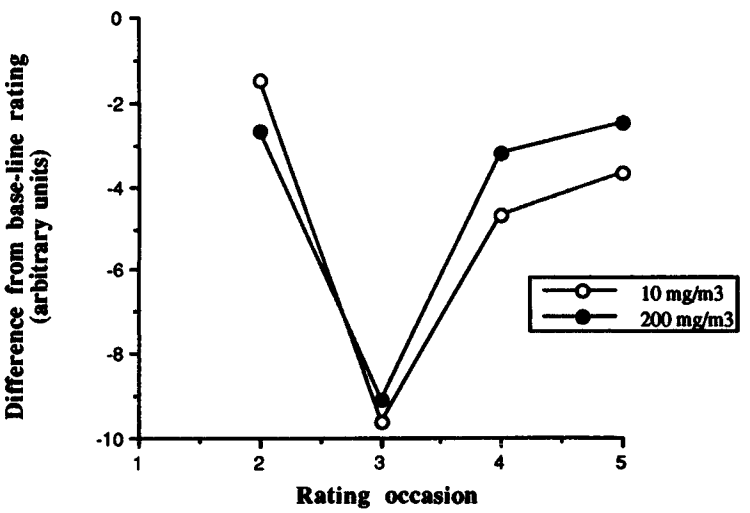

FIG. 2. Activity ratings on the mood scale over exposure time and exposure conditions. Activity scores are expressed as differences from the preexposure measurement.

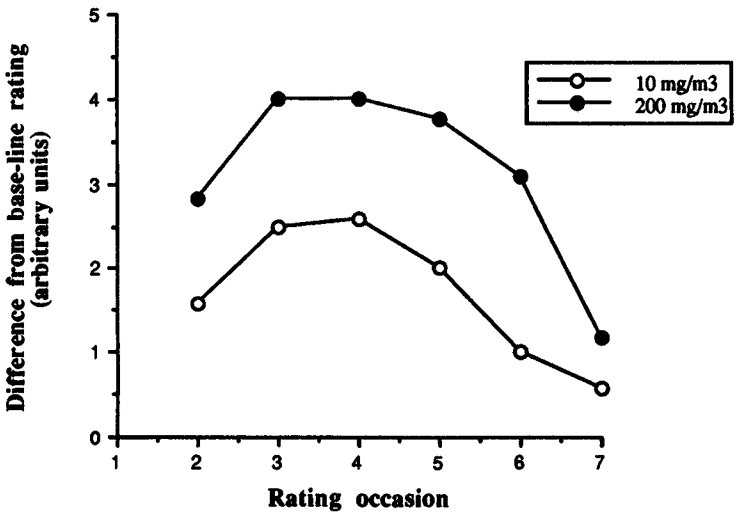

FIG. 3. Symptoms of local irritation over exposure time and exposure conditions. The symptom index is expressed as differences from the preexposure measurement.

repeated testing in the previous study. The occurrence of this training effect indicates that subjects should have been given more extensive training prior to the experiment, to reach a stable performance level.

In a previous study at our laboratory by Iregren and colleagues (Iregren *et al.*, 1986), toluene at the level of the Swedish PEL was found to decrease HR during rest, as compared to a control condition and to conditions with ethanol intake. In the study of toluene, this effect from the solvent on HR was however weak, since it disappeared during the activation induced by performance testing. No effect on HR from the solvent exposure could be demonstrated in the present study.

When comparing the present results with those from previous animal experiments, it is interesting to note the lack of performance effects in the present study.

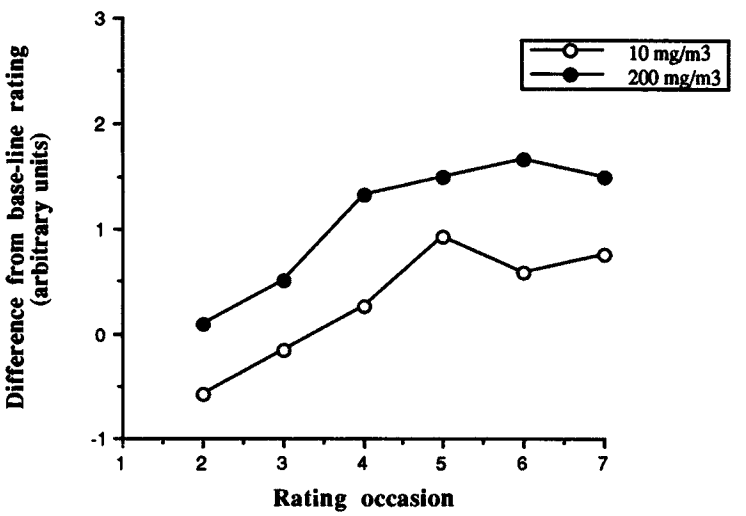

FIG. 4. Symptoms from the CNS over exposure time and exposure conditions. The symptom index is expressed as differences from the preexposure measurement.

This discrepancy in observed effects may be due to, e.g., the shorter exposure times in this study or a lower sensitivity of the performance tests applied to the human subjects. It is known that disruption of well-trained behavior in operant conditioning, as reported in the paper by Geller and colleagues (Geller *et al.*, 1979), is a very sensitive indicator of negative influences on the functioning of the CNS.

The effects found on rated symptom from the CNS and of local irritation are, on the whole, consistent with the results obtained in our previous study (Wigaeus-Hjelm *et al.*, 1990). One notable difference is, however, the lack of a statistically significant effect from the exposure on perceived irritation. There is a clear trend toward such a difference, but since the irritation level is fairly high already at exposure to 10 mg/m^3 of MIBK, the increase reported at 200 mg/m^3 was not statistically significant. This finding may be interpreted as an indication of a high potential for MIBK to induce irritation already at low concentrations.

Mood ratings were not related to the MIBK exposure in the present study. This is well in accordance with our findings in the previously mentioned study of toluene exposure and ethanol intake (Iregren *et al.*, 1986), where mood changes were induced by ethanol ingestion, while exposure to the solvent affected symptom ratings.

CONCLUSION

In the present study, 2 hr of exposure to MIBK was found to produce increased discomfort in the subjects, as measured by the symptom ratings. Performance, mood, and HR were, however, not related to exposure. Still, due to the symptoms of irritation and discomfort induced by the MIBK exposure, we would recommend a reduction of the commonly accepted ACGIH standard of 205 mg/m^3.

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Carbon Disulfide and the Central Nervous System: A 15-Year Neurobehavioral Surveillance of an Exposed Population[1]

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Carbon disulfide-induced neurobehavioral effects are well known and do not need further evidence. Carbon disulfide vasculopathy and the syndromic complex resulting in depression, loss of memory and concentration, and behavior disturbances have been widely demonstrated. Less known is the evolution of the symptomatology when the environmental conditions are consistently improved, that is, the reversibility or the progression of the dysfunctions observed. This paper reports on a neurobehavioral follow-up in a viscose rayon factory carried out, in intervals, from 1974 to 1990. Several successive improvements were implemented in the plant through the years, until finally, the most radical changes were made at the end of the Seventies and these resulted in exposure levels far below the current Threshold Limit Values. A total of 493 subjects were examined and some of them were reexamined up to six times. The last examination was completed in September, 1990. In this paper, studies by our group over the 15 years of monitoring are discussed. The results show that the general mental state, as measured by neurobehavioral methods, reflects past and current exposure. This point was explored by dividing the subjects into six groups on the basis of their length of exposure and year of examination and by comparing their performances. The results show that even exposure to levels of carbon disulfide not exceeding 8 mg/m^3 may induce absentmindedness and difficulties in perceptive abilities.

INTRODUCTION

Rayon is a synthetic fiber derived from cellulose. The main substances used in this process are caustic soda and carbon disulfide. In the viscose rayon factory where we conducted our studies, this process was carried out in the following departments: (1) the preparation department where the viscose is prepared, (2) the spinning and washing department where the viscose is transformed into the yarn, washed, and wound on the windstaffs, and (3) the processing department where the fiber is worked into the industrial products. Exposure to carbon disulfide occurs throughout the entire chemical and spinning process while noise is the main risk factor in the textile department (Tomasini and Cirla, 1981). Carbon disulfide, being a very volatile solvent, is easily inhaled, transformed, and rapidly eliminated. However, it was calculated that one-third of the daily inhaled dose can remain for a longer time in an organism and react with the cell structures by interfering with their metabolism as well as with the protein synthesis, vitamin action, chemical neurotransmitters, heme synthesis, and hormone-releasing factors, among others (Vigliani and Cazzullo, 1950; Crepet and Gobbato, 1956; Minded, 1967; Djuric and Stojadinovic, 1972; Magos, 1972; Tolonen, 1975). More

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specifically, damage to the central nervous system (CNS) can be realized according to a model which distinguishes among acute, subacute, and long-term effects (Cirla and Gilioli, 1978). Acute effects can be due to two different situations, either daily exposure to levels exceeding 1000 mg/m^3 in an unprotected environment, typical of the past century, or accidents in otherwise sufficiently safe environment; subacute effects can follow protracted exposures to levels of 500–1000 mg/m^3 characteristic of the period between 1930–1950; and long-term effects can be due to protracted exposures to progressively reduced levels but exceeding 60 mg/m^3. The reported effects vary from the acute psychoses observed 1 century ago, to the Parkinson-like symptoms seen in the first half of this century, to signs of milder, diffuse encephalopathy realizing a vasculopathic syndrome in the case of protracted medium- to low-level exposure or a psychoorganic syndrome in long-term low-level exposures. While the vasculopathic syndrome is easily identified by the behavioral correlates of an arteriosclerotic process, the psychoorganic syndrome has less-definite characteristics along a continuum going from subjective symptoms of fatigue, mild anxiety, headache, sleep disturbances, also indicated as neurasthenic syndrome, to objective signs of higher functions impairments and personality alterations. The above data derive from an abundant literature produced in the seventies on carbon disulfide effects on CNS (Mancuso and Locke, 1972; Hanninen, 1971, 1974; Hanninen *et al.*, 1978; Lilis, 1974; Knave *et al.*, 1974; Magos, 1975; Cassitto *et al.*, 1978). In the eighties, among an otherwise small number of studies (Kruse *et al.*, 1982; Peters and Levine, 1982; Putz-Anderson *et al.*, 1983; Aaserud *et al.*, 1990), only one investigation is a follow-up on long-term low-level exposed subjects (Vanhoorne *et al.*, 1984). Data on the possibility of an amelioration in mental functioning of the populations exposed, possibly due to consistent reduction of exposure levels, are scanty or nonexistent. The purpose of this paper is to report a 15-year study in a viscose rayon factory of the Lombardia province, active since 1940 for the production of fabrics for tires, transport belts, and other industrial outputs. The Institute of Occupational Health of the University of Milan initiated this collaboration with the plant in 1972. The monitoring of the behavioral indicators of carbon disulfide exposure, up to 1989, has been carried out with periodical checkups of the workers. A total of five studies were carried out either on the entire plant population or on selected groups. Only the last study, performed in 1989–1990 is reported in detail while all the other are referred to previous publications or condensed under Method and Results.

REVIEW OF THE NEUROBEHAVIORAL ACTIVITY IN THE 1975–1988 PERIOD

Airborne Concentrations

Since 1962, the working environment has substantially improved and the levels of exposure progressively reduced to the current very low values. When the first ameliorations were carried out (1962–1971), the average levels of exposure were around 60 mg/m^3 with higher peaks up to 120 mg/m^3 when specific tasks were performed. This was the situation when our Institute started its activities in the plant. Later on, other consistent changes were introduced and, by 1980, the exposure levels were around 2 mg/m^3 in the preparation department with peaks up to 16 mg/m^3, around 5 mg/m^3 in the spinning department with peaks up to 30–35

mg/m^3, and from 1 to 4 mg/m^3 in the washing department with occasional peaks to 13 mg/m^3. The last environmental and biochemical determinations were carried out in October 1988 by the Institute of Occupational Health of Pavia. The average environmental concentrations were, in the preparation department, mean 2.32, SD 2.64 mg/m^3; in the washing department, mean 4.32, SD 3.2 mg/m^3; in the spinning department, mean 8.3, SD 4.6 mg/m^3 and for the maintenance operators, mean 6.5, SD 3.4. These values were obtained by means of personal samplers carried by the workers for an entire work shift (Table 1).

Neurobehavioral Studies

Study 1. The first neurobehavioral study was carried out in 1974–1975 on 97 exposed workers and 27 controls from the same plant but working in a risk-free department. The study was part of a large medical survey on the entire population of the plant which initiated the collaboration of the Institute. The behavioral battery used consisted in nine tests, two WAIS subtests (Picture Completion and Block Design), Raven PM38, Eysenck MPI (Neuroticism and Extraversion dimensions), Cattel Anxiety Scale (ASQ), Pauli Test, Symbol Digit, and Rey RM1. This battery is heavily loaded on perceptive abilities, visuomotor coordination, memory, and personality dimensions (Table 2). The subjects were divided into five risk classes on the basis of their specific past and present tasks and length of exposure. For further details, the reader is referred to a previous publication (Cassitto *et al.*, 1978). For our present purpose, the results can be thus summarized: the first three tests, Picture Completion, Block Design, and Raven PM38, heavily loaded on perceptive abilities and reasoning, differentiated the classes of subjects, the most heavily exposed yielding the worst performances. The indexes of neuroticism and anxiety did not show important differences among the groups even though the *N* and ASQ values increased with the increasing exposure classes. The three performance tests, Pauli, Symbol Digit, and Rey Memory, showed different discrimination power among the risk classes with the least discrimination power for Rey Memory and the best discrimination power for Symbol Digit. As a whole, the visuomotor abilities, mainly tested under timing, showed a

TABLE 1
CARBON DISULFIDE MEAN EXPOSURE LEVELS IN THE DEPARTMENTS OF A VISCOSE RAYON FACTORY (1962–1988)

Years	Departments	Exposure levels[a]	Peaks
1962–1971	All[b]	60	120 mg/m^3
	Preparation[b]	2 mg/m^3	16 mg/m^3
1972–1980	Spinning[b]	5 mg/m^3	30–35 mg/m^3
	Washing[b]	4 mg/m^3	13 mg/m^3
	Preparation[c]	2.32 ± 2.64	
1988	Spinning[c]	8.30 ± 4.60	
	Washing[c]	4.32 ± 3.20	

[a] The 1962–1971 and 1972–1980 values of exposure levels are means. The 1988 values are means ± SD.

[b] The 1962–1971 and 1972–1980 values were determined by stationary sampling.

[c] The 1988 values were determined by means of personal samplers carried by the workers for an entire work shift. The determinations were performed by the Fondazione Clinica del Lavoro, University of Pavia.

TABLE 2
STUDY 1: BEHAVIORAL BATTERY USED ON 97 CARBON DISULFIDE-EXPOSED WORKERS OF A VISCOSE RAYON FACTORY (1974–1975) IN A CROSS-SECTIONAL SURVEY

Test	Functions: Main loadings
Picture Completion	Perceptive ability
Block Design	Motor perceptive ability
Raven PM 38	Abstract reasoning
Eysenck MPI (N)	Neuroticism
Eysenck MPI (E)	Extraversion
Cattell ASQ	Anxiety
Pauli Test	Vigilance
Symbol Digit	Visuomotor coordination
Rey RM1[a]	Memory recognition, short and long

[a] The Rey Memory Scale consists of seven subtests, five measuring the recognition ability for poorly structured material and two short-term and long-term recall.

progressive negative correlation with the exposure indexes. As a consequence of this study neurobehavioral monitoring was pursued on a clinical basis on single subjects sent to our Institute either as in-patients or in a day hospital regimen for disturbances suggesting possible effects of carbon disulfide exposure.

Study 2. In 1984, an analysis was carried out on the test results of 30 subjects who had been reexamined as outpatients at our Institute after their first testing in 1974–1975. All the subjects were still active in the plant; though, some of them had moved to other departments. These changes were not considered a confounding factor since all levels in all departments were low and nearly the same.

The test battery used in the clinical setting was more extensive than the one used in 1974–1975, but Block Design, Eysenck MPI, Cattel ASQ, Pauli Test, and Rey PRM1 were included. No significant differences were observed in the mean values and standard deviations of the results but the analysis of the number of subjects whose performances were better, worse, or unchanged in comparison with the first examination showed a significantly higher number of subjects with better performances at the Pauli Test and the Rey Memory subtests 3 and 4, namely at the recognition of stimuli with poor similarity with the originals. The other subtests were unchanged (Table 3). Confounding factors, such as time elapsed between the two testing sessions, age, years of exposure, education, variation in time of alcohol consumption, were controlled by means of Spearman and Pearson correlations and did not show any significant weight on the results.

Study 3. In 1988, the test results of 60 subjects, who had been employed between 1947 and 1982 and who had repeated their neurobehavioral testing twice at different intervals, were analyzed. Common measures included Eysenck MPI, Cattel ASQ, Pauli Test, protracted Reaction Time, Rey PRM, Digit Span, Benton Visual Retention and Block Design. The comparison between the first and second testing showed that the second testing had yielded better results and that these results were correlated to the age of the subjects examined (Table 4). Age–tests scores did not show such relationship for the first testing. On the contrary, a cumulative risk index (cri) inclusive of age, educational level, alcohol consumption, year of entrance in the job, and year of testing proved to be significantly related to the first testing results but not to the second ones. The improvements

TABLE 3
STUDY 2: OVERALL CHANGES OBSERVED IN THE CARBON DISULFIDE-EXPOSED SUBJECTS EXAMINED IN 1974–1975 AND 1984

Class of exposure	Subjective complaints	Mental performance	Psychomotor ability	Memory	Personality
1	Reduced	Unchanged	Unchanged	Unchanged	Unchanged
2	Reduced	Unchanged	Unchanged	Improved	Unchanged
3	Reduced[a]	Unchanged	Improved	Improved	Unchanged
4	Reduced	Unchanged	Unchanged	Unchanged	Unchanged
5	Reduced	Unchanged	Unchanged	Improved	Unchanged

Note. Class 1, nonexposed or very limited exposure for less than 3 years; Class 2, low to moderate exposure (less than 60 mg/m^3 for more than 3 years or above 60 mg/m^3 up to 1971); Class 3, heavy exposure (more than 120 mg/m^3 for more than 3 years).

Class 4, subjects with unchanged alcohol intake in 1984; Class 5, subjects with reduced alcohol intake in 1984.

[a] The subjective complaints were reduced with the exception of depression and memory.

observed at the second testing and the reduced weight of the cumulative index on these results might indeed suggest an effect of the improved environmental conditions.

Study 4. In 1988, the colleagues of the Fondazione Clinica del Lavoro, University of Pavia, carried out a new biological and environmental control by determining the urine concentrations of the workers at the end of one work shift and the ambient air concentrations by means of personal samplers carried by the workers during an entire shift. Concentration values were higher in the spinning department and lower in the washing department, but a comparison between environmental data and Threshold Limit Values (TLVs) showed that in no case had this value been reached. The lowest values were observed in the preparation department.

In parallel, new behavioral monitoring was carried out on 166 workers, 60 of them already examined. The subjective symptoms of the newly examined 106 subjects were evaluated against 106 controls, mainly bus drivers. More fatigue, loss of memory, and a general lack of energy were reported at the Subjective Symptom Questionnaire (SSQ) by the carbon disulfide-exposed workers. In the 60 previously examined workers, higher scores at the Block Design WAIS subtest and an improvement of the attention indexes (standard deviation of the protracted Reaction Time and Benton errors) were observed while memory for digits and recognition memory (Rey Memory 3) scores had decreased. These data appeared to be correlated with the age and length of exposure of the subjects, i.e., the youngest subjects with the shortest exposure showed more positive changes. Correlations between subjective symptoms, tests scores, urine concentrations, and ambient air values showed only a positive covariance of "absentmindedness" and protracted Reaction Times with ambient air and urine concentration (significance levels, 0.01 and 0.05).

Study 5: Materials and methods. The last control of the carbon disulfide-exposed population was carried out in 1989–1990. The progressive and consistent reduction of exposure to the levels seen in 1983 and the retirement from work of the majority of the subjects seen in 1975, with high past exposure, were elements

TABLE 4

STUDY 3: PEARSON *r* CORRELATIONS BETWEEN BEHAVIORAL PARAMETERS, AGE, AND CUMULATIVE RISK INDEX FOR THE FIRST AND SECOND PSYCHOLOGICAL EXAMINATION (60 SUBJECTS)

Tests	*n*	Exam I (*r*)		Exam II (*r*)	
		Age	cri[a]	Age	cri[a]
MPI N	56	−0.06	0.04	0.09	0.07
MPI E	56	−0.02	−0.04	−0.07	−0.17
CDQ-A	39	0.19	0.08	0.09	0.38*
CDQ-B	39	0.22	0.10	0.09	0.36*
RT-1′	32	0.18	0.23	0.22	0.24
RT-2′	32	0.26	0.32	0.38*	0.28
RT-3′	32	0.25	0.33	0.40*	0.18
RT-4′	32	0.21	0.38*	0.37*	0.17
RT-5′	32	0.25	0.36*	0.18	0.14
PAULI-15′	14	−0.21	−0.16	0.14	0.44
PAULI-30′	14	−0.11	−0.06	−0.04	0.42
PAULI-45′	14	−0.15	−0.12	−0.14	0.36
PAULI-60′	14	−0.20	0.02	0.18	0.47
PAULI-err	14	−0.20	−0.13	0.06	0.10
PAULI-tot	14	0.16	−0.13	0.01	0.17
Block Design	57	−0.11	−0.40**	−0.41**	−0.43**
REY PRM-2	57	−0.07	−0.20	−0.27*	−0.06
REY PRM-5	57	−0.17	−0.27*	−0.37**	−0.12
REY PRM-3	57	−0.22	−0.32*	−0.45***	−0.23
REY PRM-4	57	−0.24	−0.43***	−0.52***	0.32*
REY PRM-6	57	−0.13	−0.37**	−0.16	−0.20
REY PRM-7	57	−0.17	−0.40**	−0.17	0.008
Digit Span	40	0.001	−0.45**	−0.30	−0.28
Benton VRT, no. correct	36	−0.21	−0.29	−0.34*	−0.25
Benton VRT, errors	36	0.30	0.41*	0.33*	0.31

[a] cri, cumulative risk index. cri = (age rank − years of school attendance rank + alcohol consumption rank − year of entrance in the job rank − year of testing rank)/5.

* $P = 0.05$.

** $P = 0.01$.

*** $P = 0.001$.

which might reasonably support the hypothesis that the performances of the subjects working in a presumably safe environment would be better than those observed in 1974 and a lower number of altered mental parameters would result from the testing. The population examined consisted of 212 subjects, only 6 of whom had been examined in 1975. Of the battery administered, those tests which had been used in the 1974–1975 study were sorted out and the results compared. The possible confounding factors were considered. The factors which might justify the comparison were (a) the location of the plant in a part of the Region Lombardia where the immigration from other parts of Italy is minor and the great majority of the workers were born and live in a restricted area, about 30 miles around the plant, with very similar lifestyles; (b) the long-term monitoring of the workers carried out by our Institute within the plant or at the Institute; (c) the general acceptance and acquaintance of the workers with the testing situation. The more important confounding factors were (a) the tester effect: three persons, one of us in 1974–1975 and two other technicians trained in our laboratory administered the

tests in the eighties; (b) the changes introduced in the National Education System: most of the 1974 subjects had only 5 years of school attendance while the younger generations all have a minimum of 8 years of school; (c) the changes occurring in the small Italian province with changes in lifestyles and wider general access to information sources. The analysis was carried out on the performances of 93 subjects seen in 1974–1975 and 212 subjects seen in 1989–1990. From the battery of tests used in 1989–1990, Raven PM38, Block Design, Rey Memory Scale (subtests 2, 5, 3, 4, 6, 7), and Eysenck MPI were considered. The subjects of each group were divided into three categories, those who had been exposed for less than 5 years, those exposed for 6 to 10 years, and those exposed for more than 10 years, for a total of six groups (Table 5).

Statistical analysis is shown in Table 6. Prevalences of abnormal results in the entire population and within the groups were observed by means of percentage of results falling outside two standard deviations from the reference values used in our laboratory. Means, standard deviations, and other distribution parameters were calculated. The differences between the groups were tested by means of the nonparametric Mann–Whitney *U* test. The association of interfering factors, such as age, educational levels, alcohol, and test results were analyzed by means of Pearson correlations on the entire population and in the three length of exposure groups.

Study 5: Results. There is no trend related to the length of exposure in the percentages falling outside two standard deviations with the exception of Raven PM 38, MPI-E, and Rey 3 within the 1989–1990 group and Block Design within the 1974–1975 group. The values are in most cases around 5–10%. For Block Design only, 25% of the scores fell outside two standard deviations in the 1974–1975, more than 10 years exposure subgroup.

The Mann–Whitney *U* test performed on the two entire 1974–1975 and 1989–1990 groups showed that the 1974–1975 group performed significantly below the 1989–1990 subjects and had significant differences on school attendance and alcohol consumption (Table 7).

TABLE 5
STUDY 5: GROUPING OF SUBJECTS IN THE 1974–1975 AND 1989–1990 COMPARISON STUDY (93 AND 212 SUBJECTS)

Length of exposure	1974–1975			1989–1990		
	n	*x*	δ	*n*	*x*	δ
<5 years	22			57		
Age		32.40	7.96		26.18	5.39
Education		5.65	2.30		8.66	2.30
Alcohol		1.66	1.04		0.39	0.58
6–10 years	15			26		
Age		38.71	5.60		36.11	7.92
Education		4.92	2.53		8.65	2.60
Alcohol		2.07	1.49		0.61	0.98
>10 years	56			129		
Age		43.71	5.17		45.60	5.65
Education		5.07	2.48		6.08	2.71
Alcohol		1.97	1.40		1.50	1.09
Total	93			212		

TABLE 6
STUDY 5: STATISTICAL ANALYSIS PERFORMED ON THE TWO MAIN GROUPS AND SIX SUBGROUPS OF CARBON DISULFIDE-EXPOSED SUBJECTS

Prevalence of abnormal results in the six groups as % of measures falling outside 2 SD from the reference values.
Means, standard deviations, range, median, skewness, max score, min score.
Matched pairs within the three length of exposure groups and Mann–Whitney *U* to test the differences within the groups and on the entire population.
Pearson correlation coefficients on age, education, length of exposure, alcohol and test results.

The Mann–Whitney *U* test applied to the distribution parameters of the three matched subgroups showed significant differences. As expected, the groups are different in a longitudinal sense, i.e., the groups with the shortest length of exposure are younger, with more school attendance and better performances than those with more than 10 years of exposure. The groups are different also cross-sectionally, i.e., the descriptive parameter, age, school attendance, alcohol consumption, neuroticism, and extraversion parameters (MPI) and performance parameters showed significant differences with the exception of the Rey test (Table 8). High intercorrelations were observed for the descriptive parameters, age, educational levels, length of exposure, alcohol, and the tests results (Table 9).

DISCUSSION

Our studies indicate: (1) poorer performances by the 1974–1975 subjects compared to the 1988–1989 ones, (2) a consistent improvement in the performances of many subjects seen again in the eighties following the reduction of the exposure levels; and (3) a relatively small morbidity in the subjects seen in the years 1988–1990. Comparison with the relevance of toxic effects related in the literature shows congruence between the results of the Study 1 and those found in other industrial situations. At the time (1974), the levels of exposure were similar in

TABLE 7
STUDY 5: MANN–WHITNEY *U* TEST ON THE DIFFERENCES OF THE DISTRIBUTION PARAMETERS ON THE TWO 1974–1975 AND 1989–1990 AGE-MATCHED GROUPS (n = 73–66)

	X	SD	X	SD	z	P
						2-tailed
Age	39.29	8.19	40.28	8.55	−0.36	0.71
Educational levels	6.19	1.96	5.40	2.02	−2.35	0.01
Length of exposure	14.52	8.75	12.88	8.69	−1.01	0.31
Alcohol (code)	1.36	1.10	2.00	1.30	−2.44	0.01
						1-tailed
Raven PM38	33.93	10.52	31.01	9.04	−1.68	0.05
MPI N	16.40	10.36	18.88	9.70	−1.56	0.05
MPI E	30.29	8.41	27.14	8.25	−2.22	0.01
Block Design	22.37	7.66	19.93	7.85	−1.82	0.02
REY PRM-2	14.50	2.11	13.87	2.29	−1.89	0.02
REY PRM-5	16.98	2.27	17.30	2.06	−0.71	0.47
REY PRM-3	14.34	3.20	14.26	3.12	−0.08	0.93
REY PRM-4	12.74	4.03	11.87	4.05	−1.24	0.21
REY PRM-6	14.20	2.47	14.09	2.63	−0.17	0.86
REY PRM-7	14.25	2.75	14.07	2.92	−0.27	0.78

TABLE 8
STUDY 5: MANN–WHITNEY *U* TEST ON THE DIFFERENCES OF THE DISTRIBUTION PARAMETERS AND TESTS RESULTS IN THE THREE MATCHED PAIRS CARBON DISULFIDE EXPOSURE GROUPS

	1974–1975/1989–1990					
	<5 years		6–10 years		>10 years	
Parameters	*z*	*P*	*z*	*P*	*z*	*P*
Age	3.46	0.0005	−1.10	0.26	−2.48	0.01
Educational levels	4.39	0.0000	−3.58	0.0003	−2.76	0.005
Length of exposure	5.94	0.0000	−0.64	0.51	−0.44	0.65
Alcohol (code)	4.64	0.0000	−3.02	0.002	−1.54	0.12
Raven PM38	−2.30	0.02	−2.90	0.003	−1.32	0.18
MPI N	−0.80	0.42	−2.44	0.01	−1.95	0.05
MPI E	−2.90	0.003	−1.71	0.08	−1.16	0.24
Block Design	−3.20	0.001	−2.04	0.04	−2.27	0.02
REY PRM-2	−0.63	0.52	−1.88	0.06	−1.67	0.09
REY PRM-5	−0.02	0.97	−0.18	0.85	−0.40	0.68
REY PRM-3	−0.10	0.91	−0.02	0.97	−0.21	0.82
REY PRM-4	−1.44	0.14	0.49	0.61	−0.89	0.37
REY PRM-6	−1.05	0.29	−0.22	0.81	−0.61	0.53
REY PRM-7	−0.20	0.83	−0.84	0.39	−0.13	0.89

most of the studies reported. Perceptive and visuomotor abilities as well as reasoning were impaired in many exposed subjects in accordance with the data reported by Hanninen (1971 and 1978) and with the subjective complaints reported by Lilis (1974) and Tuttle and Wood (1977). In the successive controls (Studies 2, 3, and 4), the improvement in the performances of a relevant number of subjects occurred along with an amelioration in emotional state. In Study 4 (1988) only the

TABLE 9
STUDY 5: INTERCORRELATION MATRIX AND SIGNIFICANCE LEVELS FOR AGE, EDUCATIONAL LEVELS, LENGTH OF EXPOSURE, ALCOHOL CONSUMPTION AND TEST RESULTS IN THE ENTIRE POPULATION (n = 287)

	Age	Educational level	Length of exposure	Alcohol
Age	1			
Educational levels	−0.40**	1		
Length of exposure	0.77**	−0.32**	1	
Alcohol (code)	0.37**	−0.22**	0.34**	1
Raven PM38	−0.38**	0.43**	−0.20**	−0.22**
MPI N	0.03	−0.18**	0.02	0.01
MPI E	−0.22**	0.15*	−0.17*	−0.15*
Block Design	−0.33**	0.44**	−0.19**	−0.16*
REY PRM-2	0.07	0.05	0.02	−0.00
REY PRM-5	−0.10	0.20**	−0.08	−0.06
REY PRM-3	−0.13	0.25**	−0.13	−0.03
REY PRM-4	−0.21**	0.31**	−0.19**	−0.03
REY PRM-6	−0.14*	0.24**	−0.11	−0.04
REY PRM-7	−0.20**	0.27**	−0.11	−0.03

* P = 0.05.
** P = 0.01.
*** P = 0.001.

memory indexes both subjective and objective were still impaired. Moreover (Study 3), the relationship with the cumulative risk index was replaced by a significant relationship with age. This change was interpreted as an effect of the working environment ameliorations. Aaserud *et al.* (1990), in their study carried out on 16 subjects exposed for more than 10 years to CS_2 but away from exposure for 4 years, found a considerable neuropsychological morbidity expressed as cerebral or cerebellar atrophy and mental deterioration. The fact that the prevalence of morbidity in our subjects was low can be ascribed to their being younger than the Aaserud subjects and to our levels of exposure that, by 1974, were lower than those found in the Norwegian plant. However, it is impossible to exclude a healthy worker effect in our plant, the strongest subjects being still at work in the eighties. The last study (5) is totally different from the other four in that it compares two independent groups of subjects, one examined in 1974 and one newly examined. The two populations considered are different in terms of work environment, education, lifestyle, and test scores. The hypothesis leading to the study was that the neurobehavioral morbidity found in the 1974 workers should have been absent in the 1990 subjects working in a cleaner environment and being more educated and more informed on how to handle toxic substances. The results have shown that the hypothesis has not been entirely confirmed. The tests scores decrease with increasing length of exposure with the exception of the memory parameters which remain rather stable through the subgroups. The differences between the 1974 exposed and the 1990 exposed remain significant when age and educational levels are controlled, thus suggesting the presence of factors inside the groups which can influence the outcomes. If it is difficult to control all the variables possibly intervening in 15 years, amelioration of the work environment is certainly one of these factors resulting in the better performances of the 1990 subjects. However, also in this group, the presence of abnormal results with special reference to the memory parameters indicates that the working conditions might not be as safe as believed. The Rey parameters, where more abnormal results were found, reflect a lack of attention and reduced perceptive ability which suggest the typical, even if transient, signs of solvent-induced dysfunctions. The correlations, observed in the 1988 study (4), between biological and environmental data and the indexes of reduced vigilance as "absentmindedness" and reaction times, already discussed, seem to suggest that this might indeed be the case.

The studies carried out over these 15 years and reported in the present paper had also the objective of verifying the validity of periodical long-term monitoring as an instrument to determine the general well-being of a population exposed to neurotoxic agents through the years and the effects of introduced changes and environmental ameliorations. The experience has been extremely positive on an individual basis, thanks to the ability to sort out those subjects who present with the first signs and symptoms of central nervous system dysfunction or an increased susceptibility eventually leading to those dysfunctions. On the contrary the planning of studies comparing hundreds of subjects over a period of 15 years must consider a number of variables outside any possibility of control.

However, despite these limitations and possible sources of bias, the above reported studies indicate the trend of a progressive reduction in the incidence of neurobehavioral disturbances in subjects exposed to long-term low levels of carbon disulfide along with the ameliorations in the working ambient air. But they also call for further measures to control the possible presence of higher peaks of

exposure during the working hours and to sort out those individuals who might show increased susceptibility to the toxin.

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Adaptation of the WHO NCTB for Use in Poland for Detection of Effects of Exposure to Neurotoxic Agents[1]

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The purpose of the project was an assessment of psychometric characteristics of the WHO Neurobehavioral Core Test Battery (NCTB) (in traditional version) and the Hanninen Subjective Symptom Questionnaire adapted for Poland. The subjects were 119 greenhouse workers, 65 of them were exposed to organophosphorous pesticides and 54 were nonexposed ones. To assess the reliability, all tests from NCTB were administered twice with a 4-month interval. The results obtained in the first examination were used to determine the effects of gender, age, and education on tests performance. To determine the underlying factor structure of the test battery, the factor analysis was performed on 45 variables. The test–retest reliability coefficients ranged from 0.33 to 0.91. The lowest correlations were found for the Aiming Test—sum of errors ($r = 0.33$) and the Benton Test ($r = 0.34$). Gender appeared to be the most modifying factor of test performance. The factor analysis yielded 11 factors with eigenvalues greater than 1.0 (78.4% of variance explained).

INTRODUCTION

In recent years, there has been a growing concern regarding application of psychological tests for assessment of neurobehavioral effects of exposure to neurotoxic substances in the workplace. Many industrially used chemicals cause deterioration of central nervous system functions and a psychological test can be used for early detection of subclinical symptoms of this malfunctioning (Hanninen, 1983). In many laboratories, test batteries which consist of different tests are used. These batteries measure the similar psychological domains such as intellectual performance, memory, perceptual motor speed and accuracy, and psychomotor abilities (Cassitto, 1983). Because psychologists use different test batteries it is almost impossible to make a comparison of results obtained which allow the formulation of general conclusions. This was the reason that the group of experts sponsored by WHO and NIOSH recommended a short paper and pencil test battery which should be used as a core of every applied battery. The Neurobehavioral Core Test Battery (WHO NCTB), consisting of seven tests, is presented in detail by Johnson (1987). The methodological studies on the WHO NCTB were performed by Cassitto *et al.* (1990) and Emmen *et al.* (1988).

In Poland, we applied the WHO NCTB in several field studies and now we answer the following questions which will broaden our knowledge for applying this test battery:

1. What is the factor structure of the WHO–NCTB?
2. What is the reliability of this particular test?

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3. What are the effects of age, sex, and education on test performance and test results?

METHODS

The group of subjects consisted of 119 greenhouse workers. Sixty-five subjects were exposed to organophosphorous pesticides at their workplace. The men of this group were working as sprayers and the women were employed in cultivating flowers and vegetables. Fifty-four individuals were nonexposed subjects and were working in a canteen, office, and another part of the enterprise where pesticides were not used. The age and the years of education distributions of the subjects are presented in Table 1.

The subjects were tested with the WHO NCTB during a single test session. The sequence of the tests in the battery was the same for all subjects. They were tested twice with a 4-month interval. The first examination was carried out in winter before the extensive use of pesticides began. The tests were administered in a plant medical laboratory.

Data were analyzed using the SPSS/PC+ statistical software package. Test–retest reliability was determined using the Pearson product–moment correlation coefficient. The effect of age, education level, and sex on test results was calculated by a multiple regression analysis with an indicative variable for sex. A factor analysis was performed by using the principal components analysis program (VARIMAX).

RESULTS

The subjects were well motivated to take the tests because they were paid for participation in the study. Execution of the tests did not cause any problems for the subjects. To have more information on the test performance and to distinguish low reliable indicators we decided to use as many test measures as we could, e.g., for the Santa Ana test we used seven measures (indicators) but for the Benton test we used only one. The list of the tests measures and abbreviations labeling them is presented in Table 2.

In Table 3 mean scores for exposed and control subjects in the first and second session are presented. All cases in which both exposure and session factors affected the results as well as the significant t test for differences between the exposed and control subjects, found in the first examination, are marked. These

TABLE 1
AGE, SEX, AND EDUCATION LEVEL DISTRIBUTION OF THE SUBJECTS

	Men		Women	
	Exposed	Nonexposed	Exposed	Nonexposed
Number	39	28	26	26
Age				
Mean	31.4	35.3	35.4	33.8
SD[a]	7.7	8.8	7.9	7.8
Years of education				
Mean	11.2	11.1	10.3	11.8
SD	2.9	3.1	1.9	1.6

[a] SD, standard deviation.

TABLE 2
LIST OF TEST MEASURES (INDICATORS) AND THEIR ABBREVIATIONS SELECTED FOR STATISTICAL ANALYSIS

Name of test	Indicators	Abbreviation
POMS	TEN	Tension–anxiety
	ANG	Anger–hostility
	FAT	Fatigue–inertia
	DEP	Depression–dejection
	VIG	Vigor–activity
	CON	Confusion–bewilderment
	FRI	Friendliness
SRT	NCR	Number of correct reactions
	NOR	Number of omitted reaction
	MET	Mean time of reaction
	STA	Standard deviation
	QTR	The quickest reaction time
	LTR	The lowest reaction time
Digit Symbol (W)	DSW	Number of symbols done correctly (WAIS)
Digit Symbol (M)	DSM	Number of symbols done correctly (modified version)
Digit Span	DSF	Number of digits repeated forward
	DSB	Number of digits repeated backward
	DST	Sum of repeated digits
Santa Ana	SAP I	No. of pegs, pref. hand, 1st session
	SAP II	No. of pegs, pref, hand, 2nd session
	SAN I	No. of pegs, non-pref., 1st session
	SAN II	No. of pegs, non-pref., 2nd session
	SAPT	Sum of pegs, pref. hand
	SANT	Sum of pegs, non-pref. hand
	SAPN	Sum of pegs, pref. and non-pref.
Benton	BEN	Number of correct answers
Aiming	ANC I	Number of dots, 1st session
	ANC II	Number of dots, 2nd session
	AER I	No. of errors, 1st session
	AER II	No. of errors, 2nd session
	ANC	Sum of correct dots
	AER	Sum of errors
	AIT	Total number of dots, 1st session
	AIIT	Total number of dots, 2nd session
	ATO	Total number of dots 1st and 2nd session
Subjective Symptoms Questionnaire	FAS	Fatigue
	SDS	Sleep disturbances
	ABS	Absentmindedness
	DES	Depression
	LAS	Lability
	NVS	Neurovegetative
	GAS	Gastrointestinal
	NIS	Neurological I
	NIIS	Neurological II
	SSS	Sum of symptoms

results are shown to enable comparing them with the results obtained by other investigators. They should also be taken into account when analyzing the results presented in Tables 4 and 5.

Some dimensions of mood appeared to be sensitive to exposure to pesticides. The subjects exposed were more prone to depression and less vigorous than the

TABLE 3
THE TWO-FACTOR ANALYSIS OF VARIANCE [SESSION (S) AND EXPOSURE (E)] AND *t* TEST FOR PARTICULAR DIFFERENCES BETWEEN MEAN RESULTS FOR EXPOSED (Ex) AND CONTROL (C) SUBJECTS IN THE FIRST SESSION (n = 119)

Name of test	Indicators	1st session		2nd session		Main effects, *t* test $P < 0.05$
		Ex	C	Ex	C	
POMS	TEN	10.7	9.5	8.7	8.9	
	ANG	12.3	10.8	11.0	10.8	
	FAT	10.1	9.0	11.1	9.8	
	DEP	17.7	14.7	16.7	14.7	E
	VIG	17.7	18.8	17.5	18.7	E
	CON	6.6	6.0	5.9	5.5	
	FRI	18.5	19.4	18.6	19.3	
SRT	NCR	89.0	89.0	37.6	89.9	
	NOR	0.0	0.0	0.1	0.1	
	MET	280.0	270.0	279.0	263.0	E
	STA	57.9	51.7	60.3	58.1	
	QTR	201.0	195.0	203.0	189.0	E
	LTR	543.0	520.0	589.0	571.0	
Digit Symbol (W)	DSW	44.1	48.2	48.7	52.8	S, E
Digit Symbol (M)	DSM	78.5	84.4	85.5	95.9	S, E
Digit Span	DSF	5.0	5.2	5.2	5.3	
	DSB	3.8	4.0	4.0	4.1	
	DST	8.9	9.3	9.2	9.4	
Santa Ana	SAP I	18.8	19.0	20.4	21.0	S
	SAP II	21.3	21.7	21.8	21.8	
	SAN I	40.1	40.7	42.3	42.7	S
	SAN II	18.4	18.2	19.7	19.3	S
	SAPT	19.5	19.5	20.5	20.1	S
	SANT	38.0	37.9	40.0	39.1	S
	SAPN	78.3	78.6	82.4	81.3	S
Benton	BEN	8.3	9.0	8.8	8.7	t
Aiming	ANC I	108.0	113.0	112.0	117.0	
	ANC II	114.0	120.0	119.0	122.0	
	AER I	1.4	1.4	2.1	3.1	S
	AER II	1.8	2.8	3.0	3.9	S
	ANC	221.0	229.0	232.0	238.0	
	AER	3.2	4.1	5.0	6.8	S
	AIT	111.0	114.0	115.0	139.0	
	AIIT	116.0	125.0	122.0	126.0	S
	ATO	224.0	239.0	237.0	247.0	
Subjective Symptoms	FAS	1.8	1.9	2.1	2.1	
Questionnaire	SDS	0.8	0.8	0.5	0.6	
	ABS	1.5	1.2	1.2	1.4	
	DES	0.9	1.0	0.7	0.7	
	LAS	1.1	1.1	0.8	0.9	S
	NVS	1.4	1.2	1.4	1.4	
	GAS	0.6	0.6	0.7	0.5	
	NIS	1.0	1.1	1.1	1.2	
	NIIS	0.7	0.6	0.5	0.5	
	SSS	9.6	9.4	8.9	9.1	

controls. The former had higher mean reaction time and the quickest reaction time as well as lowered performance in two versions of the Digits Symbol and Benton tests. However, these findings should be interpreted, with respect to adverse effect of exposure, with great caution because some other important modifying factors have not been taken into account in the analysis. This problem has been presented in another paper (submitted for publication).

In Table 3 the effect of learning process on the performance of the Digit Symbol and on some indicators of Santa Ana and the Aiming tests is to be noted.

Table 4 reports the test–retest reliability coefficients of all tests measures. There were no apparent differences between the exposed and nonexposed groups in the test–retest correlations.

From two subjective methods used in the study, the POMS appeared to be more reliable than the Subjective Symptoms Questionnaire (SSQ). In the first test, the level of reliability of almost all measures exceeded 0.60. As for the SSQ only two measures (NIS, SSS) met this criterion. This means that the level of reliability of the SSQ is low. The difference in the level of reliability between the tests results from the different forms of these tests.

A mean reaction time has reached an acceptable level (0.80) among all measures of the SRT test. Two versions of the Digit Symbol test appeared to be the most reliable methods in the WHO–NCTB. The reliability level of the memory test (the Digit Span and the Benton) is very low. As for the first, the DST measure reached

TABLE 4
TEST–RETEST RELIABILITY OF THE WHO NCTB (n = 119)

Name of test	Indicators	r	Name of test	Indicators	r
POMS	TEN	0.61	Santa Ana	SAP I	.63
	ANG	.65		SAP II	.60
	FAT	.66		SAN I	.53
	DEP	.72		SAN II	.64
	VIG	.56		SAPT	.72
	CON	.73		SANT	.67
	FRI	.75		SAPN	.72
SRT	NCR	.01	Benton	BEN	.34
	NOR	.55	Aiming	ANC I	.74
	MET	.81		ANC II	.69
	STA	.39		AER I	.48
	QTR	.49		AER II	.48
	LTR	.41		ANC	.66
Digit Symbol (W)	DSW	.90		AER	.57
Digit Symbol (M)	DSM	.91		AIT	.33
Digit Span	DSF	.55		AIIT	.64
	DSB	.45		ATO	.65
	DST	.60	Subjective Symptoms	FAS	.51
			Questionnaire	SDS	.27
				ABS	.50
				DES	.54
				LAS	.36
				NVS	.55
				GAS	.55
				NIS	.72
				NIIS	.57
				SSS	.73

the relatively high level of reliability (0.60) and this test variable should be recommended for further studies. But the reliability of the Benton test is so low that one should consider excluding this test from the WHO–NCTB.

We have decided to estimate reliability for several measures of the Santa Ana test. The global measures such as the SAPT, the SANT, and the SAPN are reliable on a moderate level but they have reached the highest level among other measures of these tests. In the case of the Aiming test, we have also calculated reliability coefficients for several measures of this test. The measures based on the number of correctly placed dots are more reliable than the measures based on the number of errors. Although this level of reliability is not high it may be accepted.

In Table 5, results are presented showing the effects of age, education, and sex on test performance and tests measures. There is a positive relationship between age and the SRT measures and the FRI, the NIS, the FAS, and the SDS measures of the Subjective Symptoms Questionnaire. It means that older individuals have a longer reaction time, are more tired, have more sleep disturbances, and have more neurological symptoms than younger subjects, but the older subjects expressed a more friendly mood. Psychomotoric abilities of older people are significantly impaired. Negative relationships between age and the results of the Santa Ana test, the Aiming, and the Digit Symbol confirm that conclusion. Surprisingly, only the memory tests appeared to be free of age influence.

The education level affected the psychomotoric tests performance and memory tests results. More educated subjects performed better on psychomotoric and memory tests. We have not found any statistically significant relationship between education and performance of the SRT test. If we look at the results obtained by means of the subjective methods we can observe that the education level has been negatively correlated with the reports of neurological and neurovegetative symptoms and moods of depression, confusion, and friendliness.

The sex of the examined subjects affected subjective measures distinctly. The correlation coefficients between sex and almost all dimensions of POMS and SSQ are statistically significant. The results of two versions of the Digit Symbol, the Benton, the Aiming, and the mean score of the SRT are also positively correlated with sex. This means that mean scores for women were higher than those for men. Only in the Santa Ana test did men obtain higher scores than women.

To determine an underlying factors structure of the WHO NCTB the principal components analysis has been performed on 45 measures of the test battery. We have decided to use many measures to check the measures on particular tests loaded by the same factor. An orthogonal 11-factor solution has been accepted as the best approximate description of the factor loading the WHO NCTB. These factors have accounted for 78.4% of the total variance.

Almost all subjective measures are loaded in the factor 1. Only a few other subjective measures are loaded in other factors (the VIG and the FRI, factor 8; the SDS, factor 9; the GAS, factor 11). This means that the factor 1 deals with emotional status of subjects. The Aiming measures relating to correctly placed dots and two versions of the Digit Symbol have the highest loading in the factor 2 which represents a perceptual motor speed. The measures of errors in the Aiming are loaded in the separate factor 4 relating to precision of hand movement. All measures of the Santa Ana test, which indicate visuomotor coordination abilities, are loaded in factor 3. The Digit Symbol test and the Benton test loaded in the factor 5 represent memory ability. The measures of the Simple Reaction Time

TABLE 5
EFFECT OF AGE, EDUCATION LEVEL, AND SEX ON THE WHO NCTB RESULTS (n = 119)

Name of test	Indicators	Age r	Education r	Sex r	R^2
POMS	TEN	−0.06	0.02	0.28	0.09
	ANG	−0.11	0.03	0.18	0.05
	FAT	0.08	−0.04	0.43	0.19
	DEP	−0.04	−0.17	0.37	0.18
	VIG	0.13	−0.10	−0.08	0.03
	CON	−0.06	−0.15	0.29	0.12
	FRI	0.18	−0.17	0.00	0.05
SRT	NCR	−0.05	0.11	0.14	0.03
	NOR	0.05	−0.11	−0.14	0.03
	MET	0.18	−0.06	0.18	0.06
	STA	0.28	0.02	0.06	0.09
	QTR	0.16	−0.08	0.18	0.06
	LTR	0.29	−0.06	0.13	0.09
Digit Symbol (W)	DSW	−0.30	0.44	0.27	0.33
Digit Symbol (M)	DSM	−0.34	0.46	0.15	0.31
Digit Span	DSF	−0.04	0.31	−0.01	0.09
	DSB	−0.08	0.27	−0.02	0.07
	DST	−0.08	0.36	−0.02	0.13
Santa Ana	SAP I	−0.19	0.30	−0.15	0.09
	SAP II	−0.24	0.28	−0.14	0.14
	SAN I	−0.17	0.15	−0.16	0.13
	SAN II	−0.17	0.17	−0.07	0.05
	SAPT	−0.24	0.28	−0.09	0.06
	SANT	−0.20	0.17	−0.07	0.06
	SAPN	−0.24	0.24	−0.11	0.11
Benton	BEN	−0.08	0.20	0.17	0.07
Aiming	ANC I	−0.32	0.30	0.27	0.25
	ANC II	−0.27	0.31	0.34	0.28
	AER I	0.06	−0.13	0.07	0.02
	AER II	0.05	−0.16	0.03	0.03
	ANC	−0.23	0.23	0.34	0.22
	AER	0.06	−0.16	0.05	0.03
	AIT	−0.19	0.28	0.23	0.16
	AIIT	−0.19	0.32	0.36	0.27
	ATO	−0.26	0.22	0.33	0.23
Subjective Symptoms	FAS	0.15	0.10	0.30	0.13
Questionnaire	SDS	0.35	−0.07	0.10	0.13
	ABS	−0.16	0.06	0.36	0.17
	DES	−0.05	−0.08	0.47	0.24
	LAS	−0.13	0.13	0.37	0.18
	NVS	0.11	−0.25	0.55	0.36
	GAS	−0.13	−0.04	0.16	0.05
	NIS	0.20	−0.19	0.40	0.21
	NIIS	0.03	−0.16	0.36	0.15
	SSS	0.04	−0.10	0.55	0.31

Note. $r = 0.15$, $P < 0.05$; $R^2 = 0.06$, $P < 0.05$.

are loaded in different factors. However, the factor 6 represents this test because it includes mean reaction time, standard deviation, and the lowest reaction time. The most important trait of the measures in this factor is probably attention and response speed. The rest of the measures are loaded in specific factors. Among

them, the factor 8 is worth distinguishing because it probably represents quite a different emotional status than the rest of the POMS dimensions.

CONCLUSIONS

1. The WHO NCTB appeared to be very easy to administer. It covers a large enough range of psychological functions and therefore it may be used in field studies carried out on general population subjects.

2. The test–retest reliability of most of the tests is not high enough to take into account psychometric requirements but the level of correlation coefficients of main measures is similar to results obtained by Emmen *et al.* (1988). We have decided to continue our study because the level of test reliability could depend on the characteristics of subjects. Only the Benton test reliability appeared to be so low that we suggest excluding this test from the battery. Also, in Dutch investigation, the Benton test reliability was very low (Emmen *et al.*, 1988).

3. Age, sex, and education have effected test performance and therefore the exposed and nonexposed subjects in case-control studies must be carefully matched according to these variables.

4. The factor analysis based on detailed measures of used tests performance has revealed that the measures derived from the given test are loaded in the same factor. There are some exceptions. The most interesting exceptions are: (a) The vigor and friendliness dimensions are loaded in a factor other than the rest of the POMS measures. The same result was obtained by Cassitto *et al.* (1990). We are inclined to infer that the vigor variable relates to temperment characteristics of an individual, not to emotional state. (b) The measures derived from the Aiming test basing on the number of correctly placed dots and on the number of errors are loaded in different factors.

5. The tests measuring visual and auditory memory are loaded in the same factor. This fact supports our above-mentioned suggestion excluding the Benton test from the WHO NCTB.

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Neuropsychological Effects of Chronic Exposure to Environmental Dioxins and Furans[1]

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The environmental contamination by dioxins and furans (PCDD/PCDF) of a local area in southwest Germany due to pyrolytic processes led to a survey of health consequences in the exposed population. 2,3,7,8-TCDD (8000 ng/kg TE (ppt)) was found in the soil and up to 585000 ng/kg TE in attic dust in private homes. In a randomized study group of definitively exposed persons, a neuropsychological test battery was applied and its value as a diagnostic tool investigated. A total group of 19 persons participated in a standard neuropsychological examination including common procedures to evaluate mnestic and attentional performance and psychomotor speed (e.g., WAIS, WMS-R, TMT, and symptom and mood checklists). The range of PCDD/PCDF between 16 and 80 (mean 31) ppt did not vary substantially from blood fat values in a national sample. Results of neuropsychological testing showed only slight deviations from the expected range. Nevertheless, in a high-level exposure group, a reduction of verbal conceptualization, mnestic organization of verbal and visual stimuli, and psychomotor slowing was found. Among other correlations visual exploration speed (TMT) was most directly related to TE. Affective symptoms (such as irritability and emotional instability) were also related to exposure. Results indicate that standard neuropsychological testing can be recommended for the routine evaluation of chronic dioxin exposure.

INTRODUCTION

Changes in cognitive and affective functioning are early signs of neurotoxic damage. Patterns of behavioral change as a result of acute or chronic exposure to pesticides, organic solvents, and/or heavy metals have been main topics of the evolving research in neuropsychological toxicology (Hartman, 1988; Feldman *et al.*, 1980). Neuropsychological deficits may involve disturbances in memory, attention, visuomotor coordination, and visuoperceptual speed (Lezak, 1983). Additionally, affective symptoms such as irritability and depression as well as psychophysiological disorders frequently have been reported as concomitants of neurotoxic exposure. Neuropsychological measures may thus be interpreted as "indicators of effect." They may provide greater information about subclinical sequelae than internal dose indicators (Foa, 1982; Hartman, 1988).

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However, little is known about PCDD/PCDF uptake and metabolism in chronically exposed humans. The possible pathways are inhalation and ingestion of PCDD/PCDF-contaminated flue gas, dust particles, and food. Results of several mortality studies of employees accidentally exposed to 2,3,7,8-TCDD in the German Chemical industry showed increases of the standardized mortality rates for cancer and suicides (Thiess *et al.*, 1982; Zober *et al.*, 1990; Manz *et al.*, 1991). Studies of children who had been prenatally exposed to cooking oil contaminated with polychlorinated biphenyls and dibenzofurans reported lower birthweight and a threefold increase of developmental or psychomotor retardation, whereas no peripheral neurologic defects were registrated (Rogan *et al.*, 1990).

If a developmental delay is caused by early neurotoxic damage of the CNS, neuropsychological tests are adequate methods for evaluating particular neurotoxic effects caused by chronic exposure to PCDD/PCDF in adults, even if no overt clinical signs or neurological impairments are evident. Thus far, however, neuropsychological studies have failed to demonstrate neurotoxic effects in exposed populations (reviewed by Hartman, 1988).

The following study was initiated after a PCDD/PCDF-contaminated area had been detected in a residential area next to a metal reclamation plant in Rastatt, southern Germany. About 1500 persons lived and/or worked next to the production site for more than 20 years. They were exposed to air-borne PCDD/PCDF of flue gas and to vegetables grown on PCDD/PCDF-contaminated soil. An area of 500 m in diameter was found to be contaminated with between 50 and 100 000 ppt (ng/kg) toxic equivalency (TE). TE was calculated, relative to 2,3,7,8-TCDD, according to recommendations of the Federal Public Health Office (BGA) (Appel *et al.*, 1990).

To evaluate health effects, a cross-sectional study was performed in 450 unselected volunteers from this area (Klett *et al.*, 1991). A clinical and laboratory trial with self-reported symptoms and a comprehensive history of life-style was performed. PCDD/PCDF concentrations were analyzed from blood samples of a randomly selected sample of 22 persons from the investigated group (Wuthe *et al.*, 1990). The same persons also took part in a neuropsychological and neurobehavioral investigation reported here.

MATERIALS AND METHODS

Subjects

Twenty-two persons were randomly chosen from the population of 450 persons and 19 persons of this group (8 males, 11 females) participated in a neuropsychological examination. Three children were not tested. The mean age was 42 years (range 14–70), mean duration of exposure 21 years (5–25). The exposed group lived in the immediate vicinity of the metal reclamation plant mentioned above (mean distance 100 m). During season 11 persons consumed crops from their own vegetable garden contaminated with PCDD/PCDF (range 200–8000 ppt (ng/kg TE soil). All persons underwent a clinical and general neurological examination including a comprehensive history of medical events, life-style and environmental risk factors, and dietary habits.

Estimation of Exposure from Environmental Sources

External exposure from air and soil was evaluated by other authors retrospectively. Indoor and outdoor air pollution with PCDD/PCDF (0,1–2,2 fg/m^3 TE) cannot be regarded as a major source of PCDD/PCDF uptake. Higher PCDD/PCDF concentrations were found in soil (8000 ng/kg TE) and in the attic dust of private homes (585,000 ng/kg TE), which may have led to a considerable PCDD/PCDF exposure. Toxic equivalents were defined according to the German Public Health Office.

A reliable cumulative external exposure index could not be estimated because of the different exposure pathways including food intake, which is a major source in Germany (1,3 pg/kg bw TE; Riss *et al.*, 1989; Beck, 1990). Thus, neuropsychological test results were only correlated with blood fat PCDD/PCDF levels as an internal exposure index.

Biological Monitoring

Blood (80 ml) was drawn for PCDD/PCDF analysis. The analysis was performed by "ergo laboratories" in Hamburg (Paepke *et al.*, 1989). Levels of heavy metals were also determined. Levels of PCDD/PCDF per gram fat (ppt or ng/kg) were used as internal exposure indicators and related to the individual test results.

Neuropsychological Tests

Neuropsychological examination included common procedures for evaluating general intellectual, mnestic, attentional, and visual–motor performance.

General intelligence and cognitive functions. Using demographic and education variables, a premorbid IQ was estimated (Wilson *et al.*, 1979). Additionally, an estimated WAIS-IQ as a measure of actual overall intellectual functioning was derived from the information, similarities, block design, and picture completion subtests of the German Wechsler Adult Intelligence Scale (Wechsler, 1964; Dahl, 1972). A verbal fluency task was taken from the "Demenz-Test" (according to Kessler *et al.*, 1988).

Psychomotor speed and visual and auditory attention. Performance measures of psychomotor speed were obtained from the German version of the STROOP Test (reading, naming, and interference (seconds); Bäumler, 1985). In addition, the Trail-Making Test forms A and B were applied (seconds). As a measure of visual and auditory verbal attention, the visual and verbal span subtests of the Wechsler Memory Scale—Revised (WMS-R) were used. A short-term-memory task was administered under distracting conditions, similar to a modification of the Peterson and Peterson (1979) paradigm (Winocur *et al.*, 1984). A spatial equivalent of this task was also administered (Sullivan *et al.*, 1986). Total recall was derived as a performance measure.

Memory and learning of visual and verbal stimuli. Several memory indices were derived from the WMS-R: Logical memory stories and visual designs had to be reproduced immediately and with a 45-min delay. A Selective Reminding Test (Buschke and Fuld, 1974) was used to investigate free verbal recall. It involves the presentation of a word list which consists of 10 verbal stimuli from 10 semantically

distinct classes. This test allows a description of cognitive organization when learning a word list (total recall). A verbal, paired associate learning task was used as a measure of interference and release from proactive inhibition. The procedure was similar to that devised by Winocur and Weiskrantz (1976) (total errors).

A partial cueing procedure, the incomplete figures test first described by Gollin (1960) was applied to investigate recognition memory (recognition score and recognition memory score). Additionally, the total raw score for Kimura's Recurring Figures Test (Kimura, 1963; Hartje and Rixecker, 1978) was recorded.

Self-reported inventories. A German inventory of general health complaints (Beschwerdenliste; von Zerssen, 1975) and an attention questionnaire (Zimmermann and Poser, in preparation) with a three-factorial structure including distractability, fatigue, and drive were used. An adjective checklist (EWL-K; Janke and Debus, 1978) with the factors "well being," "deactivation," "emotional irritation," and "anxiety/depressed feelings" was used to assess mood changes (proportion of agreement). Finally, the Freiburg personality inventory (FPI-R; Fahrenberg *et al.*, 1984) was administered. In an attempt to validate findings, a behavioral rating was performed by relatives of the subjects according to Kessler *et al.* (1988).

Procedure

The clinical and neuropsychological examinations were performed on two different occasions. The clinical interview was applied in the delay period of memory tests. The sequence of test application was devised to minimize interference effects of procedures. The examiners were not aware of clinical and laboratory data. Most self-reported inventories were filled in at home.

All comparisons were made within the study group, separately for low and high PCDD/PCDF exposure (Mann–Whitney *U*-Tests). Some expected values were derived from publications on age-specific findings in the general population (age norms).

RESULTS

Physical and Neurological Examination

All subjects except one were in good general condition for age and showed no signs or symptoms suspicious for acute or chronic poisoning with PCDD/PCDF. In the total group, no signs of neurological impairment were observed.

Laboratory Tests

Results of biochemical laboratory tests were found to be within the range of normal values, except for cholesterol. The latter increased with age, but did not reach levels above 270 mg/dl. Concentrations of PCDD/PCDF, measured in relation to total fat content of blood samples, were found to be in a range between 16.1 and 80.4 ppt, with a mean value of 31.0 ppt (ng/kg TE). Blood fat values were not correlated with exposure time or distance from plant. Personal and exposure data are given in Table 1.

TABLE 1
PERSONAL AND EXPOSURE DATA[a]

	Total group	PCDD/PCDF median		P^b
		Below	Above	
Number of subjects	19	9	10	
Age (years)	41.7 (14.4)	37.6 (14.0)	45.4 (14.5)	n.s.
Sex (Male/Female)	8/11	4/5	4/6	
Estimated premorbid IQ[c]	90.3 (6)	91.1 (7)	89.7 (4)	n.s.
Education (years)	7.9 (1)	8.2 (1)	7.6 (1)	n.s.
Exposure (years)	23.6 (12)	26.3 (15)	21.2 (9)	n.s.
Distance (meters)	116 (85)	86 (80)	143 (85)	n.s.
Exposure variables[d]				
PCDDs				
2,3,7,8 Tetra-CDD	3.4 (1.3)	3.3 (1.1)	3.7 (1.5)	
1,2,3,7,8 Penta-CDD	12.5 (5.9)	9.3 (4.8)	15.4 (5.6)	0.02
Sum Hexa-CDD	59.9 (23.5)	48.0 (16.4)	70.7 (24.6)	0.02
1,2,3,4,6,7,8-Hepta-CDD	84.3 (47.6)	61.6 (32.1)	104.9 (51.4)	0.04
Octa-CDD	550.2 (453.8)	288.2 (76.2)	786.0 (525.8)	0.03
PCDFs				
2,3,7,8-Tetra-CDF	4.9 (2.5)	6.0 (2.6)	4.0 (2.2)	0.09
Sum Penta-CDF	73.9 (57.4)	42.3 (16.8)	102.5 (66.5)	0.02
Sum Hexa-CDF	107.4 (115.9)	54.1 (10.4)	155.6 (146.1)	0.01
Sum Hepta-CDF	42.2 (26.8)	30.7 (6.4)	52.7 (33.9)	
Octa-CDF	4.4 (1.8)	4.8 (0.9)	4.1 (2.3)	
Sum of PCDD and PCDFs				
PCDD	710.4 (513.8)	410.1 (116.7)	980.7 (587.1)	0.01
PCDF	232.8 (194.7)	137.7 (17.8)	318.4 (241.7)	0.05
PCDD/PCDF	943.5 (555.7)	548.0 (122.1)	1299.6 (554.3)	0.0002
High chlorinated DD/DFs	848.7 (529.4)	487.4 (116.2)	1173.9 (548.2)	0.0002
TE[e]	31.2 (19.7)	20.5 (4.4)	40.9 (23.3)	0.007

[a] Means (and standard deviations) are given.
[b] Mann–Whitney *U*-Test.
[c] According to Wilson *et al.* (1978) and Kessler *et al.* (1988).
[d] Expressed in ng/kg blood fat (ppt).
[e] Toxic Equivalents (ppt) according to recommendations of the Federal Public Health Office Berlin (Appel *et al.*, 1985).

Neuropsychological Results

Overall findings. Primary subjective complaints, as reported in the interview and health questionnaire were memory problems (63%) and concentration difficulties (38%). Frequent noncognitive complaints were fatigue (44%), irritability (42%), depressive feelings (37%), and headaches (25%). As vegetative disorders, cold feet (56%), easy blushing (31%), heat flushes (25%), and numbness (25%) were described. However, in self-report inventories, the extent of complaints was not above average when compared to a normal reference population (c.f. Table 2, footnote).

Since a control group was not yet available, age norms of neuropsychological test results were determined (as shown in Table 3). Our results indicate overall

TABLE 2
SUBJECTIVE AND SELF-REPORTED COMPLAINTS

	Total group	PCDD/PCDF median		P^a
		Below	Above	
Health complaints[b]	54.5 (10)	47.8 (10)	59.0 (9)	0.06
Anxiety	0.63	0.14	1.00	0.03
Loss of energy	0.88	0.29	1.33	0.01
Concentration	1.06	0.71	1.33	0.16
Blushing	1.00	0.29	1.56	0.01
Heat flushes	0.75	0.00	1.33	0.004
Inner tension	0.94	0.43	1.33	0.001
Attention questionnaire[c]				
Distractability	54.5 (9)	60.6 (8)	49.7 (7)	0.01
Fatigue	35.3 (5)	38.6 (2)	32.8 (5)	0.01
Drive	25.5 (4)	28.1 (2)	23.4 (4)	0.008
Personality inventory (FPI-R)[d]				
Satisfaction with life	4.7 (2)	5.3 (2)	4.3 (2)	
Inhibitedness	5.5 (2)	4.1 (2)	6.5 (2)	0.05
Excitability	4.6 (2)	3.0 (1)	6.0 (2)	0.002
Aggressiveness	4.7 (2)	5.4 (2)	4.2 (2)	
Feeling of stress	4.1 (2)	3.1 (2)	5.0 (1)	0.07
Physical complaints	4.1 (2)	3.4 (1)	4.7 (2)	
Health concern	5.5 (1)	5.1 (2)	5.8 (2)	
Extraversion	4.0 (2)	5.0 (2)	3.2 (2)	
Emotional instability	4.6 (2)	3.2 (1)	5.8 (1)	0.001
Adjective checklist (EWL)				
Well being	51.7 (23)	63.4 (17)	42.9 (25)	0.07
Deactivation	10.3 (9)	7.0 (10)	12.8 (8)	
Irritability	8.0 (10)	2.8 (4)	12.0 (11)	0.05
Anxiety/depressed feeling	11.4 (10)	7.5 (9)	14.3 (10)	

[a] Mann–Whitney *U*-Test.

[b] Age/sex norm (*T* score [average: 37–63]); Health complaint questionnaire according to von Zerssen (1975); values between 0 and 3 may be achieved in the subscales.

[c] Zimmermann and Poser (in preparation); a higher raw score indicates less complaints; maximum values are 65, 40, 30, respectively; scores were not above average compared to healthy controls (<0.5 SD).

[d] Stanine scores [average: 4–6].

average intellectual functioning. Low average performance as indicated by age norms only can be assumed in a measure of visuomotor exploration speed (TMT) and in delayed recall of verbal and visual material (WMS-R). Acquisition of a word list (SRT) and of new associations (paired associates) also appeared to be slightly lower compared to those of healthy volunteers (>0.8 SD; unpublished data).

Analysis by exposure status. Subjects were compared according to levels above (A, $n = 10$) and below (B, $n = 9$) the PCDD/PCDF median (810.6 ng/kg). As shown in Table 1 differences between both groups could not be established for 2,3,7,8-TCDD, whereas significant differences were found for PCDD, PCDF, the sum of PCDD/PCDF, and TE. Seven persons from group A had consumed self-

TABLE 3
NEUROPSYCHOLOGICAL RESULTS[a]

	Total	Norm[b]	PCDD/PCDF Median		P[c]
			Below	Above	
Cognitive functions					
WAIS estimated IQ	98.1 (13)	46	101.9 (10)	94.8 (15)	
Verbal fluency	18.8 (3)		18.2 (3)	19.4 (3)	
Information	12.3 (4)	38	12.4 (5)	12.2 (3)	
Similarities	12.8 (4)	44	15.1 (4)	10.9 (4)	0.02
Picture completion	11.9 (3)	72	13.3 (2)	10.7 (3)	0.03
Block design	21.6 (7)	55	22.7 (7)	20.8 (7)	
Gollin recognition score	18.7 (1)		18.1 (1)	19.3 (1)	0.07
Psychomotor speed, visual, and auditory attention					
Reading[d]	36.0 (17)	47	34.1 (5)	37.6 (23)	
Naming[d]	51.2 (12)	55	52.6 (7)	50.2 (16)	
Interference[d]	90.9 (18)	53	91.8 (15)	90.2 (21)	
Trail-making test, part A	51.5 (29)	32	49.0 (17)	57.2 (34)	
Trail-making test, part B	111.0 (45)	31	107.3 (37)	114.7 (54)	
Verbal span[e]	7.9 (2)	48	8.1 (2)	7.8 (2)	
Visual span[e]	8.1 (3)	49	7.9 (2)	8.3 (4)	
Peterson and Peterson (ver.)	30.0 (8)		32.9 (4)	27.4 (9)	0.20
Peterson and Peterson (vis.)	35.8 (3)		36.6 (4)	35.2 (4)	
Learning and memory					
Logical memory[e]	21.0 (5)	35	22.4 (6)	19.7 (5)	
Logical memory delay[e]	15.5 (5)	29	15.7 (5)	15.4 (6)	
Selective reminding	26.2 (10)		27.0 (9)	25.4 (12)	
Paired associates (errors)	5.0 (4)		3.6 (4)	6.6 (2)	0.01
Recogn. memory for words	4.1 (4)		6.3 (5)	2.1 (2)	0.04
Visual reproductions[e]	31.0 (6)	51	33.3 (3)	29.0 (7)	
Visual reprod. delay[e]	23.4 (9)	37	25.1 (8)	22.0 (11)	
Recurring Figures Test	19.0 (7)	20	20.7 (7)	17.4 (7)	

[a] Means (and standard deviations) of raw scores are given.
[b] Age norm values (mean percentiles [25–75, average; 9–24, low average]).
[c] Mann–Whitney *U*-Test.
[d] Stroop Test.
[e] Wechsler Memory Scale–Revised.

grown crops, in contrast to three from group B ($P = 0.06$). The groups did not differ in age ($P < 0.23$) or education. Smoking, consumption of alcoholic beverages, and dietary habits were not exceeding average. Three persons were previously employed between 10 and 21 years at the plant.

When comparing subjective symptoms of groups A and B, of 24 items of the health complaint questionnaire and the other self-reported inventories, the psychophysiological disorders (heat flushes, blushing) and noncognitive complaints (emotional instability, excitability, inner tension, fatigue, and anxiety) prevailed in group A (statistically significant, c.f. Table 2). In neuropsychological measures, group A performed less well on similarities, picture completion, paired associates,

and the recognition memory task (c.f. Table 3). Referring to the behavioral rating by relatives, most of the subjects were not judged as impaired in cognitive or affective functions.

Correlation analyses. Pertinent correlations are summarized in Table 4, some of which were found to be statistically significant when comparing 2,3,7,8-TCDD and cognitive functions, psychomotor speed, and visual memory. For example visual exploration speed as measured by the Trail-Making Test was significantly correlated with 2,3,7,8-TCDD. Similar associations could be found with respect to TE, its main factor being 2,3,7,8-TCDD. High chlorinated congeners were mainly correlated with measures of mnestic encoding and with self-reported data on health, attention, and mood.

Age, but not education, was significantly correlated with several neuropsychological test results. Age was also correlated with the sum of high chlorinated congeners ($r = 0.47$; $P < 0.04$) but not with TE. As a consequence, significant correlations were computed again, partialling out the effects of age. The association of TE, 2,3,7,8-TCDD, and neuropsychological results were unaffected by age, whereas correlations of high chlorinated congeners and test results became statistically insignificant. As shown in Table 5 the correlations of subjective complaints and high chlorinated congeners were *not* altered by age effects.

DISCUSSION

The aim of the present study was to investigate the effects of chronic exposure to PCDD/PCDF in a population not occupationally exposed. In a random sample

TABLE 4
CORRELATIONS OF NEUROPSYCHOLOGICAL TEST RESULTS AND EXPOSURE MEASURES

	PCDD/F-TE	2,3,7,8-TCDD	Penta/hexa/hepta-CDD/F	Exposure time	Age
WAIS estimated IQ	−0.30	−0.17	−0.07	0.22	0.05
Picture completion	−0.55***	−0.55***	−0.38*	0.19	−0.56***
Gollin recognition score	0.59***	0.57***	0.07	−0.15	0.15
Naming (Stroop)	0.54**	0.58**	0.12	−0.13	0.28
Trail-making test, part A	0.65***	0.76†	0.18	0.03	0.41*
Visual reproductions	−0.54***	−0.48**	−0.24	0.16	−0.38*
Visual reproductions delay	−0.29	−0.48**	−0.16	−0.02	−0.60***
Visual span	−0.36	−0.40*	0.17	0.33	−0.25
Peterson and Peterson (ver.)	0.32	−0.18	−0.65***	0.28	−0.41*
Logical memory	−0.19	0.16	−0.47**	−0.07	−0.39*
Logical memory delay	−0.17	−0.21	−0.44*	−0.34	−0.59***
Selective reminding	−0.04	−0.31	−0.37*	0.14	−0.37*
Recogn. memory for words	−0.35	−0.27	−0.37*	−0.01	−0.73†
Paired associates	0.50**	0.45*	0.24	0.09	0.16
Similarities	−0.49**	−0.21	−0.22	0.39*	−0.00

* $P < 0.10$.
** $P < 0.05$.
*** $P < 0.01$.
† $P < 0.001$.

TABLE 5
CORRELATIONS OF COGNITIVE AND EMOTIONAL COMPLAINTS AND EXPOSURE MEASURES

	PCDD/F-TE	2,3,7,8-TCDD	Penta/hexa/hepta-CDD/F	Exposure time	Age
General health complaints	0.07	−0.15	0.50**	−0.48**	0.18
Physical complaints	0.38	0.23	0.44*	−0.45*	0.24
Distractability	−0.17	−0.08	−0.51**	0.47*	−0.02
Fatigue	−0.29	−0.18	−0.54**	0.34	−0.07
Drive	−0.37	0.17	−0.63***	0.33	−0.05
Well-being	−0.07	0.00	−0.65***	0.22	−0.12
Irritability	0.31	0.13	0.72***	−0.17	0.39
Anxiety/depressive feelings	0.12	0.11	0.58**	0.00	0.16
Feeling of stress	0.22	−0.06	0.63***	−0.09	0.06
Emotional Instability	0.17	−0.11	0.71***	−0.14	0.22
Inhibitidness	−0.03	0.06	0.55**	−0.37	0.18
Aggressiveness	0.29	0.47*	−0.05	0.43*	0.16

* $P < 0.10$.
** $P < 0.05$.
*** $P < 0.01$.
† $P < 0.001$.

of definitively exposed persons, PCDD/PCDF content of blood was determined and correlated with neurobehavioral measures.

The range of TE did not vary substantially from values in a national sample (Paepke *et al.*, 1989; Beck *et al.*, 1989). However, specific congeners (penta-, hexa-, and hepta-CDF) were increased. This corresponded to the distribution found in the contaminated environment. The increase of the typical congeners in blood samples could be explained by the intake of self-grown crops and occupational exposure (Wuthe *et al.*, 1990).

Results of the neuropsychological investigation were within the range of values expected from standardized age samples. Nevertheless, chronic exposure to PCDD/PCDFs accompanied by increased blood levels was associated with a reduction of cognitive performance in verbal conceptualization, mnestic organization of verbal and visual stimuli, psychomotor slowing, and a variety of subjective complaints (e.g., affective symptoms such as irritability and emotional instability).

Given the small number of subjects and the relatively low amount of PCDD/PCDF exposure, the results of our study must be carefully interpreted. Several factors may obscure the relationship between PCDD/PCDF exposure and cognitive performance. The first problem is to clearly define internal or external exposure parameters. A basic assumption was that blood fat PCDD/PCDF levels can be interpreted as valid parameters of the body load due to the environmental PCDD/PCDF contamination. Thus, many factors that influence biological half life may also conceal the relationship between external exposure and body fat burden PCDD/PCDF.

Second, neuropsychological results are confounded by age effects. However, the association of TE or 2,3,7,8-TCDD and test results was not affected by age. Other possible confounders such as alcohol, nicotine intake, or other sources of

environmental exposure (heavy metals and pesticides) can be excluded. Another possible factor is language. German was not the mother tongue for three subjects. Nevertheless, these subjects had been living in Germany for more than 10 years and language comprehension appeared to be adequate.

No investigation was found comparing the relationship between exposure data derived from biomonitoring and neuropsychological effects in a random sample of one well-described exposed population. In those studies which exist, dioxin exposure has always been indirectly inferred from subjective reports, clinical pictures of chloracne, and/or immune system depression (Hoffman *et al.*, 1986; Korgeski and Leon, 1983; CDC, 1988). In a recent study by the Liability Corporation of the German Chemical Industry (Berufsgenossenschaft der Chemischen Industrie, 1991) on 435 of 866 exposed persons from six plants of four companies, dioxin exposure was indirectly inferred from subjective reports. A comprehensive evaluation of the results seems difficult, since neither statistical nor exposure data were given. Furthermore, selection effects cannot be excluded because neurobehaviorally impaired persons might have refused to participate voluntarily. Workers with severe intellectual deviations were excluded from analysis. No explanation was given for *why* these persons appeared to be ill-motivated or even demented. Moreover, the quality of psychometric methods used by the authors did not conform to neuropsychological standards. It is now generally accepted that intelligence scores are not appropriate to validly measure cognitive impairments of patients with possible CNS damage (Lezak, 1983). Thus, a relationship between exposure and extent of subjective complaints or psychometric results could not be ruled out.

Considering the consistencies of independent findings and the increased numbers of suicide and accidents as a cause of death in several studies, the involvement of the central nervous system in the effects of environmental dioxin exposure is a reason for concern. To quantify the relationship, more data on dose–response effects are desirable.

CONCLUSIONS

Results of the neuropsychological examination were within the range of standardized age samples. When separated into two groups of PCDD/PCDF levels above and below median, more prevalent findings were seen in the high-level group, for instance a reduction of cognitive performance in verbal conceptualization, mnestic organization of verbal and visual stimuli, psychomotor slowing, and a variety of subjective complaints (irritability and emotional instability). Thus, our results indicate that neuropsychological methods seem to be relevant tools for the evaluation of neurotoxic CNS-effects of environmental PCDD/PCDF. We recommend neuropsychological testing for the routine evaluation in chronic PCDD/PCDF exposure. Attentional and mnestic tests and computerized tests of visuomotor functions and manual dexterity can be suggested for future research.

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The Use of Visual and Chemosensory Evoked Potentials in Environmental and Occupational Health[1,2]

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The application of visual (VEP) and chemosensory evoked potentials (CSEP) in occupational and environmental health is briefly reviewed. VEPs have been used extensively in experimental neurotoxicology and play an increasingly important role in human neurotoxicity testing. The similarity of VEP waveforms in different species renders them useful for cross-species extrapolation. CSEPs, used in conjunction with traditional psychophysical tests and rating scales, offer a promising new approach to the study of indoor air pollution.

INTRODUCTION

Despite the growing application of sensory evoked potentials (EPs) in neurotoxicity testing of animals, EPs have achieved only limited use in environmental and occupational health field studies. Reasons for the limited use of EP methods, compared to behavioral methods, include the relatively high cost of equipment and the need for specially trained personnel to administer tests and interpret data. EP methods thus, are not optimally suited for field testing or workplace monitoring. However, sensory EPs have shown effects in studies of workers exposed to heavy metals, solvents, and other chemicals. Future trends in the use of visual and chemosensory EPs in human neurotoxicity testing are discussed.

VISUAL EVOKED POTENTIALS (VEP)

The vulnerability of the visual system to chemical insult is well-documented (Merigan and Weiss, 1980; Grant, 1986; Heywood, 1986). Among the 764 neurotoxicants listed by Anger and Johnson (1985), 20% were reported to alter visual function (Crofton and Sheets, 1989). Xintaras *et al.* (1966) pioneered the use of flash EPs in neurotoxicology to study the central nervous system (CNS) effects of carbon monoxide exposure. VEPs have been used extensively to study toxicant effects in animals (Boyes, 1991) and to a lesser extent in humans (Otto *et al.*, 1988). Table 1 lists more than 30 chemicals including metals, pesticides, and solvents reported to alter VEP amplitudes and/or latencies. In view of the known sensitivity of the visual system to chemical exposure, the proven utility of VEPs in clinical neurology (Regan, 1989; Chiappa, 1990; Cracco and Bodis-Wollner, 1986), and extensive application in experimental toxicology (Boyes, 1991), VEPs

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[2] This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency.

TABLE 1
CHEMICALS REPORTED TO ALTER VISUAL EVOKED POTENTIALS IN MAN AND/OR ANIMALS[a]

Solvents	Metals	Pesticides	Miscellaneous
Carbon disulfide	Cadmium	Amitraz	Carbon dioxide
Ethanol	Lead	Carbamates	Carbon monoxide
Hexane	Manganese	Chlordimefon	Formaldehyde
Sulfolane	Methyl mercury	DDT	Methylpyridines
Tetrachloroethylene	Organic mercury	Deltamethrin	Ozone
Toluene	Triethytin	DFP	Pyridine
Xylene	Trimethyltin	Dieldrin	Styrene
		Paraoxon	
		Parathion	
		Permethrin	
		Triadimefon	

[a] Adapted from Otto (1986) and Boyes (1991).

offer an important method for evaluating the toxic effects of chemical exposure which occur in the community or workplace.

A variety of stimuli including diffuse flash, simple and complex patterns, and colors can be used to elicit VEPs. Several types of VEPs commonly used in evaluating the effects of acute and chronic chemical exposure on visual function are reviewed briefly. Detailed descriptions of VEP methods are available elsewhere (Aminoff, 1992; Cracco and Bodis-Wollner, 1986; Regan, 1989; Chiappa, 1990).

Flash Evoked Potentials (FEP)

VEPs elicited by stroboscopic presentation of a diffuse flashing light have been widely used in experimental toxicology due to the ease of administering stimuli, the robustness of response in rats (Boyes, 1991), and the obviation of concerns for fixation and accommodation which must be considered when using patterned stimuli. In the method devised by Dyer and Annau (1977), rats are placed in a small chamber surrounded by mirrors and stimulated at suprathreshold intensity. Diffuse flashes stimulate the afoveal eye of the rat uniformly in all directions. At least 36 known neurotoxicants including metals, solvents, pesticides, anesthetics, and gases have been found to alter FEPs (Boyes, 1991).

Pattern-Reversal Visual Evoked Potentials

A reversing checkerboard pattern, in which light and dark squares alternate position, but maintain a constant space-averaged luminance, is widely used in clinical practice (Chiappa, 1990). According to Spehlmann (1985), pattern VEPs are preferable to flash VEPs because pattern VEPs

1. are most sensitive to visual pathway lesions;
2. exhibit less intersubject variability than flash VEPs;
3. are more convenient for selective stimulation of the left and right visual half-fields; and
4. can be used to test visual acuity.

Figure 1 shows a schematic diagram of a normal pattern-reversal VEP obtained by monocular full-field stimulation. The waveform consists of three peaks labeled

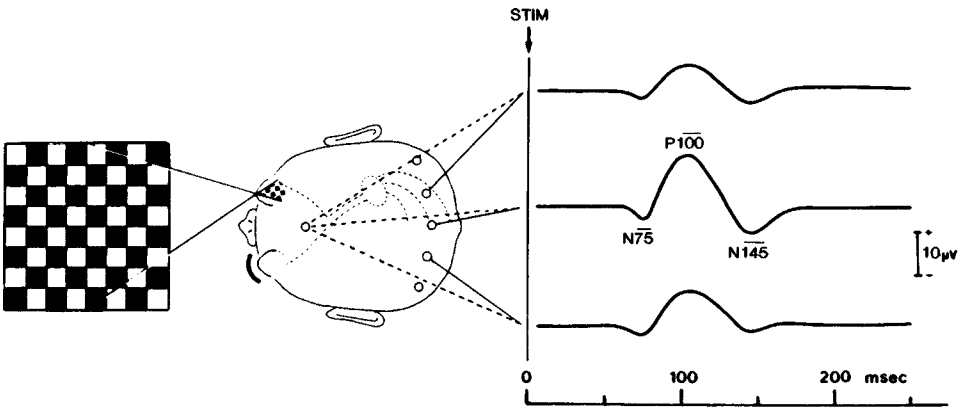

FIG. 1. Schematic diagram of normal transient pattern-reversal VEPs to monocular full-field stimulation. Stimulation of one eye produces VEPs that are distributed approximately symmetrically over both occipital areas with a maximum at the midline. They have a major positive peak (P100), preceded and followed by smaller negative peaks (N75, N145). Positivity at the occiput is plotted upward. Reprinted from R. Spehlmann (1985), with permission.

N75, P100, and N145. The prominent positive peak with a latency of about 100 msec is the feature of primary clinical importance. The pattern-reversal VEP, recorded over the occipital cortex, is normally maximal in amplitude over the midline. P100 latency is particularly sensitive to visual pathway lesions. Optic neuritis (Halliday *et al.*, 1972) and multiple sclerosis (Halliday *et al.*, 1973) cause increased P100 latency which can be observed before other clinical symptoms.

The amplitude and latency of pattern-reversal VEP components vary systematically with stimulus parameters including intensity, check size, presentation rate, and retinal locus of stimulation. Figure 2 illustrates the effect of varying presentation rate. The negative–positive–negative sequence of components which

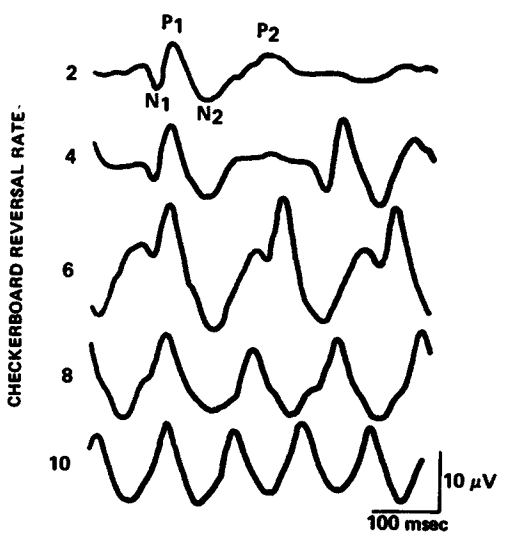

FIG. 2. Pattern VEPs evoked by 12-min checks for different alternation rates shown at left. The components in the transient waveform elicited at the lowest reversal rate begin to overlap as rate is increased, producing a steady-state waveform. Redrawn from Sokol (1980), with permission.

characterize the "transient" VEP occur at presentation rates below about 4 Hz. At faster presentation rates, responses to individual stimulus presentations begin to overlap, forming a "steady-state" response. Frequency domain (e.g., fast Fourier—the decomposition of a complex waveform into sinusoidal components) analysis methods are used to evaluate steady-state VEPs (see Regan, 1989; Chiappa, 1990; Hudnell and Boyes, 1991).

Sine-Wave Grating Visual Evoked Potentials (SWEP)

VEPs can also be elicited by sinusoidal gratings—patterns in which the transition between light and dark elements is gradual rather than abrupt as in the checkerboard pattern or square-wave grating. A Fourier decomposition of these stimuli, illustrated in Fig. 3, reveals an important difference between sinusoidal grating and checkerboard pattern which is related to the difference in transition from light to dark areas. Whereas the grating pattern contains power at only one spatial frequency and orientation, the checkerboard pattern contains power at many spatial frequencies and orientations. The visual system is thought to perform a similar analysis with neurons which respond to specific spatial frequencies

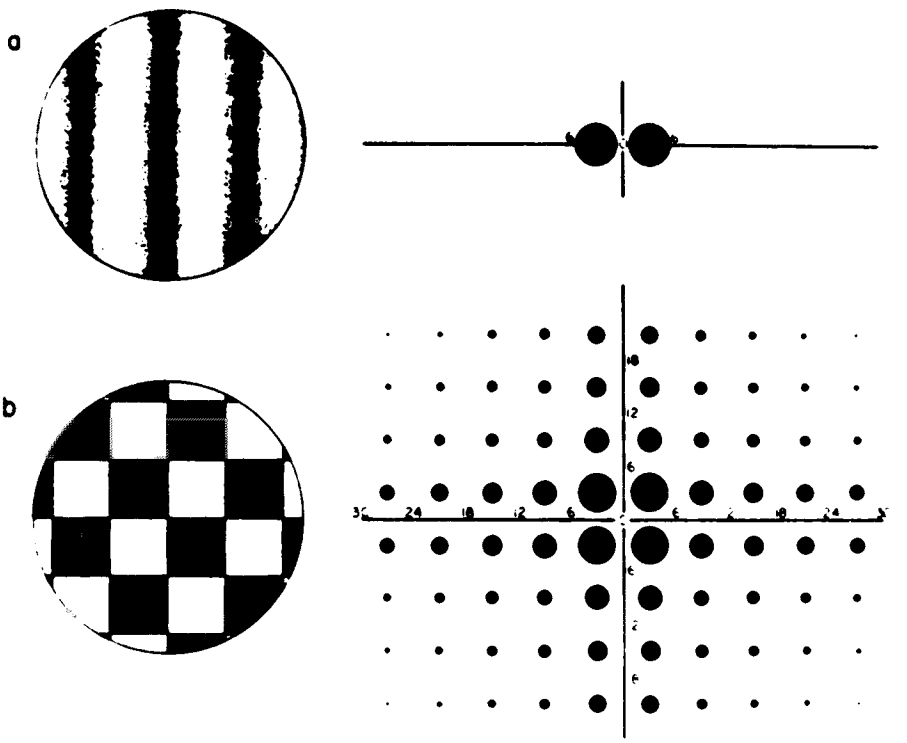

FIG. 3. Stimulus patterns of dark and light elements with gradual (a) and sudden (b) transitions between the dark and light phases and their Fourier spectra. The numbers indicate spatial frequency, and the radial dimensions represent the orientation of the spatial frequency components of each pattern. Whereas the sinusoidal grating contains energy only at the fundamental frequency and a single orientation, the checkerboard pattern contains energy at a number of frequencies and orientations. The relative contribution of each harmonic to the total energy of the pattern is indicated by the size of the dot at each spatial frequency. From I. Bodis-Wollner, M. Ghilardi, and L. Mylin (1986). The importance of stimulus selection in VEP practice: The clinical relevance of visual physiology. *In* "Evoked Potentials" (R. Cracco and I. Bodis-Wollner, Eds.), pp. 15–27. Copyright © 1986. Reprinted by permission of Wiley–Liss, a division of John Wiley and Sons, Inc.

and orientations (reviewed in Hudnell *et al.*, 1990a,b). Therefore, subpopulations of visual neurons can be selectively activated by varying the spatial frequency and orientation of sinusoidal gratings. Checkerboard patterns, on the other hand, simultaneously stimulate subpopulations of visual neurons tuned to a variety of spatial frequencies and orientations. Spatial frequency and orientation-specific effects are well known in the clinical literature (reviewed in Regan, 1989). Due to the ability of sinusoidal gratings to selectively activate neurons tuned to specific spatial frequencies and orientations (as well as temporal frequencies and orientations), SWEPs may provide the sensitivity necessary to detect abnormal function among a small percentage visual neurons.

Figure 4 shows SWEPs elicited by the onset of a grating with a spatial frequency of 4 cycles per degree (cpd) which remains constant in space-averaged luminance. The test stimulus was presented after subjects adapted to a blank field or a grating with a spatial frequency of 0.5, 1.0, 2.0, or 4 cpd. Note that amplitude of the N1 component decreases systematically as the spatial frequency of the adapting stimulus approaches that of the test stimulus. This finding demonstrates the remarkable sensitivity of a specific SWEP component to spatial frequency (Hudnell *et al.*, 1990a).

Many types of sensory evoked potentials including the SWEP can be recorded in different animal species. It is important to assess the comparability of VEPs in humans and rats since rats are typically used in experimental toxicology studies to predict health effects in humans. SWEPs can be recorded from unanesthetized rats by restraining them in a cloth harness located in front of a large test screen. Precise fixation and accommodation of the stimulus is unnecessary, since the rat has a large visual field and depth of field (Hughes, 1977), Figure 5 shows SWEP waveforms recorded in rats and humans (Hudnell *et al.*, 1990b) which demonstrate the interspecies comparability of the spatial frequency adaptation effect on the early, negative component. SWEPs are currently being used in our laboratories to assess issues of cross-species extrapolation (Hudnell *et al.*, 1991b), using neuroactive drugs and abnormal neurological conditions in humans and animal models.

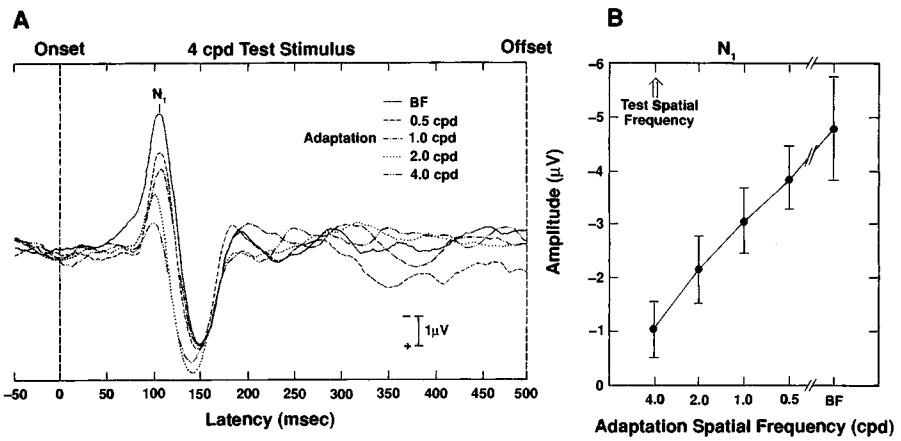

FIG. 4. (A) Pattern-onset VEPs elicited by the 4-cpd grating after subject views a blank field or one of the adaptation gratings (spatial frequency indicated on the right). (B) N1 amplitude averaged across subjects (mean ± SEM) is shown for each adaptation condition with testing at 4 cpd. A log–linear effect of adaptation on N1 amplitude was observed.

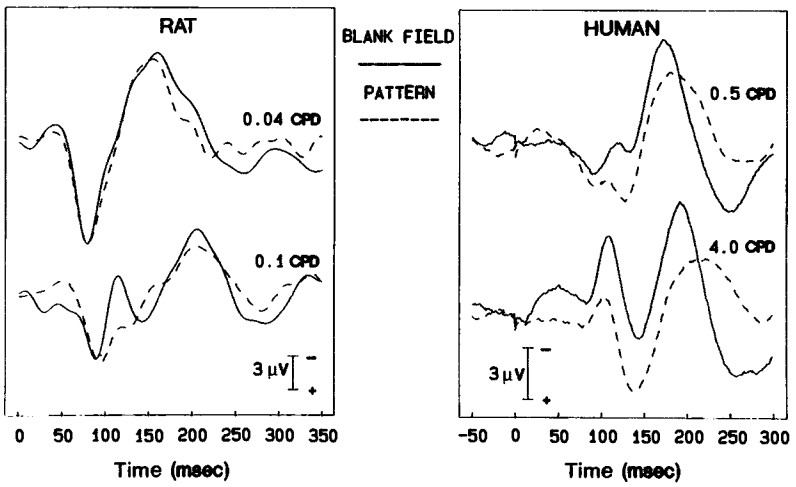

FIG. 5. Group-average pattern-onset VEPs recorded after viewing a blank field or the stationary test grating for 1 min. Pattern adaptation did not significantly affect any component amplitude at the lower spatial frequency for either species. However, the negative components at about 110 msec for both species showed significant amplitude attenuation following pattern adaptation at the higher spatial frequency. Reprinted from Hudnell *et al.* (1990b), with permission.

Application of VEPs in Occupational and Environmental Health

FEPs have been used to evaluate impaired visual function in patients with methyl mercury poisoning (Iwata, 1980) and workers exposed to *n*-hexane (Seppalainen *et al.*, 1979) and xylene (Seppalainen *et al.*, 1981). Less-dramatic changes in FEPs were observed following controlled exposure to carbon monoxide (Hosko, 1970; Groll-Knapp *et al.*, 1978), although carboxyhemoglobin (COHb) levels may have been below the threshold level for effects in visual function. Hudnell and Benignus (1989), for instance, failed to find any effect of carbon monoxide on visual function at COHb levels of 17%. FEPs have not proven to be very useful in clinical applications (cf. Chiappa, 1990), but are still used with infants and young children, comatose patients, and persons with poor visual acuity.

The use of pattern VEPs in environmental and occupational health has increased steadily during the past decade. Pattern VEPs have been used in several studies of workers exposed to solvents. Elofsson *et al.* (1980) reported that P100 amplitude increased with solvent exposure in spray painters. Chang (1987) found longer latencies in workers with subclinical as well as frank polyneuropathy resulting from *n*-hexane exposure. Urban and Lucas (1990) observed increased P100 latency and reduced amplitude in printers exposed to toluene. Of particular interest is the recent study by Altmann *et al.* (1990) in which the latencies of three pattern VEP components increased over 4 successive days of controlled exposure to 50 ppm tetrachloroethylene.

Pattern VEPs have also been used in several studies of workers exposed to lead or other heavy metals. Sborgia *et al.* (1983) reported increased P100 latency in lead workers compared to controls. Araki *et al.* (1987), Murata *et al.* (1987), and Lille *et al.* (1988) did not observe any lead-related effects on P100 latency, although other negative component latencies were reported to be prolonged in the Japanese studies.

Pattern-reversal VEPs have also been used in studies of lead-exposed children.

Winneke *et al.* (1984) reported a paradoxical relationship of P100 latency and blood lead (PbB) level in children living in the vicinity of a lead smelter. That is, P100 latency decreased as PbB increased! However, Lilienthal *et al.* (1990) failed to find any significant relationship of PbB and pattern VEPs in a follow-up study of the same children. Otto *et al.* (1985, 1989) did not find any lead-related change in P100 latency in two studies of North Carolina children, although inconsistent effects were observed on other components of pattern VEPs.

In summary, pattern VEPs appear to be sensitive to solvent exposure in workers, but the results in lead studies are inconsistent. A possible explanation is that lead selectively affects rod-mediated scotopic vision (Bushnell *et al.*, 1977; Fox and Sillman, 1979; Tessier-Lavigne *et al.*, 1985). According to Sokol (1980), pattern VEPs primarily reflect foveal (cone-mediated) vision under normal test conditions. VEPs elicited at scotopic luminance levels from dark-adapted subjects may be more sensitive to lead-induced visual dysfunction. Consistent with this hypothesis, a dose-related decrease in FEP amplitude was observed in dark-adapted monkeys (Lilienthal *et al.*, 1990) exposed to lead. On the other hand, Pb-related increases in FEP latencies were larger in monkeys under high rather than low luminance conditions.

CHEMOSENSORY EVOKED POTENTIALS

Odors and sensory irritation of the eyes, nose, and throat provide important early warning cues that alert us to the potential dangers of chemical exposure in the home and workplace. Odor perception, mediated by the olfactory nerve, and mucosal irritation, mediated by the trigeminal nerve, provide measurable endpoints of chemosensory function. Olfactory and irritation thresholds, furthermore, often occur well below the threshold for other functional or performance deficits. Symptom questionnaires or psychophysical methods are generally used to assess olfactory or irritant thresholds. Symptom questionnaires, however, are notoriously subjective in nature. Psychophysical testing methods can provide more rigorous information for health risk assessment, but other objective measures are needed to complement traditional rating scales.

Scalp-recorded potentials evoked by chemical stimulation of the nasal mucosa were described initially by Finkenzeller (1966) and Allison and Goff (1967). Kobal and Plattig (1978), however, were the first to develop an olfactometer capable of delivering chemical stimuli without exciting thermo- and mechanoreceptors as well. This objective was achieved by injecting odorants into a constantly flowing air stream with controlled temperature and humidity.

Chemosensory evoked potentials (CSEP) represent a relatively new and promising approach for the objective measurement of chemosensory response. Kobal and Hummel (1988) introduced the general term "chemosensory evoked potential" to designate scalp-recorded electrical activity elicited by chemical stimulation of the olfactory and/or trigeminal nerve. These authors distinguish two types of CSEPs. Olfactory evoked potentials (OEP) are elicited by "pure" odorants such as vanillin which anosmics are unable to perceive (Doty *et al.*, 1978). Chemosomatosensory evoked potentials (CSSEP), on the other hand, are elicited by odorless substances, such as carbon dioxide, which produce a painful sensation in the nasal mucosa. Distinguishing between olfactory and trigeminal activity, however, is difficult because the olfactory mucosa contains both olfactory and trigeminal receptors. Most olfactants stimulate both types of receptors in varying degree.

Description of CSEPs

CSEP waveforms resemble other sensory evoked potentials, although the latencies are longer since molecules must penetrate mucosal tissue before stimulating receptors. Figure 6 shows evoked potentials elicited by four different chemosensory stimuli. Vanillin and low-level acetaldehyde selectively activate olfactory receptors, while sulfur dioxide and ammonia primarily stimulate trigeminal receptors. CSEPs were obtained by averaging responses to 16 stimuli presented at 40-sec intervals. Grand means were averaged across 12 subjects. Evoked potentials elicited by the four chemicals have the same general configuration—a negative deflection (N1) followed by a large positive deflection (P2). Trigeminal stimulants tend to elicit larger amplitude, shorter latency responses than olfactory stimulants, although the amplitude and latency vary systematically with perceived intensity. Figure 7 (Kobal and Hummel, 1991) illustrates the relationship of CSEP amplitude and latency to the concentration and perceived intensity of CO_2. Amplitude increases and latency decreases as concentration increases. It should be noted, however, that suprathreshold concentrations are needed to resolve CSEPs from the background electrical noise of the brain. Therefore, olfactory and trigeminal thresholds cannot be derived from CSEPs without using extrapolation techniques (Kobal and Hummel, 1991).

Habituation of CSEPs

Habituation (or decrement of response with repeated stimulation) is a characteristic of nervous system function that is usually ignored in recording sensory evoked potentials. However, odor and irritation perception can show very rapid habituation during olfactometric presentation. For example, Cain (1974) reported that the perceived odor intensity of eugenol and propanol, at moderate concentrations, decreases by 40–50% within about 1 min as subjects breath at a natural

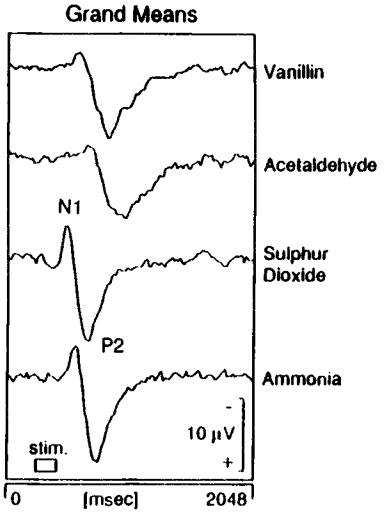

FIG. 6. Chemosensory evoked potentials (Cz) elicited by odorants (vanillin and acetaldehyde) and irritants (sulfur dioxide and ammonia). Grand means were averaged across 12 subjects. CSEPs to trigeminal irritants have larger amplitudes and shorter latencies than responses to olfactory stimulants. Adapted from Hummel and Kobal (1991), with permission.

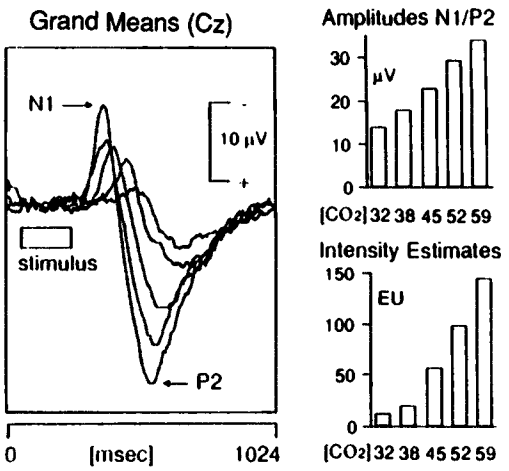

FIG. 7. Influence of different concentrations of carbon dioxide on the CSSEP (n = 20). Stimulus duration = 200 msec. Different concentrations (32, 38, 45, 52, 59%) were randomly presented 10 times each within one session. EU, estimation units according to the length of the visual analogue scale; 100 EU, standard stimulus (45%) that was presented as the first stimulus in the experiments. Reprinted from Kobal and Hummel (1991), with permission.

rate. Effects of habituation on CSEPs are shown in Fig. 8. Kobal and Hummel (1991) presented 16 series of six CO_2 pulses at interstimulus intervals (ISIs) of 2, 4, and 8 sec with an interseries interval of 50 sec. Amplitude decreased sharply between stimulus one and two, particularly at the shorter ISI (i.e., 2 sec). In an earlier study with eucalyptol, Kobal (1981) observed only a 15% reduction in amplitude when the ISI was reduced from 52 to 32 secs, but a dramatic decrease in amplitude at shorter ISIs. Kobal and Hummel (1991) recommend a minimum of 16 stimuli with an ISI of 30 sec for recording CSEPs. Although long ISIs are

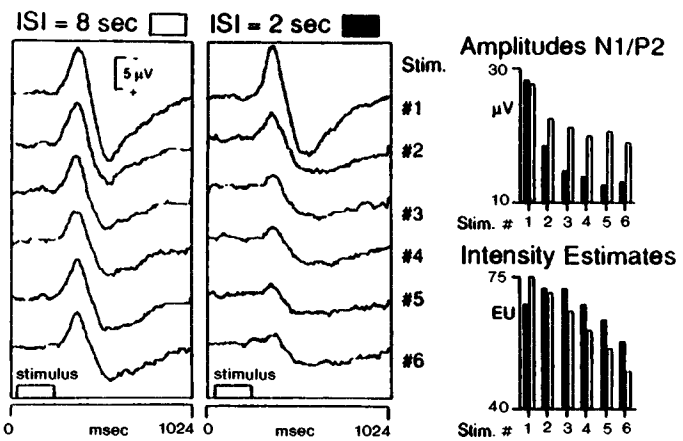

FIG. 8. Influence of stimulus repetition on the CSSEP (Cz; n = 22; 58% CO_2). Interstimulus interval was 8 and 2 sec. Interseries interval was 50 sec. The series was presented 16 times. Grand means and mean values of intensity estimates of 22 subjects. While amplitudes decreased with repeated stimulation, latencies remained unchanged. Intensity estimates decreased when the interstimulus interval of 8 sec was employed but increased for the second and third stimulus when the interstimulus interval of 2 sec was employed. This indicates some additional nociceptive activity which is not reflected in the CSSEP. Reprinted from Kobal and Hummel (1991), with permission.

required, the small number of stimuli needed for signal averaging allow reliable, average CSEPs to be collected in about 8 min.

Distinguishing OEPs and CSSEPs

As noted previously, the waveshape and electrical activity evoked by olfactory and trigeminal stimuli are similar. Kobal and Hummel (1991) have identified two distinguishing characteristics of olfactory and trigeminal CSEPs. (1) Subjects can easily localize whether the left or right nostril is stimulated by an irritant such as CO_2, but they cannot identify the nostril stimulated by an odorant such as vanillin (Kobal *et al.*, 1989). This difference in ability to localize substances which are primarily odorants or irritants is apparently reflected in differences in lateralization of the cortical responses which they elicit. Responses elicited by ammonia and sulfur dioxide show the largest amplitudes contralateral to the stimulated side. Responses to vanillin and acetaldehyde, however, show no differences between ipsilateral and contralateral recording sites (Hummel and Kobal, 1992). (2) The topographical distribution of olfactory and trigeminal evoked potentials differ. Stimulation by odorants such as vanillin or acetaldehyde produce maximal amplitude of the N1/P2 component at Pz or Pz/Cz. Stimulation by irritants such as sulfur dioxide or ammonia, on the other hand, produces a clear amplitude maxima at the vertex. The difference in topographical distribution suggests that different cortical generators are activated by input mediated through the olfactory and trigeminal nerves. This hypothesis is supported by data from a study using magnetoencephalographic techniques (Huttunen *et al.*, 1986). Responses elicited by the irritant CO_2 indicated that the generator lay in secondary somatosensory cortex. However, no generator was found in this area following stimulation with the odorant, isoamyl acetate (Hummel and Kobal, in press).

Application of CSEPs in Occupational and Environmental Health

CSEPs recorded in conjunction with psychophysical or rating scale measures of sensory irritation offer a direct means to evaluate human response to chemical exposure. A problem of growing concern in occupational and environment health which could be addressed with CSEPs is sick building syndrome (SBS)—a diffuse constellation of complaints usually including irritation of the eyes, nose, and throat—which often occurs in occupants of new or renovated buildings. Complaints of unpleasant odors often accompany other SBS symptoms. Controlled exposure to a complex mixture of volatile organic compounds (VOC) representative of new buildings consistently elicits subjective reactions of sensory irritation (Molhave *et al.*, 1986; Kjaergaard *et al.*, 1989; Otto *et al.*, 1990; Hudnell *et al.*, 1992), but objective evidence of impaired performance is more elusive. CSEPs could be used to evaluate the effects of controlled VOC exposure and perhaps to distinguish between olfactory and trigeminal components of SBS.

An extreme form of SBS is multiple chemical sensitivity (MCS), a debilitating condition in which individuals purport to be sensitive to low concentrations of a wide gamut of chemicals, e.g., cigarette smoke, perfume, cleaning agents, and paint (Cullen, 1987). A common complaint of MCS patients is hypersensitivity to odors or irritants. CSEPs offer a noninvasive, objective, physiological method to assess the reported hypersensitivity of MCS patients to chemicals.

Trigeminal nerve impairment has been reported frequently as a consequence of chronic exposure to trichloroethylene, a widely used solvent (Smith, 1966; Waters

et al., 1977). Barret *et al.* (1987) electrically stimulated the lip commissure of subjects to elicit trigeminal somatosensory evoked potentials (TSEP). Abnormal TSEPs were observed in 40/104 workers, consistent with clinical symptoms of facial hypoesthesia. Barret *et al.* concluded that TSEPs provide a reliable method to screen workers for trigeminal lesions resulting from trichloroethylene exposure.

SUMMARY

The application of visual and chemosensory evoked potentials in occupational and environmental health was briefly reviewed. Sensory EPs have been applied extensively in experimental neurotoxicology and play an increasing, though still limited, role in human neurotoxicity testing. EPs provide objective measures of sensory perception and the functional integrity of sensory pathways following chemical exposure. The similarity of EP waveforms in different species including rats and humans renders them useful for cross-species extrapolation. Chemosensory EPs, used in conjunction with traditional psychophysical methods and rating scales, offer a promising new approach to the study of indoor air pollution problems.

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Determination of Evoked Potentials in Occupational and Environmental Medicine: A Review[1]

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The measurement of cerebral evoked and event-related potentials is a promising technique for assessment of subclinical neurotoxicity and has recently been introduced into occupational and environmental medicine. Evoked potentials consist of somatosensory, visual, and auditory evoked potentials, and event-related potentials include the P300 potential. Measurement of these potentials can localize central nervous system impairments caused by exposure to a wide variety of hazardous factors in the workplace and the general environment. This paper is intended to provide an overview of research utilizing these potentials to evaluate the effects of work-related factors. The available data indicate that these potentials are sensitive and reliable methods that are easily standardized and practical to apply in the field setting. Researchers should note, however, that several covariates such as age, skin or body temperature, height, alcohol ingestion, and intelligence can influence assessment of these cerebral potentials in clinical and epidemiologic studies.

INTRODUCTION

The introduction in recent years of several technologies in neurotoxicology such as cerebral evoked potentials (EPs), event-related potentials (ERPs), computed tomography scans, and magnetic resonance imaging has enabled objective and specific evaluation of central nervous system (CNS) dysfunction causally related to a wide variety of hazardous factors in the workplace and general environment. These techniques represent substantial advances over previous diagnostic methods such as electroencephalography (EEG) or skull X rays. The driving force for much of this progress has been the rapid development of computer science. The application to neurotoxicology of computerized technologies has enabled measures of EPs/ERPs—somatosensory, visual, and auditory evoked potentials (SEP, VEP and AEP) and P300—to be introduced into the fields of occupational and environmental medicine (Otto, 1983; Cosi, 1983; Arezzo *et al.*, 1985; Harbin, 1985; Seppäläinen, 1988; Dyer, 1990). These EPs/ERPs are believed to reflect specific CNS functions and appear to be very promising methods for identifying the sites impaired due to neurotoxic factors. Furthermore, the measuring devices are portable and reasonably priced.

In this paper, we intend to provide an overview of the research that has used EPs and ERP for evaluating the effects of occupational and environmental neurotoxic factors. These work-related factors include chemical factors such as metals (e.g., lead), solvents, and other chemicals (e.g., *n*-hexane) or noxious gases and physical factors such a vibration or work with visual display terminals. Also

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we discuss some covariates related to these potentials which must be assessed in analysis to minimize misinterpretation of reported findings.

SOMATOSENSORY EVOKED POTENTIALS

Methods and Interpretation

SEPs are recorded from the human scalp after electric (or mechanical) stimulation of a sensory nerve in the upper or lower extremity or of the trigeminal nerve (Barret *et al.*, 1987). There are a wide variety of stimulating and recording techniques that can be employed to elicit SEPs, suggesting that it will ultimately be possible to vary the manner in which the test is conducted, depending on the clinical context in which it is applied. Especially in the short-latency SEP (SSEP; components with latencies of about less than 50 msec after stimulation), the conduction function on the nervous pathway, principally from the stimulating site to the somatosensory cortex, can be assessed. The components with latencies of more than 50 msec (long-latency SEP) are generally thought to arise in cerebral cortical elements and to be mediated primarily by dorsal column and lemniscal pathways in humans. They are sensitive to changes in the level of consciousness as well as other ill-defined factors (Cracco, 1972) and yet are not always specific for the impaired site.

For example, five peaks of SSEP (i.e., N9, N11, N13, N20, and P23) are recorded in a warm laboratory after electric stimulation of the median nerve at the wrist (Fig. 1). The neural origin of each of these peaks is considered as follows (Chiappa and Ropper, 1982; Jones, 1982): the N9 is generated from the brachial plexus; the N11 is from the dorsal column of the spinal cord or the entry of sensory impulse into the spinal cord; the N13 is from the gracile and cuneate nuclei or the spinal gray matter; and N20 is from the primary sensory cortex or the thalamus. The peak and interpeak latencies of SSEP are available for assessment of the neurotoxic effects on the somatosensory system. However, the interpeak latencies of SSEP are more useful than the peak latencies, because the interpeak latencies of N9–N13 and N13–N20 represent cervicospinobulbar and central conduction times, respectively, irrespective of any impairment in the peripheral nervous system. Detailed discussions regarding SEP recording methods and those meanings have been reported (Jones, 1982; Eisen and Aminoff, 1986; Kimura, 1989).

It has been reported that these SEP latencies are affected by age, body size (especially, height), and skin temperature as the covariates (Chiappa and Ropper, 1982; Jones, 1982; Murata and Araki, 1985; Eisen and Aminoff, 1986). Researchers should take note of these covariates together with the subject's past history of ossification of the posterior longitudinal ligament of the spine, trauma subsequent to traffic accident, or drug use when comparisons are made between subjects exposed to deleterious substances and unexposed controls. These covariates could be controlled by using either the partial correlation coefficient or multiple regression analysis. Also, the normal values for these SEP parameters could be established after excluding the effects of these covariates.

Application in Occupational and Environmental Medicine

Many investigators used to measure peak latencies of SEPs for assessment of the neurotoxicity of occupational and environmental factors on the somatosen-

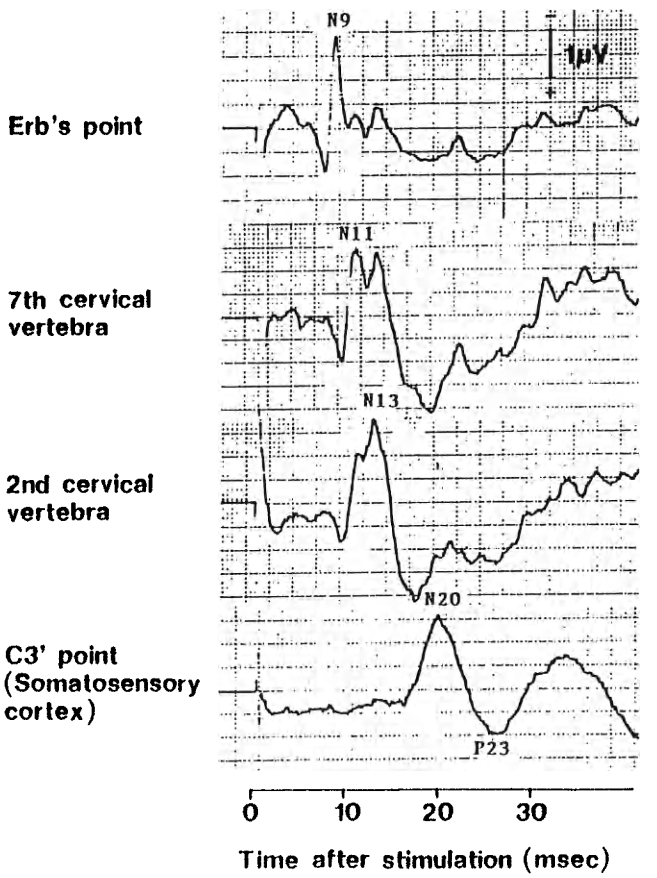

FIG. 1. Somatosensory evoked potentials recorded at the Erb's point and over the second and seventh vertebrae (C2 and C7) and the somatosensory cortex (hand area) following right median nerve stimulation at the wrist (Araki *et al.*, 1986a).

sory system. For instance, prolongation of the long-latency SEP (the conduction from the upper limb to the cerebral cortex) has been reported in vibrating tool operators (Tanabe and Kameda, 1979; Ohta *et al.*, 1979, 1985; Kusunose *et al.*, 1984). Similarly, Groll-Knapp *et al.* (1978) exposed normal subjects to carbon monoxide and found amplitude reductions but no latency changes in the long-latency SEP after stimulation of the median nerve. In these cases, however, it could not be elucidated which of the peripheral or central sites in the pathway was damaged.

Recently, considerable attention has been directed to the use of the interpeak latency on the SSEP. Its use can cancel the effect of skin temperature on the peak latencies (i.e., N9, N11, and N13) by calculating the difference (i.e., interpeak latency) between two peak latencies (Murata and Araki, 1985); nevertheless, age and height would remain as confounders of the latencies.

The interpeak latencies of SEPs have been ardently assessed since the middle 1980s. We have demonstrated that the N9–N13 interpeak latency was significantly prolonged in 20 workers exposed to lead, zinc, and copper, who had blood lead levels of 16–64 (mean 42) μg/dl (Araki *et al.*, 1986a); also, the N13–N20 interpeak latency was inversely related to the zinc level in erythrocytes. On the basis of this

result, we suggested that lead-induced conduction delay in the central nervous system might have been reversed by zinc, resulting in no significant conduction delay. Hazemann and colleagues also observed a significant conduction delay from the lumbar to the vertex (P22–P39 interpeak latency by electric stimulation of the tibial posterior nerve) in lead-exposed workers with blood lead levels of 27–240 (mean 100) μg/dl (Hazemann *et al.*, 1987; Lille *et al.*, 1988).

As shown in Table 1, SEPs have been also applied to the assessment of workers exposed to mercury, *n*-hexane, styrene, xylene, trichloroethylene, mixed solvents, carbon monoxide, and vibration, as well as to experimental studies of animals exposed to 2,5-hexanedione; in some of these studies, the adverse effects on the somatosensory system have not been found. Indeed, we failed to detect the central somatosensory dysfunction in 15 chain saw operators and in 11 styrene-exposed workers (Murata *et al.*, 1987a, 1991). Chang (1987, 1991) has confirmed the significant effects of *n*-hexane on the central nervous system by measuring the SSEP, brain stem auditory evoked potential (BAEP), and VEP together with the nerve conduction velocity; the subjects were clinical cases of polyneuropathy induced by chronic *n*-hexane poisoning. In Minamata disease (organic mercury poisoning) with a diffuse atrophy of the cerebellar hemispheres and vermis in computed tomography, all patients showed a lack of the N20 component despite the presence of the N9, N11, and N13 components (Tokuomi *et al.*, 1982); in fetal Minamata disease, a significant prolongation of N13–N20 interpeak latency was found (Inayoshi *et al.*, 1987). In an animal experiment, central conduction time of SEP was significantly delayed in rats administered 2,5-hexanedione subcutaneously (Hirata, 1989).

VISUAL EVOKED POTENTIAL

Methods and Interpretation

VEPs are recorded from the human scalp, which is prepared in the same way as that for standard EEG recording (occipital site). Two general types of visual

TABLE 1
RECENT STUDIES USING SOMATOSENSORY EVOKED POTENTIALS IN OCCUPATIONAL AND ENVIRONMENTAL MEDICINE

Work-related factors	References
Chemical	
Lead	Hirata *et al.*, 1980; Jeyaratnam *et al.*, 1985; Araki *et al.*, 1986a,b, 1987; Hazemann *et al.*, 1987; Horiguchi *et al.*, 1988; Lille *et al.*, 1988
Mercury	Tokuomi *et al.*, 1982; Lamm and Pratt, 1985; Inayoshi *et al.*, 1987; Lille *et al.*, 1988
n-Hexane	Mutti *et al.*, 1982; Chang, 1987, 1991
2,5-Hexanedione	Hirata, 1984, 1989
Mixed solvents	Hazemann *et al.*, 1987
Trichloroethylene	Barret *et al.*, 1987; Rebert *et al.*, 1991
Styrene	Murata *et al.*, 1991a
Xylene	Seppäläinen *et al.*, 1981
Carbon monoxide	Groll-Knapp *et al.*, 1978
Physical	
Vibration	Tanabe and Kameda, 1979; Ohta *et al.*, 1979, 1985; Kusunose *et al.*, 1984; Murata *et al.*, 1987a

stimuli are most often used to elicit VEPs, i.e., unpatterned flashing lights and patterned stimuli. In clinical laboratories, these are usually checkerboards. In general, pattern-reversal VEPs are considered to be more sensitive for detecting abnormalities of the visual system than the VEP elicited by flash stimuli (Sokol, 1986). Nevertheless, the flash VEPs are useful for the studies of animals, infants, and young children and dementia patients because those subjects cannot fix their eyes on the screen and therefore the pattern-reversal VEP stimulation cannot be applied to them. The VEP latencies reflect the neurological function of the long pathway between the retina and visual cortex (Sokol, 1986); these latencies may also be affected by the changes in activation and arousal functions of the cerebral cortex (Iwasaki and Kurimoto, 1988; Murata *et al.*, 1991b). The neurological interpretations for latencies and amplitudes of VEPs appear to be less definite than those for the SSEP and BAEP. In the future, measurement of the short-latency VEP should be established and standardized. In this sense, concurrent measurement of the electroretinogram (Azazi *et al.*, 1985) and VEP would be valuable. This procedure would avoid misinterpretation of the results of VEP measurement.

Figure 2 shows a VEP waveform with the N75, P100, and N145 components recorded after a pattern-reversal stimulus. The VEP latencies have been reported to be influenced by age (Lueders *et al.*, 1980), sex (Stockard *et al.*, 1979), body temperature (Hetzler *et al.*, 1988), and stimulating conditions (e.g., pattern type and reversal time) (Lueders *et al.*, 1980). The VEP amplitudes are affected by age, habituation, fatigue, level of attention, and stimulus conditions such as pattern type, reversal time, color, check size, visual acuity, contrast, and luminance (Lueders *et al.*, 1980). Each laboratory had better develop its own normative values to determine clinical abnormalities; whereas, such data may not be essential in studies where there is a sufficient range of exposure data.

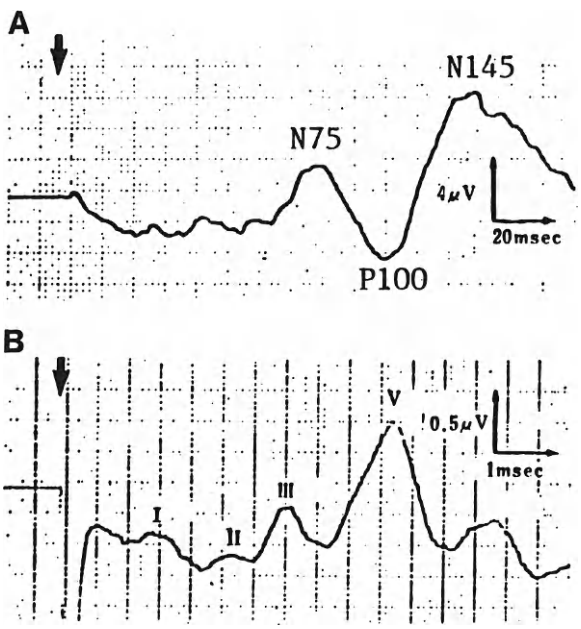

FIG. 2. Recordings of pattern-reversal visual evoked potential (A) and brain stem auditory evoked potential (B) (Murata *et al.*, 1987b).

Application in Occupational and Environmental Medicine

The number of studies using VEPs is increasing (Table 2). For example, significant changes in VEP latencies and amplitudes have been described in workers and animals exposed to lead (Fox *et al.*, 1979; Otto *et al.*, 1985; Araki *et al.*, 1987; Murata *et al.*, 1987b). Urban and Lukas (1990) have reported that the mean P100 latency was prolonged and mean amplitudes were reduced in rotogravure printers exposed to toluene. The peak and interpeak latencies of VEP were longer in both *n*-hexane polyneuropathy and subclinical cases (Chang, 1987). In volunteers exposed to 50 ppm tetrachloroethylene, the N75, P100, and N145 latencies of VEP were prolonged during the inhalation period (Altmann *et al.*, 1990). It appears that VEPs are sensitive to organic solvents such as toluene, tetrachloroethylene, *n*-hexane, 2,5-hexanedione, and xylene (Seppäläinen *et al.*, 1979, 1981; Seppäläinen, 1982; Chang, 1987, 1991; Hirata, 1989; Altmann *et al.*, 1990; Urban and Lukas, 1990).

In addition, several researchers investigated the changes in latencies and amplitudes of VEP in visual display terminal (VDT) workers, disclosing that VEP latencies are a sensitive indicator of transient visual fatigue (Gulmann *et al.*, 1979; Iwasaki and Kurimoto, 1988; Ossenblok and Spekreijse, 1988; Murata *et al.*, 1991b). Vaernes and Hammerborg (1989) described a case of a diver, who had a significant increase in P100 latency and a marked "high pressure nervous syndrome." These findings suggest that the VEP measurement is also useful for assessment of work-related visual and mental fatigue.

AUDITORY EVOKED POTENTIALS

Methods and Interpretation

The determination and understanding of AEPs have progressed considerably recently and have led to useful procedures in both occupational and environmen-

TABLE 2
RECENT STUDIES USING VISUAL EVOKED POTENTIALS IN OCCUPATIONAL AND ENVIRONMENTAL MEDICINE

Work-related factors	References
Chemical	
Lead	Fox *et al.*, 1979; Thatcher *et al.*, 1984; Otto *et al.*, 1985; Araki *et al.*, 1987; Murata *et al.*, 1987b; Lille *et al.*, 1988
Mercury	Lille *et al.*, 1988
Cadmium	Thatcher *et al.*, 1984
n-Hexane	Seppäläinen *et al.*, 1979; Chang, 1987, 1991
2,5-Hexanedione	Hirata, 1984, 1989
Mixed solvents	Seppäläinen, 1982; Poulsen and Jensen, 1986
Tetrachloroethylene	Altmann *et al.*, 1990
Toluene	Urban and Lukas, 1990
Trichloroethylene	Rebert *et al.*, 1991
Styrene	Behari *et al.*, 1986
Xylene	Seppäläinen *et al.*, 1981
Carbon monoxide	Hosko, 1970; Groll-Knapp *et al.*, 1978
Physical	
VDT work	Gulmann *et al.*, 1979; Iwasaki and Kurimoto, 1988; Ossenblok and Spekreijse, 1988; Murata *et al.*, 1991b
Diving	Vaernes and Hammerborg, 1989

tal medicine, although the AEP was recognized in the human EEG in 1939. The AEP methods are classified in Table 3. The BAEPs, usually recorded after a high-intensity click stimulus, are composed of up to seven components, commonly called components I to VII. The neurological origins of the BAEP components are understood more precisely than those of other AEP components. Further information on the recording and stimulating methods of AEP is described elsewhere (Robinson and Rudge, 1982; Kriss, 1982; Stockard *et al.*, 1986).

In our laboratory, the BAEP is measured as follows: click stimuli (0.1-msec impulses) are presented monaurally at a rate of 20/sec through shielded earphones. The intensity of click stimuli is about 80 dB hearing level (HL). The BAEP is recorded from vertex (Cz) to mastoid ipsilateral to acoustic stimulation (Fig. 2). It has been demonstrated that the I, III, and V components of BAEP primarily represent volume-conducted electrical activity from the acoustic nerve, pons, and midbrain, respectively; the interpeak latencies between these three components reflect neural conduction in the corresponding segments of the brain stem auditory pathway (Kriss, 1982; Stockard *et al.*, 1986).

Interpersonal variability of the interpeak latencies of BAEP is much smaller than those of SEP and VEP (Otto, 1986). The covariates which should be taken into account in the BAEP study are age, sex, body temperature, stimulating conditions (phase, intensity, rate, etc.), and history of drug use (Stockard *et al.*, 1986). A change in the conditions of stimulation may lead to a serious measurement bias.

Application in Occupational and Environmental Medicine

Reports on the latencies and amplitudes of the BAEP are shown in Table 4. In vibrating tool operators, the I–V interpeak latency of BAEP has been reported to be significantly prolonged (Sasaki *et al.*, 1987; Murata *et al.*, 1990). Also, Attias and Pratt (1984, 1986) have shown significant changes in the interpeak latencies of BAEP among workers exposed to a mean level of 112–117 dB (A) continuous engine noise.

As for neurotoxicants, significant delays in the I–III and I–V interpeak latencies have been observed in adults and children accidentally exposed to lead with blood

TABLE 3
METHODS OF AUDITORY EVOKED AND EVENT-RELATED POTENTIALS

Latencies	Evoked response	Origin or function
Early		
<10 msec	Electrocochleogram (ECoG)	Corti organ
	Brain stem auditory evoked potential (BAEP)	From cochlear nerve to brain stem
Middle		
10–60 msec	Middle-latency auditory evoked potential	From medial geniculate body to primary auditory cortex
Late		
>60 msec	Long-latency auditory evoked potential	
	P300	Cognition
>1.5 sec	Contingent negative variation (CNV)	Discrimination

TABLE 4
RECENT STUDIES USING AUDITORY EVOKED POTENTIALS IN OCCUPATIONAL AND ENVIRONMENTAL MEDICINE

Work-related factors	References
Chemical	
Lead	Takahashi *et al.*, 1984; Thatcher *et al.*, 1984; Otto *et al.*, 1985; Holdstein *et al.*, 1986; Murata *et al.*, 1987b; Lille *et al.*, 1988
Mercury	Lille *et al.*, 1988
Cadmium	Thatcher *et al.*, 1984
n-Hexane	Rebert and Sorenson, 1983; Chang, 1987, 1991
2,5-Hexanedione	Hirata, 1987
Mixed solvents	Antti-Poika *et al.*, 1989
Tetrachloroethylene	Altmann *et al.*, 1990
Toluene	Rebert, 1983; Rosenberg *et al.*, 1988
Trichloroethylene	Rebert *et al.*, 1991
Carbon monoxide	Groll-Knapp *et al.*, 1978
Physical	
Vibration	Sasaki *et al.*, 1987; Murata *et al.*, 1990
Noise	Attias and Pratt, 1984, 1986

lead concentrations of 30–84 (mean 49) μg/dl (Holdstein *et al.*, 1986). Significant dose–effect relationships have been found between the III and V peak latencies of BAEP and the blood lead concentrations ranging from 6 to 59 (mean 28) μg/dl in 49 children aged 6–12 years (Otto *et al.*, 1985), and between the I–V interpeak latency and the indicator of lead absorption in 20 gun metal foundry workers with blood lead levels of 16–64 (mean 41) μg/dl (Murata *et al.*, 1987b). In rats intoxicated with lead acetate, changes in the P2 peak latency and P1–N1 peak-to-peak amplitude of AEP have been observed (Takahashi *et al.*, 1984). Organic solvents such as *n*-hexane, 2,5-hexanedione, and toluene have been also related to the changes in the BAEPs (Chang, 1987; Hirata, 1987; Rosenberg *et al.*, 1988); whereas, Altmann *et al.* (1990) failed to find a significant change in the BAEP in humans exposed to tetrachloroethylene.

Shortening of I–V interpeak latencies has been reported in sensorineural hearing loss (Coats and Martin, 1977). Therefore, when the BAEP is measured in subjects with a hearing loss, the effect of noise should be taken into account as a possible confounder.

EVENT-RELATED POTENTIAL (P300)

Methods and Interpretation

The SEP, VEP, and BAEP represent direct responses of neurons to a given stimulus, and the amplitude and latency depend on the physical characteristics of the stimulus. Such "exogenous" or "stimulus-related" potentials are independent of whether the subject is attentive to or interested in the stimulus. On the other hand, there is another distinct type of cerebral potentials, i.e., the "endogenous" or "event-related" potentials (ERPs). The ERP changes only occur when the subject is selectively attentive to the stimulus and are only elicited in circumstances where the subject is required to distinguish one stimulus (the target) from a group of other stimuli. Thus, measurement of the ERP requires cooperation from subjects; the ERPs cannot be recorded when the subject is asleep.

A great number of ERP components have been identified, e.g., Nd, P165, NA,

N2, P300, P4, N400, and contingent negative variation (Goodin, 1986). Among them, the amplitude of the P300 component is highest and most easily detectable, which has a special significance in occupational and environmental medicine. Therefore, only the P300 is discussed in this review.

The P300 has been measured using the so-called "odd-ball" paradigm in many laboratories as follows: The subject is presented binaurally with a random sequence of two distinguishable stimuli, one of which occurs frequently (i.e., frequent stimulus) and the other infrequently (rare stimulus). The frequent stimulus, i.e., nontarget tone, is a 1-kHz tone burst; the rare stimulus (target tone) is a 2-kHz tone burst. These stimuli are delivered at a rate of one tone burst every 2 sec. The subjects are instructed to count mentally only the target tone. Following the target tone, the P300 component is detected (Fig. 3). The P300 component is the first maximal positive wave detected between 250 and 500 msec. As there are a wide variety of stimulating techniques that can be utilized to elicit P300, the readers can refer to the literature (Gibson, 1982; Goodin, 1986). The P300 latency corresponds to the evaluation time of target stimuli; the latency becomes longer when the task is difficult. Thus, the P300 latency has been considered to reflect cognitive function in humans; whereas, its interpretation was inferred from the behavioral data.

The covariates potentially influencing the assessment of the P300 latency and amplitude are age, sex, intelligence (or schooling years), and drugs (Goodin, 1986).

Application in Occupational and Environmental Medicine

Otto *et al.* (1981) have reported that the voltage of the slow cortical wave during sensory conditioning (ERP) varies as a linear function of blood lead concentrations (7–59 μg/dl) in children. We have also observed that the P300 latency following stimulation by target tone was significantly prolonged in gun metal foundry workers with blood lead levels of 12–59 (mean 30) μg/dl; the P300 latency was weakly correlated with blood lead and other indicators of lead absorption (Araki

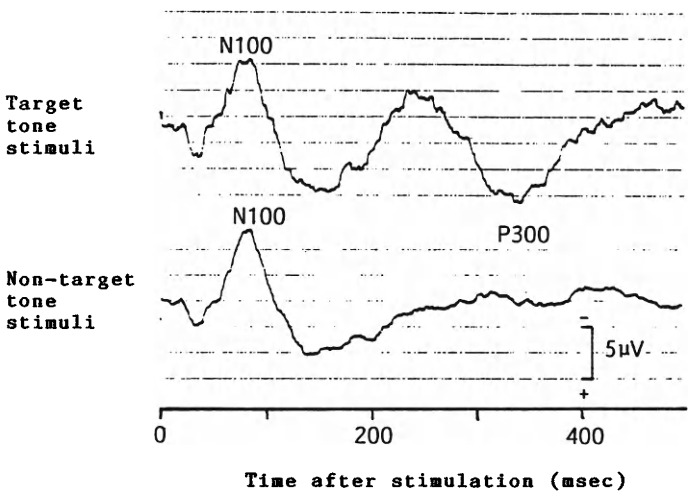

FIG. 3. P300 component of auditory event-related potential recorded from the vertex following stimulation of ears by target and nontarget tones in a 30-year-old "healthy" man. The P300 component is not elicited following stimulation by nontarget tone (Murata and Araki, 1988).

et al., 1992). According to an unexplored implication of the finding that lead causes insidious, asymptomatic injury to the central nervous system, some fraction of cases of dementia or of other late onset neurological illness may be related to chronic exposure to lead (Landrigan, 1989). Further studies are needed to assess the effects of lead exposure in subjects with chronic neurological diseases.

Vaernes and Hammerborg (1989) observed prolongation of the P300 latency by more than 2 standard deviations from predive results in three of six divers during a heliox dive to 360 meters in seawater, indicating the occurrence of impaired cognitive function at deeper depths. We have found that acute alcohol ingestion affected the P300 latency in healthy adults aged 20–26 (mean 23) years, in whom the latency was significantly delayed 2 hr after acute ingestion of 200 ml spirits (containing 25% ethanol) (Murata and Araki, 1988, Fig. 4). Clear change in the P300 has been demonstrated neither in workers exposed to organic solvents (Massioui *et al.*, 1987) nor in subjects exposed to low-level carbon monoxide (Harbin *et al.*, 1988).

DISCUSSION AND CONCLUSION

Table 5 summarizes recent studies using cerebral evoked potentials. Further expanded studies are needed to reach a definite conclusion on dose–effect relationships for each factor. The EPs and ERP with both short (SSEP, BAEP, etc.) and relatively long latencies (VEP, P300, etc.) appear to be sensitive and reliable methods for evaluating chronic effects of work-related factors on the central nervous system. Measurements of these potentials should be valuable for comprehensive interpretation of the nervous system effects of occupational and environmental factors. The measurement of EP/ERP should be useful also for as-

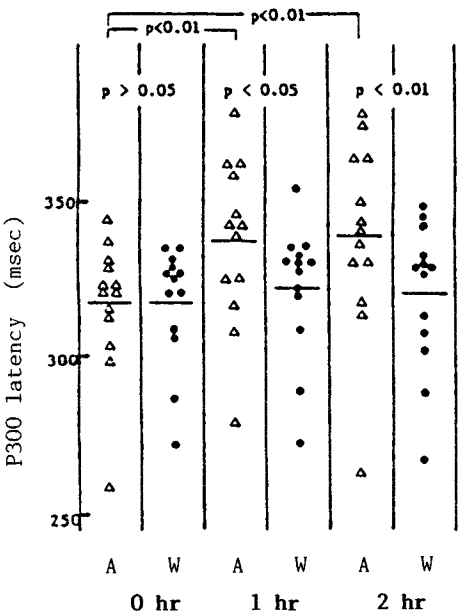

FIG. 4. Changes in P300 latency before and 1- and 2-hr after ingestion of 200 ml spirits containing 25% ethanol (A) and of 200 ml water ingestion (W) in 13 healthy young adults (Bonferroni multiple comparison) (Murata and Araki, 1988).

TABLE 5
SUMMARY OF RECENT STUDIES USING CEREBRAL EVOKED POTENTIALS

Work-related factors	Changes reported in literature			
	SEP	VEP	AEP	ERP
Chemical				
Lead	H(+), A(+)	H(?), A(+)	H(+), A(+)	H(+)
Mercury	H(+)	H(?)	H(−)	
Cadmium		H(?)	H(?)	
n-Hexane	H(+)	H(+)	H(+), A(+)	
2,5-Hexanedione	A(+)	A(+)	A(+)	
Mixed solvents	H(−)	H(?)	H(−)	H(?)
Tetrachloroethylene		H(+)	H(−)	
Toluene		H(+)	H(+), A(+)	
Trichloroethylene	H(?), A(−)	H(+), A(−)	A(+)	
Styrene	H(−)	H(−)		
Xylene	H(−)	H(+)		
Carbon monoxide	H(?)	H(?)	H(−)	H(−)
Physical				
Vibration	H(?)		H(+)	
VDT work		H(+)		
Diving		H(+)		H(+)
Noise			H(+)	

Note. SEP, somatosensory evoked potential; VEP, visual evoked potential; AEP, auditory evoked potential; ERP, event-related potential. H, human study; A, animal study. +, significant change in evoked potentials; −, no significant change in evoked potentials; ?, conflicting or unclear in reports.

sessment of central nervous system dysfunction following acute exposure to neurotoxicants (Iwasaki and Kurimoto, 1988; Murata and Araki, 1988; Murata *et al.*, 1991b). The number of EP/ERP studies in occupational and environmental medicine is expected to increase further in the near future.

When the EP/ERP measurement is applied to subjects exposed to work-related factors, effects of several covariates (age, height, skin temperature, education, intelligence, alcohol ingestion, etc.) should be eliminated. Also, control subjects should be carefully selected in order to avoid sampling bias; sometimes, selection of control subjects is more difficult than that of the exposed subjects. It is possible that measurements have to be performed under a time limitation imposed by employers or employees. In this case, the investigator must decide on the most appropriate and effective combination of EP/ERP measures that can be done within the limited time. Researchers should be fully familiarized with these kinds of difficulties apart from usefulness of these tests.

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Nd and P300 in Healthy Volunteers[1]

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We attempted to standardize values of the attention-related negative potential (Nd) and the P300 in 100 healthy volunteers (50 females, 50 males) who were given the task of making dichotic syllable discriminations requiring key-press responses. Ages ranged between 18 and 59 years. Nd was found to be maximum in the Fz region, P300 being maximum in the Pz region. The means and standard deviations of the Nd and P300 areas in their maximum regions were 554.1 ± 307.8 μV · msec and 2148.5 ± 1261.5 μV · msec, respectively. After being transformed into logarithmic values, the distribution patterns of the Nd and P300 areas followed a Gaussian distribution. When the lower limit of normal values was tentatively assigned to mean − 2 SD using logarithmically transformed data for both Nd and P300, 94% of the subjects were found to display values above the lower normal limit for Nd, and 95% for P300. Neither Nd nor P300 areas correlated with age, while P300 latencies displayed a weak positive correlation with age. Females displayed relatively larger values than males for Nd and P300 areas and P300-peak amplitudes. Females and males showed nearly equal P300-peak latencies.

INTRODUCTION

Development of valid, reliable, and noninvasive methods for evaluation of neurotoxic insults due to chemicals such as solvents and pesticides is strongly required in the field of occupational medicine. Event-related potentials (ERPs) have recently attracted much attention as a significant electrophysiological index among candidates of evaluation methods for the purpose as described above (Otto, 1983; Harbin, 1985). Individuals with neurobehavioral effects of toxicants display various cognitive impairments, i.e., attention deficit, memory loss, etc., which are assessed by measuring ERPs. However, as Otto (1983) and Harbin (1985) suggest, normative values of ERPs in healthy subjects should be clearly set in order to make ERP application possible for evaluation of neurotoxic insults. Therefore, we attempted to standardize values of two components in ERPs, namely the attention-related negative potential (Nd) and the P300, in normal populations. Nd refers to negative components of ERPs, as reported by Näätänen *et al.* (1978, 1981) and Hillyard *et al.* (1971, 1973), which start at approximately 70–100 msec, peaking at approximately 200 msec after stimulus onset. Nd is thought to emerge on occasions of differential discrimination between task-relevant and task-irrelevant stimuli, thus implicating selective attentional functioning. P300 is a complex of positive components of ERPs elicited by novel, rare, or task-relevant stimuli at approximately 300 msec after stimulus onset. Sutton *et al.* (1965) first explained the possible cognitive meaning of P300 as the resolution of uncertainties. Later Donchin *et al.* (Magliero *et al.*, 1984) hypothesized that

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P300 reflects context updating or situation evaluation. P300 has been regarded as a complex waveform reflecting higher cognitive functions.

SUBJECTS AND METHODS

Subjects

One hundred healthy adult volunteers (50 males and 50 females aged 18–59, mean 32.3 ± 11.3, years) participated in this study. All subjects were free from any hearing disability, and none had a history of neurological or psychiatric disorders. The mean education was 14.4 ± 2.3 (range 8–22) years. There were no significant differences between female and male subjects in age or years of education.

Methods

In the dichotic syllable discrimination tasks employed in the present study, stimuli consisted of the syllables (/te/ and /ga/) spoken in male and female voices and presented to subjects via headphones, with the male voice being presented to one ear and the female voice to the other ear with no changes between the stimuli within a session. The frequency ratio in appearance of /te/ and /ga/ was 2.5:7.5. The stimuli were applied in a random, alternating pattern to one side or the other during a session. Subjects were required to press one of two response keys to differentiate between "targets," that is, the syllable /te/ in one designated ear, and other categories of stimuli. The duration of each stimulus was 150 msec; the stimulus intensity being set approximately at 60 dBSL. Interstimulus intervals varied randomly between 1800 and 2000 msec. The number of the target stimuli for each run was 30.

Subjects were seated in a soundproof anechoic room with eyes closed. According to the International 10–20 Electrode System, EEGs were recorded at Fz, Cz, and Pz monopolarly referenced to linked earlobe electrodes. Vertical and horizontal eye movements from the right eye were also recorded. EEGs were amplified using DC preamplifiers (bandpass down 6 dB at 0.15 and 300 Hz) and processed with a sampling frequency of 250 Hz. EEG data contaminated by potentials with peak-to-peak amplitudes of more than 100 μV or accompanied by EOGs of more than 150 μV during the period 40 msec prestimulus to 800 msec poststimulus were eliminated from averaging. After a smoothing process using a digital filter with a window width of 33.3 msec, averaged ERP waveforms for individual subjects were averaged separately into four categories: (1) target syllables in the attended ear, (2) nontarget syllables in the attended ear, (3) target syllables in the nonattended ear, and (4) nontarget syllables in the nonattended ear.

Nd was defined as the negative component of a difference wave, 0–400 msec after stimulus onset, between ERPs elicited by nontarget syllables in the attended ear and those for nontarget syllables in the nonattended ear. For the index to represent Nd, those areas were employed which were defined as negative areas between 0–400 msec in the difference wave described above. P300 was defined as the positive component of ERP, 260–600 msec after stimulus onset. P300 areas, P300 amplitudes, and P300 latencies for targets in the attended ear were employed as indices representing P300. P300 areas were defined as the positive areas of target ERPs between 260 and 600 msec, with P300 amplitudes and P300 latencies corresponding to those measures of the most positive peaks in the relevant latency periods.

RESULTS

ERP Waveforms

The grand average of ERP waveforms for 100 subjects is shown in Fig. 1. Waveforms for the four stimulus categories are shown separately in the figure along with the wave differences. The P300s in Fig. 1 are most clearly elicited by the targets in the attended ear, particularly at the Pz region. Figure 1 also reveals that Nd waves are most prominent at the Fz region in which two peaks are identified. In the Cz and Pz regions, the first peak is clearer than the second one. The Nd for the Fz region and P300 for the Pz region are utilized in the following analyses.

Relationship between Nd and P300

The means and SDs of the Nd area at Fz and P300 at Pz were 554.1 ± 307.8 and 2148.5 ± 1261.5 μV · msec, respectively. The relationship between Nd and P300 areas was not significant (Pearson's correlation coefficient, $r = 0.07$, not significant).

Distribution of Nd Areas

Since the distribution pattern of absolute Nd area values was asymmetric, with the number of smaller Nd subjects greater than that of the larger Nd subjects (skewness = 0.296), the distribution pattern of logarithmically transformed Nd values could be obtained. A histogram depicting these values is displayed in Fig. 2. This distribution was regarded as Gaussian ($X^2(15) = 30.16$, $0.10 > P > 0.05$). The mean and SD of the log-transformed Nd areas were 2.6 (398.1 μV · msec) and 0.3 (2.0 μV · msec), respectively. When the lower limit of the normal Nd was defined as the mean − 2 SD in the log-transformed Nd areas, it was 2 (100.0 μV · msec), a value which 94 of 100 subjects exceeded.

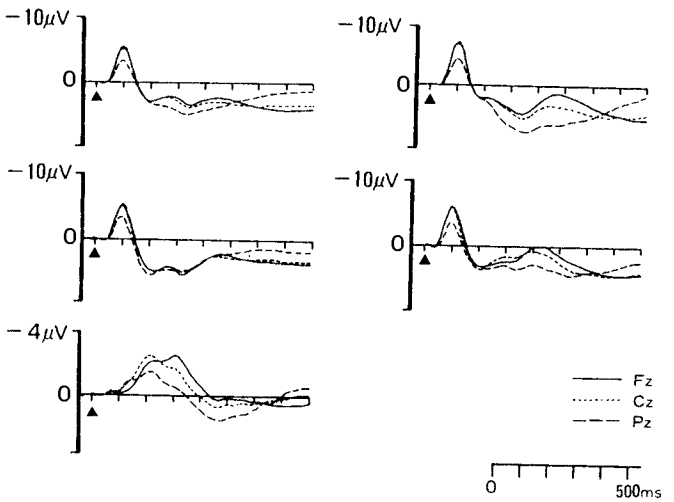

FIG. 1. The grand average ERP waveform for each region and stimulus category. (Upper left) Attended nontarget; (upper right) attended target; (middle left) nonattended nontarget; (middle right) nonattended target; (lower left) difference wave.

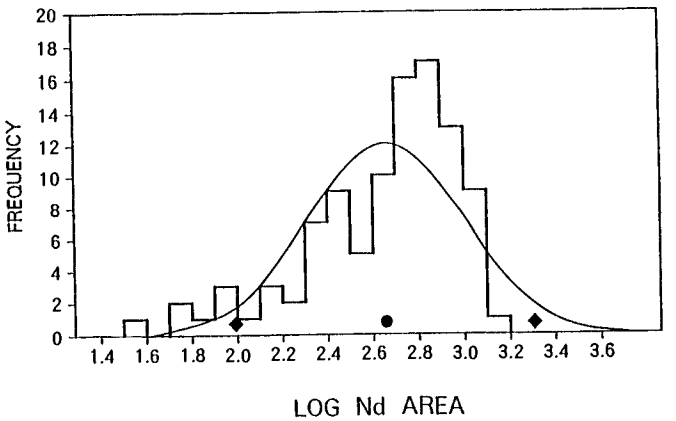

FIG. 2. The distribution of log-transformed Nd areas at Fz. The closed circle indicates the mean and the closed diamond indicates the mean ± 2 SD.

P300-peak Amplitudes and Latencies

The means and the SDs of P300-peak amplitudes and latencies were 8.5 ± 4.7 μV and 343.3 ± 31.6 msec, respectively. The kurtosis and skewness of the P300-peak amplitudes distribution were 3.821 and 0.829, respectively. While, the kurtosis and skewness for P300-peak latencies were 2.473 and 0.094, respectively. Neither the amplitudes nor the latencies fit the Gaussian distribution model.

Distribution of P300 Areas

Examination of the distribution pattern of log-transformed P300-area values revealed that they fit the Gaussian distribution model ($X^2(12) = 17.65$, $0.20 > P > 0.10$; see Fig. 3). The mean and the SD of log-transformed P300 areas were 3.2 (1584.9 μV · msec) and 0.3 (2 μV · msec), respectively. When the lower limit of normal P300 was defined as the mean − 2 SD in log-transformed P300 areas, it was 2.6 (398.1 μV · msec), a value which 95 of 100 subjects exceeded.

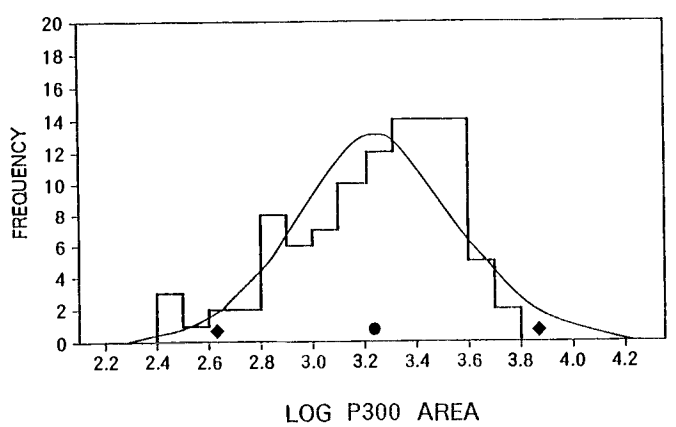

FIG. 3. The distribution of log-transformed P300 areas at Pz. Symbols are explained in Fig. 2 legend.

Age, Gender and ERPs

When we checked the relationship between ages of the subjects and log-transformed Nd or P300 areas, we found no significant relationships. We also calculated the correlation coefficients between the ages of the subjects and the amplitudes or latencies of the P300 peaks. Only P300-peak latencies exhibited a weak but significant relationship with age ($r = 0.287$, $P < 0.01$). Each year's advance in age produces a 0.8-msec prolongation in the P300-peak latency.

We also checked possible gender-related differences in Nd and P300 areas as well as amplitudes and latencies of P300 peaks. Although females demonstrated relatively larger values than males in all indices of the Nd areas (females, 569.2 ± 274.4 μV · msec; males, 539.0 ± 340.1 μV · msec), P300 areas (females, 2324.1 ± 1139.4 μV · msec; males, 1972.9 ± 1361.8 μV · msec), and P300-peak amplitudes (females, 9.1 ± 4.1 μV; males, 7.8 ± 5.2 μV), not all of the differences were statistically significant. Furthermore, the females and the males displayed nearly equal values of P300-peak latencies (females, 340.2 ± 30.7 msec; males, 346.5 ± 32.5 msec).

DISCUSSION

Area Measurement as an Index of P300s

In the present study, we employed area as an index to represent P300s in addition to using peak amplitudes and peak latencies. Squires *et al.* (1973) and Johnson and Donchin (1978) employed area as part of their P300 indices, too. These authors found (1) area to be less affected by variance in P300 latencies than conventionally employed amplitudes and (2) that area correlates better with behavioral data. In addition, we suggest that measuring area is especially advantageous when P300s are applied to psychiatric patients in which P300 peaks cannot be identified clearly, producing difficulties in employing peak amplitudes and latencies as indices for P300s.

Relationship between P300 Latency and Age

P300 latencies displayed a weak positive correlation with age; 1-year increments in age produced 0.8-msec prolongations in latencies. This result is very similar to that of many previous studies and is fairly consistent with reports of an approximately 1-msec prolongation in the P300 latency with 1-year increments in age (Goodin *et al.*, 1978; Gordon *et al.*, 1986; Pfefferbaum *et al.*, 1984). Considering many previous studies which employed the conventional oddball paradigm to elicit P300s, the result obtained here suggests that the P300 latency prolongation with age occurs approximately similarly across different tasks. Concerning P300 areas, we found no significant relationship with ages. This result is consistent with previous results of other investigators who reported no relationship between P300-peak amplitudes and ages. The lack of age association in P300-peak amplitudes is thought to be due to a larger individual than age variation.

Distributions of Nd and P300 in Healthy Subjects

While distribution patterns of Nd so far have not been reported, Polich (1986) reported that P300 amplitudes distributed normally, and Blackwood *et al.* (1990) reported a normal distribution of P300 latencies. Both of them used a large number of healthy subjects in their samples. According to Polich (1986), P300 amplitudes had a sharp Gaussian distribution with a narrow range, which is different from the

results in the present study. We speculate that this difference is due to the relatively greater homogeneity of samples employed in Polich's study than in ours. Though not described in detail, the kurtosis and skewness data suggested that the distribution pattern of P300 latencies might come close to the Gaussian distribution, if the number of subjects was enlarged, thereby reproducing the result of Blackwood *et al.* (1990).

Concerning gender-related differences in the ERPs, Josiassen *et al.* (1990) reported that PCA analyses revealed larger components corresponding to N100 and P300 in females than in males. Though not reaching statistical significance, our results regarding gender differences are consistent with those of Josiassen *et al.* (1990).

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International Comparison of Odor Threshold Values of Several Odorants in Japan and in The Netherlands[1]

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The purpose of this paper is to compare the published odor threshold values of six odorants. In Japan, all of the odor threshold values used in the Offensive Odor Control Law (enacted in 1972) were determined in an odor-free room (4 m^3) by a trained panel (20 men, ages 30–45 years who were perfumers) who sniffed the odors directly and made absolute judgments of odor quality and intensity. In The Netherlands, sensorial odor concentration measurements were made with an olfactometer in a mobile sniffing car with eight panelists, four men and four women, ages 18–40 years. Such presentations are repeated with different dilution ratios. Comparison of the threshold data for the six different compounds given as the barely perceptible concentration level revealed striking similarities for hydrogen sulfide (in Japan 0.0005 ppm/in The Netherlands 0.0003 ppm), phenol (0.012/0.010), styrene (0.033/0.016), toluene (0.92/0.99), and tetrachloroethylene (1.8/1.2) but not for *m*-xylene (0.012/0.12). Such a similarity was not found with any other literature sources.

INTRODUCTION

In Japan, the Offensive Odor Control Law was enacted in 1972 (Environment Agency, 1972). In 1991, there were 12 controlled odorants: ammonia, methyl mercaptan, hydrogen sulfide, dimethylsulfide, dimethyl disulfide, trimethylamine, acetaldehyde, styrene, propionic acid, *n*-butyric acid, *n*-valeric acid, and isovaleric acid. The ranges of ambient air-controlled concentrations from factories are 0.0009 ppm (*n*-valeric acid) to 5 ppm (ammonia). These concentrations correspond to 2.5 to 3.5 on a 6-point odor intensity scale: no odor, barely perceptible or detectable, faint or recognizable, easily noticed, strong, and very strong (Katz and Talbert 1930). The actual concentrations of odorants in the air were determined by gas chromatography with the cold-trapping (liquid oxygen) and the adsorption-trapping methods with porous polymer beads, such as Tenax-GC (for styrene), and chemical reaction by strontium hydroxide on glass beads (for lower fatty

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acids) followed acid–base chemical reaction for regeneration (Hoshika *et al.*, 1981a; Hoshika, 1982).

Odor threshold values of odorants in air in odor test systems vary widely, depending on such factors as the observer, the technique for low background level, and the purity of the odorants.

Of the experimental parameters used to measure odor threshold values, the determination of actual concentrations of odorants in the odor test systems is one of the more important.

Leonardos *et al.* (1969) used an odor test room which had a volume of 13.2 m^3 to determine the odor threshold values of 53 odorants. Smith and Hochstettler (1969) reported the determination of odor threshold values in air using ^{14}C-labeled compounds and scintillation counting to monitor concentrations. Mills *et al.* (1963) also used an odor-free room for quantitative odor measurements. However, in their work, the actual concentrations of the odorants in the odor test room were not determined by reliable methods. Fluck (1976) used an odor test room that had a volume of approximately 28.2 m^3 and a low odor background to determine the odor threshold of phosphine. Concentrations of the phosphine were determined by using detector tubes. Whisman *et al.* (1978) evaluated ethanethiol and tetrahydrothiophene as odorants in propane, measuring concentrations in air in four testing modes by gas chromatography. Buttery *et al.* (1981) reported the odor threshold of thiamine odor compounds using odor-free Teflon squeeze bottles equipped with Teflon tubes.

Hoshika and Muto (1982) reported the determination of the actual concentrations of 46 odorants prepared in air in a 10-m^3 stainless-steel odor test room. The 46 odorants tested were 4 sulfur compounds, 10 lower aliphatic carbonyl compounds, an aromatic hydrocarbon, 7 lower aliphatic monoalcohols, 11 phenols, 6 lower fatty acids, and 7 indoles.

The recoveries of the odorants having boiling points lower than about 150°C were quantitative, but those having boiling points higher than 160°C gave recoveries of about 50%, except for the phenols, which had much lower recovery levels. Unfortunately, odor threshold measurements depend largely on the purity of the odorants; for example, the reported odor threshold concentrations of hydrogen sulfide vary from 0.65 to 1400 $\mu g/m^3$ (Sullivan, 1969).

However, there are few reports on the determination of threshold values of a wide variety of odorants in an odor test system. This paper describes a comparison of published threshold data for six different compounds given as barely perceptible or detectable concentration level by subjects in two countries, Japan and The Netherlands.

MATERIALS AND METHODS

Reagents

Hydrogen sulfide (of 99.6% minimum purity) was obtained from Matheson Gas Products (East Rutherford, NJ). The standard solution of hydrogen sulfide was prepared by dissolving the pure gas (50 ml) in 50 ml of water. The hydrogen sulfide solution was standardized by iodometric titration. Phenol (of 98% minimum pu-

rity) was obtained from Wako Pure Chemical Industries Ltd., and a standard solution was prepared by dissolving 1 g of phenol in 100 ml of ethanol. Styrene, minimum assay 95% was stabilized with 0.003–0.004% of 4-*tert*-butylcatechol (chemical grade).

m-Xylene (of 98% minimum purity), toluene (of 98% minimum purity), and tetrachloroethylene (of 99% minimum purity) were obtained from Wako Pure Chemical Industries Ltd. All reagents were of guaranteed or reagent grade.

Odor Threshold Test System

In Japan, all of the odor threshold values used in the Offensive Odor Control Law (enacted in 1972) were determined in an odor-free room (4 or 10 m^3) by a trained panel (20 male perfumers, ages 30–45 years) who sniffed the odors directly and made absolute judgments of odor quality and intensity. The odor gases were prepared in the room on alternate days using directly static method (regress analysis). The odor test room, standard sample gas preparation in the odor-free test room, and gas chromatography for the determination of the actual concentrations of several odorants prepared in the odor-free room have been described previously (Environment Agency, 1980; Hoshika and Muto, 1982).

In The Netherlands, sensorial odor concentration measures are made with an olfactometer. The TNO sniffing car is mobile, with a cabin in which odorous air is mixed with clean odor-free room air and subsequently presented to panelists for assessments (Barker and Barker 1988; Don 1986; Miedema *et al.*, 1985; Roos *et al.*, 1984). Such presentations are repeated with different dilution ratios. The instrument was equipped with three sniffing cups (the so-called forced-choice triangle test method), with a minimal flow of 15 liters/min. There were eight panelists, four men and four women, ages 18–40 years.

From statistical analysis of a large number of scores with 50% correct response of the panel, odor concentrations are expressed in odor units/m^3.

In this paper, mg/m^3 is converted to parts per million (ppm) by using the equation

$$\text{ppm (vol/vol)} = \frac{22.4 \times A}{\text{MW} \times V \times \dfrac{273}{273 + t} \times \dfrac{P}{760}},$$

where A is the quantity of the odorant in milligrams, MW is the molecular weight of the odorant, V is the volume (in m^3) of the sample gas meter, t is 20°C of the gas meter in the sample gas, P is the barometric pressure (in mm Hg) of the sample gas, and 22.4 is constant.

RESULTS

The comparison of the published threshold data for the six different compounds is shown in Fig. 1. The odor threshold data used in this figure are given as barely perceptible or detectable concentrations in partial recognition threshold data. As shown in Fig. 1, the comparison of the published odor threshold data for the six different compounds given as barely perceptible or detectable concentration lev-

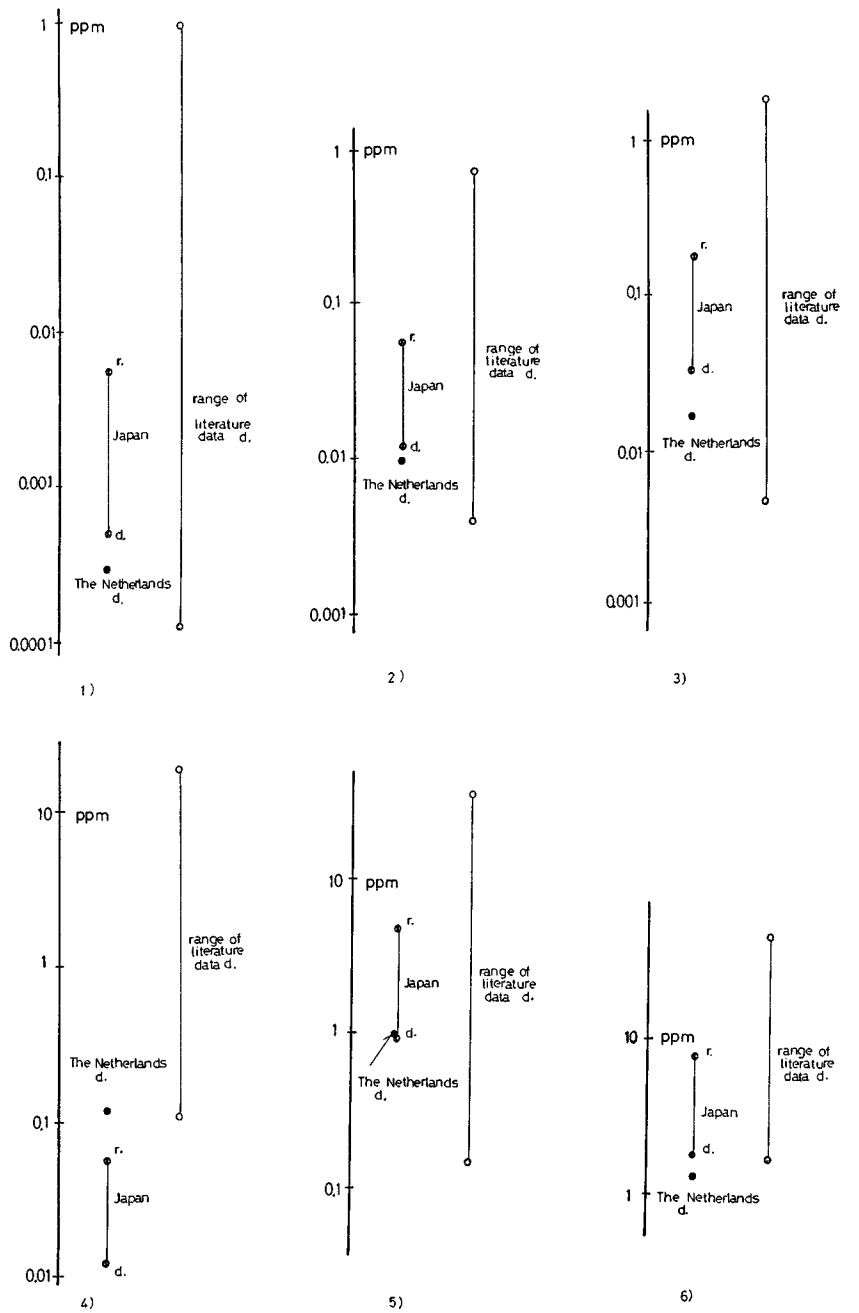

FIG. 1. Comparison of odor threshold values. (1) Hydrogen sulfide in Japan and in The Netherlands, and ranges of literature data: d, barely perceptible or detectable odor threshold; r, recognizable odor threshold. (2) Data on phenol: d and r are the same as in part 1. (3) Data on styrene: d and r are the same as in part 1. (4) Data on *m*-xylene: d and r are the same as in part 1. (5) Data on toluene d and r are the same as in part 1. (6) Data on tetrachloroethylene: d and r are the same as in part 1.

els revealed striking similarities for hydrogen sulfide (in the Japanese static method with odor-free test room the level is 0.0005 ppm and in The Netherlands dynamic flow method it is 0.0003 ppm), phenol (0.012 ppm/0.010 ppm), styrene (0.033 ppm/0.016 ppm), toluene (0.92 ppm/0.99 ppm), and tetrachloroethylene (1.8 ppm/1.2 ppm) and none for *m*-xylene (0.012 ppm/0.12 ppm). However, the origin of the remarkable difference in the *m*-xylene data between Japan and The Netherlands is unclear.

DISCUSSION

Such striking similarities were not found with any other literature sources (Van Gemert and Nettenbreijer, 1977). The data from literature before 1960 have been omitted. In Japan, the odor threshold values of odorants in air in odor test room vary widely, depending on such factors as the test procedure and technique, the observer, and the purity of the odorants.

The test odorants used must be of high purity, and a static air dilution system utilizing an air dilution medium that has a low background level of odors must be employed. The determination of the odor threshold values of the odorants onto the surface of the apparatus and from diffusion loss (Hoshika and Muto, 1982) must be made.

Of the experimental parameters used to measure odor threshold value, the determination of actual concentrations of odorants in odor test room air is one of the more important. The recoveries of hydrogen sulfide, phenol, and styrene were 95, 14, and 98%, respectively.

In The Netherlands, in each of the eight panelist's cabins in the sniffing car there are three sniffing cups where the air is presented for examination; diluted odorous air comes from one of the three cups, while odorless air comes from the other two. The panelists must determine which one of the three is different (the so-called forced-choice triangle test). From a statistical analysis of a large number of panel scores the odor concentrations are revealed by on-line data processing in a personal computer. The values of this parameter are by definition equal to the dilution ratio (Don, 1986), which correspond with 50% correct responses of the panel after correction for guessing. The mean value of at least five measurements was used.

CONCLUSION

It is suggested that at the barely perceptible concentration level, hydrogen sulfide, phenol, styrene, toluene, and tetrachloroethylene odors are perceived at the same levels Japan and in The Netherlands.

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A Reproducible Trigger for the Measurement of Trigeminal Latencies Elicited by a Glabellar Tap[1]

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In order to develop a screening test of trigeminal latencies which could be used in the field, a stimulator which delivered a "tap" consisting of a focused puff of air to the glabella was compared with a tap delivered by means of a lightweight hammer with a piezoelectric trigger similar to that described by Shahani (Shahani and Young, 1972, *Neurology* 22, 149–154). Results were similar for both methods although the mechanical tap failed to elicit a response from the left side in one subject. The benefit of the air tap is in the reproducible force of the tap and better aim than is possible with the hammer. © 1993 Academic Press, Inc.

INTRODUCTION

Environmental and occupational exposure is an area of great and growing concern. In order to associate effect with exposure, it is often necessary to study large numbers of subjects and controls in field settings. Such testing requires rapid, reproducible, and cost-effective methodology which can be used successfully by nonspecialists. This paper describes an attempt to simplify and standardize a laboratory procedure for use in field surveys.

MATERIALS AND METHODS

Blink reflex was studied using a tap from a lightweight hammer with a piezoelectric trigger and a focused puff of air to produce a glabellar tap. Surface electromyographical recordings were made using surface EMG electrodes placed on the outer canthus superior and inferior to the orbicularis oculi muscle with a ground placed on the neck 5 cm anterior to the angle of the jaw. Data were collected and stored by a computerized system consisting of battery-powered amplifiers, an analog-to-digital converter, and a microcomputer.

The hammer consisted of a round pencil eraser 3 cm in length held lightly against a piezoelectric element, both mounted on a 30-cm aluminium shaft. The force generated by striking the glabella with the tip of the pencil eraser was transmitted to the piezoelectric element creating a signal which triggered the recording system. Tapping a second piezoelectric element with this device and recording both responses on a dual-trace oscilloscope confirmed that there was no measurable delay in response at the 0.25 msec sampling rate of the recording system.

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The air tap was produced by opening an electrically actuated solenoid which permitted air from a pressure reservoir to flow through a 60-cm length of small bore tubing (~1 mm) one end of which was held 1 cm from the surface of the skin at the glabella. the delay from the computer keystroke which triggered the solenoid to the arrival of the wavefront of the puff of air at the glabella was measured by placing a pressure transducer with its diaphragm 1 cm from the tip of the tubing. Because the cross section of the tubing is small, flow within the tubing is laminar and the force of the puff is reproducible as long as the reservoir is kept above the threshold at which flow no longer increases with increasing driving pressure. This pressure independence was confirmed by means of the above-mentioned pressure transducer.

The air solenoid which controls the start and duration of the puff was silenced by wrapping it in foam rubber. Because the sound of the puff at the catheter tip produced no measurable response by itself, no attempt was made to muffle it.

Ten 250 msec traces were saved to disk for each type of stimulus. The traces were averaged digitally and plotted (Fig. 1), and the latencies for air and hammer stimulus were compared by Student's *t* test using STATA statistical software (Computing Resource Center, Los Angeles, CA).

RESULTS

Results (average of 10 trials) are presented in Tables 1 and 2. Because one

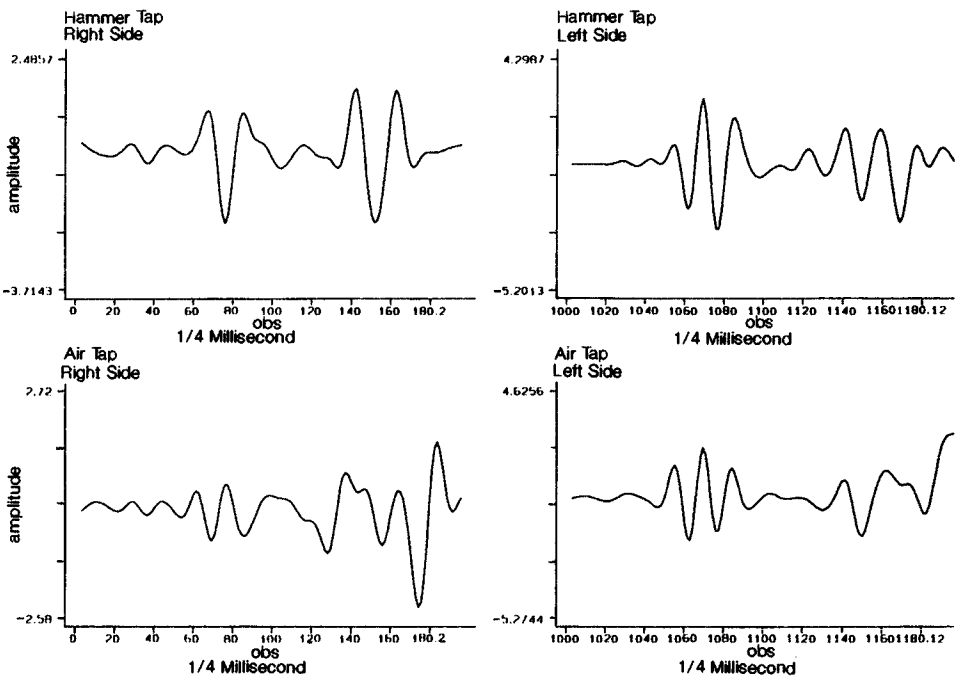

FIG. 1. Subject R. Plots of surface EMG response to hammer and focused air puff stimulus. Each plot is the mean of the responses to 10 stimuli. The first major vertical deflections from the left (beginning at 60 and 1060 on the ordinate) represent R1. The second major deflection in each trace represents R2.

TABLE 1
RESULTS FOR INDIVIDUAL SUBJECTS IN MILLISECONDS

id	Hammer tap left	Hammer tap right	Air tap left	Air tap right
1	15.1	15.0	15.1	15.1
2	14.6	16.1	14.5	15.16
3	13.9	17.8	14.8	21.1
4	[a]	13.9	13.3	13.3
5	15.1	16.0	14.2	13.3

[a] No response.

subject had no R1 response by hammer tap on the left, only the four subjects with complete data are compared for the left response.

The means of air and hammer tap are not different for both the left and the right side ($P = 0.93$ for the left and $P = 0.898$ for the right).

DISCUSSION

In a study of subjects exposed to trichloroethylene in well water, Feldman *et al.* (1988) found differences between exposed and control subjects, suggesting the value of electrophysical measurement of the blink reflex as an epidemiological tool. We found that the use of an electrical stimulator as described by Kimura (1983) was not always well tolerated by our subjects and was cumbersome in the field. A glabellar tap technique utilizing a phonographic cartridge as a hammer (Fisher *et al.*, 1979) was better tolerated and simpler to use in the field. We found however that striking the glabella accurately and with reproducible force was difficult, particularly with seated rather than supine subjects.

A generalized puff of air to the face is reported to produce only a second component of blink (R2). Our experience using both a generalized puff and a puff aimed at the cornea (unpublished observation) confirms that only a second component is produced. However, a sharply focused puff of air to the glabella produces an R1 response.

Our data for latency of the first component response in these clinically normal subjects are within the range reported by others (Shahani and Young, 1972; Fisher *et al.*, 1979) but, despite Shahani's suggestion that there should be no difference between tap and electrical stimuli, our mean values for these volunteer subjects

TABLE 2
COMPARISON OF R1 BY GLABELLAR TAP USING HAMMER AND PUFF OF AIR IN MILLISECONDS (MSEC)

	n	Mean R1	SD	Min	Max
Hammer tap, left[a]	4	14.66	0.577	13.86	15.10
Air tap, left[a]	4	14.62	0.383	14.16	15.06
Hammer tap, right	5	15.76	1.471	13.86	17.83
Air tap, right	5	15.55	3.225	13.25	21.08

[a] Only subjects with complete data are compared for the left response.

are longer than those of Feldman's referents by 2 msec. It may be that the extremely long left-side latencies measured for subject 3 (Table 1) are early R2 responses or that subject 3 is abnormal.

Further investigation is needed to determine whether tap, electrical stimulation, flash of light, and focused puff of air produce different latencies. Focused puff of air stimulus to the glabella appears to produce a first component response comparable to that produced by tap with equal dependability.

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Assessment of Sympathetic Nerve Activity Controlling Blood Pressure in the Elderly Using Head-Up Tilt[1]

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In order to assess age-related changes in sympathetic nerve activity controlling blood pressure, we recorded muscle sympathetic nerve activity, blood pressure, and heart rate during head-up tilt in 10 healthy elderly (69–75 years) and 16 healthy young (19–23 years) subjects. The elderly had significantly lower responsiveness of muscle sympathetic nerve activity to postural change than did the young subjects. In the elderly, marked rise in blood pressure without increase in muscle sympathetic nerve activity was observed in nearly upright position during head-up tilt, whereas this phenomenon was not observed in the young. We conclude that neural control function of blood pressure during head-up tilt in the elderly differs from that in the young, which may be due to age-related change in baroreflex function.

INTRODUCTION

Recent advances in microneurographic technique have enabled direct observation of peripheral sympathetic nerve discharges in humans (Hagbarth and Vallbo, 1968). The activity of postganglionic sympathetic efferent nerve fibers to skeletal muscles (muscle sympathetic nerve activity) identified by this technique plays an important role in the control of blood pressure (Wallin, 1981, 1983).

Many previous studies on sympathetic nerve function used indirect indices such as heart rate, blood pressure, and perspiration. Plasma catecholamine is also an indirect index. We predict that the utility of microneurograms will increase in examination of sympathetic nerve function for elderly subjects who have variable age-related changes of all organs.

The purpose of this study was to assess age-related changes in sympathetic nerve activity controlling blood pressure using head-up tilt.

SUBJECTS AND METHODS

Ten healthy elderly (69–75 years) and 16 healthy young (19–23 years) subjects were examined. All subjects were informed of the purpose and of the procedure used in this study and gave their consent to participate in the experiment. This study was conducted under the guidelines proposed by the Japan Microneurography Society and was approved by the Ethical Committee on Human Research of the Research Institute of Environmental Medicine, Nagoya University.

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The subjects were asked to lie down on a tilt table in a horizontal position. Muscle sympathetic nerve activity was recorded as described elsewhere (Mano, 1990) from the tibial nerve at the popliteal fossa by a microneurographic technique with a tungsten microelectrode with a tip diameter of about 1 μm and an impedance of 3–5 MΩ.

The identification of muscle sympathetic nerve activity is based on the presence of the following discharge characteristics reported by Mano (1990): (1) pulse-synchronous and rhythmic efferent burst discharges recorded from muscle nerve fascicle, (2) modulation by respiration, and (3) enhancement by maneuvers increasing intrathoracic pressure such as Valsalva's maneuver. The number of bursts per minute (burst rate) in muscle sympathetic nerve activity was used as a quantitative index.

During the experiment, heart rate was determined by ECG monitoring (Nihon Koden, Hyperventilation Detector; MZW6100) and arterial blood pressure was measured by the oscillometric method (Nihon Colin, BP-203Y) every minute.

Figure 1 shows the protocol of the experimental procedure. Each subject was studied during the day between 10:00 and 17:00. After the identification of muscle sympathetic nerve activity, the subject lay quietly in the supine position for at least 30 min, and parameters were measured at 0° tilt for the following 15 min. The table was then tilted in a passive and graded fashion to upright standing. Parameters were recorded for 3 min at each tilt angle.

Statistical analysis of differences between the two groups was performed with Student's *t* test. Probability values of less than 0.05 were considered significant.

RESULTS

The average values of muscle sympathetic nerve activity, heart rate, and blood pressure at rest in the supine position in both age groups are summarized in Table 1. The average values of burst rate in muscle sympathetic nerve activity and blood pressure at rest were significantly higher in the elderly than in the young.

Figure 2 shows changes in burst rate of muscle sympathetic nerve activity during head-up tilt in both age groups. In both age groups, burst rate of muscle sympathetic nerve activity increased linearly to the sine function of the tilt angle. During head-up tilt from 0° to 90°, burst rate of muscle sympathetic nerve activity increased from 51.7 (bursts/min) to 63.6 in the elderly and from 13.7 to 36.7 in the young. The increase in burst rate by orthostasis from the supine position to upright standing was significantly smaller in the elderly than in the young.

Figure 3 shows changes in heart rate during head-up tilt in both age groups. In the elderly, heart rate increased from 63.8 to 77.8 by orthostasis and in the young from 60.2 to 95.8. Heart rate during head-up tilt in the elderly was less increased compared with that in the young.

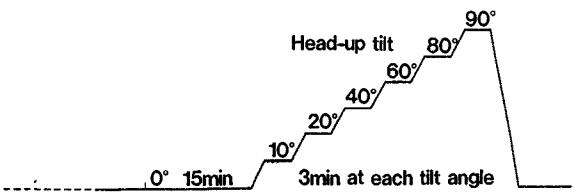

FIG. 1. Protocol of the experimental procedure.

TABLE 1
MUSCLE SYMPATHETIC NERVE ACTIVITY, BLOOD PRESSURE, AND HEART RATE AT REST IN THE SUPINE POSITION IN ELDERLY AND YOUNG SUBJECTS

	Elderly		Young		
	Mean	SD	Mean	SD	P^a
Burst rate (burst/min)	51.7	8.3	13.7	11.1	<0.01
Systolic BP (mmHg)	121.1	15.0	109.5	10.4	<0.05
Diastolic BP (mmHg)	66.4	8.8	54.1	6.5	<0.01
Heart rate (beats/min)	63.8	7.1	60.2	7.0	NS

[a] *P*, Statistical significance of difference between the two age groups, using Student's *t* test.

Figure 4 shows the relationship between changes in burst rate in muscle sympathetic nerve activity and blood pressure during head-up tilt in both age groups. In the elderly, rises in both systolic and diastolic blood pressure without increase in muscle sympathetic nerve activity were observed when the subjects were in a nearly upright position, whereas the phenomenon was not observed in young subjects.

DISCUSSION

Sundlöf and Wallin (1978) and we (Iwase *et al.*, 1990) observed that resting muscle sympathetic nerve activity increased with advancing age. In this study, burst rate in muscle sympathetic nerve activity was also found to be higher in the elderly than in the young. If the findings of Wallin (1981, 1983) which indicate that muscle sympathetic nerve activity is composed mainly of vasoconstrictor impulses are taken into consideration, our results suggest that peripheral sympathetic nerve activity controlling blood pressure may increase in the elderly at rest.

Bruke *et al.* (1977) and we (Iwase *et al.*, 1990) reported an increase in muscle

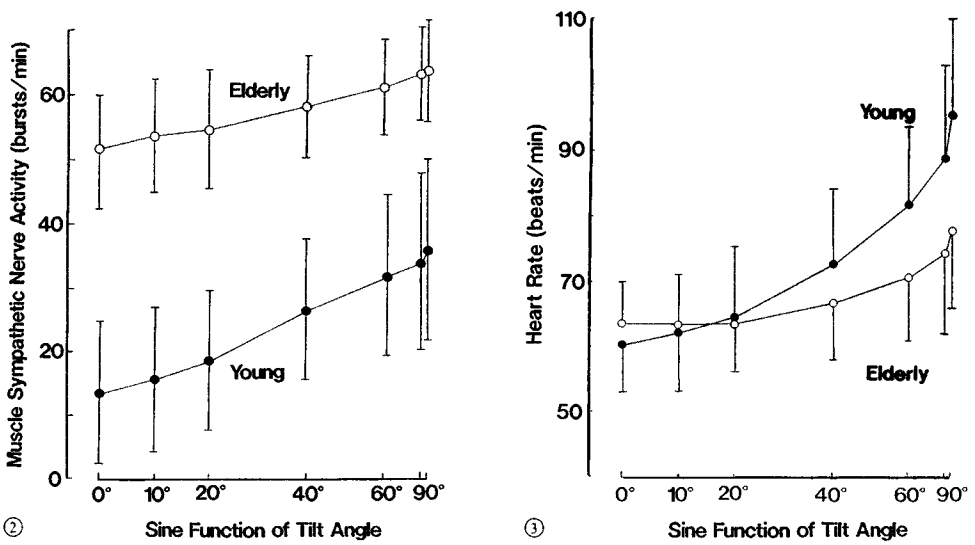

FIG. 2. Changes in burst rate of muscle sympathetic nerve activity during head-up tilt in elderly (○) and young (●) subjects.

FIG. 3. Changes in heart rate during head-up tilt in elderly (○) and young (●) subjects.

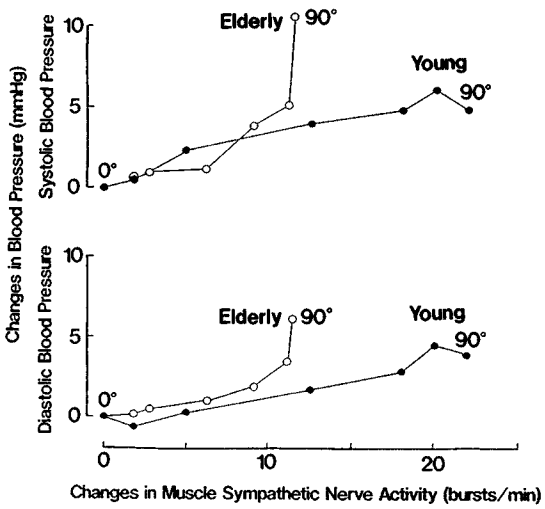

FIG. 4. Relationship between changes in burst rate of muscle sympathetic nerve activity and blood pressure during head-up tilt in elderly (○) and young (●) subjects.

sympathetic nerve activity due to postural change. In this study, burst rate in muscle sympathetic nerve activity also increased due to postural change, and the amount of increase in muscle sympathetic nerve activity during head-up tilt was smaller in the elderly than in the young, which suggests that the responsiveness of muscle sympathetic nerve activity to orthostasis may decline with advancing age.

In the above-mentioned studies, however, the relation of muscle sympathetic nerve activity to blood pressure during orthostasis was not examined. In the present study, blood pressure during head-up tilt did not prominently change in the young, while in the elderly a marked rise in blood pressure, which was not accompanied by an increase in muscle sympathetic nerve activity, was observed when subjects were in a nearly upright position.

In this position, return blood volume to the heart is presumably recovered by the pumping action of muscles in the lower extremities by which cardiac output may be increased. However, increase in heart rate in this standing position in the elderly was not so marked, compared with that in the young. Baroreflex function is modified by aging (Karemaker *et al.*, 1989). Under this condition, reduced function of the baroreflex in the elderly might cause the observed rise in blood pressure.

In this analysis, we used only the average value of muscle sympathetic nerve activity and blood pressure for 3 min. Because transient changes in muscle sympathetic nerve activity and blood pressure were not examined, it is unknown whether the same phenomenon can also be observed after 3 min in the upright position. Our results merely suggest that the response time of neural mechanisms controlling blood pressure may be delayed in the elderly.

In summary, we conclude that neural control function of blood pressure during head-up tilt in the elderly differs from that in the young, which may be due to the age-related change in baroreflex function.

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ENVIRONMENTAL RESEARCH 60, 145–159 (1993)

Vibrotactile Threshold Testing in Occupational Health: A Review of Current Issues and Limitations[1]

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Quantitative cutaneous vibrotactile threshold measurement has been proposed as a useful method for assessing peripheral nervous system function in occupational and environmental neuroepidemiology. It allows rapid, quantitative, and nonaversive assessment of peripheral nervous system function. Acceptance of this method is currently limited, however, because of poor standardization of methods, the lack of data regarding the effects of age, gender, and other covariates, and minimal demonstration of association between vibrotactile threshold and conventional measures of peripheral nerve function. Data from a series of validation studies intended to address some of these problems are presented and relevant literature is discussed. Specifically, results of studies in which reliability and time efficiency of testing protocols were measured, the effects of covariates on measurement of vibrotactile threshold were estimated, and vibrotactile thresholds were compared to physical examination and electrophysiologic evaluation are discussed. Recommendations are made regarding choice and standardization of testing protocol, covariates, and issues for future research.

INTRODUCTION

The peripheral nervous system is vulnerable to injury following exposure to chemical substances such as solvents, pesticides, and heavy metals and to physical agents such as high-frequency hand–arm vibration and ergonomic factors such as force and repetition. Possible reasons for selective vulnerability of the nervous system have been reviewed elsewhere (OTA, 1990; Thomas, 1980) but include the inability to replace lost neurons and the need of neurons to transport cellular products over long distances. Objective and reliable methods of assessing peripheral nervous system integrity are needed for epidemiologic study of groups with occupational and environmental exposure to neurotoxicants, for screening and surveillance of workers at risk of neurologic dysfunction, and for assessment of individuals with such exposures.

Measurement of vibrotactile threshold has been proposed as a useful method for evaluating peripheral nervous system dysfunction or disease (Bove *et al.*, 1986; Bleecker, 1986). This method allows for rapid, nonaversive, and quantitative assessment of the integrity of the somatosensory pathways that convey information induced by vibratory stimulation. While these characteristics render vibrotactile thresholds especially useful measures of outcome in occupational and environmental neurotoxicology, their acceptance has been limited by a lack of

[1] Presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

standardization of testing protocols; use of poorly defined units for reporting thresholds; limited data on the effects of age, gender, and other covariates; and minimal demonstration of associations between vibrotactile threshold and accepted, commonly used methods of assessing peripheral nerve function, especially physical examination and electrophysiologic evaluation. In this paper these issues are reviewed, recommendations for standardization are made, and areas in need of additional research are identified.

Overview of Neurologic Assessment

The two most commonly used methods for objective assessment of peripheral nervous system function are clinical neurological examination and electrophysiologic evaluation including nerve conduction velocity measurement and electromyography. These methods are not well suited for use in epidemiologic studies for assessment of workers exposed to potentially neurotoxic agents or for use in screening and surveillance programs of individuals at risk of neurologic dysfunction. Methods of greatest utility in occupational neuroepidemiology are those that provide outcomes that are objective, quantitative, reliable, standardized, and nonaversive; can be administered rapidly with portable, inexpensive equipment by minimally trained testers; and have readily available normative data. Comparison of the relative strengths and weaknesses of physical examination, electrophysiologic evaluation, and vibrotactile threshold testing is made in Table 1.

Clinical examination typically results in a categorical outcome, which is "adjusted" for the effects of covariates such as age based on the examiner's personal experience. Intra- and interexaminer variability has not been well established. While the method is rapidly administered using inexpensive tools and is readily accepted by subjects, it must be administered by a trained clinician.

Nerve conduction velocity testing is a well-established measure of peripheral nerve function that results in continuous outcomes which can be adjusted for the effects of covariates such as age, height, and limb temperature. Because the test

TABLE 1
CHARACTERISTICS OF PHYSICAL EXAMINATION (PxEx), ELECTROPHYSIOLOGIC MEASURES (NCV/EMG), AND VIBROTACTILE THRESHOLD MEASUREMENT (VT)

Characteristic	PxEx	NCV/EMG	VT
Objective	−	+	±
Quantitative	−	+	+
Reliable	?	+	+
Standardized	±	+	−
Norms	−	+	±
Rapid	+	−	±
Nonaversive	+	−	+
Portable	+	±	+
Inexpensive	+	−	±
Little tester training required	−	−	+

Note. +, True or available for the method; −, false or not available for the method; ±, true or false depending on the specific test protocol; ?, unknown.

measures nerve function directly, it is not subject to deliberate manipulation by the subject. The method, however, is aversive, using mildly noxious stimulation with electric current to evoke responses from nerve and muscle. In addition, the testing equipment is expensive, a highly trained technician is required, and the procedure is relatively time consuming to administer.

Quantitative cutaneous vibrotactile threshold testing provides an alternate method for assessment of peripheral nerve function. The outcome of this type of measurement is continuous and may, therefore, be adjusted for the effects of age and other covariates. In addition, use of continuous measures of outcome allows more precise estimates of function than do the dichotomized results of physical examination. Measurement of vibrotactile thresholds can be performed rapidly by a technician. The method is nonaversive and is therefore suitable for repeated testing over time or for testing asymptomatic volunteer subjects who might object to unpleasant stimulation.

It has become customary to refer to vibrotactile threshold testing as a measure of peripheral nerve function. Indeed, as will be discussed in this paper, vibrotactile testing is useful for assessing potential peripheral nerve dysfunction. However, these measures are dependent upon the integrity of the entire somatosensory pathway, not just the peripheral nerves.

The somatosensory system includes components of both the peripheral and central nervous systems. The sensory components of the peripheral nervous system consist of the peripheral nerves and their associated sensory endings. The peripheral nerves that mediate perception of touch, pressure, and vibration are myelinated and 10–15 μm in diameter. They are designated as "A-beta" fibers. Their sensory endings are associated with a variety of mechanoreceptors that transduce mechanical stimulation into neural impulses (Dellon, 1981).

The A-beta class of fibers and their associated mechanoreceptors can be classified according to their adaptation to constant pressure stimulation. Slow adaptation is mediated by Merkel's cells and Ruffini endings, which are referred to as intensity detectors. Moderately rapid adaptation is mediated by Meissner corpuscles, which are referred to as velocity detectors, and very rapid adaptation is mediated by Pacinian corpuscles, which are referred to as acceleration detectors. The Pacinian corpuscle is the mechanoreceptor responsible for transduction of vibratory stimuli in the range of 100 to 200 Hz (Mountcastle, 1980; Schmidt, 1986).

The neural impulses generated by the receptors are conducted along the nerve through the dorsal root into the spinal cord, where a synapse occurs with second-order afferent neurons in the dorsal column nuclei. Fibers from these second-order neurons pass through the medial lemniscus to the thalamus, where a second synapse occurs. Fibers from these third-order neurons pass to the sensory cortex, where at least one additional synapse occurs. At all stages of this relay process a spatial mapping of the skin is preserved. This representation is proportional to dermal receptor density rather than actual skin surface area. Thus, the area represented by the head, hands, and feet is disproportionately large.

Disturbances in sensory function can be caused by dysfunction of any component of the somatosensory system. For example, exposure to hand–arm vibration likely causes injury to both the Pacinian corpuscle and the distal-most projections

of the sensory nerve fibers (Brammer *et al.*, 1987; Cherniack, 1990). Exposure to a variety of hazards, including certain hexacarbon solvents, organophosphates, and heavy metals, is associated with axonal degeneration of myelinated peripheral nerves (Schaumburg *et al.*, 1983). Injury to the central nervous system can also produce disturbances in sensory function. Stroke or space-occupying lesions can produce dramatic alterations in sensation. Toxic exposures may also selectively affect central components of the somatosensory system, as is thought to occur following exposure to organic mercury compounds (Berlin, 1986). Since damage to any component of the somatosensory pathway can result in changes in vibration perception, measurement of vibrotactile thresholds is likely to be more useful as a screening tool for neurologic effects of exposure than for localization of those effects to a specific component of the somatosensory pathway.

Uses of Vibrotactile Thresholds in Occupational Medicine

Studies of the effects of exposure to chemical agents, such as solvents, and to physical agents, such as segmental hand–arm vibration, have been performed using vibrotactile thresholds as a measure of outcome. In addition, of relevance to occupational neuroepidemiology is the potential use of vibrotactile thresholds for detection of carpal tunnel syndrome.

Chemical exposure. Vibrotactile thresholds have been used in studies of workers exposed to organic solvents and other chemicals in the United States and Europe. Eloffson *et al.* (1980) observed elevated vibrotactile thresholds in a cross-sectional study of spray painters exposed to organic solvents. As part of a screening of acrylamide-exposed workers for peripheral nerve dysfunction, Arezzo *et al.* (1983) used vibrotactile thresholds. Halonen *et al.* (1986) measured vibrotactile thresholds in a screening of shipyard workers exposed to organic solvents. In a group of painters exposed to organic solvents, Bove *et al.* (1989) found elevated vibrotactile thresholds. McConnell *et al.* (1990) found elevated thresholds among agricultural workers with a history of organophosphate pesticide poisoning.

Hand–arm vibration. Many studies of workers with occupational exposure to hand–arm vibration have employed vibrotactile thresholds as an outcome measure (Farkkila *et al.*, 1985; Brammer *et al.*, 1987; Lundborg *et al.*, 1987; Ekenvall *et al.*, 1989; Cherniack *et al.*, 1990). Elevated thresholds were observed among vibration-exposed workers in most studies. A consensus is emerging in this area of research that the neurological effects of exposure to hand–arm vibration are more readily detected with measurement of cutaneous sensory thresholds than with nerve conduction velocity determination or physical examination.

Carpal tunnel syndrome. Few studies are currently available regarding the use of vibrotactile thresholds for the detection of carpal tunnel syndrome (Szabo *et al.*, 1984; Borg and Lindblom, 1986, 1988; Lundborg *et al.*, 1986; Merchut *et al.*, 1990; Jetzer, 1991). Although they suggest a role for vibrotactile thresholds in detecting carpal tunnel syndrome, several of these studies are limited by poor definition of carpal tunnel syndrome or by inadequate study design. No studies are currently available in which vibrotactile thresholds are reported for a working population at risk of carpal tunnel syndrome.

CRITIQUE OF THE LITERATURE

Several problems emerge upon review of the literature describing the use of vibrotactile thresholds in occupational neuroepidemiology. Ideally, a method of determining vibrotactile thresholds should (1) be standardized regarding both testing protocol and reporting of results, (2) be well characterized with respect to covariates such as age and gender, and (3) have known relationships to other methods for assessing peripheral nerve function.

Standardization

Because of differences in hardware, test protocol, and other factors, it is not currently possible to compare vibrotactile threshold results across studies nor to pool data from similar populations studied by different investigators.

Hardware. Vibrotactile thresholds obtained with at least five commercially available devices have been reported in the English language occupational health literature. These include the Biothesiometer (Biomedical Instruments, Newbury, OH), Vibratron II (Physitemp, Clifton, NJ), Optacon (Telesensory Systems, Palo Alto, CA), Somedic Vibrometer (Somedic, Stockholm, Sweden), and the B&K Vibrometer (Bruel and Kjaer, Naerum, Denmark). In addition, published papers are available for several investigator-developed devices (Dyck *et al.*, 1984; Muijser *et al.*, 1986; Brammer *et al.*, 1987; Maurissen and Chrzan, 1989).

The lack of standardization due to differences in hardware will be difficult to overcome. Factors such as diameter of the contactor and whether it has a rigid surround vary from device to device and may limit comparability of results (Verillo, 1963; Lamoré and Keemink, 1988). One remediable problem resulting from differences in hardware configuration is the lack of standardized reporting units for vibrotactile threshold. Thresholds are variously reported as "vibration units" (Vibratron), volts delivered to the transducer (Biothesiometer), and acceleration (B&K Vibrameter). Investigators have also reported thresholds in log micrometers of peak-to-peak displacement (Gerr *et al.*, 1990; Dyck *et al.*, 1984), and decibels relative to a reference value, typically 1 μm (Bolanowski *et al.*, 1988).

Vibration can be characterized completely by describing the shape of the waveform, its frequency, and its amplitude. Virtually all electromechanical vibrometers produce sinusoidal stimulation; therefore, the shape of the waveform is not a barrier to the comparison of results. Frequency is usually fixed at either 100 or 120 Hz although some devices produce vibration over a range of frequencies. Whereas the choice of reporting units is somewhat arbitrary, it seems most useful to report thresholds in universal units of amplitude that are not idiosyncratic to a particular vibrometer. Specifically, micrometers of displacement may be the most intuitively understandable to readers and are universal measures of displacement independent of the hardware used to generate them. We advocate the use of the common logarithm of micrometers of peak-to-peak displacement because a logarithmic transformation linearizes the relationship between vibrotactile threshold and age (Gerr *et al.*, 1990; Dyck *et al.*, 1984) and yields a threshold easily converted to decibels relative to 1 μm.

Using a calibrated accelerometer, we have observed differences in actual vibration amplitude among Vibratrons displaying the same vibration unit value. It seems, therefore, that blind acceptance of the manufacturer's calibration may also result in noncomparability of results. Experiences with other devices may be similar. Periodic measurement of actual vibration amplitude with a calibrated accelerometer will improve the quality of reported amplitude data and reduce systematic differences between studies, even those using the same type of equipment.

Test protocol. In addition to the diversity of hardware in use, a variety of testing protocols have also been used. The major issue in protocol development has centered around the use of "forced-choice" *versus* "yes–no" procedures. These terms refer to the responses required of the subject. In a forced-choice procedure a stimulus is presented in only one of two or more intervals within each trial. The stimulus is always presented on each trial, but varied randomly among the intervals, and the subject is required to choose which interval contained the stimulus. In a yes–no procedure the subject is required to respond as to whether he/she felt the stimulus or not.

Yes–no methods are prone to "criterion bias," i.e., differences between individuals in the criterion that a subject uses to decide whether a stimulus was in fact present or absent. "Criterion shift" refers to a change over time within individuals in criterion for responding positively. Thus, a subject might require greater intensity stimulation on one occasion than another to declare that he/she feels the same stimulus. Forced-choice methods are not prone to criterion bias. However, forced-choice methods are prone to considerable estimation bias and require lengthy protocols to provide reliable estimates. Several papers reviewing the theoretical difficulties with forced-choice algorithms are available (Kershaw, 1985; Rose *et al.*, 1970). Although criterion bias is a concern in psychophysical laboratory experiments of healthy volunteers, the relative importance of criterion bias and estimation bias in time-constrained epidemiologic and clinical studies has not been evaluated empirically.

Another important aspect of the psychophysical protocol for estimating a threshold is determining how the stimulus intensity is to be varied. One commonly used method, the method of limits, involves monotonically increasing or decreasing stimulus intensity. The familiar Békésy method used in audiometry is an automated, continuous method of limits presentation of stimulation with a yes–no type of response. Thus, when a stimulus that is ramping up in intensity becomes perceptible, the subject pushes a button, which causes the stimulus to ramp down in intensity until the subject no longer perceives the stimulus and stops pushing the button, which causes the stimulus to begin ramping up in intensity, and so on. Most commonly used forced-choice procedures use so-called "staircase" methods of stimulus presentation. Staircase procedures use the subject's prior response(s) to stimuli to determine the intensity of the subsequent stimulation to efficiently concentrate most trials near the subject's threshold. The theory for some staircase estimators is well established (Wetherill and Levitt, 1965).

In the context of evaluating a commercially available fixed-frequency vibrome-

ter, we compared the forced-choice and staircase stimulus presentation procedure recommended by the manufacturer of the device to a simpler yes–no method of limits procedure. This comparison was performed in two samples: healthy ambulatory volunteers (Gerr and Letz, 1988) and diabetic patients (Gerr *et al.*, 1990). Test–retest correlation coefficients and time efficiency for both testing methods were calculated. In both groups, the method of limits protocol was of comparable or better reliability and was much more time efficient. We concluded that the method of limits procedure was more desirable than the manufacturer's recommended forced-choice procedure for use in occupational medicine field investigations in which time efficiency is critical. In addition, the particular forced-choice protocol recommended by the manufacturer does not yield a threshold with a precise psychophysical definition. Other forced-choice procedures are available that are well grounded psychophysically and have been compared to manual and automated method of limits procedures.

Muijser *et al.* (1986) tested the distal volar surface of the nondominant index finger of a group of workers with no history of exposure to toxic chemicals. Subjects were tested twice, 1 week apart with two different vibrometers, using both a two-interval forced-choice protocol as well as a method of limits procedure in which the stimulus strength was increased from below threshold to greater than threshold for seven trials, followed by seven trials of descending stimulation amplitude from suprathreshold levels. They observed that ". . . unexpectedly, the forced-choice method did not produce lower standard deviations than did the much simpler method of limits." However, because several subjects were not able to identify an extinction point on the descending method of limits trials, the authors recommended use of the forced-choice method.

Dyck *et al.* (1990) compared several algorithms for use in automated measurement of vibrotactile threshold of the index finger in 20 healthy subjects and the great toe in 20 patients with mild neuropathy. In addition to using a forced-choice algorithm, they also tested a so-called "linear ramp" procedure as well as several Békésy-type algorithms that differed from each other in rate of change of stimulus intensity. Subjects were tested on two occasions, and the difference between the tests was calculated. Among healthy subjects, all non-forced-choice algorithms were found to have equivalent or better repeatability than the forced-choice algorithm. In addition, the time efficiency for the Békésy algorithms were substantially better than for the forced-choice procedure. Specifically, the forced choice protocol required an average administration time of 9.1 minutes while the most lengthy of three Békésy protocols required 1.3 min.

Among the patients with mild neuropathy, Dyck *et al.* also found that repeatability was not different among algorithms. In addition, although administration times were longer for this group, the Békésy algorithms were again more time efficient, requiring between ½ to ⅓ as much time to administer as the forced-choice algorithm. Dyck *et al.* stated: "By the criteria of accuracy, repeatability, and speed, the Békésy algorithms with null stimuli . . . provided an accurate and fast threshold." The authors concluded that forced-choice algorithms may be preferable to the Békésy algorithm when the patient is unable to depress the

response key or slow to respond. Furthermore, they caution about the use of non-forced-choice procedures when the subject has symptoms such as paresthesias that are difficult to discriminate from the vibratory stimuli.

Other factors. Little information is available regarding the optimal anatomic site(s) for determination of vibrotactile thresholds. Clearly, for detection of distal axonal disease, the distal portion of the lower extremity is preferable to the upper extremity. However, there is little agreement regarding the properties of thresholds from different sites. For example, the pad of the digit is quite distal, is not subject to bone transmission, and has high receptor density. However, it is prone to callosities which likely affect the measurement, although currently to an unknown degree. Likewise, great heterogeneity exists regarding the size of the stimulator surface used as well as the effect of variation in contact pressure. These variables are known to affect vibrotactile threshold measurement (Goldberg and Lindblom, 1979; Harada and Griffin, 1991). Additional research is needed to clarify these important issues, particularly concerning their effects on the detection of disease or subclinical pathology.

Covariates

Covariates of interest include age, height, gender, skin temperature, and alcohol consumption. Control of covariates allows for greater precision of estimates and more powerful statistical comparisons. A number of studies are available in which the effects of a variety of covariates on vibrotactile thresholds are evaluated.

Age and height. We have evaluated the effects of age and height as well as other potential covariates on vibrotactile thresholds determined with our method of limits protocol in a group of 132 asbestos-exposed construction trades workers (Gerr *et al.,* 1990). Regression equations relating age and height to vibrotactile threshold were calculated. The relationships between age and vibrotactile threshold, and between height and vibrotactile threshold are depicted in Figs. 1 and 2, respectively. Partial residuals are presented because in this population, age and height were slightly negatively correlated. Age and height were significantly related to finger and toe thresholds. Age had a greater effect than height on both finger and toe thresholds. The proportion of variance of vibrotactile threshold accounted for by age and height was calculated and was substantially greater for the lower extremity than the upper. The presence of medical illness or a history of heavy alcohol use was not associated with elevated thresholds. However, the number of such subjects was small and the power to detect such effects was low.

Many other investigators have found a strong effect of age on vibrotactile threshold (Goff *et al.,* 1965; Goldberg and Lindblom, 1979; Verrillo, 1979; Dyck *et al.,* 1984; Era *et al.,* 1986; Halonen, 1986; Muijser *et al.,* 1986; Sosenko *et al.,* 1989; Gerr *et al.,* 1990). The relationship is well established to be log-linear (age vs threshold expressed in log micrometers). The need to account for the effect of age is well accepted among investigators.

Only a few studies have investigated the effects of height on vibrotactile thresholds. In addition to our work, only Sosenko *et al.* (1989) have reported regression parameters for both age and height. They found a significant association between

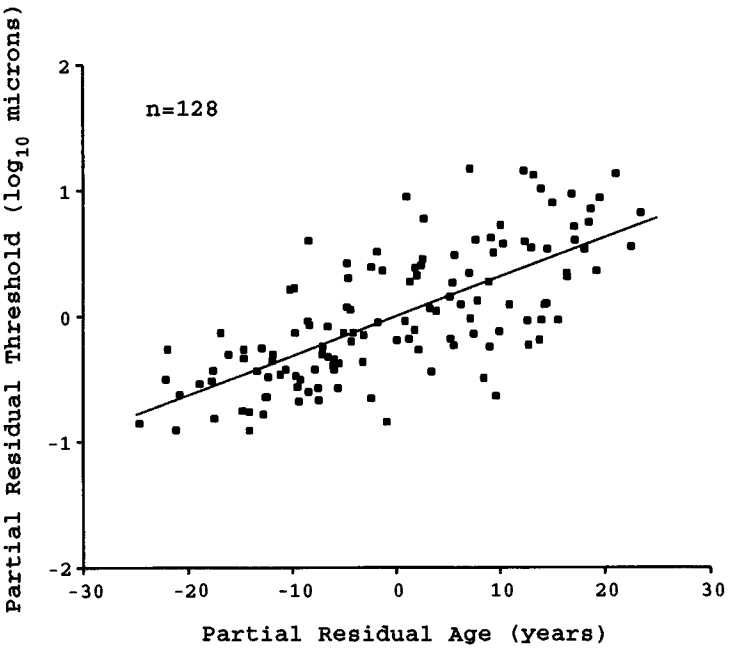

FIG. 1. Partial residual of great toe vibrotactile threshold vs partial residual of age.

height and toe, but not finger, thresholds. Era *et al.* (1986) observed a significant effect of height on vibrotactile thresholds obtained at the medial malleoli but did not report an estimate of its magnitude. Halonen (1986) reported nonsignificant effects of height on lower extremity vibrotactile thresholds when the study population was stratified by gender. When male and female subjects were pooled, a significant effect for height was found. Halonen (1986), however, cautioned that confounding by gender might have occurred and caused the apparent association.

Gender. Gender effects have also been studied, although published data are not as consistent as they are for the effects of age and height. Sosenko *et al.* (1989) found no significant differences in vibrotactile threshold between male and female subjects, but they apparently did not adjust for the 12-cm average greater height in male subjects in their study. Dyck *et al.* (1984) also reported finding no differences in finger or toe threshold between male and female subjects. Halonen (1986) found that males had higher lower extremity thresholds than did females and that the difference increased with increasing age. Interestingly, Halonen (1986) was concerned that gender was confounding the relationship between height and threshold, but did not mention height as a potential confounder for estimates of the effect of gender on threshold. Conversely, Goff *et al.* (1965) found that female subjects had higher index finger thresholds than did males at all frequencies tested except 50 Hz. Clearly, this issue is far from resolved. Future research regarding differences in gender must account for the potentially confounding effect of height.

Skin temperature. Several studies are available in which the effect of temper-

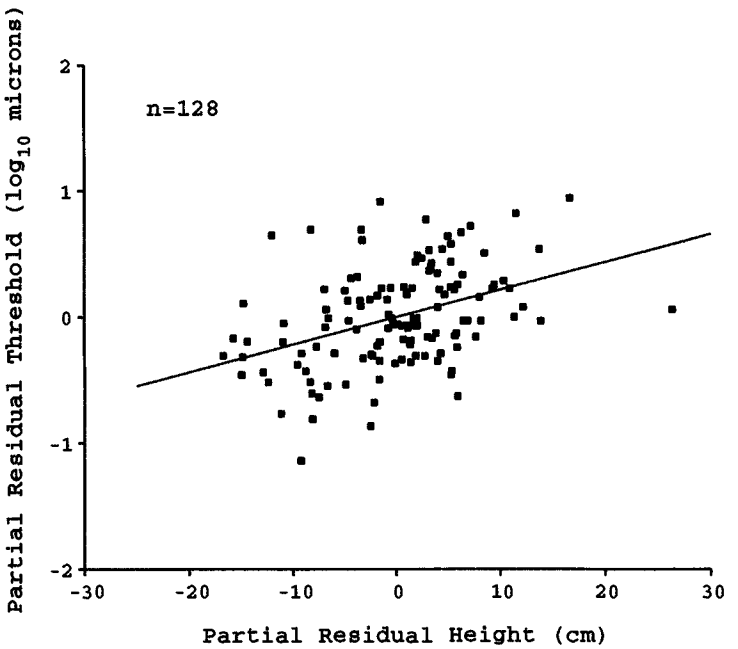

FIG. 2. Partial residual of great toe vibrotactile threshold vs partial residual of height.

ature on vibrotactile threshold was determined. Three studies involved experimental manipulation of skin temperature by controlling room temperature (Harada and Griffin, 1991), controlling the temperature of the vibrating contactor (Bolanowski and Verrillo, 1982), and by immersing the hand in ice water and allowing it to warm during vibrotactile threshold measurement (Halonen, 1986). Skin temperatures between 20 and 40°C had no effect on the 100- to 125-Hz vibrotactile threshold. At 100–125 Hz an increase in threshold at 15°C relative to higher temperatures was observed in all studies. The effect of temperature increased with higher frequency stimulation. These studies indicate that vibrotactile thresholds are not affected by skin temperature over the range usually encountered in study subjects tested at normal ambient temperature.

In addition to these experimental studies, baseline skin temperature was measured as a potential covariate at the time of vibrotactile threshold determination in two cross-sectional studies of normal subjects (Halonen, 1986; Sosenko *et al.*, 1989). These studies have produced conflicting results. Halonen (1986) found that increased skin temperature was nearly significantly associated with decreased vibrotactile threshold in men. Conversely, Sosenko *et al.* (1989) found that increase skin temperature was significantly associated with *increased* great toe vibrotactile threshold. These results require replication in a larger population. In any event, because the results from experimental studies indicate that changes in an individual subject's skin temperature per se have little effect on vibrotactile threshold, temperature should not be used as a covariate in cross-sectional analyses. Indeed, if differences in baseline temperature are caused by differences in

neurologic integrity (and thus are associated with vibrotactile threshold), inclusion of skin temperature as a covariate may bias associations of other factors with vibrotactile threshold toward the null.

Alcohol. Alcohol consumption is commonly presumed to affect neurologic performance and vibrotactile threshold measurement. Review of the literature yielded only four studies in which the effects of alcohol on vibrotactile threshold were assessed. In a study by Melgaard *et al.* (1986) the effect of alcohol consumption on vibrotactile threshold was assessed in 468 male subjects, all 45 years old. No significant difference in the proportion of elevated vibrotactile thresholds was found for those admitting to daily consumption of alcohol versus those without daily consumption. In addition, the responses to questions about drinks in the past week, drinks in the past day, and years with current drinking pattern were not significantly related to vibrotactile threshold. Only the group of subjects who experienced major health or social consequences of drinking (e.g., alcohol withdrawal seizures, morning shaking, conflicts with the law, or use of professional assistance for drinking problems) had a significantly increased proportion of subjects with elevated thresholds. In two studies of normal subjects (Era *et al.*, 1986; Sosenko *et al.*, 1989) no effect of alcohol consumption was found on vibrotactile threshold after deletion of those with a diagnosis of alcoholism. Finally, in a study of hand–arm vibration syndrome (Farkkila *et al.*, 1985), no association was observed between alcohol consumption and vibrotactile threshold. In summary, despite widespread belief to the contrary, reported alcohol consumption in nonalcoholics has not been associated with elevated vibrotactile thresholds. These results may be limited by the potential lack of reliability of reported alcohol intake. Nevertheless, only those subjects with serious social or medical consequences of ethanol consumption have been found to have elevated vibrotactile thresholds.

Comparison with Physical Examination and Measures of Nerve Conduction Velocity

Several studies are available comparing vibrotactile threshold to the well-established procedures of physical examination and measurement of nerve conduction velocity. These studies were undertaken to determine the relationship between vibrotactile threshold and methods with established utility for the detection of peripheral nerve dysfunction. Such comparisons are part of a "validation" program for demonstrating that vibrotactile thresholds are a meaningful measure of the integrity of the peripheral nervous system.

We have compared vibrotactile thresholds to physical examination and electrophysiologic parameters in a group of unselected patients referred for diagnostic electromyography (Gerr *et al.*, 1991). Vibrotactile thresholds correlated well with physical examination of vibration perception. In addition, when compared to a number of electrophysiologic measures, vibrotactile thresholds were most strongly correlated with late responses (F-wave and H-reflex) of lower extremity nerves. Specifically, correlation coefficients between late responses and vibrotactile threshold ranged from 0.56 to 0.68 in the lower extremity. The relationship between vibrotactile threshold and tibial nerve F-wave latency is depicted graph-

ically in Fig. 3. Correlations between other electrophysiologic parameters and vibrotactile threshold were poorer. Analyses were also performed to assess the utility of measurement of vibrotactile threshold for detecting electrophysiologically proven distal axonopathy. With specificities set at 90 and 95%, age- and height-adjusted thresholds were abnormal in 86 and 71%, respectively, of those patients with distal axonopathy.

Other investigators have also observed modest correlations between vibrotactile thresholds and nerve conduction measurements (Tegner and Lindholm, 1985; Dyck *et al.*, 1987; Sosenko *et al.*, 1987). These results indicate that vibrotactile thresholds do not measure the same neurologic attributes as nerve conduction velocity and electromyography and, thus, are not fully equivalent to electrophysiologic evaluation.

CONCLUSIONS AND RECOMMENDATIONS

1. To facilitate comparison of results, thresholds should be reported as the common logarithm of micrometers of peak-to-peak displacement of the vibrating stimulus. The frequency of vibration should be stated and investigators should specify when the waveform of the stimulation is not sinusoidal.
2. Instruments should be calibrated with accepted techniques and equipment.
3. Protocols that have been formally evaluated for reliability, compared to conventional measures of peripheral nerve function, and for which the effects of age and height are established should be used. Application of unproven protocols should be avoided.
4. Users of forced-choice protocols should be prepared to perform at least 50 trials to obtain reasonably precise thresholds.

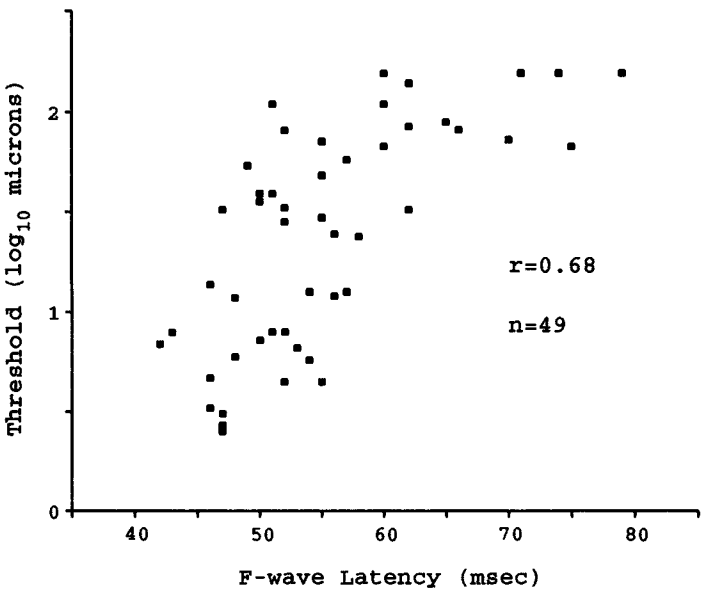

FIG. 3. Tibial nerve F-wave latency (maximum) vs great toe vibrotactile threshold.

5. Efforts should be made among investigators to standardize test site, contactor application force, contactor surface area, and other factors that may contribute to noncomparability between studies and laboratories.

6. The potentially confounding effects of age and height should be controlled in epidemiologic studies using vibrotactile threshold as outcomes. The establishment of normal values for all methods must take these covariates into account.

7. A study of vibrotactile threshold differences between males and females should be performed with the confounding effect of height controlled.

8. Skin temperature should be maintained above 20°C during measurement of vibrotactile threshold. Skin temperature should not be used as an independent variable in analyses in which vibrotactile threshold is the dependent variable.

9. Large, carefully performed studies of working subjects in which the relationship between alcohol consumption and vibrotactile threshold is estimated are needed.

10. The utility of vibrotactile thresholds for detection of carpal tunnel syndrome should be investigated in groups in which both clinical and electrodiagnostic methods have been used to establish the diagnosis of CTS.

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ENVIRONMENTAL RESEARCH 62, 325–332 (1993)

Assessment of the Effects of Occupational and Environmental Factors on All Faster and Slower Large Myelinated Nerve Fibers: A Study of the Distribution of Nerve Conduction Velocities[1]

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To assess the effects of occupational and environmental factors on all faster and slower large myelinated peripheral nerve fibers, we measured the distribution of nerve conduction velocities (DCV) in men exposed to local vibration, lead, thallium, styrene, mixed solvents, and alcohol as well as in diabetic patients. The results indicated that conduction velocities of faster large myelinated nerve fibers are more sensitive to most toxic chemicals and physical factors than those of the slower fibers. Further studies are needed to investigate which of the DCV and the conventional peripheral nerve conduction velocity is a more sensitive indicator of the subclinical effects of these factors and whether zinc and copper antagonize the effects of lead on the DCV.

INTRODUCTION

Peripheral neuropathy is one of major human diseases of occupational and environmental origins (Friedlander and Hearne, 1980; WHO Study Group, 1980; Johnson, 1987). Early detection and prevention of subclinical changes in the peripheral nerves are essential in occupational and environmental medicine.

Peripheral nerve conduction velocity has been widely used for evaluation of peripheral neuropathy. However, the conventional methods for measuring conduction velocities yield only two discrete values that reflect the maximal and the slower conduction velocities of fibers in the nerve trunk; the function of the vast majority of fibers cannot be directly assessed.

Several groups of investigators have introduced new and different techniques to derive the distribution of conduction velocities (DCV) of large myelinated nerve fibers (alpha fiber group) by means of computer analysis. The techniques are classified into sensory DCV (double conduction distance method, Barker *et al.*, 1979; single fiber axon potential method, Kovacs *et al.*, 1979; deconvolution method, Cummins *et al.*, 1979; spectral analysis method, Hirose *et al.*, 1986) and motor DCV (mean motor unit action potential method, Lee *et al.*, 1975; deconvolution method, Caddy *et al.*, 1981; collision method, Leifer, 1981). It has been demonstrated that the DCV has a good correlation with the distribution of the fiber diameters in the sural nerve of a healthy man (Cummins *et al.*, 1981) and of a *n*-hexane poisoning patient with mild axonal degeneration and focal demyelination (Yokoyama *et al.*, 1990b). The close correlation has been also obtained in peripheral nerves of normal monkey and cat (Caddy *et al.*, 1981; Milner *et al.*, 1981) and of 2,5-hexanedione poisoned cat (Sax *et al.*, 1984).

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Since 1985, we have had a technique to derive the DCV by means of computer analysis and collected new findings regarding the effects of various occupational and environmental factors on peripheral nerves (Araki *et al.*, 1986, 1988; Yokoyama *et al.*, 1990a,b; Murata *et al.*, 1991a,b,c; Fujimura *et al.*, 1993; Sata *et al.*, 1993). In this paper, we intend to clarify which of the faster or slower large myelinated nerve fibers are more sensitive to local vibration, lead, thallium, styrene, mixed solvents, alcohol, and diabetes mellitus.

SUBJECTS AND METHODS

Subjects

Nine groups of subjects were examined: vibrating tool operators (two groups), lead smelters, gun metal foundry workers (exposed to lead, zinc, and copper), an acute thallium-poisoning patient, organic solvent workers, styrene workers, alcoholic patients, and diabetic patients. They were all males. Table 1 shows characteristics of these subjects. Control subjects were healthy males whose ages were not significantly different from those of the exposed subjects.

Electrophysiological Studies

Electrophysiological studies were conducted in a warm laboratory (28–32°C) using a two-channel electromyograph (Medelec MS-92); skin temperature was maintained in the range of 30–36°C for all subjects. The DCV was measured by the same method reported previously by us (Araki *et al.*, 1986, 1988; Yokoyama *et al.*, 1987), a modified method of Barker *et al.* (1979). After electrical stimulation of the right median nerve at the second digit with a 70- to 100-V square wave pulse of 0.2 msec, the compound action potential was picked up at the wrist and elbow (Fig. 1). The DCV was calculated between the velocities of 30.0 and 77.5 m/sec by the double-conduction distance method using a NEC PC9801 microcomputer. Figure 2 exemplifies the DCV calculated. The calculated DCV was expressed by the conduction velocities below which 10, 20, . . . , 90% of active nerve fibers lie (V10, V20, . . . , V90 velocities). Conventional maximal sensory nerve conduction velocity (SCV) of the forearm segment of median nerve was calculated by using the same method reported previously (Araki and Honma, 1976).

RESULTS

The DCV and SCV for the nine groups of subjects are shown in Table 2. All parameters of the DCV (V10 to V90 velocities) together with the SCV were significantly slowed in the 10 chain saw operators (Group 1). On the other hand, only the faster velocities of the DCV (V60 to V90 velocities) were significantly slowed in another group of vibrating tool operators (Group 2) with and without a history of vibration-induced white finger attack.

The DCV and SCV were measured in two lead smelters (Group 3) 11 and 19 times successively at 1-month intervals. Values lower than the lower limit for controls were more frequent for the V80 and V90 velocities than those for the V10 and V20 velocities.

In the high lead group of gun metal foundry workers (Group 4), the V10 velocity and the SCV were significantly slowed; no significant reduction was observed in the V20 to V90 velocities ($P > 0.05$). In the low lead group (Group 4), there was no significant slowing in the V10 to V90 velocities and the SCV ($P > 0.05$). In the 20 gun metal foundry workers, the V10 velocity was inversely correlated with the blood lead concentration and 24-hr mobilization yield of lead in urine by CaEDTA

TABLE 1
CHARACTERISTICS OF SUBJECTS EXAMINED

Group	Number[a]	Age (years) Mean (range)	Exposure/disease	Indices of exposure
1	10 (10)	53 (41–59)	Local vibration (Chain saw operators)	Length of exposure, 16–26 (mean 22) years History of white finger attack, 7 subjects
2	24 (17)	51 (37–63)	Local vibration (Chain saw, rock drill, grinder, tie tamper, concrete vibrator, hand hammer, impact wrench operators)	Length of exposure, 1–36 (mean 19) years History of white finger attack, 13 subjects
3	2 (11)	57, 51	Lead (smelters)	Blood lead, 70–121 (mean 93) and 63–85 (mean 54) μg/dl for Smelter 1 and 2, respectively, during measurement period (20 and 11 months)
4	20 (20)	48 (34–59)	Lead, zinc and copper (gun metal foundry workers)	Blood lead, 22–59 (mean 39 μg/dl; 40 or above in 9 subjects (high lead group) and below 40 in 11 subjects (low lead group) Plasma zinc, 73–111 (mean 88) μg/dl Plasma copper, 64–126 (mean 91) μg/dl
5	1 (16)	31	Thallium (ingestion of rodenticide)	Urinary thallium, 3.5 mg/dl
6	11 (11)	37 (21–53)	Mixed solvents, mainly toluene (painters, solvent mixers, pressmen)	Length of exposure, 3–30 (mean 15) years Urinary hippuric acid (end of shift), 0.2–6.0 (mean 2.6) g/liter
7	11 (11)	40 (22–61)	Styerene monomer (laminating workers in a FRP-boat factory)	Urinary mandelic acid (end of shift), 0–855 mg/g Cn
8	23 (23)	50 (30–64)	Ethanol (alcoholic patients)	Drinking more than 10 years Severe dependency (DSM-III-R, APA)
9	10 (10)	44 (22–63)	Diabetes mellitus	Fasting blood sugar, 105–363 (mean 219) mg/dl Length of suffering from disease, 0.2–30 (mean 11) years

[a] Number of controls in parentheses.

(MPb) ($r = -0.595$, $P < 0.01$); the V20 velocity was also inversely correlated with MPb ($r = -0.449$, $P < 0.05$). On the other hand, the V90 velocity was positively correlated with the amount of zinc excreted in urine for 24 hr (UZn) ($r = 0.482$, $P < 0.05$); the V80 velocity and the SCV were positively correlated with UZn ($r = 0.459$ and 0.455, respectively, $P < 0.05$).

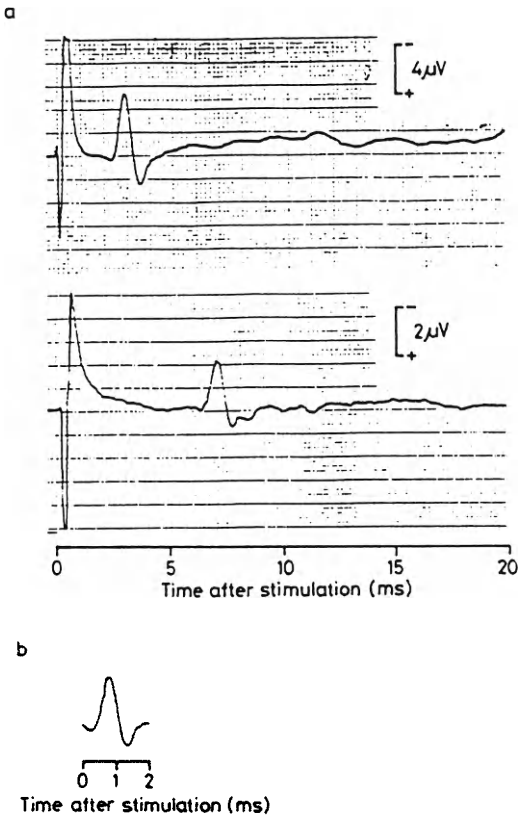

FIG. 1. (a) Compound action potentials (CAPs) recorded from median nerve at the wrist (top) and elbow (bottom) after stimulation of the second finger in a chain saw operator and (b) calculated single-fiber action potential (SFAP, relative amplitude) corresponding to conduction velocity of 60.0 m/sec.

The DCV was measured in an acute thallium-poisoning patient (Group 5) 2 and 11 months after the onset of clinical sensorimotor neuropathy. In the first examination, the V70 to V90 velocities and the SCV were lower than the normal lower limit. In the second examination, the V70 to V90 velocities and SCV returned to a normal level and all parameters of the DCV including slower fiber velocities were higher than the initial levels.

In 11 workers exposed to mixed solvents (Group 6), V60 to V90 velocities as well as the SCV were significantly lower than those in age-matched control subjects. In 11 styrene workers (Group 7), the V80 velocity and SCV were significantly slower than those of controls.

The V40 to V90 velocities and the SCV in 23 alcoholic patients (Group 8) were significantly slowed. The V30 to V90 velocities in 10 diabetic patients (Group 9) were slower than controls.

DISCUSSION

The major finding drawn from the above results is that conduction velocities of faster fibers of large myelinated fibers are more sensitive to local vibration, lead, thallium, mixed solvents, styrene, and alcohol as well as diabetes mellitus

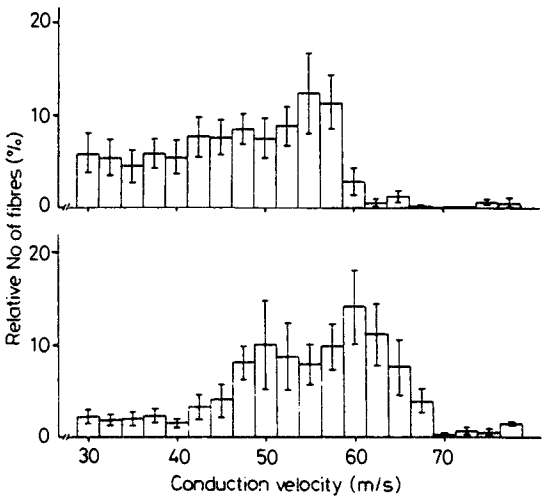

FIG. 2. Distribution of conduction velocities in 10 chain saw operators (top) and in the same number of matched controls (bottom; means and standard errors).

than those of slower fibers. This agrees with most of the following observations obtained by the DCV methods other than the double-conduction distance method:

Single-Fiber Action Potential Method

The DCV was measured in 63 workers exposed to *n*-hexane at levels less than 100 ppm in air at their workplace (Sax *et al.*, 1981). The Vmax velocity (the conduction velocity below which 95% of active nerve fibers lie) was significantly decreased. Similarly, the DCV in the sural nerve was measured by the single-fiber axon potential method in 3 workers with a clinical sensorimotor neuropathy due to *n*-hexane poisoning (Yokoyama *et al.*, 1990b). Their V50 to V90 velocities were lower than "normal" lower limits and returned to a normal level 3 years after the initial measurement.

Deconvolution Method

The DCV was measured in 29 diabetic patients without symptoms or signs of polyneuropathy or with mild clinical neuropathy (Dorfman *et al.*, 1983). The Vmax, Vpeak (the mode of the conduction velocities), and Vmin (the conduction velocity below which 5% of active nerve fibers lie) were significantly decreased in the patients.

Collision Method

All parameters of the DCV velocities were significantly decreased in 9 patients suffering from myotonic dystrophy, indicating that both faster and slower fibers were affected in this disease. Furthermore, it appears that subclinical effects of the occupational and environmental factors are reversible after termination of exposure and the recovery of faster fibers is more delayed than that of slower fibers.

Finally, in workers exposed to lead, zinc, and copper, only the V10 velocity was significantly affected in the present study; V80 and V90 velocities were pos-

TABLE 2
DISTRIBUTION OF CONDUCTION VELOCITIES (V10–V90) AND SENSORY NERVE CONDUCTION VELOCITY (SCV) IN NINE GROUP OF SUBJECTS AND CONTROLS[a]

Group		Conduction velocities (m/s)[b]	
		Exposed group	Controls
1	V10	36.7 (30.0–52.3)*	42.7 (32.0–55.4)*
	V50	47.0 (38.0–55.4)**	55.3 (49.8–61.3)**
	V90	56.7 (50.2–60.5)**	63.2 (52.9–72.5)**
	SCV	50.2 (45.7–55.6)**	55.4 (48.5–60.2)**
2[c]	V60	54.1 (44.7–59.0)*	56.7 (51.4–61.5)*
	V90	57.3 (49.1–63.8)**	61.0 (55.9–64.9)**
	SCV	55.3 (43.9–61.4)*	59.4 (50.7–61.2)*
3			
Smelter 1	V10	48.1 (38.0–62.1)	45.0 ± 8.6
(20 times of	V50	54.2 (42.9–64.8)	55.6 ± 5.6
measurement)[d]	V90	58.7 (49.4–71.9)	61.4 ± 3.6
	SCV	55.6 (48.5–61.1)	60.4 ± 5.2
Smelter 2	V10	47.6 (41.7–53.0)	45.0 ± 8.6
(11 times of	V50	52.0 (46.5–57.0)	55.6 ± 5.6
measurement)[d]	V90	56.5 (51.3–62.3)	61.4 ± 3.6
	SCV	53.5 (49.2–61.1)	60.4 ± 5.2
4 (high lead	V10	37.4 (31.6–49.7)*	42.2 (32.0–55.4)*
group)	SCV	44.6 (38.7–53.0)*	42.2 (36.0–32.9)*
5[e]	V70	50.3[+]	60.4 ± 4.4
	V90	52.6[+]	65.3 ± 5.4
	SCV	47.4[+]	56.4 ± 3.5
6	V60	58.1 (51.0–64.9)*	61.0 (54.5–67.6)*
	V90	60.7 (53.4–67.6)*	63.9 (59.5–72.0)*
	SCV	57.2 (50.0–62.6)***	62.2 (58.7–65.2)***
7	V80	60.5 (53.2–67.8)*	63.3 (59.0–69.5)*
	SCV	55.1 (51.3–60.0)*	56.9 (49.2–60.0)*
8	V40	49.8 (45.0–53.6)*	52.6 (48.6–56.6)*
	V70	52.9 (48.5–57.3)***	57.6 (53.9–61.2)***
	V90	55.8 (51.2–60.4)***	60.7 (57.2–64.2)***
	SCV	53.1 (37.0–59.8)***	60.2 (51.5–66.6)***
9	V50	54.0 (41.3–66.0)**	47.7 (36.5–55.6)**
	V70	58.5 (48.1–71.2)***	51.0 (39.9–61.4)***
	V90	63.2 (51.2–75.3)***	55.4 (46.0–67.1)***

[a] Group numbers as well as number of subjects are the same as those in Table 1.

[b] Mean with range (mean ± SD for controls of lead and thallium).

[c] Vibrating tool operators with a history of white finger attack (VWF). The V60–V90 and SCV were also significantly decreased in those without attack ($P < 0.05$).

[d] In Smelter 1, the V90, V50, and V10 velocities were below the normal lower limit (mean − 2 SD of controls) 11, 2, and 0 times (55, 10, and 0%), respectively; the SCV was below the limit nine times (45%). In Smelter 2, the V90, V50, V10, and SCV were below their lower limit 8, 3, 0, and 8 times (73, 27, 0, and 73%), respectively. In the two subjects, the values below the lower normal limit for the V80, V90, and SCV were significantly more frequent than those for the V10 and V20.

[e] First measurement.

*,**,*** Significantly different at $P < 0.05$, 0.01, and 0.001, respectively.

[+] Below the normal lower limit.

itively correlated with urinary zinc excretion. Therefore, the effects of lead on the faster fibers might have been antagonized by zinc, resulting in a normal level of conduction velocities for the faster fibers. This hypothesis should be confirmed by further studies.

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ENVIRONMENTAL RESEARCH 61, 323–336 (1993)

Assessment of Central, Peripheral, and Autonomic Nervous System Functions in Lead Workers: Neuroelectrophysiological Studies[1]

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To assess the effects of lead on central, peripheral, and autonomic nervous systems, the visual-, short-latency somatosensory-, and brainstem auditory-evoked potentials (VEP, SSEP, and BAEP), event-related potential (P300), distribution of nerve conduction velocities (DCV), and electrocardiographic R–R interval variability (CV_{RR}), together with conventional median and radial nerve conduction velocities (NCV), were measured in the lead workers. The lead workers consisted of 22 gun metal foundry workers occupationally exposed to lead, zinc, and copper. In the lead workers with blood lead concentrations below 65 μg/dl, the latencies of the VEP (from the retina to the visual cortex), SSEP (from the brachial plexus to the brainstem), and P300 (which reflects cognitive function) were significantly prolonged when compared with the sex- and age-matched controls. All these latencies and the BAEP latencies (from the cochlear nerve to the brainstem) were significantly correlated with the indicators of lead absorption among these workers. The CV_{RR} (especially, a component of parasympathetic activity) was significantly depressed in the lead workers. The slower (V10) velocity of the DCV, the motor, and sensory NCVs were also significantly slowed. These findings suggest that lead affects not only peripheral nerve but also the central and autonomic nervous functions at a subclinical level; zinc may antagonize the neurotoxic effects of lead.

INTRODUCTION

Considerable concern has been directed to the subclinical neurotoxicity of lead in occupationally and environmentally exposed persons (WHO Study Group, 1980; Landrigan, 1989). The adverse effects of lead on the central and peripheral nervous system have been widely documented through demonstration of abnormalities in visual-, somatosensory-, and auditory-evoked potentials (Jeyaratnam *et al.*, 1985; Otto *et al.*, 1985; Holdstein *et al.*, 1986) and of slowing of peripheral nerve conduction velocities (Seppäläinen *et al.*, 1975, 1983; Araki and Honma, 1976; Landrigan *et al.*, 1976; Feldman *et al.*, 1977; Buchthal and Behse, 1979; Araki *et al.*, 1980; Ashby, 1980; Singer *et al.*, 1983; Triebig *et al.*, 1984). Impairment of psychological performance and reduction in intelligence has also been shown in lead-exposed workers with blood lead (BPb) concentrations below 70 μg/dl (Mantere *et al.*, 1984; Baker *et al.*, 1984; Araki *et al.*, 1986b; Jeyaratnam *et al.*, 1986; Yokoyama *et al.*, 1988).

The autonomic nervous system has been shown to be vulnerable to environmental insults, e.g., styrene and vibration (Murata *et al.*, 1991a,b). On the other

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hand, apart from studies of the association between lead and autonomic symptoms such as abdominal pain, constipation, and diarrhea (Cullen *et al.*, 1983), only two previously published reports have examined the autonomic nervous effects of lead. In one of these prior studies, Teruya *et al.* (1991) indicated that workers with BPb concentrations over 30 μg/dl had a significant decrease in variability of the electrocardiographic R–R intervals (CV_{RR}) during deep breathing. Also, Araki *et al.* (1990b) observed a significant relationship between the BPb concentration and CV_{RR} variation in a follow-up study of a lead-smelting worker.

Peripheral nerve trunks consist of several thousand myelinated nerve fibers with slower and faster conduction velocities in parallel to the fiber diameters. The conventional methods for determination of conduction velocities yield only two discrete values that reflect the maximal and slower velocities of fibers in the nerve trunk; the function of the vast majority of fibers cannot be directly evaluated. In recent years, the application of computerized technologies has enabled a noninvasive measure of the distribution of nerve conduction velocities (DCV) in step with the development of computer sciences (Barker *et al.*, 1979). Using this technique, we have found that the faster large myelinated nerve fibers are more affected than the slower fibers in subjects exposed to thallium, *n*-hexane, styrene, and local vibration (Yokoyama *et al.*, 1990a,b; Murata *et al.*, 1991a,b). Accordingly, it would be necessary to clarify which of the faster or slower fibers of the large myelinated nerve are affected by lead.

Since the early 1980s, we have introduced several electrophysiological methods into our laboratory for evaluation of central, peripheral, and autonomic nervous systems. Namely, we have measured pattern-reversal visual-, short-latency somatosensory-, and brainstem auditory-evoked potentials (VEP, SSEP, and BAEP), event-related potential (EPR, especially P300), DCV, and CV_{RR} in workers exposed to a variety of occupational and environmental factors. In this study, we intend to summarize our data in workers exposed to lead with BPb concentrations below 65 μg/dl (Araki *et al.*, 1986a,c, 1987, 1990a; Murata *et al.*, 1987a,b; Murata and Araki, 1991). Also, a possible hypothesis on the interactive effects among lead, zinc, and copper is addressed in the light of causation of neurotoxicity.

SUBJECTS

The study group (lead workers) consisted of 22 male gun metal foundry workers with BPbs of 12 to 64 μg/dl. Concentrations of zinc and copper in the plasma (PZn and PCu) ranged from 66 to 148 μg/dl and from 46 to 136 μg/dl, respectively. The gun metal was composed of lead (5%), zinc (5%), copper (85%), and tin (5%). None of the subjects were exposed in their workplace to arsenic or solvents. They had been employed at the factory for 1 to 18 years, and their ages ranged from 32 to 59 years. Workers' alcohol consumption (100% ethanol equivalent) ranged from 0 and 630 ml per week. None of them had specific signs and symptoms indicative of clinical lead poisoning or of other neurologic, endocrinological, or cardiovascular disorders; there was no evidence that they had subclinical radiculopathy or spondylosis. The nature of the procedure in this study was fully explained to all subjects, and the study was carried out with their informed consent during the 5-year period.

Control subjects, matched to each lead worker by age (same 5-year span), were selected from "healthy" men who lived in the same residential area as the lead

workers. Controls were excluded from the study if they had occupational exposure to lead or if they had endocrinological, neurologic, or cardiovascular disorders. There were no significant differences in age, height, skin temperature, alcohol consumption, or years of schooling between the workers and control subjects on a group basis (paired-sample t test, $P > 0.05$).

METHODS

The VEP, SSEP, BAEP, P300, DCV, radial, and median nerve conduction velocities and CV_{RR} were conducted in a warm laboratory (28–32°C) by the use of two-channel electromyograph (Medelec MS-92), videostimulator (Medelec VS-6), audiostimulator (Medelec ST-10), ECG-amplifier (NEC-Sanei 1271SP), and microcomputer (NEC PC9801UV2) with an analog-to-digital converter (Neolog PCN-2198); skin temperature remained in the range of 30 to 35°C for all subjects.

Pattern-Reversal Visual-Evoked Potential

The VEP with binocular full-field stimulation was conducted in a darkened room (Sokol, 1986; Araki *et al.*, 1987; Murata *et al.*, 1987b). Subjects sat in front of a TV screen quietly and fixated the center of the TV screen. Visual-evoked potential (i.e., N75, P100, and N145 components) was recorded using standard electrodes fixed to the occipital cortex, the right mastoid. These latencies are considered to reflect the visual conduction from the retina to the visual cortex (Sokol, 1986). The daily variations (coefficient of variation) in VEP latencies were below 5.9% (Araki *et al.*, 1987).

Short-Latency Somatosensory-Evoked Potential

The SSEP was measured by a modified Jones' method (Jones, 1977; Araki *et al.*, 1986c, 1987). The N9 component was recorded at the Erb's point after stimulation of the right median nerve at the wrist; the N13 component was recorded at the second cervical vertebra; and the N20 and P23 components were on the scalp overlying the left sensory cortex. As the SSEP latencies are significantly affected by age, height, and skin temperature, the latencies measured in control subjects were strictly adjusted to the age, height, and skin temperature of the matched lead worker by means of the partial regression coefficients obtained from 72 healthy men by using multiple regression analysis (Araki *et al.*, 1986c). The N9–N13 and N13–N20 interpeak latencies represented cervicospinobulbar and central conduction times, respectively. The daily variations in the SSEP latencies were between 0.7 and 3.6% (Araki *et al.*, 1986c).

Brainstem Auditory-Evoked Potential

The BAEP was measured using the method described by Kriss (1982). Click signals with an intensity of about 80 dB (HL) were presented to the right ear through earphones. The BAEP was recorded using standard electrodes fixed to the vertex and right mastoid ipsilateral to stimulation after amplification and filtration. The I, III, and V components of the BAEP are considered to primarily represent volume-conducted electrical activity from the acoustic nerve, pons and midbrain, respectively (Kriss, 1982). The daily variations in the I, III, and V

latencies of the BAEP in a 23-year-old male student for 16 days were 6.6, 2.5, and 1.3%, respectively (Murata *et al.*, 1987b).

Event-Related Potential (P300)

The P300 was conducted using the method described by Goodin (i.e., "oddball" paradigm) (Goodin, 1986; Araki *et al.*, 1990a). The subject was presented with a random sequence of two distinguishable stimuli, one of which occurred frequently (nontarget tone of 1 kHz, 240 trials) and the other occurred infrequently (target tone of 2 kHz, 60 trials). These stimuli were delivered at an intensity of 90 dB and at a rate of 1 tone burst every 2 sec. The subjects were instructed to count mentally the target tone only. Cerebral responses to the two stimuli were recorded at the vertex and linked mastoids, and averaged separately. The P300 component, detected with the target tone stimuli, was the first maximal positive wave of between 250 and 500 msec. The daily variation in the P300 latency in a 32-year-old male subject for 14 days was 4.3% (Araki *et al.*, 1990a).

Distribution of Conduction Velocities

The DCV was measured by a modified method of Barker *et al.* (Barker *et al.*, 1979; Araki *et al.*, 1986a). After electrical stimulation of the right median nerve at the second finger, compound action potentials were recorded at both the wrist and elbow. The DCV was calculated by the double conduction distance method. The calculated DCV was expressed by the following parameters: the conduction velocities below which 10, 20, . . ., 80, and 90% of active nerve fibers lie (V10, V20, . . ., V80, and V90 velocities). The daily variations in these DCV parameters were below 4.2% (Araki *et al.*, 1986a).

Peripheral Nerve Conduction Velocities

Maximal motor nerve conduction velocity (MCV) of the distal radial nerve was conducted using the method described by Jebsen (1966a); similarly, the sensory conduction velocity (SCV) in the forearm segment of the radial nerve was measured by the method of Downie and Scott (1967). The MCV and SCV of the right median nerve were measured using standard techniques (Kimura, 1989). The daily variations in radial and median nerve conduction velocities were below 4.4% (Murata *et al.*, 1987a).

Electrocardiographic R–R Interval Variability

One hundred R–R intervals on electrocardiogram were continuously measured and stored on a floppy disk in real time (sampling time, 1 msec), after the subject had lain quietly supine for 10 min (Murata and Araki, 1991). The CV_{RR} was defined as the ratio of the standard deviation of the R–R intervals to their average value (RR_{mn}, msec). The power spectrum of R–R intervals was calculated by autoregressive spectral analysis. The spectrum of each two components, i.e., respiratory sinus arrhythmia (RSA) and Mayer wave-related sinus arrhythmia (MWSA), was separated by component analysis. Each component coefficient of variation (i.e., $C\text{-}CV_{RSA}$ and $C\text{-}CV_{MWSA}$) was defined as the ratio of the square root of each component power (P_k, $msec^2/c/b$) to the RR_{mn} (k = RSA or MWSA). The $C\text{-}CV_{RSA}$ and $C\text{-}CV_{MWSA}$ are considered to reflect parasympathetic and sym-

pathetic activities, respectively (Pagani *et al.*, 1986; Hayano *et al.*, 1990; Murata *et al.*, 1991b). The daily variation in the CV_{RR} was 7.5% (Murata and Araki, 1991).

Analyses of Blood and Urine Samples

Blood samples were collected just before these measurements and the start of 24-hr urine collection. Calcium disodium ethylenediamine tetraacetate (CaEDTA) was then injected intravenously (Araki *et al.*, 1984); 24-hr mobilization yields of lead, zinc, and copper in urine (MPb, MZn, and MCu) were measured. The concentrations of lead in whole blood, plasma, and erythrocytes (BPb, PPb, and EPb), concentrations of zinc and copper in plasma and erythrocytes (PZn, EZn, PCu, and ECu) and the amount of each metal spontaneously excreted in urine for 24 hr (UPb, UZn, and UCu) were measured by atomic absorption spectrophotometry (Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer Z-8000) by the methods described in our previous report (Araki *et al.*, 1984). Similarly, the intraerythrocytic enzyme δ-aminolevulinic acid dehydratase (ALAD) activity, 24-hr spontaneous urinary excretion of δ-aminolevulinic acid (ALA), and coproporphyrin (CP) were measured (Araki *et al.*, 1984); the blood hemoglobin (Hb) and packed cell volume (Ht) by the cyanmethemoglobin method and the hematocrit method, respectively. The reproducibility of these analyses has been described (Araki *et al.*, 1984). BPb, PZn, and PCu could be measured only in 10 of the control subjects; their BPb, PZn, and PCu concentrations, i.e., 14 ± 4 (SD), 75 ± 14, and 72 ± 16 μg/dl, respectively (Murata *et al.*, 1987a).

Statistical Analysis

The paired-sample *t* test was used to determine the significance of the matched differences between the lead workers and age-matched controls. Dose–effect relationships were tested between indicators of lead, zinc, and copper absorption and the electrophysiological measurements by simple or age-adjusted (i.e., partial) correlation coefficients. Moreover, the stepwise multiple regression analysis was conducted when some variables in bloods and urines were significantly correlated with these electrophysiological measures (the variables were entered and removed from the equation at a significant level of $P < 0.05$). All analyses were performed using the Statistical Package for the Biosciences (SPBS) (Uni-Science Co.).

RESULTS

The N75 and N145 latencies of the VEP were significantly prolonged in the 19 lead workers (Table 1); also, the N9 peak and N9–N13 interpeak latencies of the SSEP were prolonged. The N145 latency was inversely correlated with Ht in the 19 lead workers, and was positively correlated with duration of employment (Table 2). Similarly, the N9 latency was positively correlated with CP (Table 2); the N9–N13 interpeak latency was inversely correlated with Ht. The alteration in N145 latency of the VEP during the 1-year period was positively correlated with the corresponding change in EPb in the 19 lead workers ($r = 0.525$, $P < 0.05$), and was inversely correlated with the change in ECu ($r = -0.556$, $P < 0.05$). By contrast, the N13–N20 latency of the SSEP was inversely correlated with indicators of zinc absorption (Table 2).

The I–V interpeak and V peak latencies of the BAEP were significantly related

TABLE I

RESULTS OF VISUAL-, SHORT-LATENCY SOMATOSENSORY-, AND BRAINSTEM AUDITORY-EVOKED POTENTIALS (VEP, SSEP, BAEP), EVENT-RELATED POTENTIAL (P300), RADIAL AND MEDIAN NERVE CONDUCTION VELOCITIES (NCV), AND R-R INTERVAL VARIABILITY IN LEAD-EXPOSED WORKERS AND AGE-MATCHED CONTROLS[a]

	Number of pairs	Lead workers (mean and range)	Matched controls (mean and range)	Matched difference[b]
VEP (msec)				
N75	19	79.9 (68.0–89.6)	74.2 (63.2–88.8)	5.7 ± 7.2**
P100	19	105.7 (84.8–114.4)	103.1 (85.6–112.0)	2.6 ± 9.4
N145	19	139.8 (120.4–159.2)	133.1 (117.6–152.0)	6.7 ± 13.4*
SSEP (msec)				
N9	19	9.1 (8.2–9.9)	8.8 (8.1–9.5)	0.3 ± 0.6*
N9–N13	19	3.7 (3.2–4.7)	3.3 (2.8–4.2)	0.4 ± 0.5**
N13–N20	19	6.2 (4.8–7.8)	6.6 (5.4–7.3)	−0.4 ± 1.1
N20–P23	19	5.9 (3.3–7.8)	6.2 (3.1–9.9)	−0.3 ± 2.2
BAEP (msec)				
I	20	1.48 (1.24–1.88)	1.52 (1.28–1.80)	−0.04 ± 0.18
III	20	3.77 (3.36–4.12)	3.82 (3.46–4.36)	−0.05 ± 0.50
V	20	5.74 (5.28–6.20)	5.71 (5.12–6.00)	0.03 ± 0.34
P300 (msec)	22	321 (294–346)	307 (262–342)	14 ± 26*
Radial NCV (m/sec)				
MCV	20	56.5 (51.9–62.0)	59.7 (53.0–68.6)	−3.2 ± 4.9**
SCV	20	57.6 (49.6–66.2)	58.9 (51.1–65.2)	−1.3 ± 6.0
Median NCV (m/sec)				
MCV	20	56.5 (51.4–61.5)	59.1 (53.8–62.8)	−2.6 ± 3.4**
SCV, forearm	20	61.0 (56.6–69.7)	63.0 (58.0–66.9)	−2.0 ± 4.2*
SCV, palm	20	46.6 (39.5–55.4)	48.1 (38.2–60.7)	−1.5 ± 6.7
R–R interval variability (%)				
CV_{RR}	16	2.57 (1.60–4.77)	3.98 (1.86–5.79)	−1.41 ± 1.04***
C-CV_{RSA}	16	1.45 (0.34–3.54)	2.08 (1.18–4.52)	−0.63 ± 1.05*
C-CV_{MWSA}	16	1.11 (0.40–2.10)	1.48 (0.52–4.37)	−0.37 ± 0.95

[a] Abbreviations used are as in the text.

[b] Means and standard deviation of matched differences.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$ (paired-sample t test).

to Ht in the 20 lead workers (Table 2), whereas no significant differences in the BAEP latencies were found between the 20 lead workers and age-matched controls (Table 1).

The P300 latency of the ERP was significantly prolonged in the 22 lead workers (Table 1). The P300 latency in these workers was significantly correlated with BPb and UPb as well as with working years, UZn, and age (Table 2); UPb was the only variable which was selected by stepwise regression analysis. No significant correlation was found between the P300 latency and peripheral nerve conduction velocities of the radial and median nerves in the 22 lead workers ($P > 0.05$).

The DCVs in the 20 lead workers and in the 20 age-matched controls are illustrated in Fig. 1. The V10 velocity of the DCV was significantly slowed in the high lead group of 9 lead workers (BPb > 40 μg/dl) (Student's t test, $P < 0.05$). There were no significant differences in other DCV parameters between the high lead group and control subjects; similarly, in all parameters between the low-lead

TABLE 2

FACTORS SIGNIFICANTLY CORRELATED WITH LATENCIES OF VISUAL-, SHORT-LATENCY SOMATOSENSORY-, AND BRAINSTEM AUDITORY-EVOKED POTENTIALS (VEP, SSEP, BAEP) AND EVENT-RELATED POTENTIAL (P300), RADIAL AND MEDIAN NERVE CONDUCTION VELOCITIES (NCVs), DISTRIBUTION OF CONDUCTION VELOCITIES (DCV), AND R–R INTERVAL VARIABILITY IN LEAD-EXPOSED WORKERS[a]

	Number of workers	Factors (simple correlation coefficient r in parentheses)
VEP (msec)		
N145	19	Ht (−0.651**), years employed (0.497*)
SSEP (msec)		
N9	19	CP (0.502*)
N9–N13	19	Ht (−0.537*)
N13–N20	19	EZn (−0.780***), PZn (−0.503*)
BAEP (msec)		
V	20	Ht (−0.523*)
I–V	20	Ht (−0.449*)
P300 (msec)	22	BPb (0.447*), UPb (0.507*), years employed (0.492*), UZn (0.438*), Age (0.430*)
Radial NCV (m/sec)		
MCV	20	UZn (0.487*)
SCV	20	ALA (−0.674*), CP (−0.577**), PZn (0.478*)
Median NCV (m/sec)		
MCV	20	EZn (0.526*)
SCV, palm	20	ECu (0.447*), PCu (0.584*)
DCV (m/sec)		
V10	20	BPb (−0.459*), MPb (−0.542*), PCu (−0.497*)
V20	20	MPb (−0.449*), PCu (−0.459*)
V80	20	UZn (0.482*)
V90	20	UZn (0.514*)
R–R interval variability (%)		
$C\text{-}CV_{MWSA}$	16	PZn (0.528*)

[a] Abbreviations used are as in the text.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$ (t test).

group and controls ($P > 0.05$). The V10 and V20 velocities were inversely correlated with indicators of lead absorption (BPb and MPb) as well as PCu in the 20 lead workers (Table 2). On the other hand, the V80 and V90 velocities were positively correlated with UZn (Table 2). PCu was positively correlated with MPb in the 20 lead workers ($r = 0.536$, $P < 0.05$).

The MCVs in the radial and median nerves and the SCV in the median nerve were significantly slowed in the 20 lead workers (Table 1). Indicators of lead absorption (ALA and CP) were inversely correlated with the SCV in the radial nerve while, on the other hand, indicators of zinc absorption (PZn and UZn) were positively correlated with the MCV and SCV in the radial nerve (Table 2). Results of stepwise multiple regression analysis indicated that not only indicators of zinc absorption but also the indicator of copper absorption were positively related to radial nerve conduction velocities (Table 3); also, indicators of zinc and copper absorption were positively related to median nerve conduction velocities.

In the 16 lead workers, the CV_{RR} and $C\text{-}CV_{RSA}$ were significantly reduced as compared to the matched controls (Table 1). The $C\text{-}CV_{MWSA}$ was significantly

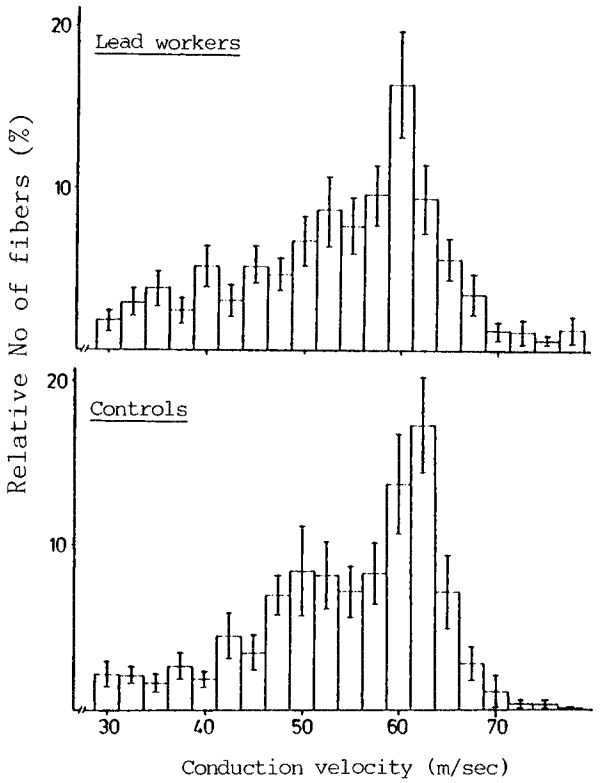

FIG. 1. Distribution of conduction velocities in 20 lead workers and in 20 control subjects (averages and standard errors).

correlated with PZn in the lead workers (Table 2); its age-adjusted correlation coefficient was also significant ($r = 0.526$, $P < 0.05$). None of the CV_{RR}, C-CV_{RSA}, or C-CV_{MWSA} was significantly correlated with the median nerve conduction velocities in the 16 lead workers ($P > 0.05$).

TABLE 3
RELATIONSHIPS BETWEEN INDICATORS OF LEAD, ZINC, AND COPPER ABSORPTION AND RADIAL AND MEDIAN NERVE CONDUCTION VELOCITIES IN 20 LEAD WORKERS: STEPWISE MULTIPLE REGRESSION ANALYSIS[a]

Conduction velocities	Multiple correlation coefficient	Variables selected (standard regression coefficient)
Radial nerve		
MCV	0.487	UZn (0.487*)
SCV	0.875	ALA (−0.522**), CP (−0.298*), ECu (0.351*), PZn (0.318*)
Median nerve		
MCV	0.526	EZn (0.526*)
SCV, forearm	—	None
SCV, palm	0.584	PCu (0.584**)

[a] Abbreviations used are as in the text; the significance level for entering and removing variables in the regression analysis was $P < 0.05$.

* $P < 0.05$ and ** $P < 0.01$.

DISCUSSION

Central Nervous System Effects of Lead

In the present study, the N9–N13 interpeak latency of the SSEP and the N145 latency of the VEP were significantly affected by the indicators of lead, zinc, and copper absorption; these latencies were significantly prolonged. These findings agree with those using somatosensory and visual evoked potentials (Otto *et al.*, 1985; Jeyaratnam *et al.*, 1985). Therefore, it is suggested that lead affects the central nervous system function including the somatosensory pathway from the brachial plexus to the medulla oblongata and the visual pathway from the retina to the cerebral cortex.

In addition, a dose–effect relationship in the I–V interpeak latency of the BAEP was found to be significant despite the absence of significant differences in the BAEP latencies in the lead workers. Otto *et al.* (1985) have shown the linear relationship of the BAEP latency and BPb levels. Also Holdstein *et al.* (1986) have reported I–III interpeak latency of the BAEP was significantly delayed in lead-exposed children as compared to their control group. Thus, these observations suggest that the brainstem auditory pathway is probably influenced by lead.

The P300 of the ERP is elicited only in circumstances where the subject is required to distinguish one stimulus (target) from a group of other stimuli (non-target). The P300 latency corresponds to the evaluation time of target stimuli; when the task is difficult, the latency becomes longer. The P300 has been considered to reflect cognitive function in humans (Kutas *et al.*, 1977). Therefore, results of the present study suggest that lead affects cognitive function. This finding coincides with the observation on the ERP in children by Otto *et al.* (1981) and also with the data on impairment of psychological performance and reduction in intelligence in lead workers by many investigators (Mantere *et al.*, 1984; Baker *et al.*, 1984; Araki *et al.*, 1986b; Jeyaratnam *et al.*, 1986; Yokoyama *et al.*, 1988).

Peripheral Nervous System Effects of Lead

The radial and median nerve conduction velocities were significantly slowed in the lead workers used in this study. The results confirm the bulk of previous observations of lead-induced subclinical peripheral nerve dysfunction (Araki and Honma, 1976; Buchthal and Behse, 1979; Ashby, 1980; Triebig *et al.*, 1984; Jeyaratnam *et al.*, 1985).

The V10 and V20 velocities of the DCV were significantly correlated with two or three of all the indicators of lead, zinc, or copper absorption examined; these indicators were consistent (MPb, PCu, and BPb). These results, as well as a significant slowing of the V10 velocity, suggest that the slower sensory fibers were inversely affected by lead, resulting in a conduction delay at the BPb level of 40–60 μg/dl. Significant correlations of BPb and MPb with conventional MCV and SCV have been shown in some studies (Araki and Honma, 1976; Seppäläinen *et al.*, 1979; Ashby, 1980; Araki *et al.*, 1980). The inverse relation of PCu to the V10 and V20 velocities is considered to reflect lead effects on the slower fibers, as PCu was positively correlated with the MPb.

A significant reduction in the MCV of the proximal radial nerve (i.e., arm segment) has been observed in battery workers with BPbs of 60 ± 15 μg/dl (Ashby, 1980), in workers suffering from "clinical lead poisoning" with BPbs of

27 to 180 (mean 72) μg/dl (Vasilescu, 1973), and in secondary lead smelters with BPbs of 30 to 110 μg/dl (Lilis *et al.*, 1977). However, it is often difficult to exclude the measurement error of a nerve distance between two stimulation points (Jebsen, 1966b), and no significant association between nerve conduction velocities and indicators of lead absorption has been detected (Ashby, 1980; Lilis *et al.*, 1977). Therefore, the MCV in the distal radial nerve, as assessed in this study, might have represented a more sensitive effect of lead on peripheral nerves. Direct comparison between the effects on the proximal and distal nerves is needed to clarify this hypothesis.

Seppäläinen *et al.* (1972, 1975) have shown that the conduction velocity of the slower motor fibers of the ulnar nerve was particularly sensitive to lead. It appears that both motor and sensory slower nerve fibers are sensitive to lead. Histologic studies are needed to verify the changes underlying these physiological alterations.

Autonomic Nervous System Effects of Lead

In the present study, the CV_{RR} and C-CV_{RSA} were found to be significantly reduced in the lead-exposed workers. This finding agrees with a previous observation made in lead workers by Teruya *et al.* (1991), who showed a significant decrease of CV_{RR} during deep breathing in workers with BPb concentrations over 30 μg/dl when compared with workers with BPb levels less than 20 μg/dl. Also, it is consistent with our previous report that the CV_{RR} was significantly correlated with BPb in a 12-month follow-up study of a lead-smelting worker with BPb levels of 80–120 μg/dl (Araki *et al.*, 1990b). All of these findings suggest that lead influences the autonomic nervous system function (i.e., CV_{RR}) mainly through depression of parasympathetic activity (i.e., C-CV_{RSA}). Thus, these data extend our range of understanding of the syndrome of subclinical lead toxicity.

Interaction of Lead, Zinc, and Copper on the Central, Peripheral, and Autonomic Nervous Systems

Evidence was found in this study that zinc has an effect antagonistic to that of lead in the upper somatosensory pathway (Table 2). Thus, lead-induced conduction delay in the central nervous system (prolongation of N13–N20 interpeak latency of the SSEP) may have been reversed by zinc, resulting in no significant conduction delay.

The possibly antagonistic effects of both zinc and copper on lead-induced slowing of nerve conduction velocities in the present study agree with the observation in animals made by Klauder and Petering (1975), who showed that the adverse effects of lead on growth and hematopoietic indicators were minimized when dietary zinc, copper, and iron were adequate. However, the present result sharply contrasts with two other recent observations in animals, in which lead toxicity was found to be exaggerated by dietary copper (Cerklewski and Forbes, 1977; Malhotra *et al.*, 1982).

The faster (V80 and V90) velocities of the DCV were positively correlated with UZn, suggesting that zinc antagonized a lead-induced conduction delay in these nerve fibers. These data coincide with the present observation that zinc possibly antagonized the effects of lead on the conventional motor and sensory conduction velocities of the radial nerve (Table 2). As no indicator of lead or copper absorption was significantly correlated with indicators of zinc absorption in the

present study, the effects of zinc on the nerve conduction velocities are considered to be independent of the effects of those metals.

The $C\text{-}CV_{MWSA}$ in the lead-exposed workers was positively related to the PZn irrespective of age. This finding is consistent with the hypothesis that zinc has an effect antagonistic to that of lead in the autonomic nervous system, as well as in the central and peripheral nervous system. Thus, lead-induced autonomic nervous system dysfunction might have been reversed in these workers at least in part by zinc, resulting in the observed absence of a significant difference in the $C\text{-}CV_{MWSA}$.

On the basis of our results and the previous literature, therefore, zinc appears to antagonize the neurotoxic effects of lead. On the other hand, the interactions between copper and lead, even in the tissue/body levels, are disputable (Miller *et al.*, 1990); whereas our results showed the competitive relation between lead and zinc on the peripheral nerve conduction. The size of our study group, however, might not have been sufficient to demonstrate such interactive effects among lead, zinc, and copper. Further studies, with an adequate animal model or with larger populations, will be needed to confirm these hypotheses.

Interrelation among the Central, Peripheral, and Autonomic Nervous System Effects of Lead

There was no significant relationship between the P300 latency and peripheral nerve conduction velocities. Also, no significant relations were found between any of the ECG parameters and the nerve conduction velocities in the lead-exposed workers. A possible interpretation for these findings is that the effects of lead on the central, autonomic, or peripheral nervous system functions may be independent of each other. Indeed, there has been few information on the complex "clinical lead poisoning" including encephalopathy, extensor muscle palsy drop, and colic. Thus, the action of lead on the visceral autonomic nervous system may manifest itself primarily through changes in the tone of the visceral smooth muscle (Janin *et al.*, 1985); whereas we had no direct evidence linking the ECG parameters and gastrointestinal symptoms due to lead exposure (abdominal pain, constipation, or diarrhea). Additional study will be required to clarify the mechanisms of lead toxicity on the central, peripheral, and autonomic nervous system functions.

CONCLUSION

These electrophysiological findings suggest that lead affects not only peripheral nerve conduction but also the central and autonomic nervous system functions even when clinically overt effects are absent; zinc may antagonize the neurotoxic effects of lead.

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Effects of Lead Exposure on Neurophysiological Parameters[1]

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To clarify the chronic effect of lead exposure on the central and peripheral nervous systems (CNS and PNS), we performed neurophysiological tests on 41 lead-exposed male workers. Unexposed workers (controls, $N = 39$) were examined for auditory brain stem response (ABR), and their ABR parameters were compared with those of 15 lead-exposed workers age-matched to the controls. Neurophysiological tests included those of motor and sensory nerve conduction velocity of the radial nerve (MCV, SCVwa and SCVfw), electroretinograms, pattern reversal visual evoked potential (VEP), ABR, and short-latency somatosensory evoked potentials (SLSEP). Neurophysiological parameters were analyzed by regression analysis [independent parameters: age, exposure duration, and current and time-weighted average lead concentration in whole blood (PbB and TWA-PbB)]. ABR parameters were also tested by Student's t test. Significant negative correlations were found between radial MCV and TWA-PbB and SCVwa and PbB, while significant positive correlations were found between the latency of component N145 of VEP and exposure duration and between the latency of component N20 of SLSEP and PbB. The mean of interpeak latency between component III and V of ABR of 15 lead-exposed workers was significantly prolonged compared with that of the control group. These results suggested that lead exposure has a greater effect on the conduction function in the PNS than in that of the CNS in somatosensory and auditory pathways, and inversely in visual pathway.

INTRODUCTION

Disorders in the central nervous system (CNS) and the peripheral nervous system (PNS) due to lead exposure are well-known as lead encephalopathy and lead neuropathy. There have been many reports on subclinical lead neuropathy studied by nerve conduction velocity (NCV) (Seppäiläinen, 1975, 1979; Paurev, 1979; Bord, 1982; Singer, 1983; Ehele, 1986; He, 1988). However, only a few reports exist on subclinical lead neuropathy studied by radial NCV or encephalopathy by neurophysiological methods.

With the development of computer technology, we can now detect the subclinical effect on the nervous system of lead exposure using the evoked potential technique which utilizes a computer averaging method. The "long-latency" evoked potential has been used in studies by Hirata *et al.* (1980) and Jeyaratnam (1985). Recently, far field potentials such as auditory brain stem response (ABR) in the auditory ascending pathway and short-latency somatosensory evoked potential (SLSEP) in the somatosensory ascending pathway have been utilized in clinical neurological examinations. Araki *et al.* (1986a,b, 1987) reported the ABR and SLSEP effects due to lead exposure.

In 1980, we reported the effects of lead exposure in NCV and the long-latency

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somatosensory evoked potentials (SEP) stimulated at the wrist (the median nerve) and the ankle (the posterior tibial nerve) among lead-exposed Japanese workers in four factories (Hirata *et al.*, 1980). In that survey, however, the long-latency SEP test could not identify the lesion in the CNS caused by lead exposure because long-latency SEP included the peripheral part of the somatosensory ascending pathway.

Since surgical and experimental studies have revealed the origins of the main components of far field potentials including ABR and SLSEP, we investigated the effects on the CNS of lead exposure among lead-exposed Japanese workers in four factories by using interpeak latencies (IPL) between the main components of ABR and SLSEP. Also, radial NCV, electroretinogram (ERG), and pattern reversal visual evoked potential (VEP) were used to clarify the effect of chronic lead exposure on the PNS and CNS. In the present study, we mainly examined the correlation between neurophysiological parameters and lead exposure indicators [exposure duration, current lead concentration in whole blood (PbB), and time-weighted average of lead concentration in whole blood (TWA-PbB)] and age as factors affecting neurophysiological parameters.

SUBJECTS AND METHODS

Subjects

We randomly selected 41 lead-exposed male workers from four Japanese factories involved in the manufacture of lead–glass-based colors (factory A, $N = 20$), the production of lead electrode plates (factory B, $N = 8$), the casting of lead–bronze (factory C, $N = 4$), and the casting of lead pipes and plates (factory D, $N = 9$). The exposed workers ranged from 19 to 58 years old (40.4 ± 11.3), and the duration of exposure to lead from 8 months to 29 years (16.6 ± 9.1). Thirty-nine unexposed workers, ranging from 41 to 51 years old (46.3 ± 3.38), were randomly selected from a nylon manufacturing factory for the examination ABR values. The selection procedure excluded workers who had diseases, consumed alcohol (more

TABLE 1
THE DESCRIPTION OF THE SUBJECTS

	Lead-exposed		
	Total	Comparison of ABR	Unexposed (comparison of ABR)
Number	41	15	39
Age	40.4 ± 11.3 years (19–58)	46.6 ± 4.2 years (40–52)	46.3 ± 3.38 years (41–51)
Exposure duration	12.9 ± 9.5 years (0.67–29)	16.6 ± 9.1 years (3.75–29)	—
PbB	43.3 ± 17.9 μg/dl (13–70)	42.4 ± 15.6 μg/dl (13–67)	—
TWA-PbB	43.5 ± 15.3 μg/dl (12.8–70.1)	30.9 ± 12.9 μg/dl (17.7–70.1)	—
Skin temperature	33.7 ± 0.93°C (31.6–35.6)	—	—

Note. Parentheses indicate range; PbB, current lead concentration in whole blood; TWA-PbB, Time-weighted average of lead concentration in whole blood in the past 5 years.

than 81 ml/day), or had a history of diseases and injuries which could cause neurological disorders (such as diabetes mellitus and cerebral injury).

Lead exposure level. The levels of lead concentration in ambient air in the workplace by personal sampling ranged from 0.024 to 1.66 mg/m^3 in factory A, from 0.265 to 1.35 mg/m^3 in factory B, from 0.026 to 2.69 mg/m^3 in factory C, and from 0.010 to 1.95 mg/m^3 in factory D in 1985 and 1986, when the present study was conducted. Internal lead exposure levels in the workers were represented by the current lead concentration in whole blood (PbB) and the time-weighted average of lead concentration in whole blood in the past 5 years (TWA-PbB). PbB of 41 lead-exposed workers ranged from 13 to 70 μg/dl (43.3 ± 17.9) and TWA-PbB from 12.8 to 70.1 μg/dl (43.5 ± 15.3). PbB and TWA-PbB of 15 lead-exposed workers, whose ABR parameters were compared with those of the unexposed controls, ranged from 13 to 67 μg/dl (42.4 ± 15.6) and 17.7 to 70.1 μg/dl (30.9 ± 12.9), respectively.

Table 1 shows the characteristics of the subjects.

Methods

Neurophysiological tests. Neurophysiological tests were conducted in an electrically shielded and air-conditioned room (temperature, 22–26°C). AVB-9-type amplifier (Nippon Kohden Co. Japan), 3F46-type electric stimulator (also as a trigger discharger, San-ei Sokki Co., Japan), 7S07-type two-channel averager (Nippon Denki Sanei Co., Japan), and 3036-type XY recorder (Yokogawa Electric Works Co., Japan) were commonly used in the neurophysiological tests.

1. Radial NCV study was conducted according to the description of Smorto and Basmajan (1980). Motor nerve conduction velocity (MCV) was calculated from the difference between latencies of evoked motor action potential (MAP) of the extensor indices muscle stimulated at the 3- to 4-cm proximal point of the forearm and a point 6 to 7 cm proximal to the lateral epicondyle of the upper arm. Sensory nerve conduction velocity from the index finger to the wrist (SCVfw) was calculated from the latency of the sensory nerve action potential (SNAP) at the tendon of the extensor pollicis longus muscle orthodromically stimulated at the index finger. Sensory nerve conduction velocity from the wrist to the forearm (SCVwa) was calculated from the latency of SNAP at the same point by antidromic stimulation at the flexor surface close to the cephalic vein of the mid-forearm. Muscle action potential and SNAPs were amplified with a bandpass of 1.5 Hz to 3 kHz. A total of 32 SNAPs were averaged for 10 msec analysis time. In the NCV study, skin tempreature was measured using an MGA-III 129-type thermistor thermometer (Shibaura Electrics Co., Japan) with its tip attached to the mid-point of the forearm.

2. ERG was recorded at the right inferior eyelid through a disk electrode. Ordinary ERG including a and b waves was amplified with a bandpass of 0.5 Hz to 3 kHz, and oscillatory potentials (OP) were recorded with a bandpass of 50 Hz to 3 kHz by strobo flash photic stimulation with 20 J of intensity (3G21P-type retinograph stimulator, San-ei Sokki, Japan). With the ERG, four oscillatory potential responses were averaged for 50 msec of analysis time. The latencies of the a and b waves and the first oscillatory potential that stably appeared were statistically analyzed.

3. VEP was recorded through a disk electrode attached at the Oz point according to a 10–20 electrode system with a bandpass of 0.5 to 300 Hz by pattern

reversal stimulation (ST-5-type click pattern stimulator, Medelec Co., UK). Another electrode attached at Fz served as the reference electrode. A total of 128 responses were averaged for 200 msec of analysis time. Latencies of components N75, P100, and N145 were statistically analyzed.

4. For ABR, click sound stimulations of 126 dB sound pressure level (duration 0.1 msec) were given to the right ear of subjects through a headphone by a ST 5-type click pattern stimulator (Medelec Co., UK). A needle electrode subcutaneously inserted into the scalp at 3 cm anterior from the vertex served as the active electrode. A disk electrode attached to the right earlobe served as the reference electrode. Another disk electrode attached at the left earlobe served as the ground electrode. Responses were amplified with a bandpass of 15 Hz to 3 kHz. A total of 1024–2048 responses were averaged for 10 msec analysis time. Latencies of components I, III, and V and IPL between component I and V (IPL I–V) and component III and V (IPL III–V) were statistically analyzed.

5. SLSEPs were recorded at Erb's point (the supraclavicular fossa) for component N9 through a disk electrode, at the second and seventh cervical vertebrae for component N11 and N13, and at C3 using a 10–20 electrode system for component N20 with needle electrodes. A disk electrode attached at Fz according to the 10–20 electrode system served as the reference electrode and another one attached at the right earlobe as the ground electrode. Enough supramaximal electric stimulation to evoke a thumb twitch was applied to the median nerve at the wrist. A total of 512–1024 responses was amplified with a bandpass of 1.5 Hz to 3 kHz and was averaged for 20 msec of analysis time. Latencies of components N9, N11, N13, and N20 and IPL between N11 and N13 (IPL N11–13), N11 and N20 (IPL N11–20), and N13 and N20 (IPL N13–20) were statistically analyzed.

PbB measurement. Blood was collected by venopuncture from lead-exposed subjects in periodical health examinations to check for lead poisoning. After 0.1 ml of blood had been diluted by 4 ml of 0.5 *N* nitric acid and centrifuged at 3000 rpm, 0.1 ml of the diluted blood was injected into the carbon furnace of an 845-type flameless atomic absorption spectrophotometer (Nippon Jarrel Ash, Japan) (Kosaka, 1983).

Statistical analysis. Neurophysiological parameters were examined by correlation analysis and the stepwise method of multiple regression analysis (independent variables: age, exposure duration, PbB and TWA-PbB; dependent variables: neurophysiological parameters), in which independent variables were entered and removed from the regression equation at a significant level of $P < 0.05$. Since neurophysiological parameters are affected by aging, we calculated partial correlation coefficients between neurophysiological parameters and exposure duration, PbB, and TWA-PbB by age adjustment. The comparison between parameters of ABR of lead-exposed and unexposed workers was examined by Student's *t* test.

RESULTS

Significant negative correlations by correlation analysis were observed between age and exposure duration ($P < 0.01$), PbB and TWA-PbB ($P < 0.01$), MCV and age ($P < 0.01$), MCV and PbB ($P < 0.05$), MCV and TWA-PbB ($P < 0.01$, Fig. 1), SCVwa and PbB ($P < 0.05$), and SCVwa and TWA-PbB ($P < 0.01$, Fig. 2). Significant positive correlations by correlation analysis were observed between SCVfw and skin temperature ($P < 0.01$), the latency of component N145 of VEP and exposure duration ($P < 0.01$, Fig. 3), IPL III–V of ABR and age ($P < 0.05$),

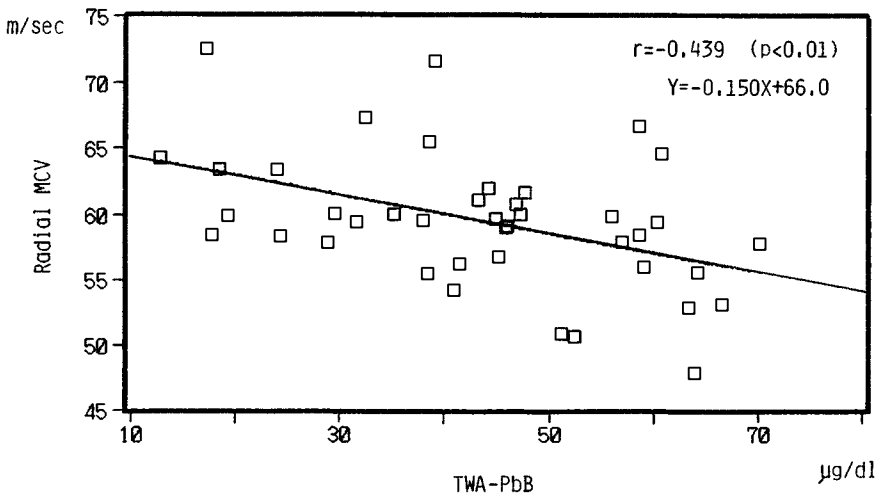

FIG. 1. Relationship between radial motor nerve conduction velocity (MCV) and time-weighted average lead concentration in whole blood (TWA-PbB) of lead-exposed workers.

the latency of component N20 in SLSEP and PbB ($P < 0.05$), and IPL N13–20 in SLSEP and PbB ($P < 0.05$, Fig. 4).

Table 2 shows partial correlation coefficients between neurophysiological parameters and exposure duration, PbB, and TWA-PbB by age adjustment. Significant negative correlations were observed between MCV and TWA-PbB ($P < 0.05$) and SCVwa and PbB ($P < 0.05$). Significant positive correlations were observed between the latency of component N145 and VEP and exposure duration ($P < 0.01$) and the latency of component N20 and PbB ($P < 0.05$).

After removal of age and insignificant variables by the stepwise method of multiple regression analysis, SCVwa showed significant correlation with TWA-PbB and exposure duration ($P < 0.05$), the latency of component N145 of VEP with exposure duration and PbB ($P < 0.01$), and the latency of component N20

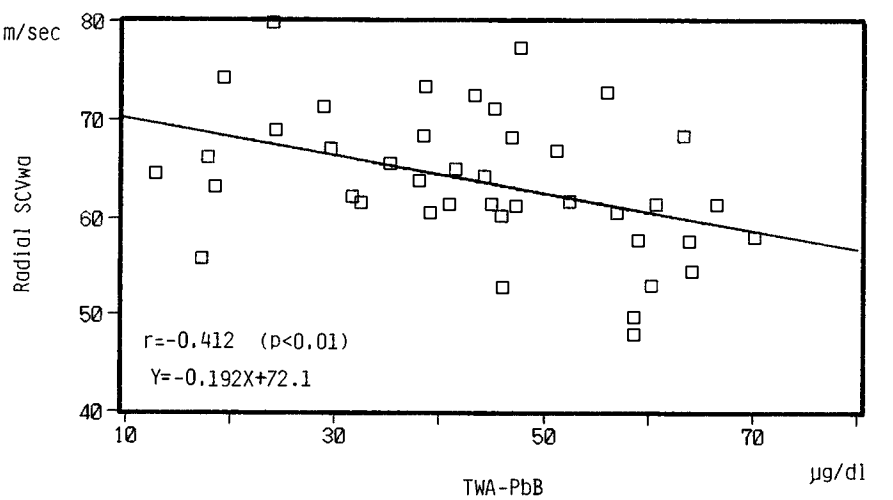

FIG. 2. Relationship between radial sensory nerve conduction velocity from the wrist to the forearm (SCVwa) and TWA-PbB of lead-exposed workers.

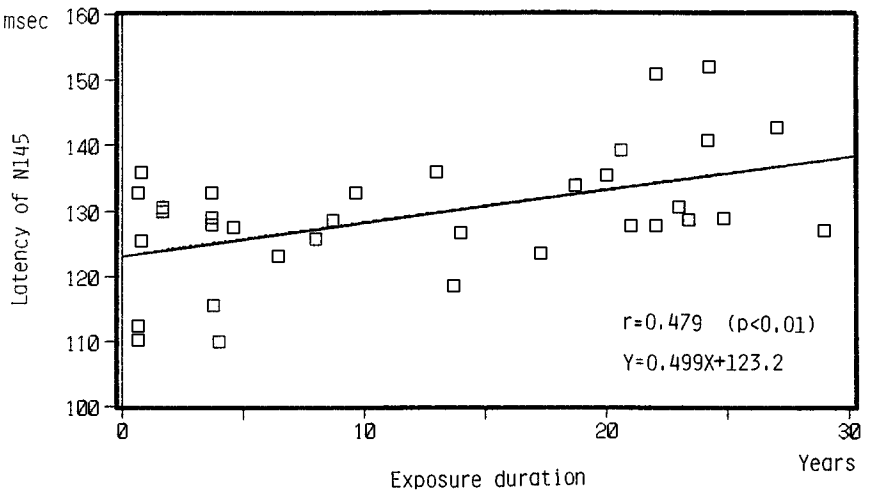

FIG. 3. Relationship between latency of component N145 of visual evoked potential (VEP) and lead exposure duration of exposed workers.

and IPL N11–20 and N13–20 of SLSEP with PbB and TWA-PbB ($P < 0.01$, respectively) (Table 3).

Table 4 shows the comparison of latencies of the three main components and IPL I–V and IPL III–V of ABR. Mean latencies of components I and III and IPL III–V of ABR of 15 lead-exposed workers were significantly delayed in comparison with those of unexposed workers ($P < 0.05$, $P < 0.01$, $P < 0.05$, respectively).

DISCUSSION

In the present study, we observed a significantly negative correlation between radial NCV and lead exposure indicators and a significant positive correlation

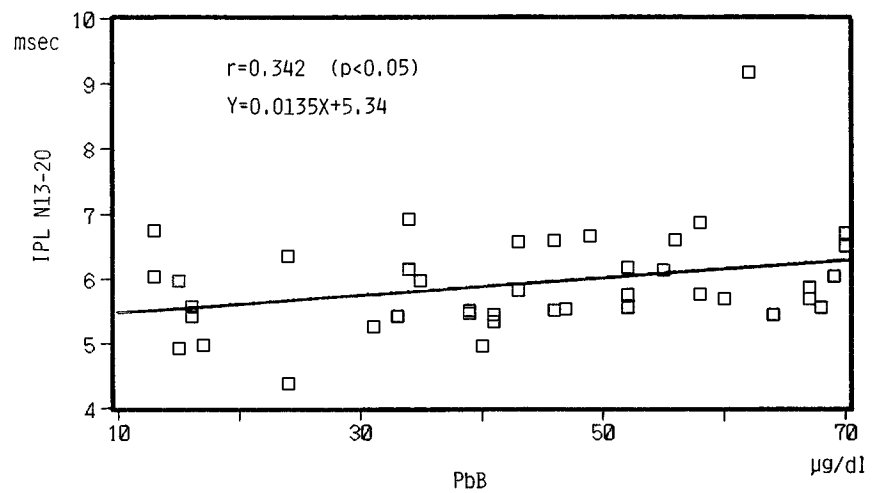

FIG. 4. Relationship between interpeak latency between component N13 and N20 (IPL N13–20) of short-latency somatosensory evoked potentials (SLSEP) and current lead concentration of whole blood (PbB) in lead-exposed workers.

TABLE 2
PARTIAL CORRELATION COEFFICIENTS BETWEEN NEUROPHYSIOLOGICAL PARAMETERS AND EXPOSURE DURATION, PbB, AND TWA-PbB BY AGE ADJUSTMENT

	Exposure duration	PbB	TWA-PbB
Radial NCV			
MCV	0.031	−0.304	−0.367*
SCVwa	0.103	−0.368*	−0.016
SCVfw	−0.075	−0.304	−0.035
ERG latency			
a wave	0.080	−0.047	0.053
b wave	−0.022	−0.073	−0.040
OP 1	0.244	−0.299	−0.267
VEP latency			
N75	−0.195	0.210	0.144
P100	0.058	−0.126	−0.023
N145	0.406**	−0.294	−0.132
ABR			
Latency			
I	0.284	0.216	0.261
V	−0.118	−0.026	0.067
IPL			
I–V	−0.169	−0.098	−0.012
III–V	−0.123	0.085	0.163
SLSEP			
Latency			
N9	−0.170	0.110	0.051
N11	−0.075	0.216	0.224
N13	−0.050	0.087	0.115
N20	−0.275	0.329*	0.108
IPL			
N11–13	0.029	−0.177	−0.142
N11–20	−0.271	0.194	−0.075
N13–20	−0.263	0.270	0.005

Note. PbB, TWA-PbB, same as Table 1; NCV, nerve conduction velocity; MCV, motor nerve conduction velocity; SCVfw: sensory nerve conduction velocity from the index finger to the wrist; SCVwa, sensory nerve conduction velocity from the wrist to the forearm; ERG, electroretinogram; OP, oscillatory potential of ERG; VEP, visual evoked potential by pattern reversal stimulation; ABR, auditory brain stem response; IPL, interpeak latency; SLSEP, short-latency somatosensory evoked potentials.

* $P < 0.05$.

** $P < 0.01$.

between lead exposure indicators and the latencies of the late component of VEP and SLSEP and IPL of SLSEP. Furthermore, latencies of early components and IPL III–V of ABR of lead-exposed workers were significantly delayed compared with those of unexposed workers.

Although radial palsy is a characteristic sign in lead neuropathy, radial NCV has rarely been utilized in the investigation of subclinical lead neuropathy using NCV testing because of the difficulty of measurement or of attaining agreement in cases using needle electrode. Murata *et al.* (1987) reported the reduction of MCV of lead-exposed gun-metal workers and a significant negative correlation between SCV (same as SCVwa in the present study) and urinary δ-amino-levulinic acid and coproporphyrin. This suggested that chronic lead exposure results in a decrease of the radial nerve conduction function. Since negative correlation between NCV

TABLE 3
CORRELATION BETWEEN NEUROPHYSIOLOGICAL PARAMETERS AND LEAD EXPOSURE INDICATORS BASED ON STEPWISE MULTIPLE REGRESSION ANALYSIS

Neurophysiological parameters	Selected independent variables		Multiple correlation coefficients (R)
SCVwa	TWA-PbB	(−0.378*)	0.442*
VEP-N145	Exposure	(0.479**)	0.540**
SLSEP N20	PbB	(0.357*)	0.557**
IPL N11–20	PbB	(0.247)	0.509**
IPL N13–20	PbB	(0.342*)	0.529**

Note. Abbreviations same as those in Tables 1 and 2. Parentheses indicate standard correlation coefficient.
* $P < 0.05$.
** $P < 0.01$.

and lead exposure indicators implies that an increase of PbB reduces the conduction function of the radial nerve, the results of the present study offer support for reduction in the conduction function in the radial nerve due to lead exposure. However, the present results do not suggest a dose–effect relationship between lead exposure and radial NCV, perhaps because of the relatively small population of lead-exposed workers.

Since ERG parameters showed insignificant correlations among lead exposure indicators, the PNS of the visual ascending pathway may not be involved in chronic lead exposure. On the other hand, we observed a significant positive correlation between the latency of the component N145 of VEP and lead exposure duration, that is, the latency is delayed with increased duration. Araki *et al.* (1987) also reported that the latencies of the N1 and N2 components (the same as components N75 and N145 in the present study) were significantly prolonged in comparison with the matched controls. Since the cerebral cortex is considered as the origin of discharged component N145 (not clarified in detail) (Halliday, 1982), these facts indirectly suggest that chronic lead exposure affects the CNS but not the PNS functions.

There are few reports on ABR and lead exposure. ABR in humans is considered to consist of three main components generated from the acoustic nerve (component I), the superior olivary nucleus (component III), and the inferior colliculus (component V) (Robinson and Rudge, 1982). Consequently, the latency of component I indicates the conduction time from the onset of stimulation to the acous-

TABLE 4
ABR PARAMETERS IN LEAD-EXPOSED AND UNEXPOSED WORKERS (MEAN ± STANDARD DEVIATION)

ABR parameters	Lead-exposed (N = 15)	Unexposed (N = 39)	t value	P
Latency I	1.42 ± 0.159 msec	1.52 ± 0.107 msec	2.57	<0.05
III	3.65 ± 0.187	3.82 ± 0.157	3.44	<0.01
V	5.69 ± 0.117	5.77 ± 0.17	1.67	NS
IPL I–V	4.27 ± 0.166	4.20 ± 0.191	1.17	NS
III–V	2.04 ± 0.129	1.95 ± 0.191	2.03	<0.05

Note. Abbreviations same as those in Table 2. NS, not significant by Student's t test.

tic nerve and component III to the superior olivary nucleus, and IPL III–V reflects the conduction time from the superior olivary nucleus to the inferior colliculus, that is, the conduction function of the brain stem. Significant delay of the latency of component I and IPL III–V of ABR of lead-exposed workers in the present study suggests that chronic lead exposure reduces the conduction function of the acoustic nerve and the brain stem, respectively. The discrepancy between regression analysis and exposed-control analysis cannot be explained by the interaction effect of age. The insignificance in the regression analysis suggests that the dose–response relationship between ABR parameters and lead exposure indicators is not linear. Further investigation will be needed to confirm the lead exposure effect on ABR.

Components N9, N11, N13, and N20 of SLSEP are considered to be generated from the brachial plexus, the dorsal column of the spinal cord, the posterior horn of the spinal cord, and the somatosensory area of the cortex, respectively (Jones, 1982; Drechsler, 1985). Consequently, the latency of N20 and IPL N13–20 indicates conduction times from the wrist to the cerebral cortex (the same as the early component of long-latency SEP) and from the posterior horn to the cortex, respectively. Araki *et al.* (1987) observed no significant prolongation of IPL N13–20 in the comparison between lead-exposed gun-metal workers and age-matched controls, but significant correlation with urinary coproporphyrin concentration which is increased by lead exposure. Although the present study differs in the number of subjects and statistical analysis from Araki's report, the correlation between lead exposure indicators and IPL N11–20 and N13–20 suggests that the conduction function from the spinal cord to the cortex decreases with the increase of PbB, that is, chronic lead exposure decreases the conduction function of the somatosensory ascending pathway in the CNS.

Generalization of the results for the PNS and CNS in the somatosensory ascending pathway suggests that the conduction function in the PNS (SCVwa and MCV) was more seriously affected by chronic lead exposure than that of the CNS (IPL N11–20 and N13–20). Similarly, the larger t value of latency component I indicates more involvement of the PNS than the CNS in the auditory ascending pathway (IPL III–V). On the other hand, the involvement of the visual pathway appeared less directly in the CNS with a significant positive correlation between the latency of component N145 and lead exposure duration and was not found in the PNS (ERG).

CONCLUSIONS

In the present study, we observed that radial MCV and SCVwa, the latency of component N145 of VEP, and the latency of component N20 of SLSEP showed a significant correlation with lead exposure indicators. The latencies of component I and IPL III–V of ABR of lead-exposed workers were significantly prolonged compared with those of unexposed workers. These results suggest that the conduction functions of the CNS and PNS are decreased by chronic lead exposure.

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Relations between Lead Exposure and Peripheral Neuromuscular Functions of Lead-Exposed Workers—Results of Tapping Test[1]

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In this study, four experiments using the tapping test were conducted to evaluate the possible subclinical effects of lead exposure on the neuromuscular systems of lead workers at some transfer printing factories in Japan. Decreases in the tapping ability appeared coincidentally with higher blood lead levels. The recovery of the tapping ability after 30 sec rest in the groups of 30–45 μg/dl and above 45 μg/dl PbB was worse than that in the group with less than 29 μg/dl PbB. The recovery of the decreased tapping ability after 60 sec rest was better even in the group with 30–45 μg/dl PbB. The tapping ability for 0–10 sec at the first tapping test was sustained after 30 or 60 sec rest in the group with the PbB below 29 μg/dl; however, the tapping ability at the second and third tapping test decreased in the two groups with the PbB level above 30 μg/dl. The decreased finger tapping speed may be functional evidence of low-grade motor neuropathy among the workers with higher levels of lead absorption.

INTRODUCTION

It is well known that patients with typical lead poisoning demonstrate neurological signs and symptoms such as paralysis of extensor muscles of extremities, sensory disorders, hand and finger tremor, and decreased grasping power (Browning, 1961). The diagnostic importance of weakened extensors of the wrist and fingers of the hand most used for work has been greatly emphasized. Some observers believe that even a slight difference in strength between the two hands is the best early diagnostic sign of plumbism (Finkel, 1983). However, recent improvements in occupational hygiene as well as regular medical supervision of lead-exposed workers have remarkably reduced such typical neurological manifestations in industrial lead poisoning (Seppalainen and Hernberg, 1972). Despite this change, it has been recently reported that subclinical nerve damage may possibly occur (Sessa *et al.*, 1965; Simpson *et al.*, 1964; Seppalainen and Hernberg, 1972, 1980; Takeuchi *et al.*, 1975; Araki and Honma, 1976; Ashby, 1980). There have been a few reports on the relation between tapping ability and blood lead (Landrigan *et al.*, 1975; Winnecke *et al.*, 1983). Both were concerned with neurophysiological dysfunction in children with chronic low-level lead absorption. The authors conducted a study including four experiments using the tapping test in order to evaluate the possible subclinical effects of lead exposure on the neuromuscular system among lead workers at some transfer printing factories in Japan.

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TABLE 1
SUBJECTS AND AVERAGE ROOM TEMPERATURE DURING THE EXPERIMENTS

Experiment	Male subjects (age)	Room temperature (°C)
1	48 (18–59)	24.5 ± 0.5
2	32 (18–59)	18.5 ± 5.1
3	19 (21–58)	21.5 ± 2.8
4	27 (18–58)	25.6 ± 8.2

SUBJECTS AND METHODS

The subjects were working in the transfer printing companies as operators of various printing machines and were exposed to lead contained in powdered colors; however, many workers had no definite workplace and frequently alternated between jobs because they were required to cover all the processes of the transfer printing in small businesses. The age and average room temperatures during the experiments are shown in Table 1.

The first experiment was undertaken with 48 men (18–59 years old), the second with 32 men (18–59 years old), the third with 19 men (21–58 years old), and the fourth with 27 men (18–58 years old). Tapping ability was measured throughout the four experiments with Tapping Test Counter No. 1347 (Roken type, made by Takei Instrument Co., Ltd.) (Kimotsuki, 1967). When tapping tests were conducted, the Tapping Test Counter was placed on a desk at a height adequate for the subjects who were in a standing position (Kimotsuki, 1969). The blood lead concentration was measured by the flameless AAS method (Fukaya, 1982).

The design of the four experiments is shown in Fig. 1. Each experiment had its own purpose, but the aim of the four experiments as a whole was to determine how lead poisoning affects the decrease in tapping ability and how the length of the rest interval can facilitate recovery of the decreased tapping ability. The aim of the first experiment was to determine whether the decreased tapping ability would appear coincidentally with the higher blood lead levels. The aim of the second experiment was to determine whether the recovery of the decreased tapping ability would appear after 30 sec of rest in each group, and whether it would

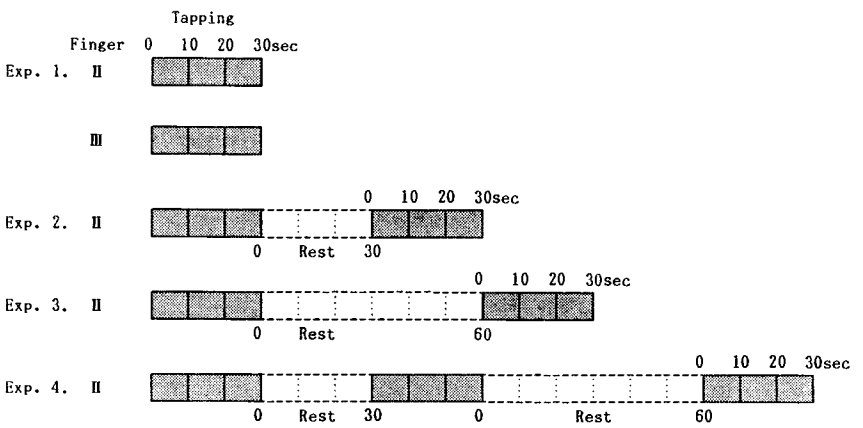

FIG. 1. Experimental designs for the tapping ability tests.

TABLE 2
AVERAGE AGES OF THE SUBJECTS IN THE THREE GROUPS

	≦29 μg/dl PbB		30 ~ 44 μg/dl PbB		≧45 μg/dl PbB	
Expt	*N*	Mean age ± SD	*N*	Mean age ± SD	*N*	Mean age ± SD
1	16	33.6 ± 11.79	17	36.6 ± 13.69	15	46.9 ± 11.68
2	10	41.8 ± 12.04	8	41.6 ± 17.86	14	42.2 ± 11.43
3	6	44.0 ± 11.68	6	47.5 ± 9.23	7	43.6 ± 14.02
4	7	43.6 ± 10.81	13	44.6 ± 13.43	7	45.0 ± 12.73

be better in the group with the lower level of PbB. The aim of the third experiment was to determine whether the recovery of the decreased tapping ability would appear after 60 sec of rest in each group, and whether it would be better in the group with the lower level of PbB. The aim of the fourth experiment was to determine whether the recovery of the decreased tapping ability after 30 sec of rest and 60 sec of rest after the second series of tapping would appear in each group, and whether it would be better in the group with low levels of PbB.

Experiment 1. Tapping ability was measured on the second and the third finger of the right hand for 30 sec. The number of taps was counted and recorded at 10-sec intervals: 0–10, 10–20, and 20–30 sec (the second finger: TII-10, TII-20, and TII-30; the third finger: TIII-10, TIII-20, and TIII-30, respectively).

Experiment 2. Tapping ability was measured on the second finger of the right hand for 30 sec, and after a 30-sec rest, it was measured again for 30 sec. The number of taps was counted and recorded at 10-sec intervals.

Experiment 3. Tapping ability was measured on the second finger of the right hand for 30 sec, and after a 60-sec rest, it was measured again for 30 sec.

Experiment 4. Tapping ability was measured on the second finger on the right hand for 30 sec, and after a 30- and a 60-sec rest each tapping ability was measured for 30 sec. The tapping experiment was measured and recorded every 30 sec at intervals of 10 sec each.

RESULTS

Subjects were divided into three groups according to the blood lead levels, namely, below 29, 30–44, and 45 μg/dl or more for analysis. The average ages, average experience with the transfer printing, and average blood lead concentrations of the subjects in each experiment are shown in Table 2, 3, and 4, respectively.

TABLE 3
YEARS OF EXPOSURE OF THE SUBJECTS TO THE THREE GROUPS

	≦29 μg/dl PbB		30 ~ 44 μg/dl PbB		≧45 μg/dl PbB	
Experiment	*N*	Av. exposed years[a]	*N*	Av. exposed years	*N*	Av. exposed years
1	16	3.9 ± 8.62	17	9.2 ± 9.32	15	19.6 ± 13.01
2	10	5.7 ± 10.01	8	10.1 ± 10.87	14	17.8 ± 10.61
3	6	9.8 ± 11.65	6	12.9 ± 9.71	7	22.6 ± 12.12
4	7	9.4 ± 11.85	13	13.4 ± 11.07	7	22.1 ± 10.25

[a] Means ± SD.

TABLE 4
AVERAGE BLOOD LEAD CONCENTRATIONS OF THE SUBJECTS TO THE THREE GROUPS

	≦29 μg/dl PbB		30 ~ 44 μg/dl PbB		≧45 μg/dl PbB	
Exp.	*N*	Av. PbB[a]	*N*		*N*	
1	16	15.5 ± 4.95	17	40.1 ± 3.09	15	56.2 ± 9.48
2	10	15.6 ± 6.06	8	38.3 ± 4.33	14	58.0 ± 12.87
3	6	14.5 ± 5.54	6	36.7 ± 0.82	7	58.0 ± 13.95
4	7	14.9 ± 6.49	13	34.7 ± 3.92	7	56.6 ± 3.95

[a] Means ± SD.

Experiment 1. As shown in Table 5, the average tapping ability in the group of ≧45 μg/dl PbB was significantly lower than that in the group of ≦29 μg/dl (TII-10, TII-20, TIII-20, TIII-30). The higher PbB level groups were relatively older than the lower PbB level ≦29 μg/dl group. The authors searched for the partial correlation coefficients controlling for age. The partial correlations between TIII-20 or TIII-30 and PbB level were significantly negative (Table 6).

Experiment 2. The average age of the subjects was 41.8 ± 12.04 in the ≦29 μg/dl group (n = 10), 41.6 ± 17.86 in the 30–44 μg/dl group (n = 8), and 42.2 ± 11.43 in the of ≧45 μg group (n = 14) in PbB. As shown in Fig. 2, the average tapping ability in the ≧45 μg/dl group showed a tendency to decrease for 0–10 and 10–20 sec and was significantly lower than that in the ≦29 μg/dl group for 20–30 sec at the first tapping and every 10 sec at the second tapping.

Figure 3 shows the results when the count number for 0–10 sec of the first tapping and each count number for the other 10 sec were compared by paired t test. The count number for the 10 sec for the second tapping was not significantly difference from that of the first tapping in the group with ≦29 μg/dl PbB.

However, the count number of the second tapping in the ≦44 μg/dl group and that in the ≧45 μg/dl group, respectively, were significantly different from the values for 0–10 sec. These values were significantly different from the values for 0–10 sec at the first tapping.

Experiment 3. The average age of the subjects was 44.0 ± 11.68 in the ≦29 μg/dl group (n = 6), 47.5 ± 9.23 in the 30–44 μg/dl group (n = 6), and 43.6 ± 14.02 in the ≧45 μg/dl group (n = 7). As the blood lead level became higher, the tapping

TABLE 5
EACH VALUE OF THE TAPPING ABILITY IN THE THREE PbB LEVEL GROUPS

	≦29 μg/dl PbB[a] (n = 16)	30 ~ 44 μg/dl PbB (n = 17)	≧45 μg/dl PbB (n = 15)
TII-10	47.4 ± 9.82	46.9 ± 5.43	40.9 ± 7.49*
TII-20	41.8 ± 9.43	39.8 ± 6.34	35.3 ± 7.15*
TII-30	35.4 ± 11.08	33.3 ± 8.29	28.9 5.52
TIII-10	44.6 ± 8.25	45.4 ± 4.60	39.7 7.30
TIII-20	40.6 ± 8.38	39.4 ± 6.11	34.7 ± 5.55*
TIII-30	35.2 ± 9.95	34.2 ± 6.58	27.9 ± 6.20*

[a] Means ± SD.
* Statistically significant ($P < 0.05$) compared with the group of ≦29 μg/dl PbB.
** The same at $P < 0.01$.

TABLE 6
PARTIAL CORRELATION BETWEEN PbB AND TAPPING ABILITY BY CONTROLLING FOR AGE

n = 48	Correlation coefficient
TII-10	−0.2034†
TII-20	−0.1986†
TII-30	−0.1692
TIII-10	−0.1023
TIII-20	−0.2529*
TIII-30	−0.2455*

* Statistically significant at $P < 0.05$.
† The same at $P < 0.10$.

ability decreased (Fig. 4). Controlling for age and years of exposure, the partial correlation coefficients were calculated and similar results were obtained.

Tapping ability for 0–10 sec at the first tapping and the count number for 0–10 sec at the second tapping had a statistically significant difference in the ≥45 μg/dl group (Fig. 5).

Experiment 4. The average age of the subjects was 43.6 ± 10.81 for 30–44 μg/dl and 45.0 ± 12.73 in the ≥45 μg/dl group (n = 7). As the PbB level went higher, tapping ability decreased (Fig. 6). The count numbers of tapping 0–10 sec at the first tapping and those for 0–10 sec after 30 or 60 sec rest, respectively, had a significant difference by paired t test in the two groups above 30 μg/dl in PbB level. However, this is not true for the group at a level below 29 μg/dl (Fig. 7).

DISCUSSION

Some researchers have pointed out that a slight delay in motor nerve conduction velocities occurs in lead-exposed workers at PbB levels of above 45 μg/dl (Seppalainen and Hernberg, 1972; Takeuchi *et al.*, 1975) and confirmed that a neuropathy may precede all other signs of lead poisoning. Feldman and his colleagues (1977) have suggested that measurements of motor nerve conduction velocity would be useful in the diagnosis of subliminal or otherwise unrecognized

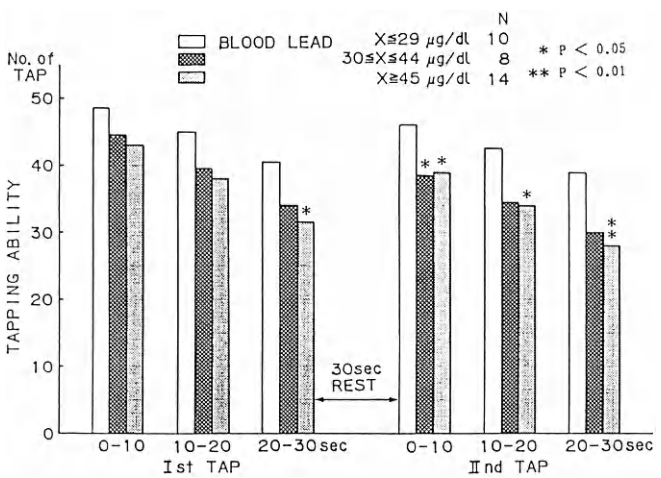

FIG. 2. The relationships between PbB levels and the neuromuscular functions.

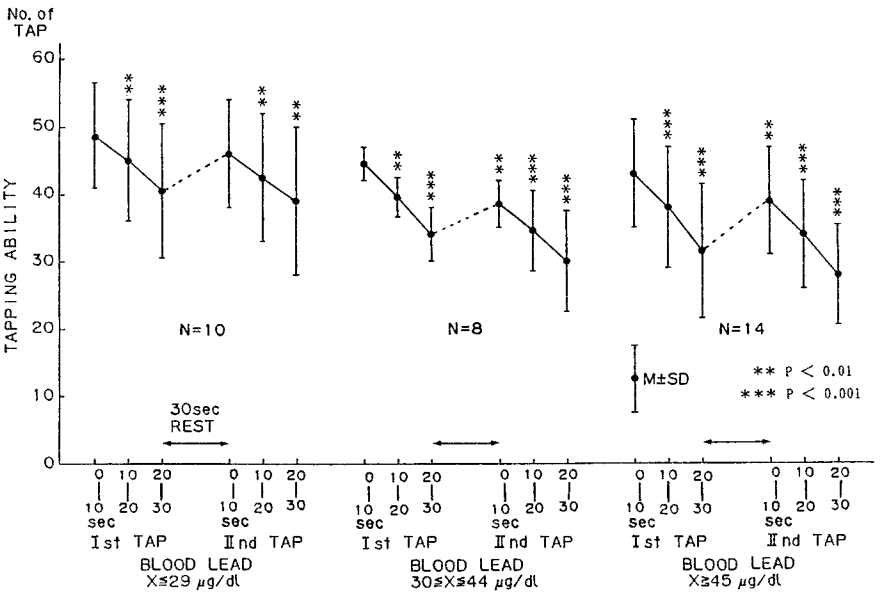

FIG. 3. Each value of the tapping ability compared with the value for the first 10 sec in the three PbB level groups.

toxic effects of lead. However, there have been few reports on the relations between tapping ability and blood lead. According to Landrigan *et al.* (1975) symptom-free children ages 3–15 years with blood lead concentrations of 40–68 μg/dl had significant slowing in a finger–wrist tapping test. Winneke *et al.* (1983) also found a near-significant, inverse relationship between finger–wrist tapping speed and Pb blood in school-age children. According to the report by Kimotsuki (1969), the effect of the temperature upon the velocity of tapping with a finger was negligible in our four experiments because the average room temperature was above 18°C.

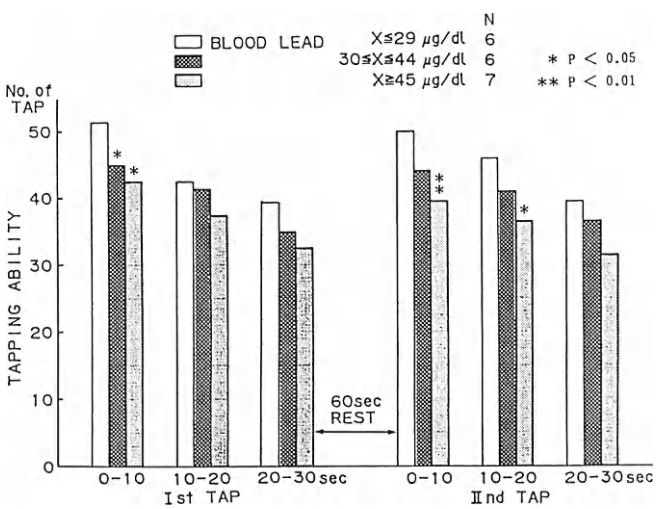

FIG. 4. The relationships between PbB levels and neuromuscular functions.

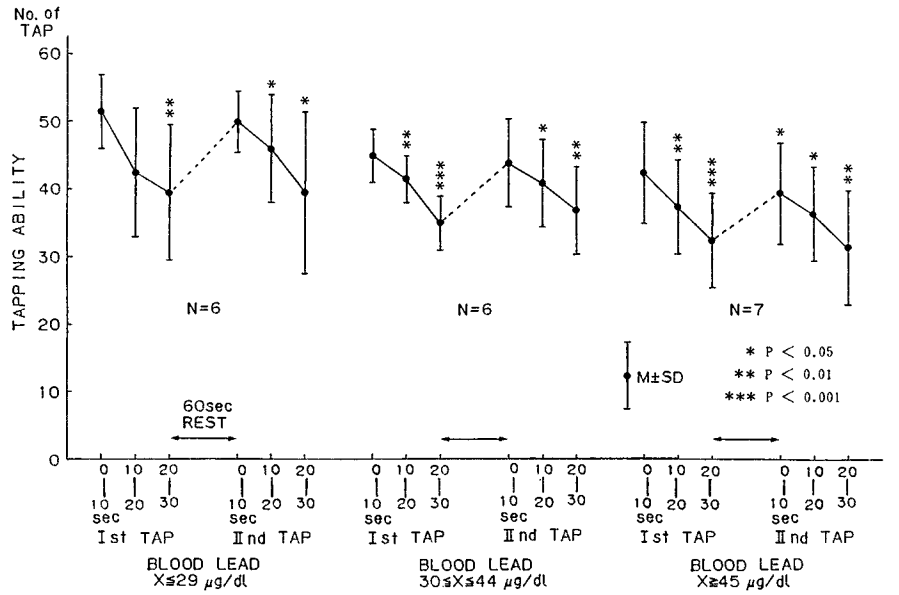

FIG. 5. Each value of the tapping ability compared with the value for the first 10 sec in the three PbB level groups.

The major findings of the four experiments can be summarized as follows:

1. The decrease in the tapping ability appeared coincidentally with higher blood lead levels.

2. Recovery of the tapping ability after 30 sec rest in the 30–45 and above 45 μg/dl PbB groups was worse than that in the group with less than 29 μg/dl PbB.

3. Recovery of the decreased tapping ability after 60 sec rest was better even in the group with 30–45 μg/dl PbB.

4. The tapping ability for 0–10 sec for the first tapping was sustained after 30 or 60 sec rest in the group with the PbB below 29 μg/dl; however, the tapping ability

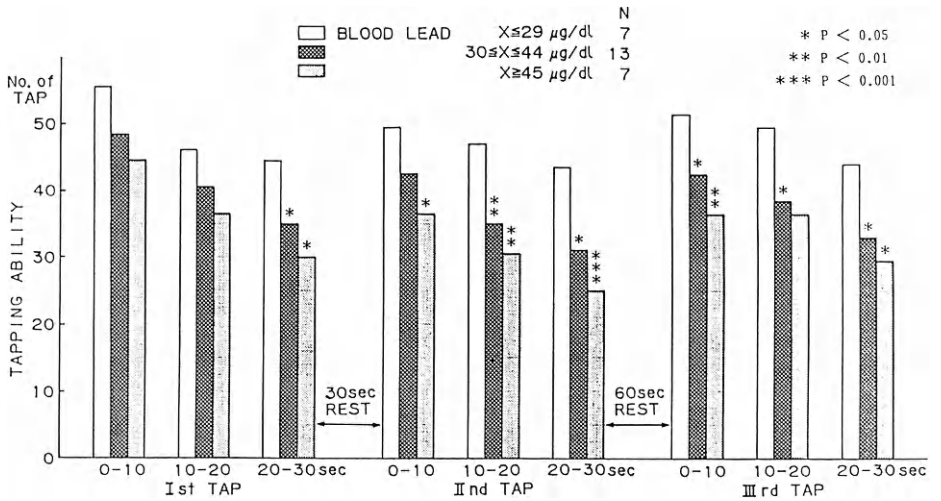

FIG. 6. The relationships between PbB levels and neuromuscular functions.

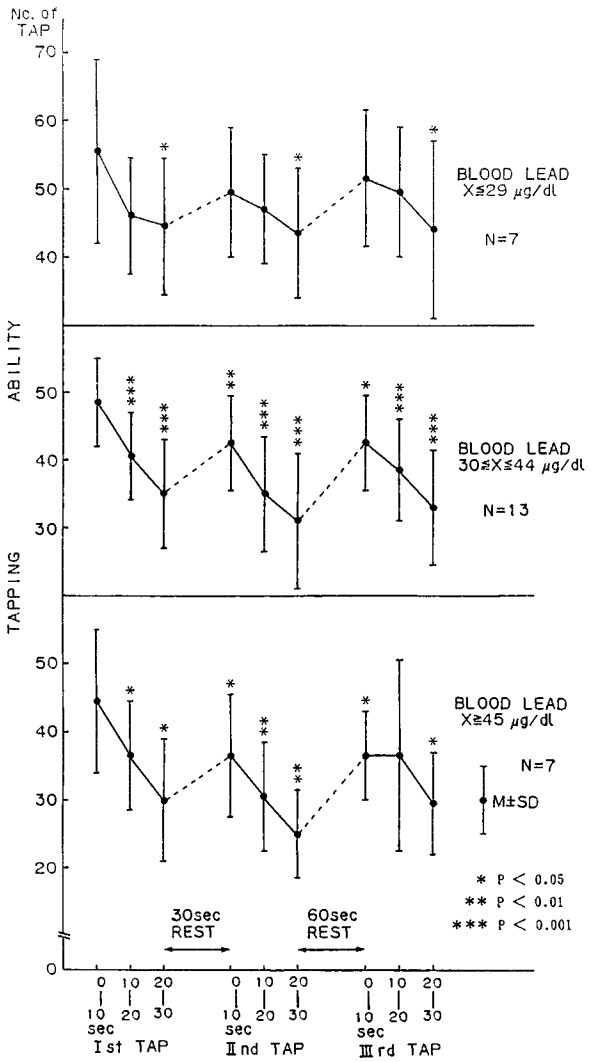

FIG. 7. Each value of the tapping ability compared with the value for the first 10 sec in the three PbB level groups.

for the second and third tapping was decreased in the two groups of the PbB level above 30 μg/dl.

5. The decreased finger tapping speed may be functional evidence of low-grade motor neuropathy among the transfer printing workers with higher levels of lead absorption.

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ENVIRONMENTAL RESEARCH 61, 308–316 (1993)

Radial and Median Nerve Conduction Velocities in Workers Exposed to Lead, Copper, and Zinc: A Follow-Up Study for 2 Years[1]

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To evaluate the interactive effects of lead, zinc, and copper on the peripheral nervous system in man, we measured maximal motor and sensory conduction velocities (MCV and SCV) in the distal radial and median nerves in 19 gun metal foundry workers with asymptomatic increased absorption of these metals twice at a 12-month interval. The workers' initial blood lead (BPb) concentrations ranged from 16 to 64 (mean, 42) μg/dl. The principal findings in the present study indicated that (1) radial and median nerve conduction velocities were significantly slowed in the gun metal foundry workers; (2) indicators of lead absorption were inversely related to radial nerve conduction velocities, whereas indicators of copper and zinc absorption were positively correlated with the radial and median nerve conduction velocities; and (3) yearly changes in MCV in the radial nerve and in SCV in the median nerve were positively correlated with the changes in indicators of copper and zinc absorption. These findings suggest that zinc and copper antagonize the subclinical neurologic effects of lead. Also, the radial and median nerve conduction velocities provide important indicators of subclinical lead toxicity.

INTRODUCTION

Lead is a potent neurotoxin, and the effects of lead on the central and peripheral nervous system are dose related. Evidence has developed, however, that the dose-related severity of lead toxicity may be reduced as levels of zinc and/or copper increase in the human body. Zinc antagonizes the inhibitory effect of lead on the intraerythrocytic enzyme δ-aminolevulinic acid dehydratase (ALAD) (Thomasino *et al.*, 1977; Meredith and Moore, 1980; Araki *et al.*, 1984). Also, we observed in two previous studies on the short-latency somatosensory-evoked potentials, and on the distribution of conduction velocities in sensory fibers of the median nerve in lead workers, that zinc may antagonize the effects of lead on the central and peripheral nervous systems (Araki *et al.*, 1986a,b). Furthermore, we found in another study that lead-induced conduction delay in the visual pathway of the central nervous system may be antagonized by copper (Araki *et al.*, 1987). Thus, we hypothesize that the antagonistic effects of zinc and copper on lead toxicity may be more extensive in man than currently recognized.

Slowing of peripheral nerve conduction velocities in workers with asymptomatic increased absorption of lead has been demonstrated in the majority of previous studies, indicating that median nerve conduction velocities are particularly sensitive to lead exposure (Seppalainen *et al.*, 1975, 1983; Araki and Honma,

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1976; Repko *et al.*, 1978; Buchthal and Behse, 1979; Ashby, 1980; Bordo *et al.*, 1982; Singer *et al.*, 1983; Rosen *et al.*, 1983). On the other hand, radial nerve conduction velocities have rarely been examined, especially for motor conduction velocity in the forearm segment of the radial nerve. This is probably due to the technical difficulty of measuring radial nerve conduction in the forearm; because of the presence of a volume-conducted response from distal muscles, a needle electrode must be carefully inserted into the deep muscle, e.g., the extensor indicis muscle, to record the action potentials (Jebsen, 1966a). As wrist drop is a distinguishing manifestation of peripheral neurotoxicity of lead in "classical" lead poisoning, the motor conduction velocity in the forearm, if measured with a reliable and valid technique, might provide a sensitive measure of subclinical lead toxicity.

In this study, we measured motor and sensory conduction velocities in the foreman segment of the radial and median nerves in gun metal foundry workers twice at a 12-month interval. These workers had been exposed simultaneously to lead, copper, and zinc. We analyzed the data using stepwise regression analysis to assess the interactive effects of these metals on peripheral nerve conduction velocities.

SUBJECTS AND METHODS

Subject

The 19 subjects were male gun metal foundry workers with blood lead (BPb) concentrations of 16 to 64 (mean, 42) μg/dl in the initial year of the examination and their BPb concentrations ranged from 25 to 59 μg/dl (mean, 39) in the second year. Their initial plasma copper and zinc (PCu and PZn) concentrations ranged from 64 to 136 μg/dl and from 75 to 148 μg/dl, respectively (Table 1). Gun metal is composed of lead (5%), zinc (5%), copper (85%), and tin (5%). These workers had been employed at the metal factory for 1–16 (mean, 9) years; their ages ranged from 33 to 58 (mean, 47) years. They drank alcohol equivalent to 0–630 (mean, 216) ml of 100% ethanol per week in the initial year and to 0–760 (mean, 287) ml in the second year. None of them had specific signs or symptoms indicative of

TABLE 1
BLOOD AND URINARY LEVELS OF LEAD, COPPER, AND ZINC IN THE FIRST AND SECOND YEARS OF EXAMINATION IN 19 GUN METAL FOUNDARY WORKERS (MEANS ± SD)

	First year	Second year	Matched difference (*P*)[a]
Plasma (μg/dl):			
Lead	0.8 ± 0.2	0.7 ± 0.2	NS
Copper	103 ± 19	91 ± 18	<0.05
Zinc	96 ± 16	89 ± 10	<0.05
Erythrocytes (μg/dl):			
Lead	109 ± 29	99 ± 22	<0.05
Copper	101 ± 21	91 ± 16	<0.05
Zinc	821 ± 94	917 ± 201	NS
Urine (μg/liter, SG 1.020):			
Lead	117 ± 60	79 ± 31	<0.01
Copper	20 ± 30	16 ± 4	<0.05
Zinc	1621 ± 813	1020 ± 400	<0.01

[a] Paired-sample *t* test (NS, not significant).

clinical lead poisoning or neurologic, hematological, or other endocrinological disorders; none were exposed in their workplace to such neurotoxic chemicals as arsenics or solvents.

The control subjects, matched to each gun metal foundry worker by age were selected from 41 "healthy" men for the neurophysiological study. None were occupationally exposed to lead, copper, or zinc, nor had neurological disorders. They ingested alcohol equivalent to 0–1100 (mean, 262) ml of 100% ethanol per week. The mean (±standard deviation) values of BPb, PCu, and PZn for the controls were 14 (±4), 72 (±16), and 75 (±14) μg/dl, respectively. Their ages ranged from 35 to 62 years; there were no significant differences in age, skin temperature, and alcohol ingestion between them and the gun metal foundry workers in either of the two examinations (paired sample t test, $P > 0.05$).

A local exhaust ventilation system was introduced into the workplace of the gun metal foundry workers after the conduction of electrophysiological studies in the first year of the investigation. The geometric mean air lead concentration in their workplace decreased from 63.4 to 44.7 $\mu g/m^3$ over the 12-month interval. This resulted in a decrease in workers' mean urinary lead, copper, and zinc (UPb, UCu, and UZn) concentrations in the second year as shown in Table 1.

Methods

The measurement of radial and median nerve conduction velocities in the right forearm was conducted in a warm laboratory (26–32°C) by the use of Medelec MS-92 two-channel electromyograph; skin temperature was maintained in the range of 31–35°C for all subjects. The maximal motor nerve conduction velocity (MCV) in the distal radial nerve was measured by the method described by Jebsen (1966a); similarly, sensory nerve conduction velocity (SCV) in the radial nerve was measured by the antidromic method of Downie and Scott (1967). The MCV of the median nerve was measured by the method reported by Araki and Honma (1976); the SCV in the forearm (elbow to wrist) and in the palmar (wrist to finger) segments of the median nerve were measured by the antidromic technique (Smorto and Basmajian, 1979).

To ascertain daily variation in the radial and median nerve conduction velocities, we performed the measurement repeatedly over a period of 15 days in a 23-year-old "healthy" medical student. We found that the coefficients of variation were 4.4 and 4.2% for the MCV and SCV of the radial nerve, respectively, and 3.0, 3.3, and 3.6% for the MCV, SCV (forearm), and SCV (palm) of the median nerve, respectively.

Blood samples were collected just before the measurement of nerve conduction velocities and the start of the 24-hr urine collection. BPb, erythrocyte and plasma concentrations of lead (EPb and PPb), UPb, erythrocyte concentration of copper (ECu), PCu, UCu, erythrocyte concentration of zinc (EZn), PZn, and UZn were measured by atomic absorption spectrophotometry (Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer 180-80) using the same methods as described in our previous report (Araki *et al.*, 1984). Similarly, the activity of ALAD, urinary concentrations of δ-aminolevulinic acid and coproporphyrin (ALA and CP), and the blood hemoglobin (Hb) were measured by the methods described previously (Araki *et al.*, 1983, 1984). The reproducibility of these analyses has also been described previously (Araki *et al.*, 1983, 1984).

Differences in nerve conduction velocities between the gun metal foundry workers and the same number of matched controls were tested by the paired sample *t* test. The effects of indicators of lead, copper, and zinc absorption (BPb, PPb, EPb, UPb, ALAD, ALA, CP, Hb; PCu, ECu, UCu; PZn, EZn UZn) and other factors (duration of employment, alcohol ingestion, and age) on the nerve conduction velocities in the first and second years, together with the effects in the yearly alterations, were examined by stepwise regression analysis, in which a total of 17 variables were entered and removed from the regression equation at a significance level of $P < 0.05$.

RESULTS

In the radial nerve of 19 gun metal foundry workers, the MCV was significantly slowed in both the first and second years; also the SCV was significantly slowed in the second year (Table 2, Fig. 1). In the median nerve, the MCV and SCV (forearm) were significantly slowed in the first year; the SCV (palm) was slowed in the second year (Table 2, Fig. 1). Figure 2 illustrates the relationships between the nerve conduction velocities and indicators of both zinc and copper absorption.

Results of stepwise regression analysis indicate that the SCV of the radial nerve was significantly related to indicators of lead adsorption (ALA or Hb) in the first and second years (Table 3); this neurologic result was positively related to indicators of copper and zinc absorption (PCu, ECu, UCu, or PZn). Similarly, the MCV, SCV (forearm), and SCV (palm) of the median nerve in the 2 years were positively related to indicators of copper and zinc absorption (PCu, ECu, or EZn) (Table 3).

The yearly change in the MCV of the radial nerve was positively related to the yearly change in the level of copper absorption (PCu); the change in the SCV (forearm) of the median nerve was positively related to the change in the level of zinc absorption (EZn) (Table 4). On the other hand, yearly changes in the MCV of the radial nerve and in the SCV (forearm) of the median nerve were inversely related to the change in alcohol ingestion (Table 4).

TABLE 2
DIFFERENCES IN THE RADIAL AND MEDIAN NERVE CONDUCTION VELOCITIES BETWEEN THE FIRST AND SECOND YEARS OF EXAMINATION IN 19 GUN METAL FOUNDRY WORKERS AND THE SAME NUMBER OF MATCHED CONTROL SUBJECTS (MEANS ± SD)

	First year[a]		Second year	
Conduction velocities	Gun metal foundry workers	Controls	Gun metal foundry workers	Controls
Radial nerve (m/sec):				
MCV	56.8 ± 2.9**	59.9 ± 2.8**	57.0 ± 4.3**	59.9 ± 2.8**
SCV	57.3 ± 4.4	59.6 ± 3.8	56.3 ± 3.6**	59.6 ± 3.8**
Median nerve (m/sec):				
MCV	56.5 ± 3.1**	59.1 ± 2.6**	57.9 ± 3.0	59.1 ± 2.6
SCV (elbow to wrist)	61.1 ± 3.4*	63.5 ± 2.7*	61.1 ± 3.4	63.5 ± 2.7
SCV (wrist to finger)	46.2 ± 4.2	48.5 ± 4.8	44.0 ± 4.0**	48.5 ± 4.8**

[a] Published in Araki *et al.*, 1987.
* $P < 0.05$.
** $P < 0.01$ (paired-sample *t* test).

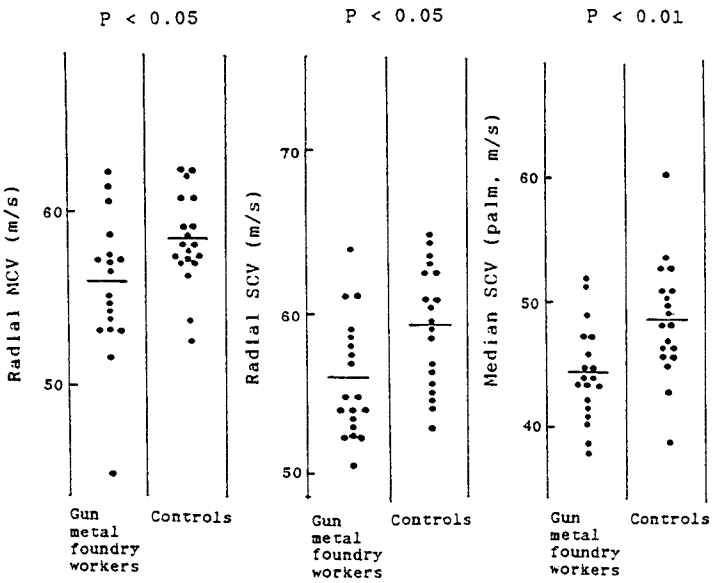

FIG. 1. Radial and median nerve conduction velocities in the second year of examination in nineteen gun metal foundry workers and the matched controls. Average values (transverse lines) are compared by the paired-sample t test. There are no differences in MCV and SCV (forearm) of the median nerve between two groups ($P > 0.05$).

DISCUSSION

The principal findings in the present study were as follows: (1) radial and median nerve conduction velocities were significantly slowed in the gun metal foundry workers with BPbs less than 65 μg/dl; (2) indicators of lead absorption were inversely related to the radial nerve conduction velocities, whereas indicators of copper and zinc absorption were positively correlated with the radial and median nerve conduction velocities in the 2 years of examination; and (3) yearly changes in the MCV of the radial nerve and in the SCV (forearm) of the median nerve were inversely related to the change in alcohol ingestion.

The findings in this study suggest that zinc and copper antagonize the subclinical effects caused by lead in relation to peripheral nervous system function. Similarly, in our previous study, changes in the N145 latency of VEP and in the N9–N13 latency of SSEP were positively correlated with changes in indicators of lead absorption and inversely correlated with changes of indices of zinc and copper absorption (Araki *et al.*, 1987); the changes in N9 and N13–N20 latencies of SSEP suggest that lead interferes with both peripheral and central nerve conduction but that zinc and copper appear to antagonize strongly the lead-induced conduction delay in the upper central nervous system while antagonizing only weakly the effects induced by lead in the lower central and peripheral nervous system.

In addition, the antagonistic effects of both zinc and copper on lead-induced slowing of nerve conduction velocities are consistent with the following other observations: (1) Antagonistic effects of zinc and vitamin C on lead induced neurotoxicity in lead workers (Sohler *et al.*, 1977; Papaioannou *et al.*, 1978). (2) The adverse effects of lead on growth and on hematopoietic indicators were minimized when dietary zinc, copper, and iron were adequate in animals (Klauder

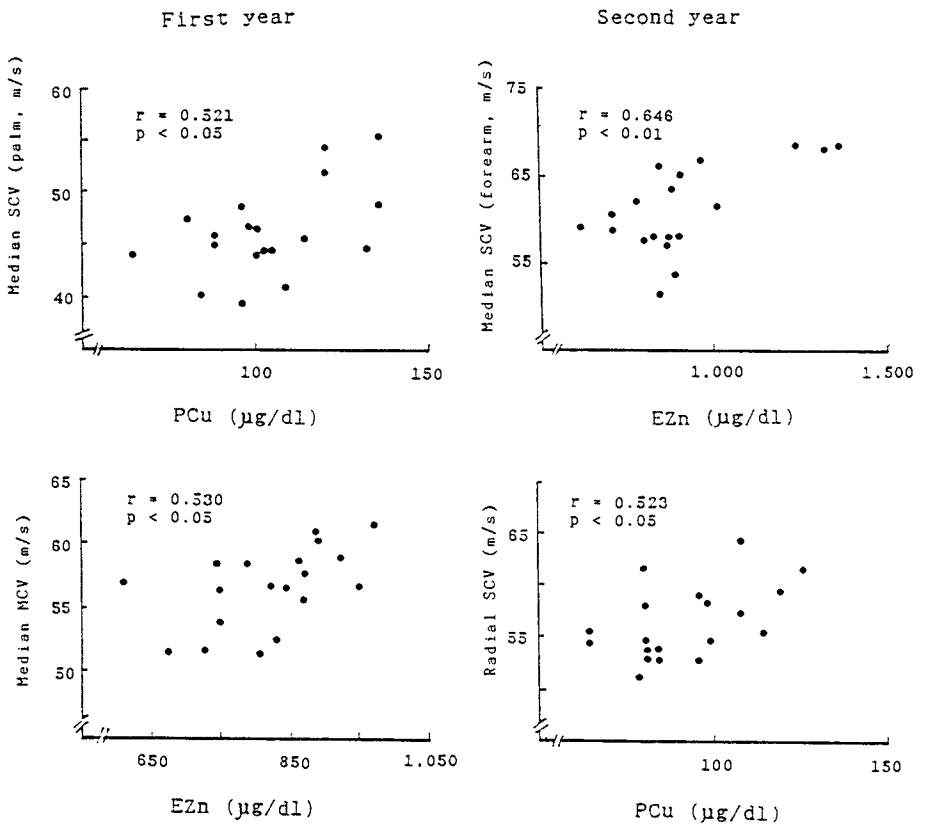

FIG. 2. The effects of copper and zinc absorption on nerve conduction velocities in 19 gun metal foundry workers in the first and second years. Abbreviations used are as in the text.

and Petering, 1975). (3) The lead–zinc interactions in the central nervous system have been reviewed by Petit (1984). (4) Copper deficiency leads to a defect in the process of myelination in the spinal cord of animals (Underwood, 1971). However, the present results contrast sharply with two other recent observation in animals, in which lead toxicity was found to be exaggerated by dietary copper (Cerklewski and Forbes, 1977; Malhotra *et al.*, 1982).

In the present study, the MCV in the forearm segment of the radial nerve was significantly slowed in the gun metal workers in both examination years; also the SCV was slowed in the second year. On the other hand, a significant reduction in the MCV of the radial nerve in the arm segment has been observed previously in lead workers with average BPbs of about 60–75 μg/dl, including workers suffering from "clinical lead poisoning" (Vasilescu, 1973), secondary lead smelters (Lilis *et al.*, 1977), and battery workers (Ashby, 1980). All of these studies have been made in the arm segment of the radial nerve, where it is often difficult to exclude error in the measurement of nerve distance between two stimulation points (Jebsen, 1966b), and no significant association between nerve conduction velocities and indicators of lead absorption has been detected (Lilis *et al.*, 1977; Ashby, 1980). Further studies are needed to clarify whether or not the MCV and SCV of the radial nerve in the forearm segment are more sensitive indicators of the subclinical neurotoxic effects caused by lead than those in the arm segment.

TABLE 3
EFFECTS OF INDICATORS OF METAL ABSORPTION AND OTHER FACTORS ON NERVE CONDUCTION VELOCITIES IN 19 GUN METAL FOUNDRY WORKERS IN THE FIRST AND SECOND YEARS: STEPWISE REGRESSION ANALYSIS[a]

	First year		Second year	
Conduction velocities	R^b	Indicators selected (t value)	R^b	Indicators selected (t value)
Radial nerve				
MCV		None		None
SCV	0.830	ALA (−3.960), ECu (2.652) PZn (2.356)	0.822	PCu (4.793), Hb (4.205) UCu (2.994)
Median nerve				
MCV	0.530	EZn (2.577)	0.844	Age (−5.734), ECu (3.129) Alcohol (−2.881)
SCV (elbow to wrist)		None	0.646	EZn (3.489)
SCV (wrist to finger)	0.521	PCu (2.515)		None

[a] The significance level for entering and removing the 17 variables in the regression analysis was $P < 0.05$; abbreviations used are as in the text.
[b] Multiple correlation coefficient.

A significant slowing of the SCV was found not only in the median nerve but also in the radial nerve in gun metal foundry workers with mean BPb levels of around 40 μg/dl in the present study. On the other hand, Seppalainen *et al.* (1983) found no clear relationship between the SCV of the radial nerve and lead exposure among lead workers with mean BPb levels of 30 μg/dl or so. Therefore, a slowing of the SCV and MCV in the upper limbs may occur for a lead-exposed group with BPbs in a critical range between 35 and 40 μg/dl. This might be consistent with data of Schwartz *et al.* (1978), showing a thresholds for lead-induced slowing of the peroneal MCV in the children with the BPb levels of 20–30 μg/dl.

Alcohol ingestion was inversely related to the MCV of the radial nerve and to the SCV of the median nerve (Table 4). It is suggested that alcohol ingestion is contraindicated in the course of therapy for lead-induced neurotoxicity.

Indicators of lead absorption did not affect the median nerve conduction velocities in this study. Nevertheless, it is essential that investigators presume an antagonistic effect of zinc and/or copper on lead neurotoxicity when no reduction

TABLE 4
EFFECTS OF YEARLY CHANGES IN INDICATORS OF METAL ABSORPTION AND ALCOHOL INGESTION ON THE CORRESPONDING ALTERNATIONS IN THE RADIAL AND MEDIAN NERVE CONDUCTION VELOCITIES IN 19 GUN METAL FOUNDRY WORKERS: STEPWISE REGRESSION ANALYSIS[a]

Conduction velocities	Multiple correlation coefficient (R)	Indicators selected (t value)
Radial nerve		
MCV	0.689	Alcohol (−3.057) PCu (2.323)
Median nerve		
SCV (elbow to wrist)	0.676	Alcohol (−2.873) EZn (2.656)

[a] The significance level for entering and removing 15 variables, $P < 0.05$; abbreviations used are as in the text.

in nerve conduction velocities is found in lead-exposed workers. A few investigators have failed to find significant reductions in peripheral nerve conduction velocities in workers with asymptomatic increased lead absorption (Paulev *et al.*, 1979; Baloh *et al.*, 1979; Spivey *et al.*, 1980; Nielsen *et al.*, 1982). Of them, indeed, Paulev *et al.* (1979) reported the presence of simultaneous exposure to lead and zinc.

The amounts of lead used in industry have been decreasing. On the other hand, the number of patients with acute lead toxicity and with subclinical lead intoxication appear not to be decreasing abruptly. Therefore, the essential problem is to detect the subclinical neurotoxicity of lead prior to the appearance of acute toxicity. Multiple approaches are needed to detect such subtle toxicity including stable markers of low lead exposure, education of the dangers of lead, and biological monitoring in high-risk populations.

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Are Faster or Slower Large Myelinated Nerve Fibers More Sensitive to Chronic Lead Exposure? A Study of the Distribution of Conduction Velocities[1]

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To determine which of the faster and slower large myelinated nerve fibers (alpha fiber group) are more sensitive to chronic lead exposure, the distribution of nerve conduction velocities (DCV) as well as conventional sensory nerve conduction velocity (SCV) were measured once a month for 20 and 11 months in two male lead workers with blood lead concentrations of 70 to 121 and of 63 to 85 μg/dl, respectively. Differences in the frequency beyond the "normal" ranges between conduction velocities of faster nerve fibers (V80, V90, or SCV) and those of slower fibers (V10 or V20) were analyzed by the McNemar test. In the two lead workers, the values below the lower normal limits for the V80 and V90 velocities were more frequent than those for the V10 and V20 velocities; similarly, lower values for the SCV were more frequent than those for the V10 and V20 velocities ($P < 0.05$). It was suggested that faster nerve fibers are more sensitive to chronic lead exposure than slower nerve fibers. These findings agree with our published data on the effects of local vibration, thallium, *n*-hexane, styrene, mixed organic solvents, alcohol dependency, and diabetes mellitus.

INTRODUCTION

The cellular target of lead in the peripheral nervous system is still unclear. Seppäläinen *et al.* (1975) showed that slower and maximal motor nerve conduction velocities decrease at levels of occupational lead exposure previously considered "safe," i.e., at blood lead (BPb) concentrations below 70 μg/dl. Also, we reported that the sensory nerve conduction velocity (SCV) is affected in adults with asymptomatic increased lead absorption (Araki and Honma, 1976). A reduction in peripheral nerve conduction velocities in workers exposed to lead has been confirmed by many investigators (Repko *et al.*, 1978; Buchthal and Behse, 1979; Ashby, 1980; Bordo *et al.*, 1982; Rośen *et al.*, 1983; Seppäläinen *et al.*, 1983; Singer *et al.*, 1983; Chen *et al.*, 1985; Jeyaratnam *et al.*, 1985; Murata *et al.*, 1987).

Peripheral nerve trunks consist of several thousand myelinated nerve fibers with slower and faster conduction velocities in parallel to the fiber diameters. The conventional methods for determining conduction velocities yield only two discrete values that reflect maximal and slower velocities of fibers in the nerve trunk; the function of the vast majority of fibers cannot be directly assessed. Three research groups independently developed new and different transcutaneous techniques to derive the distribution of conduction velocities (DCV) of large myelinated fibers (alpha fiber group) by means of computer analysis (Barker *et al.*, 1979;

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Cummins *et al.*, 1979; Kovacs *et al.*, 1979). We have introduced Barker *et al.*'s, method into our laboratory (Araki *et al.*, 1986).

To determine which of the faster and slower nerve fibers are principally involved, we examined the DCV in humans exposed to various occupational and environmental factors and have found that the faster nerve fibers of the peripheral nerves are more sensitive to chain saw operation (Araki *et al.*, 1988; Murata *et al.*, 1991a), acute thallium poisoning (Yokoyama *et al.*, 1990a), *n*-hexane (Yokoyama *et al.*, 1990b), styrene (Murata *et al.*, 1991b), mixed organic solvents (Murata *et al.*, 1991c), alcohol dependency (Fujimura *et al.*, 1993), and diabetes mellitus (Araki and Yokoyama, 1988).

Also, we measured the DCV in 20 workers exposed to lead, zinc, and copper with BPb concentrations of 22 to 59 μg/dl (Araki *et al.*, 1986) and found that the V10 velocity was significantly showed in the workers with BPb concentrations of 40 μg/dl and above. In these workers, the V10 velocity was inversely correlated with the BPb; the V80 and V90 velocities were positively correlated with urinary zinc excretion. These data suggested that conduction velocities of both the faster and the slower fibers were inversely affected by lead at BPb concentrations of 40 to 59 μg/dl and that the effects of lead on the faster fibers were antagonized by zinc absorption.

In the present study, we measured the DCV and the SCV in two male lead workers to clarify which of the faster or slower nerve fibers are more sensitive to chronic lead exposure.

SUBJECTS AND METHODS

Subjects

The subjects were 2 male lead workers aged 57 and 51 years (Workers 1 and 2, respectively). They had been exposed to lead for 28 and 20 years, respectively. Electrophysiological measurement was conducted once a month for 20 months (Worker 1) and 11 months (Worker 2). During these periods, their BPb concentrations ranged between 70 and 121 (mean 93) μg/dl in Worker 1 and between 63 and 85 (mean 74) μg/dl in Worker 2. Control subjects were 11 unexposed men aged 45–65 (mean 54) years.

Electrophysiological Studies

Electrophysiological studies were conducted in a warm room (28–32°C) using a two-channel electromyograph (Medelec MS-92); skin temperature was maintained in the range 30–34°C for all subjects. The DCV was measured by the same method reported previously by us (Araki *et al.*, 1986, 1988), a modified method of Barker *et al.* (1979). After electric stimulation of the right median nerve at the second finger with an 80- to 110-V square wave pulse of 0.2 msec, two compound action potentials were recorded at the wrist and the elbow. The DCV was calculated between the velocities of 1.25 and 108.75 m/sec by the double-conduction distance method, using an NEC PC9801LS5 microcomputer. The calculated DCV was expressed by the following parameters: the conduction velocities below which 10, 20, 30, 40, 50, 60, 70, 80, and 90% of active nerve fibers lie (V10, V20, V30, V40, V50, V60, V70, V80, and V90 velocities). The SCV of the forearm segment was calculated by using the same compound action potentials and distance as used for the DCV measure. Daily variations (coefficients of variation) in the DCV and SCV

have been described previously by us (Araki *et al.*, 1986). "Normal" ranges of the parameters of the DCV and the SCV were determined as the values between mean −2 standard deviation and mean +2 standard deviation in 11 unexposed controls. Differences in the frequency beyond the normal ranges between conduction velocities of faster nerve fibers (V80, V90, or SCV) and those of slower fibers (V10 or V20) were analyzed by the McNemar test.

RESULTS

The parameters of the DCV and the SCV for 2 lead workers and 11 controls are shown in Table 1. The V10, V50, and V90 velocities of the DCV, SCV, and BPb concentration during the study period for Workers 1 and 2 are illustrated in Figures 1 and 2. In Worker 1, the V90 velocity was below the lower normal limit 11 times, i.e., 11 months (55%) during the study period; the V80, V70, V60, V50, V40, V30, V20, and V10 velocities were also below the limits 7, 3, 2, 2, 2, 1, 1, and 0 times (35, 15, 10, 10, 10, 5, 5, and 0%), respectively; and the SCV was below the limit 9 times (45%). Similarly, in Worker 2, the V90, V80, V70, V60, V50, V40, V30, V20, and V10 velocities and the SCV were below their lower normal limits 8, 7, 6, 4, 3, 2, 1, 0, 0, and 8 times (73, 64, 55, 36, 27, 18, 9, 0, 0, and 73%), respectively. In the 2 subjects, the values below the lower normal limits for the V80 and V90 velocities were significantly more frequent than those for the V10 and V20 velocities; lower values for the SCV were also significantly more frequent than the latter values ($P < 0.05$). On the other hand, there was no significant difference in the frequency below the lower normal limits between the SCV and either V80 or V90 velocity.

DISCUSSION

The major finding in this study was that the values below the lower normal limits for the V80 and V90 velocities were more frequent than those for the V10 and V20 velocities in two lead workers. This finding suggests that chronic lead exposure affects mainly faster nerve fibers among the large myelinated nerve fibers.

TABLE 1
DISTRIBUTION OF NERVE CONDUCTION VELOCITIES (DCV) AND SENSORY NERVE CONDUCTION VELOCITY (SCV) IN THE MEDIAN NERVE FOR 2 LEAD WORKERS AND 11 UNEXPOSED CONTROLS[a]

	Worker 1 (20 monthly measurements)	Worker 2 (11 monthly measurements)	Controls
DCV (m/sec)			
V10	48.1 ± 6.1	47.6 ± 3.4	45.0 ± 4.3
V20	50.2 ± 5.7	48.7 ± 3.5	48.9 ± 3.4
V30	51.5 ± 5.3	49.9 ± 3.4	52.2 ± 3.5
V40	52.8 ± 5.0	51.0 ± 3.3	54.1 ± 3.1
V50	54.2 ± 4.6	52.0 ± 3.3	55.6 ± 2.8
V60	55.6 ± 4.6	53.0 ± 3.2	57.0 ± 2.4
V70	56.6 ± 4.8	53.9 ± 3.1	58.4 ± 2.1
V80	57.6 ± 4.6	55.0 ± 3.1	59.8 ± 2.0
V90	58.7 ± 4.4	56.5 ± 3.0	61.4 ± 1.8
SCV (m/sec)	55.6 ± 3.5	53.5 ± 3.4	60.4 ± 2.6

[a] Mean and standard deviation.

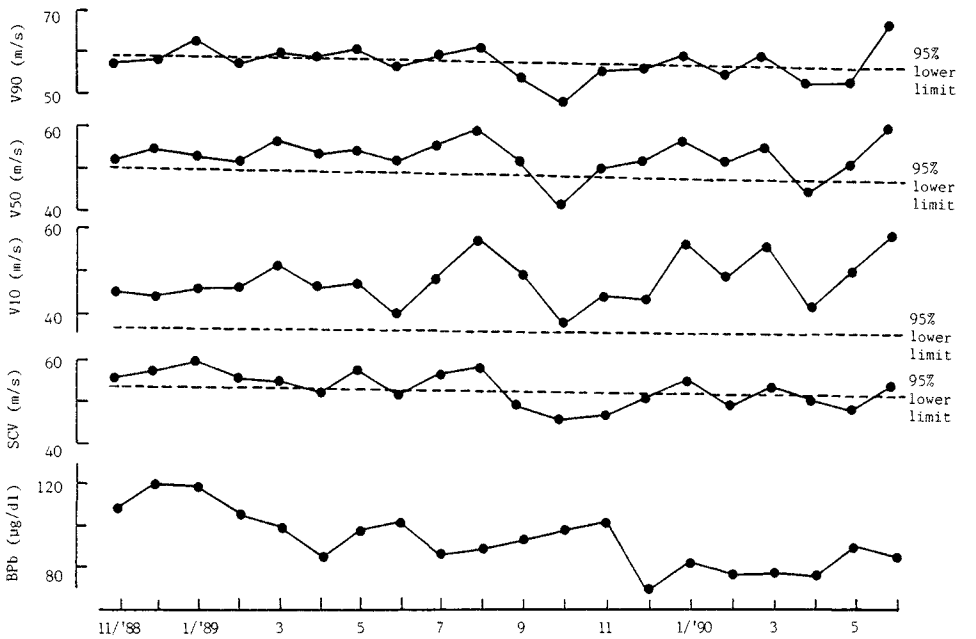

FIG. 1. The V10, V50, and V90 velocities of the distribution of nerve conduction velocities; the sensory nerve conduction velocity (SCV); and blood lead (BPb) concentrations during a study period for Worker 1.

The present results agree with the data on the DCV effects of local vibration, acute thallium poisoning, *n*-hexane, styrene, mixed organic solvents, alcohol dependency, and diabetes mellitus as followed: All parameters of the DCV (V10 to V90 velocities) were significantly slowed in chain saw operators (Araki *et al.*, 1988) and conduction velocities of the faster nerves (V60 to V90 velocities) were significantly slowed in another group of vibrating-tool operators (Murata *et al.*, 1991a). Only the V70 to V90 velocities were significantly slowed in a patient with acute thallium poisoning (Yokoyama *et al.*, 1990a). In workers exposed to *n*-hexane at an ambient level of less than 100 ppm, only faster fibers were significantly affected (Sax *et al.*, 1981); at a probably higher exposure level of *n*-hexane, all nerve fibers were affected to a greater extent (Yokoyama *et al.*, 1990b). In styrene workers, the V80 velocity was significantly slowed (Murata *et al.*, 1991b). In workers exposed mixed organic solvents, the V60 to V90 velocities were significantly slowed (Murata *et al.*, 1991c). Similarly, the V40 to V90 velocities in alcoholics and the V50 to V90 velocities in diabetics were significantly delayed (Fujimura *et al.*, 1993; Araki and Yokoyama, 1988).

By contrast, in workers exposed to lead, zinc, and copper, only the V10 velocity was significantly slowed (Araki *et al.*, 1986); the V90 velocity was positively correlated with the urinary zinc excretion. Therefore, the effects of lead on the faster fibers were probably antagonized by zinc, resulting in a normal level of conduction velocities for the faster fibers.

In the present study, values below the lower normal limit for the SCV were more frequent than those for the V10 and V20 velocities; there was no significant difference in the frequency below the lower normal limits between the SCV and either V80 or V90 velocity. The SCV represents conduction velocities of faster

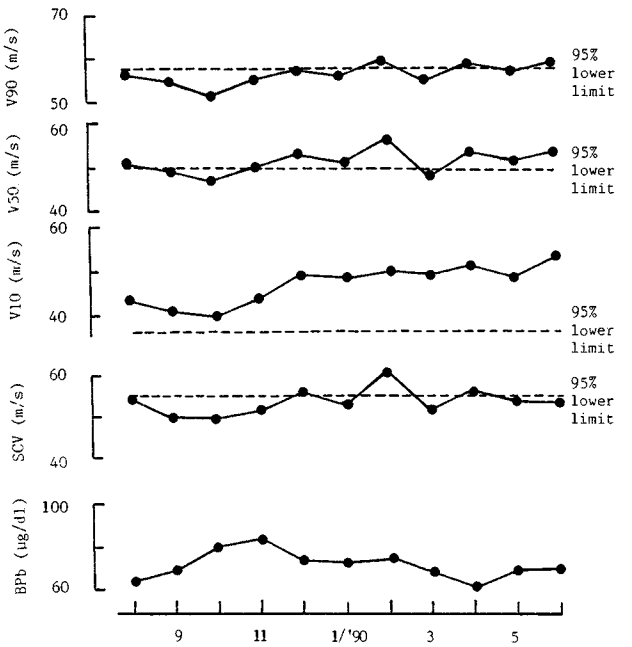

FIG. 2. The V10, V50, and V90 velocities of the distribution of nerve conduction velocities; the sensory nerve conduction velocity (SCV); and blood lead (BPb) concentrations during a study period for Worker 2.

nerve fibers. Therefore, these findings confirm that faster nerve fibers are affected mainly by chronic lead exposure.

The present findings represent the results only in two lead workers. To confirm the sensitivity of the conduction velocities of faster nerve fibers and the SCV in chronic lead exposure, we need further studies with a greater number of lead workers free from zinc exposure.

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ENVIRONMENTAL RESEARCH 61, 357–361 (1993)

Measurement of Vibratory Perception Threshold (VPT) in Workers Exposed to Organic Solvents[1]

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Vibratory perception threshold (VPT) of 437 workers exposed to organic solvents in various industries was measured using an automated device, Vibrometer TM-31A, at the medial malleolus of the right leg. There were statistically significant correlations between age and VPT ($r_s = 0.378$, $P < 0.01$) and job experience and VPT ($r_s = 0.125$, $P < 0.01$). Fifty-five out of 437 examinees showed abnormally higher VPT than the previously reported control values, suggesting an existence of potential neuropathy in these individuals. There was no significant correlation between the amount of urinary metabolites and VPT. Neither specific exposure-related subjective symptoms nor clinical neurological signs were found in any workers. The study also discusses the validity of and the future problems in measuring VPT in workers exposed to solvents.

INTRODUCTION

Early detection of neuropathy induced in workers exposed repeatedly to organic solvents is of great importance for their health administration. It is known that measurement of sensitivity to vibration, in parallel with other neurophysiological methods and traditional neurological examinations, is useful in diagnosing peripheral neuropathies such as those in diabetes mellitus or uremia. In this paper, we report the results of studying the vibratory perception threshold (VPT) in workers handling various organic solvents in Ishikawa Prefecture, Japan.

SUBJECTS AND METHODS

Subjects. A total of 437 workers exposed to solvents (394 males; 43 females). were examined. Ages ranged from 16 to 72 years old and job experience from 1 month to 38 years (Tables 1 and 2).

VPT measurement. An apparatus was used (Vibrometer, TM-31A, Check Co. Ltd., Tokyo) in which a metal vibrator (90 × 10 × 4 mm) was oscillated at 125 Hz with automatically increasing amplitudes from 0 to 150 μm/50 sec. The VPT was measured three times at the medial malleolus of the right leg (Figs. 1 and 2), and the mean of three measurements was taken as VPT value (μm). The mean coefficient of variation (CV) of the three measurements was 12.6%.

Urinary metabolites. Values of hippuric acid (105 subjects) and methylhippuric acid (80 subjects) were reported from some industries.

Exposure levels. Concentration of the main solvents in the work place atmosphere was reported from each industry.

[1] Presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

TABLE 1
AGE DISTRIBUTION

Age	Male	Female	Total
16~	18	1	19
20~	86	13	99
30~	77	6	83
40~	144	13	157
50~	69	10	79
	394	43	437

RESULTS

The main organic solvents used in industries are summarized in Table 3. Thinners, which consist mainly of xylene and toluene, are most widely used in industries in such operations as painting, washing, and binding. There were statistically significant correlations between age and VPT (Spearman $r_s = 0.429$, $P < 0.01$) and between job experience and VPT ($r_s = 0.194$, $P < 0.01$) (Tables 3 and 4).

Abnormally higher VPT values were observed in 55 workers out of the 437 examined than in those reported in 1030 normal subjects, who were all male clerical workers in one industry (Kamon 1990). Neither specific exposure-related subjective symptoms nor clinical neurological signs were found in any workers.

The reported values of urinary metabolites are shown in Table 5. No statistically significant correlation between the amounts of metabolites and VPT was found. According to the reports from industries, the exposure levels to solvents in the workplace were below the TWA values in Japan.

DISCUSSION

The measurement of VPT has been frequently used to detect subclinical sensory dysfunction associated with conditions such as diabetes mellitus and uremia (Williamson, 1905; Collens *et al.*, 1946; Steiness, 1957a; Nielsen, 1971a,b; Edwards, 1973). Steiness (1957b) has reported that the VPT values in normal subjects increase with age.

A variety of neurophysiological test methods is available for assessing the effects of exposure to chemicals on the worker's nervous system (Johnson, 1987). Of these methods, some sensory tests are known to be suitable for screening the

TABLE 2
EXPERIENCE IN WORK WITH SOLVENTS

Experience (years)	Male	Female	Total
0~	25	1	26
1~	88	22	110
5~	71	6	77
10~	78	8	86
20~	101	5	106
30~	31	1	32
	394	43	437

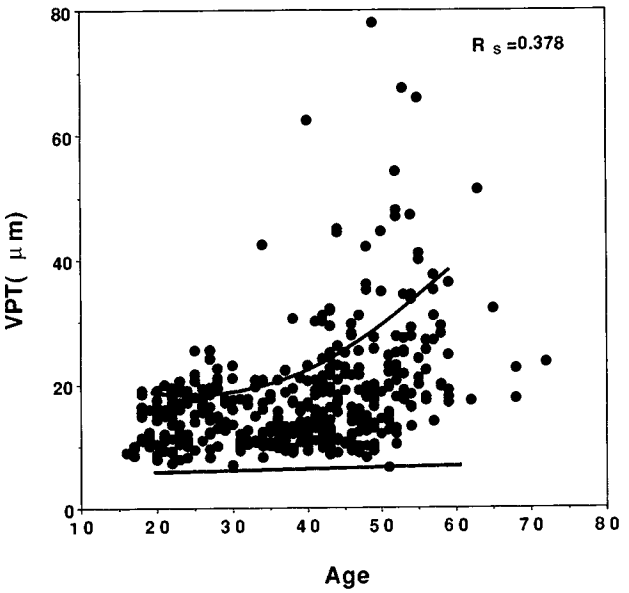

FIG. 1. Scatter diagram of age and vibratory perception threshold (VPT) in 437 solvent workers. The two lines indicate the lowest and highest limits of the 95% confidence interval of the normal VPT (Kammon, 1990).

neurotoxic effects on peripheral nerves. Arrezo and co-workers (1980, 1983) and Maurissen and Weiss (1980) have shown that the VPT method is useful as a first stage screening of individuals with potential exposure to certain neurotoxicants such as acrylamide. Halonen *et al.* (1986) found that 4 men out of 90 workers

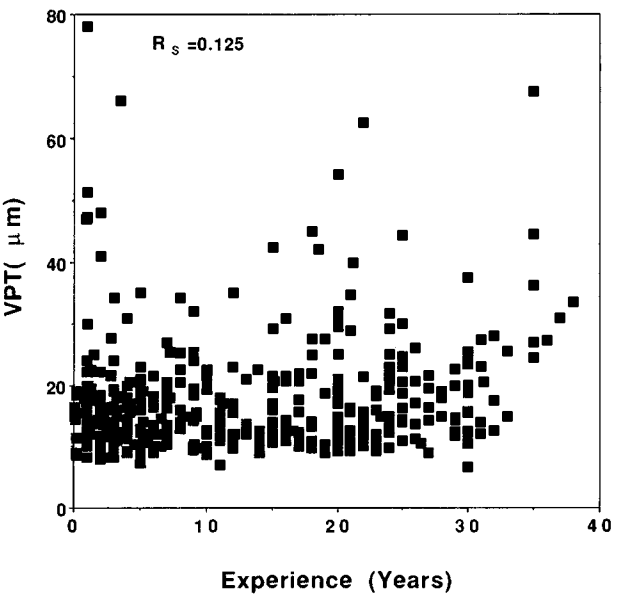

FIG. 2. Scatter diagram of job experience (years) and VPT in 437 workers exposed to solvents.

TABLE 3
SOVENTS FREQUENTLY USED IN INDUSTRIES

Thinner
G-5100
1,1,1-Trichloroethylene
Xylene
Toluene
Gasoline
Methylchloride

exposed to solvents had increased VPT in the lower limbs and abnormalities in laboratory investigations and stated that the VPT procedure was useful in monitoring the peripheral nervous system in workers exposed to toxic chemicals, although the increased VPT may not necessarily have been associated with exposure.

The present study showed that the VPT values were significantly and positively correlated to both age and job experience, but especially to age, in workers treating organic solvents in various industries. Increased VPT was also found in some workers exposed to solvents who had neither subjective nor clinical neurological symptoms, nor increased urinary excretion of metabolites due to solvents, suggesting a possibility of sensory disturbances in these individuals.

The VPT measured three times in a subject using the present apparatus (TM-31A) was found to be reproducible as already shown by Kamon (1990). The time needed for the measurement was less than 3 min per person, making this a suitable method for screening large populations.

Although VPT measurement is capable of detecting sensory dysfunction, further studies in more workers are needed, as are repeated examinations of the present subjects, especially in those who showed abnormal VPT. The purpose of these further studies would be to elucidate the mechanism and background for the abnormal values, and to determine the validity as well as the usefulness of the technique for assessing neurological impairment in workers exposed to solvents.

TABLE 4
NUMBER OF WORKERS SHOWING ABNORMALLY HIGH VPT VALUES

Age	*N*	High VPT subject	Percentage	Normal range[a] (upper values, mm)
16~	19	—	—	—
20~	55	8	14.5	18.3
25~	44	8	18.2	19.6
30~	36	3	8.3	20.8
35~	47	3	6.4	20.9
40~	92	12	13.0	23.1
45~	65	7	10.8	27.8
50~	47	10	21.3	33.4
55~	32	4	12.5	38.4
	437	55	12.6	

[a] Kamon (1990).

TABLE 5
URINARY METABOLITES

Hippuric acid (g/dl)	*N*	Methyl hippuric acid (g/dl)	*N*
<1	82	<0.5	66
1–2.5	22	0.5–1.5	14
>2.5	1	>1.5	0
	105		80

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ENVIRONMENTAL RESEARCH 61, 84–92 (1993)

Nervous System Effects of Occupational Exposure to Styrene: A Clinical and Neurophysiological Study[1]

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Quantitative EEG of 99 workers occupationally exposed to styrene was analyzed and compared to exposure parameters. All of the workers came from reinforced plastics industry. The workplaces and factories were previously evaluated, and the exposure levels were known rather accurately. The exposure information form workplaces and the individual exposure data were combined to form an Exposure Index, which reliably reflected long-term exposure to styrene in various occupational settings. All of the subjects underwent careful medical, neurological, and neurophysiological examinations. Quantitative EEG was recorded from 19 channels and analyzed for absolute and relative power, asymmetry, coherence, frequency distribution, and statistical normative database comparisons (Neurometrics). The EEG data showed a significant increase of abnormal EEG classifications in workers with higher exposure. When the workers were divided into two groups, based on the exposure data, those with higher exposure had higher absolute EEG power in alpha and beta bands in the frontotemporal regions of the brain. The findings indicate that abnormalities in cerebral function can be demonstrated even with relatively low mean exposure levels. It is concluded that the efforts to lower the administrative hygienic levels of styrene have been in the right direction.

INTRODUCTION

Neurotoxic effects of occupational styrene exposure have been reported in several studies (Seppäläinen and Härkönen, 1976; Härkönen, 1977; Rosen *et al.*, 1978; Mutti *et al.*, 1984; Triebig *et al.*, 1985, 1989; Cherry and Gautrin, 1990). There has been a trend to lower the administrative levels of styrene in many countries. In Finland, the level was reduced from 100 to 50 ppm in 1981, and to 20 ppm in 1988. The last reduction was based on demonstration of chromosome abnormalities. The actual air concentrations in workplaces have followed the regulations. During the 1970s, the average styrene air concentration was 100 ppm, and in the 1980s the average level was 64 ppm. In a recent study in Finland, the real exposure levels were still higher in many work places, despite the administrative reduction of the values (Pfäffli, 1990). The mean of 8 hr weighted level of styrene was 34 ppm, significantly above the administrative level of 20 ppm.

Earlier reports of neurotoxicity of styrene were based on visual analysis on EEG, symptom questionnaires, and psychological tests. While the last two are very important and useful in neurotoxicological work, more sensitive techniques have replaced conventional EEG. In this study we have used quantitative spectral

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and frequency analysis of EEG to estimate the effects of modern, relatively low levels of styrene on the central nervous system.

MATERIALS AND METHODS

Subjects

One hundred workers were examined at the Institute of Occupational Health, Helsinki, Finland. The workers came from workplaces and factories where careful hygienic and cytogenetic studies had previously been performed. Thus, the exposure levels of these factories were known. The general criteria for inclusion were informed consent and male sex. Also, workers with a history of neurological disease, brain injury, diabetes or other endocrinological disease, renal or hepatic disease, neurotic or psychotic psychiatric disorder, or cancer were excluded. After initial agreement with the companies, the factories were visited and each worker was reviewed at this stage for the above exclusion criteria.

The workers were given an occupational, medical, and neurological examination. The occupational and medical survey consisted of a careful evaluation of work conditions and methods and a clinical general medical examination. The neurological examination was performed in a standardized way in each case. Consumption of alcohol was estimated by an alcohol abuse evaluation scheme (Juntunen *et al.*, 1988). Usage of beer, wine, and distilled spirits was evaluated separately. Total yearly amount of alcohol consumption was calculated from these figures.

EEG Methods

EEG was recorded using the international 10/20 system with a Cadwell Spectrum 32 apparatus. The recording was done to get an adequate sample of artifact-free EEG for quantitative analysis. This usually meant recording for about 10–15 min of awake EEG. The subjects were instructed to close the eyes, imagine looking at something straight in front, and try to remain awake. A technologist performing the recording kept close watch on the signal and spoke to the subject, if signs of lowering of vigilance were detected. Thus, no complete conventional EEG recordings with various activations and possibly states of different levels of wakefulness were made. One recording could not be evaluated for technical reasons.

The EEG signal from 19 channels was stored on a compact disk (laser disk), and the analyses were done using the stored signal. The sampling rate was 200 Hz. All of the recordings were evaluated conventionally by visual analysis. Quantitative analyses were then performed, including calculation of absolute power, relative power, asymmetry, coherence, frequency analysis (mean frequency, median frequency, peak frequency), and Neurometrics analysis. The epochs for quantitative analysis were collected under visual control, avoiding all artifacts, such as eye or lid movements, head movements, muscle artifact, etc. Otherwise, the epochs were collected consecutively, avoiding, however, periods of obvious drowsiness. The quantitative analysis was performed by a trained technologist. All of the epochs were printed and later evaluated and accepted by a clinical neurophysi-

ologist. A total of 48 epochs, each 2.5 sec of duration, was collected, if possible. In some cases, this could not be achieved because of muscle or other artifacts, but in all cases, more than 36 epochs could be used for analysis.

Statistical Analysis

The results of the quantitative EEG analyses were statistically evaluated using the SAS version 6.04 software for microcomputers. The Student's t test and analysis of variance were used as parametric tests; χ^2 test was used as a nonparametric test. Logarithmic transformation was used for raw QEEG data evaluations to reach normal distribution.

In the Neurometrics analysis, an individual quantitative analysis result was compared with a normative database (John *et al.*, 1987). This gives Z-score estimations from 19 electrodes, and multivariate Z scores from different brain regions. A discriminant score analysis gives a statistical estimation of the normality of the overall recording, and the results were defined as normal, abnormal, or not classifiable, based on these statistical calculations.

Estimation of the Exposure

The estimation of the exposure was based on the hygienic and biologic measurements, general exposure information regarding the profession, job classification, and detailed work method analysis. Occupational hygienic measurements were done in all workplaces for styrene, styrene oxide, acetone, and dust. In this paper, only the styrene values will be considered. The concentrations were measured at breathing level of each worker using either active or passive sampling. The measurement was done during a whole 8-hr working day. From urine samples, levels of mandelic acid, phenylglyoxylic acid, and parahydroxymandelic acid were measured. These samples were collected after a work day. An Exposure Index (EI), giving an overall estimation of occupational exposure, was formed from these data. Four different parameters were evaluated for the EI: (1) the working method, (2) the number of years of exposure, (3) level of daily exposure, and (4) time-weighted mandelic acid level. The details of the classifications are presented in Table 1. The working method has significant influence on the ambient air concentration, and was scored accordingly. The current method of the company was used in the classification. However, if the method had been changed recently, the old method was used. This appeared to be uncommon in the companies involved, and was not considered to influence the results. Duration of the exposure in years is also important for the total styrene load. The level of daily exposure was calculated by multiplying the measured air concentration by the number of the laminating hours, which was considered to improve the accuracy of the "dose" estimation. Mandelic acid can react quite rapidly to increase in exposure, and erroneous estimation of exposure can result from single measurements, which can show the result of rapid peak exposure. Taking the number of daily laminating hours into account (by multiplying mandelic acid score by the laminating hours score) was found to decrease this risk, and was adopted in the formation of EI. After calculating the index, it was to divided by 4 to increase its illustrativeness.

TABLE 1
PRINCIPLES IN THE CLASSIFICATION OF PARAMETERS FOR THE EXPOSURE INDEX

1. Working method	FA	CP	OP	ML	SL
Score	1	2	3	4	5
2. Years at work	<5	<10	<15	<20	>20
Score	1	2	3	4	5
A. Laminating hours per day	Occasionally	<4	<6	>6	Often overtime
Score	1	2	3	4	5
B. Ambient air concentration (ppm)	<20	<50	<100	<150	<150
Score	1	2	3	4	5
C. Mandelic acid, mmol/liter	<3.2	<7.0	<10	<15	>15
Score	1	2	3	4	5

Note. Work method, used principally: FA, fully automatic; CM, closed process; OM, open process; ML, manual lamination; SL, spray lamination.

RESULTS

Exposure Data

The average age of the subjects was 38 years (range 20–60 years). Their average duration of styrene exposure was 12.8 years (range 0.5–32 years). Laminating was the main task for 52 workers, the rest performed other tasks. The mean length of laminating work was 3 hr per day. The characteristics of the exposure parameters are given in Table 2.

For statistical evaluations, the studied group was divided into three groups on the basis of the EI. Subjects with EI between 0 and 2.5 ($N = 44$) formed Group 1; those with EIs between 2.6 and 3.5 ($N = 35$) were in Group 2; and the rest, with EIs above 3.5 ($N = 30$) were in Group 3. The mean age (±SD) in Group 1 was 37.9

TABLE 2
STATISTICAL CHARACTERISTICS OF THE EXPOSURE PARAMETERS

Variable	*N*	Minimum	Maximum	Mean	SD
High exp.	100	0	9	2.9	2.6
Work years	100	0.1	32.0	12.7	7.5
Styr_8_H	100	0.4	183.0	29.5	38.1
Styr_8_H2	100	0	175.0	5.8	20.2
Styr_MAN	100	0.1	39.2	2.9	5.7
Styr_MAN2	100	0.2	13.8	1.4	2.6
Exp. Index	100	1.0	10.5	3.8	2.0

Note. Abbreviations used: High exp., hours of laminating per day; Work years, number of working years with styrene exposure; Styr_8_H, Airborne styrene concentration (8 hr) ppm; Styr_8_H2, Airborne styrene concentration, measurement 2, ppm; Styr_MAN, Urinary mandelic acid level, mmol/liter; Styr_MAN2, Urinary mandelic acid level, measurement 2, mmol/liter; Exp. Index, Exposure Index.

(±10.0) years; in Group 2 it was 41.2 (±9.2) years; and in Group 3 it was 40.4 (±9.4) years. The differences in the average ages were not statistically significant.

Neurological Data

Long-term symptoms were common among the cohort. The most common chronic symptoms were tiredness (28%), forgetfulness (28%), tension neck (27%), memory disturbances (24%), headache (24%), and excessive sweating (20%). Temporary symptoms experienced during workday were more common: 49% of the subjects had excessive tiredness; 20% had difficulties in concentration; and 14% had felt short-term nausea.

Some of the symptoms were more common among subjects with higher exposure (EI > 3) (Table 3). The difference was most significant in memory disturbances. Temporary feelings of dizziness were significantly more common among the higher exposure group (25/6%, $P < 0.05$). People in laminating work complained of more fatigue than those in other types of work (51/26%, $P < 0.05$). In clinical neurological examination, 26% had slight abnormalities, mostly in balance and coordination, but they did not correlate with the exposure index.

Quantitative EEG

Power spectrum analysis showed an increase of the total EEG power in all electrode regions in alpha and beta bands in relation with EI. This was most obvious in the frontal and temporal regions of the brain (Table 4). When the low EI group and the high EI group were compared, the differences were almost significant at 5% level. The total power of delta and theta bands did not differ significantly in the two groups. QEEG power asymmetry and coherence did not correlate with the exposure, expressed as EI, measured airborne concentration, or urinary mandelic acid level.

In Neurometrics analyses, the QEEG was classified normal in 38 cases, abnormal in 30 cases, and it could not be classified in 31 cases. The average simple exposure parameters in different QEEG groups are shown in Table 5. Both the airborne styrene concentration and urinary mandelic acid levels were higher among those with abnormal QEEG; however, the differences were not statistically significant. Distribution of abnormal QEEG classifications were different among the three exposure groups (Table 6). The overall distribution was not statistically significant ($P = 0.079$), but the trend in the highest exposure group

TABLE 3
DISTRIBUTION OF SOME NEUROLOGICAL SYMPTOMS IN LOW AND HIGH EXPOSURE GROUPS

Symptom	% Low exposure (EI ≤ 3, $N = 51$)	% High exposure (EI > 3, $N = 49$)	P
Headache	12	35	<0.05
Memory disturbances	8	39	<0.001
Forgetfulness	12	41	<0.01
Sensory symptoms in lower and higher extremities	2	18	<0.05
Excessive sweating	10	29	<0.05

TABLE 4
EEG ABSOLUTE POWER (AFTER log10 TRANSFORMATION) IN LOW AND HIGH EXPOSURE GROUPS

Region	Low exposure (EI ≤ 2.5)	High exposure (EI > 3.5)	*P*
Left frontal	1.11 + 0.37	1.27 + 0.40	0.09
Right frontal	1.13 + 0.37	1.28 + 0.40	0.10
Left frontotemporal	0.84 + 0.33	1.00 + 0.36	0.05
Right frontotemporal	0.86 + 0.33	1.02 + 0.35	0.06
Left temporal	0.81 + 0.31	0.95 + 0.34	0.07
Right temporal	0.80 + 0.32	0.95 + 0.31	0.06
Left temporoparietal	1.19 + 0.44	1.33 + 0.46	0.17
Right temporoparietal	1.28 + 0.47	1.35 + 0.48	0.57
Left parietal	1.36 + 0.44	1.48 + 0.45	0.27
Right parietal	1.37 + 0.46	1.47 + 0.45	0.37
Left occipital	1.52 + 0.51	1.58 + 0.52	0.63
Right occipital	1.58 + 0.51	1.57 + 0.52	0.99

showed that there was an increase of abnormal QEEG classifications. The average alcohol consumption was not significantly different in the QEEG groups (Table 4).

DISCUSSION

In this study we have demonstrated QEEG changes in workers occupationally exposed to styrene. A slight exposure–effect relationship was also shown between exposure and QEEG findings and neurological symptoms. In this study we used a calculated exposure estimate, which took measured exposure levels, type of work, duration of exposure, and biological exposure indicators into account. This exposure index has been found to be a good model of styrene exposure (Pfäffli, 1990), but it is, of course, an estimate. However, correlation of effects with air concentration measurements, blood styrene levels, or urinary mandelic acid levels, which are common ways to estimate exposure, is not necessarily more reliable for chronic effects because of the cross-sectional nature of these measurements. Long-term exposure evaluation data are not very common in workplaces. Thus, it can be argued that an estimate including all possible relevant exposure

TABLE 5
THE MAIN EXPOSURE PARAMETERS (MEANS ± STANDARD DEVIATION) AMONG QEEG GROUPS

	QEEG		
	Normal	Not classifiable	Abnormal
Laminating	3.03 ± 2.59	3.31 ± 2.33	2.55 ± 2.88
Styrene level	26.6 ± 41.6	29.9 ± 28.0	33.6 ± 43.5
U-mandela	2.40 ± 4.51	2.87 ± 4.40	3.77 ± 7.97
Alcohol	8870 ± 8990	7069 ± 7991	7280 ± 7178

Note. Laminating, number of laminating hours per day; Styrene level, 8-hr weighted individual styrene airborne; concentration after a working day; U-mandela, urine mandelic acid concentration after a working day; Alcohol, alcohol consumption, g/year.

TABLE 6
DISTRIBUTION OF QEEG IN THE THREE EXPOSURE GROUPS

	EI = 0–2.5	EI = 2.6–3.5	EI ≥ 3.5
Normal	20	10	4
Not classifiable	8	13	4
Abnormal	10	8	12

data can be a better way to express long-term exposure than single measurements, or even a series of measurements.

Increased neurological symptoms have been described in styrene workers (Härkönen, 1977). The symptoms were nonspecific, but essentially similar to those found previously: memory disturbances, concentration difficulties, irritability, and dizziness. In Härkönen's study, the symptoms did not correlate with urinary mandelic acid levels, and no exposure–response relationship could be demonstrated. This is in contrast to our results, which do suggest such a relationship. The explanation to this discrepancy is that our Exposure Index estimates long-term exposure, and it is weighted toward chronic effects, whereas mandelic acid levels indicate short-term exposure.

EEG alterations in occupational styrene exposure have been documented in several studies (Klimkova-Duetschova *et al.*, 1973; Hrube *et al.*, 1975; Seppäläinen and Härkönen, 1976). These reports were based on conventional visual evaluation of EEG. The exposure levels were mainly relatively high. Because of these earlier works, the hygienic levels have been brought down in the recent past. The average 8-hr weighted average styrene concentration in this study was now 32 ppm, which is still much higher than the administrative hygienic level in Finland of 20 ppm. In the present study, the number of abnormal EEGs in visual inspection was not increased, and did not correlate with the exposure. Quantitative analysis showed consistent, although slight, changes in the more highly exposed group. All of the workers were healthy; they had not sought medical advice for any symptoms. However, when specifically asked, those with higher exposure had more neurological symptoms than those with lower exposure. Thus, in QEEG analysis, exposure was associated with symptoms as well as EEG changes.

The nature of the QEEG is unclear. In this study, increased absolute power in alpha and beta bands was detected. This could be seen maximally in frontal regions, but was obvious also temporally and parietally. Only in occipital regions was this power increase with exposure not seen. Basically, this kind of change could be caused by lowered vigilance (Santamaria and Chiappa, 1987). On the other hand, similar changes have been described in solvent exposure (Orbæk *et al.*, 1988), although these authors found increased power in all frequency bands. In the present study, all or the recordings were done in the morning, which could be presumed to decrease the effects of drowsiness. Also, the criteria of selection of epochs were identical in all cases. This power increase can be an initial neurotoxic phenomenon, nonspecific in nature.

The QEEG findings in discriminant analysis are statistical in nature. A statistical abnormality does not mean neurological disease—some uncommon normal

EEG variants are outside of statistical limits of normality (for example, at 5% level), but still are medically within normality. The changes found do not thus suggest increased cerebral disease in the workers. The present data are not sufficient for such a conclusion, but in individual cases they show a statistically significant difference.

CONCLUSIONS

The critical point after this and other studies is: What is a safe hygienic limit for occupational styrene exposure? These results do not give a direct answer to this question. Our method of evaluating long-term exposure does not allow reliable evaluation of the safety of airborne exposure levels. Some extrapolations can be made, but the study design was not optimum for this purpose. However, the results show central nervous system effects, both symptomatic and neurophysiological, associated with styrene exposure. Although the workers were considered healthy, the increased number of neurological symptoms and QEEG abnormalities forces one to reevaluate the occupational hygienic limits of styrene in work places. If an effect is considered to precede illness, which is quite conceivable, it is important to consider TLVs in this light. Although no decisive limit of safety cannot be point out from these results, it seems rational to state that the efforts to lower the administrative styrene levels in workroom air have been in the right direction.

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Assessment of the Distribution of Nerve Conduction Velocities in Alcoholics[1]

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To assess the effects of chronic alcohol ingestion on the faster and slower nerve fibers (alpha fiber group) in the peripheral nervous system, the distribution of nerve conduction velocities (DCV) and conventional maximal motor and sensory nerve conduction velocities (MCV and SCV) in the median nerve were measured in 23 male patients with severe alcoholic dependency (DSM-III-R), aged 30–64 (mean, 50) years, and in 23 age-matched healthy men. The DCV was expressed by the conduction velocities below which 10, 20, . . . , 80, and 90% of active fibers lie (V10, V20, . . . , V80, and V90 velocities). The V40 to V90 velocities of the DCV were significantly slower in the alcoholics than in the control subjects; the SCV and MCV in the alcoholics were also significantly slowed. These findings suggest that the faster large myelinated nerve fibers are more sensitive to chronic alcohol ingestion than the slower large myelinated nerve fibers.

INTRODUCTION

Alcoholic neuropathy is one of the most common forms of peripheral neuropathy; it accounts for nearly 30% of all cases of polyneuropathy (Kemppainen *et al.*, 1982; Shields, 1985). Histopathological studies have clarified that large myelinated nerve fibers are affected at an early stage of alcoholic neuropathy (Walsh and McLeod, 1970; Ohnishi *et al.*, 1972; Tredici and Minazzi, 1975; Behse and Buchthal, 1977; Takatsu, 1980). Electrophysiologically, mild to moderate degrees of slowing in motor and sensory nerve conduction velocities have been reported in alcoholic patients (Mawdsley and Mayer, 1965; Worden, 1976; Willer and Dehen, 1977; Victor, 1984; Shields, 1985). As the peripheral nerve trunk consists of several thousand large myelinated nerve fibers with faster and slower conduction velocities in parallel to the fiber diameters, it is essential to clarify whether faster or slower fibers of the large myelinated nerve are affected at an earlier stage of alcoholic neuropathy.

Recently, we have introduced a noninvasive technique to derive the distribution of conduction velocities (DCV) of the large myelinated fibers (alpha fiber group) by means of computer analysis (Araki *et al.*, 1986a, 1988; Yokoyama *et al.*, 1987). Using this technique, we have found that the faster large myelinated nerve fibers are more affected than the slower fibers in subjects exposed to lead, thallium, *n*-hexane, styrene, and local vibration (Araki *et al.*, 1986a, 1988; Yokoyama *et al.*, 1990a,b; Murata *et al.*, 1991a,b; Sata *et al.*, 1991). In the present study, this technique is applied to 23 patients diagnosed as alcoholic dependent.

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SUBJECTS AND METHODS

Subjects

Subjects were 23 male patients who had been diagnosed as severely alcoholic dependent by the criteria of "Diagnostic and Statistical Manual of Mental Disorders, Third Edition–Revised" (DSM-III-R) (American Psychiatric Association, 1987). All subjects had drunk alcohol over 10 years. Their ages ranged from 30 to 64 (mean 50) years. None of the patients had suffered from endocrinological disorders or had been occupationally exposed to heavy metals or solvents. Four patients had subjective symptoms of sensory abnormalities such as paresthesias and pain in their legs at the time of the examination.

Control subjects were 23 healthy men, matched to each patient by age (3 years span). There were no significant differences in age or skin temperature between the patients and the controls (paired-sample t test, $t = 0.92$ and 0.12, respectively; $P > 0.05$).

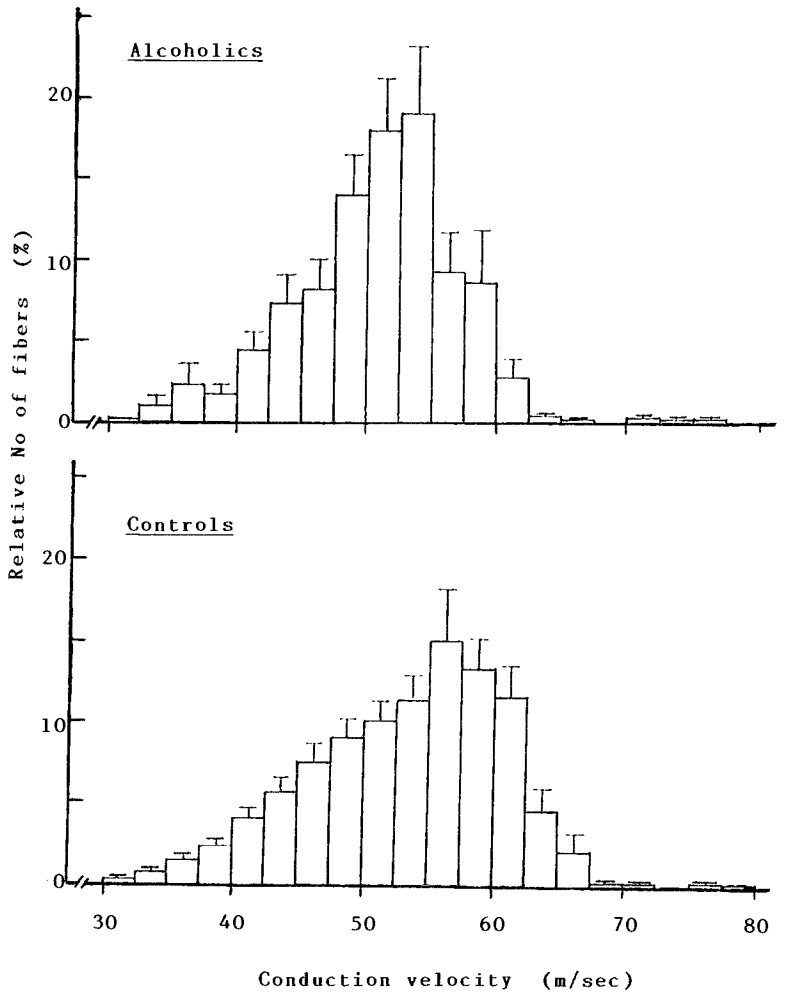

FIG. 1. Distribution of conduction velocities in 23 patients with alcoholic dependency and the same number of age-matched controls (average and standard error).

The nature of the procedure in the present study was fully explained to all subjects, and the study was carried out with their informed consent.

Methods

The DCV was measured by the same method reported previously by us (Araki *et al.*, 1986a, 1988; Yokoyama *et al.*, 1987), i.e., a modified method of Barker *et al.* (1979). The study was conducted in a warm laboratory (26–30°C) using a two-channel electromyograph (Medelec MS-92); skin temperature was maintained in the range of 30–34°C for all subjects. The right median nerve was stimulated repeatedly with stainless ring cathode and anode electrodes, tied around the proximal and distal interphalangeal joints of the second finger, respectively, with an 80–120 V square wave pulse of 0.2 msec duration. The compound action potentials (CAPs) were recorded at the wrist and the elbow with surface electrodes. The responses were averaged 100 to 250 times. The DCV was calculated by the double conduction distance method (Barker *et al.*, 1979) between the velocities of 1.25 and 108.75 m/sec with an interval of 2.5 m/sec from the two CAPs, using a NEC PC-9801LS5 microcomputer. The calculated DCV was expressed by the following parameters: the conduction velocities below which 10, 20, 30, 40, 50, 60, 70, 80, and 90% of active nerve fibers lie (V10, V20, V30, V40, V50, V60, V70, V80, and V90 velocities). When we performed the measurement over a period of 15 days in a 24-year-old healthy student, the daily variations (coefficients of variation) ranged from 2.0 to 4.7% for the V10 to V90 velocities (Araki *et al.*, 1986a).

Maximal motor median nerve conduction velocity (MCV) of the right forearm (between the elbow and wrist) segment was measured using standard techniques (Kimura, 1989). In addition, sensory median nerve conduction velocities (SCV) of the forearm (elbow to wrist) and palm (wrist to second finger) segments were

TABLE 1

DIFFERENCES IN DISTRIBUTION OF NERVE CONDUCTION VELOCITIES (DCV, m/sec) AND MAXIMAL MOTOR AND SENSORY NERVE CONDUCTION VELOCITIES (MCV AND SCV, m/sec) IN THE MEDIAN NERVE BETWEEN 23 PATIENTS WITH ALCOHOLIC DEPENDENCY AND THE SAME NUMBER OF MATCHED CONTROLS

	Patients[a]	Controls[a]	Matched differences[b]
DCV			
V10	45.2 (39.9–50.5)	44.1 (40.0–48.1)	1.1 ± 6.9
V20	47.1 (42.2–52.0)	47.8 (43.9–51.6)	−0.7 ± 6.2
V30	48.7 (43.7–53.7)	50.6 (46.5–54.8)	−1.9 ± 6.1
V40	49.8 (45.0–53.6)	52.6 (48.6–56.6)	−2.8 ± 5.8*
V50	50.9 (46.3–55.5)	54.4 (50.6–58.2)	−3.5 ± 5.4**
V60	51.9 (47.4–56.3)	56.0 (52.3–59.7)	−4.1 ± 5.1***
V70	52.9 (48.5–57.3)	57.6 (53.9–61.2)	−4.7 ± 4.9***
V80	54.2 (49.8–58.6)	59.0 (55.4–62.6)	−4.8 ± 4.9***
V90	55.8 (51.2–60.4)	60.7 (57.2–64.2)	−4.9 ± 4.9***
Nerve conduction velocities			
MCV	53.6 (37.0–59.8)	60.2 (51.5–66.6)	−4.1 ± 4.3***
SCV, palm	41.2 (29.5–49.7)	45.8 (35.0–51.9)	−4.6 ± 7.6**
SCV, forearm	53.1 (37.0–59.8)	60.2 (51.5–66.6)	−7.1 ± 5.5***

[a] Means with ranges in parentheses.

[b] Mean and standard deviation of matched differences.

* $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$ (paired-sample t test).

antidromically measured in the usual manner (Kimura, 1989). The daily variations in these peripheral nerve conduction velocities were between 3.0 and 3.6% (Araki *et al.*, 1986b).

The paired-sample *t* test was conducted to determine the significance of the matched differences between the patients and control subjects using the Statistical Packages for the Biosciences (SPBS) (Uni-Science Co.).

RESULTS

The DCV in 23 patients with alcoholic dependency and in the same number of control subjects is shown in Fig. 1, in which the DCV is shifted toward the slower conduction velocities in the former subjects due mainly to a relative decrease in faster fibers with conduction velocities of 55.0–67.5 m/sec. The V40 to V90 velocities of the DCV were significantly slowed in the patients; the SCV of the palm and forearm segments and the MCV of the forearm segment were also significantly slowed (Table 1). Figure 2 exemplifies the differences in DCV parameters between the patients and control subjects.

DISCUSSION

The major finding of this study is that the V40 to V90 velocities of the DCV as well as the median SCV and MCV were significantly slowed in alcoholic patients.

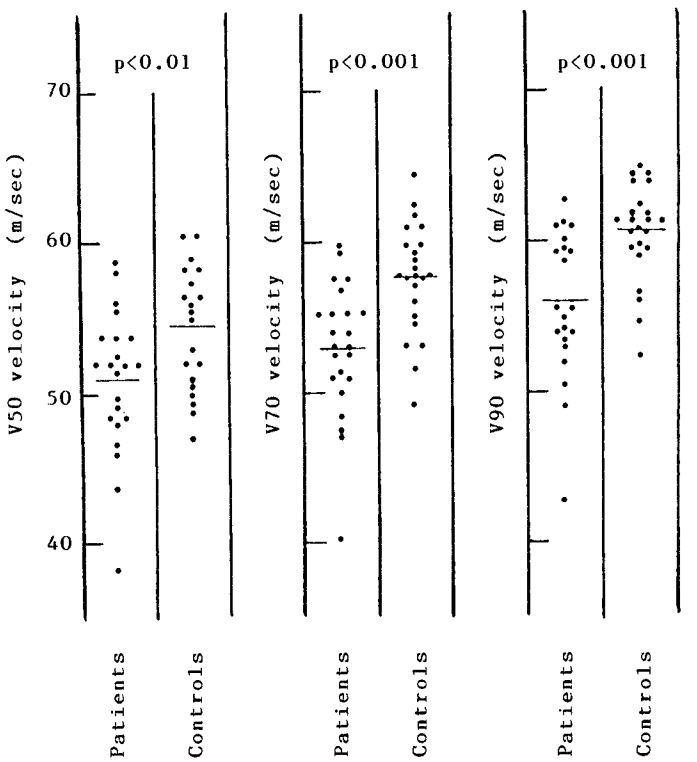

FIG. 2. Differences in distribution of conduction velocities (V50, V70, and V90 velocities, m/sec) between 23 patients with alcoholic dependency and the same number of matched controls (paired-sample *t* test). Transverse lines indicate mean values.

It is suggested that chronic alcohol ingestion affects the faster large myelinated nerve fibers more than the slower ones.

This finding agrees with the following observations by us: (1) conduction velocities of all faster and slower large myelinated median nerve fibers (V10 to V90 velocities, Araki *et al.*, 1988) and of the faster nerve fibers (V70 to V90 velocities, Murata *et al.*, 1991b) were significantly slowed in chain saw and other vibrating tool operators; and (2) conduction velocities of faster nerve fibers were significantly slowed in a patient with acute thallium poisoning (Yokoyama *et al.*, 1990a), in workers exposed to *n*-hexane (Yokoyama *et al.*, 1990b), in styrene workers (Murata *et al.*, 1991a), and in lead workers (Sata *et al.*, 1991). Thus, it appears that conduction velocities of the faster fibers are affected earlier than the slower fibers by many occupational and environmental factors.

In the present study of alcoholic patients, the relative proportion of large myelinated nerve fibers with the conduction velocities of 55.0–67.5 m/sec was decreased (Fig. 1). Using the conversion coefficient of 6 m/sec per 1 μm of a fiber diameter (Yokoyama *et al.*, 1990b), the diameters of these nerve fibers are calculated as approximately 9–11 μm. The calculated diameters are compatible with the histopathological data by Behse and Buchthal (1977), who demonstrated that the nerve fibers with diameters of 9 to 13 μm are especially impaired in patients with alcoholic neuropathy with a mild loss of nerve fibers. Behse and Buchthal (1977) have described that the sural SCV can be predicted from the histogram of fiber diameters in case of axonal degeneration. A comparative study of histopathological and physiological data is required to examine the validity of the DCV method.

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Assessment of Central, Peripheral, and Autonomic Nervous System Functions in Vibrating Tool Operators: Neuroelectrophysiological Studies[1]

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To evaluate the effects of vibrating tool operation (i.e., combined stressors of local vibration, noise, cold climate, and heavy work) on the central, peripheral, and autonomic nervous systems, the short-latency somatosensory and brain stem auditory evoked potentials (SSEP and BAEP), the distribution of sensory median nerve conduction velocities (DCV), conventional median nerve conduction velocities (NCV), and the electrocardiographic R-R interval variability (CV_{RR}) were measured in three groups of male vibrating tool operators and age-matched male healthy adults. Two components of the CV_{RR} reflecting parasympathetic activity (C-CV_{RSA}) and sympathetic activity (C-CV_{MWSA}) were also examined. In the first group of vibrating tool operators (15 chain saw operators), all parameters of DCV (V10–V90 velocities) and sensory and motor nerve conduction velocities of NCV were significantly slowed. All peak latencies of SSEP were significantly prolonged, while no significant differences were found in the interpeak latencies of SSEP. The N9 peak latency of SSEP was significantly related to total working days. In the second group of the operators (12 chain saw and 8 brush saw operators), the I–V interpeak and V peak latencies of BAEP were significantly prolonged in the 12 chain saw operators; the I–V interpeak latency of BAEP was significantly correlated with the working years in the 8 brush saw operators. In the third group of vibrating tool operators, i.e., 13 operators with a history of vibration-induced white finger (VWF group) and 11 operators without VWF (non-VWF group), both the CV_{RR} and C-CV_{RSA} were significantly reduced in the VWF group; only the CV_{RR} was significantly reduced in the non-VWF group. Similarly, the faster velocities of DCV (V70, V80, and V90 velocities) were significantly slowed in both the VWF and non-VWF groups. In conclusion, it is suggested that vibrating tool operation affects the faster sensory and motor nerve fibers, the parasympathetic activity, and the auditory pathway from the acoustic nerve to the brain stem.

INTRODUCTION

It has been reported that vibrating tool operation may affect the central nervous system. For example, the following data have been demonstrated in Japan: a delay in digital plethysmographic responses to auditory stimuli (autonomic nervous system dysfunction) (Matoba *et al.*, 1975), abnormal waveform patterns in the electroencephalogram (EEG) (Hitora, 1977; Arikawa *et al.*, 1978), brain stem dysfunction in the repetitive evoked electromyogram (Hitora, 1977), and changes in hormonal secretion in the limbic system (Matoba *et al.*, 1985). Using the somatosensory evoked potential, it has been shown that conduction time from the upper limb to the cerebral cortex is prolonged in vibrating tool operators (Tanabe and Kameda, 1979; Ohta *et al.*, 1985). Moreover, Sasaki *et al.* (1987a) have indicated that the interpeak latencies of brain stem auditory evoked potential (BAEP) (a measure of conduction in the pontine and midbrain portion of the

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auditory pathway) were significantly delayed in patients with occupational vibration disease. The application of newly developed electrophysiologic techniques such as the study of variability in R-R intervals (CV_{RR}) (Heinonen *et al.*, 1987; Kobayashi *et al.*, 1987; Sasaki *et al.*, 1987b; Harada *et al.*, 1989) coupled with measurements of the short-latency somatosensory evoked potential (SSEP) and BAEP might enable objective evaluation of the effects of vibrating tool operation on the nervous system.

The cellular targets of the central and autonomic nervous system possibly affected by vibration have not been determined. With respect to the peripheral nerves, on the other hand, a demyelinating neuropathy with a pronounced loss of nerve fibers was observed in the pneumatic vibrating tool operators (Takeuchi *et al.*, 1986). In an experimental animal study, furthermore, the electromicroscopic findings suggested that the diameter of myelin sheaths disrupted by vibration varied from 2 to 12 μm and that the extent of the myelin disruption is proportional to the cumulative vibration dose (Ho and Yu, 1989). We have recently introduced a noninvasive method to derive the distribution of nerve conduction velocities (DCV) of several thousand large myelinated nerve fibers (alpha fiber group) in peripheral nerve trunks (Araki *et al.*, 1986a, 1988), and determination of the DCV will throw light on the evaluation of conduction velocities of all these fibers in vibrating tool operators. For comprehensive evaluation of the etiology, it would be useful to connect these data on the adverse effects of vibration on the peripheral nervous system with comparable data on the central and autonomic nervous systems.

In this study, we intend to summarize our data on the effects of vibrating tool operation on the central, peripheral, and autonomic nervous system functions (Murata *et al.*, 1987, 1990, 1991; Araki *et al.*, 1988). The SSEP, BAEP, DCV, and R-R interval variability as well as conventional peripheral nerve conduction velocities have been measured in three groups of vibrating tool operators exposed to local vibration, noise, cold climate, and heavy work.

SUBJECTS AND METHODS

Subjects

Three groups of male vibrating tool operators were examined. The nature of the procedures used in this study was explained fully to all subjects, and the study was carried out with their informed consent.

Group 1 was composed of 15 chain saw operators and 15 age-matched male healthy controls. The chain saw operators had engaged in chain saw operations for 16 to 34 (mean 22) years and worked for 50–203 (mean 124) days. Their age, skin temperature, alcohol ingestion, and height were not significantly different from those in the age-matched controls (paired sample t test, $P > 0.05$).

Group 2 consisted of 12 chain saw and 8 brush saw operators and age-matched male healthy controls for each. The 12 chain saw operators, aged 44 to 63 (mean 56) years, had worked for 4 to 24 (mean 16) years. Similarly, the 8 brush saw operators, aged 23 to 56 (mean 43) years, had worked for 1 to 29 (mean 12) years. No significant differences in age, alcohol ingestion, or skin temperature were found between the 12 chain saw operators and the matched controls (paired sample t test, $P > 0.05$) or between the 8 brush saw operators and the matched controls ($P > 0.05$).

Group 3 was composed of 24 vibrating tool operators, of whom 13 had a history of vibration-induced white finger (VWF group) and 11 did not have such a history (non-VWF group). The VWF group, aged 38 to 63 (mean 51) years, had worked for 3 to 40 (mean 18) years; also, the non-VWF group, aged 37–62 (mean 52), had worked for 1–36 (mean 19) years. There were no significant differences in age or years of exposure between the VWF and non-VWF groups (Student's t test, $P > 0.05$). The number of vibrating tool operators by type of vibrating tools were as follows: 6 chain saw operators, 5 rock drill operators, 3 grinder operators, 3 tie tamper operators, 3 concrete vibrator operators, 2 hand hammer operators, and 2 impact wrench operators. The vibration acceleration for these vibrating tools has been reported to be within 144 to 171 dB (AL) (Futatsuka *et al.*, 1984). The control subjects were 17 healthy men aged 35 to 63 (mean 52) years. There were no significant differences in age, alcohol consumption, or skin temperature either between the VWF and control groups or between the non-VWF and control groups (Student's t test, $P > 0.05$).

None of these vibrating tool operators or the healthy control subjects were occupationally exposed to neurotoxic substances such as heavy metals and organic solvents; none had ever suffered from cardiovascular, neurologic, endocrinologic, or other potentially confounding disorders such as pneumoconiosis and otitis.

Methods

Electrophysiological measurements were conducted using a two-channel electromyograph (Medelec MS-92), audiostimulator (Medelec CK-63), ECG-amplifier (NEC-Sanei 1271SP) and a microcomputer (NEC PC-9801UV2) with an analog-to-digital converter (Neolog PCN-2198; sampling time, 1 msec).

Four peak latencies of SSEP were measured by the same method reported previously by us (a modified Jones' method) (Jones, 1977; Araki *et al.*, 1986b, 1987). After electrical stimulation of the right median nerve at the wrist, the N9 peak was recorded at the Erb's point above the clavicle; the N13 peak was recorded at the second cervical vertebra; and N20 and P23 peaks were on the scalp overlying the sensory cortex contralateral to the stimulated limb. The interpeak latencies of N9–N13 and N13–N20 represented cervicospinobulbar and central conduction times, respectively. Daily variation (coefficient of variation) in the SSEP latencies was between 1.1 and 3.6% (Araki *et al.*, 1987).

The BAEP was measured by the method described by Kriss (Kriss, 1982; Murata *et al.*, 1990). Click signals were presented to the right ear through earphones. The intensity of click stimuli was about 80 dB hearing level for each subject. The BAEP was recorded using three standard EEG electrodes fixed to the vertex (Cz) and right mastoid ipsilateral to stimulation. The responses were averaged 2000 times after amplification and filtration. Daily variations in the I, III, and V latencies of BAEP in a 23-year-old male student for 16 days were 6.6, 2.5, and 1.3%, respectively (Murata *et al.*, 1990). It has been shown that the I, III, and V components of BAEP primarily represent volume-conducted electrical activity from the acoustic nerve, pons, and midbrain, respectively (Kriss, 1982).

The DCV was measured by a modified method of Barker *et al.* (1979) (Araki *et al.*, 1986a, 1988). After electrical stimulation of the right median nerve at the second finger, compound action potentials were detected at both the wrist and elbow. The DCV was calculated by the double-conduction distance method. The

calculated DCV was expressed by the following parameters: the conduction velocities below which 10, 20, · · · , 80, and 90% of active nerve fibers lie (V10, V20, · · · , V80, and V90 velocities). The daily variations in these DCV parameters were below 4.2% (Araki *et al.*, 1986a). The maximal motor nerve conduction velocity (MCV) of the forearm segment and sensory nerve condution velocities (SCV) of the forearm and palm segments were measured in the right median nerve using standard techniques (Kimura, 1989). The daily variation in peripheral nerve conduction velocities was between 3.0 and 3.6% (Araki *et al.*, 1986b). Skin temperature remained in the range of 30–34°C for all subjects.

After the subject had lain quietly supine for 10 min, one hundred R-R intervals on the ECG were continuously measured and stored on a floppy disk (Murata *et al.*, 1992). The CV_{RR} was defined as the ratio of the standard deviation of the R-R intervals to their average value (RR_{mn}, msec). The power spectrum of R-R interval variability was calculated by autoregressive spectral analysis. The spectrum of each of two components was separated by component analysis: respiratory sinus arrhythmia (RSA) and the Mayer wave-related sinus arrhythmia (MWSA). Each component coefficient of variation (i.e., $C\text{-}CV_{RSA}$ and $C\text{-}CV_{MWSA}$) was defined as the ratio of the square root of each component power ($msec^2$/cycle/beat) to the RR_{mn} (Hayano *et al.*, 1990; Murata *et al.*, 1991). The $C\text{-}CV_{RSA}$ and $C\text{-}CV_{MWSA}$ have been considered to reflect parasympathetic and sympathetic activities, respectively (Akselrod *et al.*, 1981; Eckberg, 1983; Pomerantz *et al.*, 1985; Pagani *et al.*, 1986; Hayano *et al.*, 1990; Murata *et al.*, 1992). The CV_{RR} is composed of the $C\text{-}CV_{RSA}$, $C\text{-}CV_{MWSA}$, and other components. The CV_{RR} was measured repeatedly during an 18-day period in a healthy 30-year-old man; daily variation was found to be 7.5% (Murata *et al.*, 1992).

The paired sample *t* test was used to determine the significance of the matched difference between the vibrating tool operators and the age-matched controls; also, Student's *t* test was done to compare the two groups. The dose–effect relationship was tested between the period of exposure and the electrophysiological measurements by the Pearson's product moment correlation coefficient (*t* test). All analyses were performed using the Statistical Packages for the Bioscience (SPBS) (Uni-Science Co.).

RESULTS

Short-Latency Somatosensory Evoked Potential, Distribution of Nerve Conduction Velocities, and Peripheral Nerve Conduction Velocities in Chain Saw Operators

Eleven of fifteen chain saw operators had a history of VWF. All parameters of the DCV were significantly slowed in the 11 chain saw operators (Table 1). The MCV and SCV in the median nerve were significantly slowed in the 15 chain saw operators (Table 1). Also, all peak latencies of the SSEP were significantly prolonged in the operators; however, no significant difference in the interpeak latency of the SSEP was found between the chain saw operators and the matched controls (Table 1). The N9 and P23 peak latencies were significantly correlated with total working days in the 15 chain saw operators ($r = 0.589$ and 0.645, respectively, $P < 0.05$).

TABLE 1

DIFFERENCES IN THE LATENCIES OF SHORT-LATENCY SOMATOSENSORY EVOKED POTENTIALS (SSEP LATENCIES, msec), DISTRIBUTION OF NERVE CONDUCTION VELOCITIES (DCV, m/sec), AND MAXIMAL MOTOR AND SENSORY MEDIAN NERVE CONDUCTION VELOCITIES) (MCV AND SCV, m/sec) BETWEEN 15 CHAIN SAW OPERATORS AND AGE-MATCHED CONTROLS[a]

	Chain saw operators[b]	Matched controls[b]	Matched difference[c]
SSEP latencies			
N9	9.8 (8.9–11.0)	9.1 (8.5–9.8)	0.7 ± 0.7**
N13	13.4 (11.9–14.4)	12.7 (11.9–13.5)	0.7 ± 0.9**
N20	19.9 (18.6–20.8)	19.0 (17.9–20.1)	0.9 ± 0.9**
P23	26.5 (24.0–29.2)	25.3 (23.6–26.8)	1.2 ± 1.3**
N9–N13	3.6 (2.9–4.5)	3.6 (3.0–4.2)	0.0 ± 0.7
N13–N20	6.6 (5.7–8.2)	6.3 (5.3–6.9)	0.3 ± 0.8
N20–P23	6.5 (4.4–9.4)	6.3 (5.3–7.6)	0.2 ± 1.1
DCV[d]			
V10	36.7 (30.0–52.3)	42.7 (32.0–55.4)	−6.0 ± 8.2*
V20	40.4 (31.2–53.3)	47.7 (35.5–58.8)	−7.3 ± 7.6*
V30	43.4 (34.0–54.1)	51.4 (43.6–59.5)	−8.0 ± 8.1*
V40	45.3 (35.5–54.6)	53.8 (48.1–60.2)	−8.5 ± 8.1**
V50	47.0 (38.0–55.4)	55.3 (49.8–61.3)	−8.3 ± 7.7**
V60	49.3 (42.1–56.3)	57.4 (50.8–64.0)	−8.1 ± 7.1**
V70	51.5 (45.3–57.4)	58.8 (51.2–64.8)	−7.3 ± 6.4**
V80	54.1 (48.7–58.5)	61.1 (52.1–66.3)	−7.1 ± 6.1**
V90	56.7 (50.2–60.5)	63.2 (52.9–72.5)	−6.5 ± 5.9**
Nerve conduction velocities			
MCV	54.8 (49.6–61.5)	58.5 (55.2–63.9)	−3.7 ± 4.9*
SCV, forearm	56.6 (49.0–61.7)	64.3 (58.4–68.5)	−7.7 ± 4.6***
SCV, palm	44.4 (38.9–49.4)	49.9 (38.2–61.3)	−5.5 ± 8.1*

[a] Abbreviations same as in text.
[b] Means with ranges in parentheses.
[c] Mean and standard deviation of matched differences.
[d] The number of matched pairs was 11.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ (paired sample t test).

Brain Stem Auditory Evoked Potential and Peripheral Nerve Conduction Velocities in Chain Saw and Brush Saw Operators

Six of the twelve chain saw operators had a history of VWF; however, none of the 8 brush saw operators had any VWF history. In the chain saw operators, V peak and I–V interpeak latencies of BAEP were significantly prolonged; however, no significant prolongation of BAEP latencies was found in the brush saw operators (Table 2). Peripheral nerve conduction velocities were significantly slowed in both the chain saw and brush saw operators (Table 2). The I–V interpeak latency and the MCV in the brush saw operators were significantly correlated with working years ($r = 0.776$ and -0.752, respectively, $P < 0.05$). Moderate hearing loss was observed in all frequency regions of the audiogram in both the chain saw and brush saw operators; hearing levels at 4 and 8 kHz were significantly lower in chain saw operators than in brush saw operators (Table 2).

TABLE 2

DIFFERENCES IN THE LATENCIES OF BRAIN STEM AUDITORY EVOKED POTENTIALS (BAEP LATENCIES, msec), MAXIMAL MOTOR AND SENSORY MEDIAN NERVE CONDUCTION VELOCITIES (MCV AND SCV, m/sec) AND HEARING LEVELS (dB) BETWEEN 12 CHAIN SAW OPERATORS AND AGE-MATCHED CONTROLS AND BETWEEN 8 BRUSH SAW OPERATORS AND AGE-MATCHED CONTROLS[a]

	Chain saw operators[b]	Matched controls[b]	Matched differences[c]	Brush saw operators[b]	Matched controls[b]	Matched differences[c]
BAEP latencies						
I	1.69 (1.46–1.88)	1.61 (1.36–1.80)	0.08 ± 0.22	1.65 (1.36–1.88)	1.59 (1.40–1.92)	0.06 ± 0.26
III	4.01 (3.64–4.48)	3.78 (3.24–4.36)	0.23 ± 0.44	3.91 (3.72–4.24)	3.84 (3.48–4.40)	0.07 ± 0.37
V	5.93 (5.52–6.20)	5.59 (5.08–5.92)	0.34 ± 0.42*	5.84 (5.52–6.08)	5.74 (5.52–6.00)	0.10 ± 0.26
I–V	4.24 (3.92–4.60)	3.98 (3.44–4.28)	0.26 ± 0.38*	4.19 (3.92–4.48)	4.14 (3.76–4.40)	0.05 ± 0.20
Nerve conduction velocities						
MCV	54.5 (49.5–58.5)	58.5 (54.7–62.5)	−4.0 ± 4.3**	54.4 (47.0–59.9)	59.2 (55.0–62.0)	−4.8 ± 6.0
SCV, forearm	57.8 (47.8–63.0)	62.2 (58.0–65.4)	−4.4 ± 5.0*	58.9 (54.8–70.3)	64.0 (61.1–68.5)	−5.1 ± 3.9**
SCV, palm	43.3 (36.7–47.4)	46.4 (38.2–54.9)	−3.1 ± 7.1	44.0 (30.1–51.5)	53.4 (46.5–60.9)	−9.4 ± 10.3*
Hearing level						
500 Hz	30 (10–55)			34 (10–60)		
1000 Hz	32 (10–60)			25 (5–60)		
2000 Hz	38 (15–65)			23 (5–60)		
4000 Hz	55 (20–75)[d]			33 (−10–60)[d]		
8000 H	53 (15–80)[d]			28 (−5–60)[d]		

[a] Abbreviations same as in text.

[b] Means with ranges in parentheses.

[c] Mean and standard deviation of matched differences.

[d] Differences between 12 chain and 8 brush saw operators are significantly different (Student's t test, $P < 0.05$).

* $P < 0.05$.

** $P < 0.01$ (paired sample t test).

R-R Interval Variability, Distribution of Nerve Conduction Velocities, and Peripheral Nerve Conduction Velocities in VWF and Non-VWF Operators

The CV_{RR} and C-CV_{RSA} in 13 VWF operators were significantly smaller than those in 17 control subjects; the CV_{RR} in 11 non-VWF operators was also significantly smaller than that in 17 control subjects (Table 3). The V70 to V90 velocities of the DCV and the SCV of the forearm and palm segments in the 13 VWF operators were significantly slower than those in the 17 control subjects; the V70 and V80 velocities and the SCV of the forearm segment in the 11 non-VWF operators were also significantly slower than those in the 17 control subjects (Table 3). There were no significant differences in the R-R interval variability, the DCV, or the peripheral nerve conduction velocities between the 13 VWF operators and the 11 non-VWF operators (Student's t test, $P > 0.05$).

The C-CV_{MWSA} in the 24 vibrating tool operators, i.e., 13 VWF operators and 11 non-VWF operators, was significantly correlated with all of the DCV parameters ($r = 0.411$ to 0.575, $P < 0.05$) and with the MCV ($r = 0.520$, $P < 0.05$) and the SCV of the forearm ($r = 0.439$, $P < 0.05$). The partial correlations of the C-CV_{MWSA} with all parameters of the DCV and with peripheral nerve conduction parameters, with the effects of age eliminated, were also statistically significant, except in the case of the correlations with the V90 velocity and the SCV of the

TABLE 3

DIFFERENCES IN THE ELECTROCARDIOGRAPHIC R-R INTERVAL VARIABILITIES (CV_{RR}, C-CV_{RSA} AND C-CV_{MWSA}, %), DISTRIBUTION OF NERVE CONDUCTION VELOCITIES (DCV, m/sec) AND MAXIMAL MOTOR AND SENSORY MEDIAN NERVE CONDUCTION VELOCITIES (MCV AND SCV, m/sec) BETWEEN VIBRATING TOOL OPERATORS WITH AND WITHOUT VIBRATION-INDUCED WHITE FINGER (VWF AND NON-VWF GROUPS) AND CONTROL GROUP[a]

	VWF group[b] (N = 13)	Non-VWF group[b] (N = 11)	Control group[b] (N = 17)
R-R interval variabilities			
CV_{RR}	2.25 ± 0.75***	2.61 ± 0.58**	3.70 ± 1.07
C-CV_{RSA}	1.12 ± 0.33*	1.55 ± 0.64	1.65 ± 0.92
C-CV_{MWSA}	1.38 ± 0.70	1.26 ± 0.62	1.60 ± 0.99
DCV			
V10	47.4 ± 4.0	44.6 ± 3.8	46.1 ± 5.4
V20	49.6 ± 3.7	48.7 ± 3.7	49.5 ± 4.3
V30	51.0 ± 3.8	50.6 ± 3.5	52.4 ± 4.1
V40	52.2 ± 3.9	52.0 ± 3.3	54.0 ± 3.8
V50	53.1 ± 4.0	53.3 ± 3.0	55.4 ± 3.4
V60	54.1 ± 3.9	54.4 ± 3.0	56.7 ± 3.1
V70	55.0 ± 3.9*	55.6 ± 3.1*	58.0 ± 2.9
V80	56.0 ± 4.0**	56.9 ± 3.3*	59.4 ± 2.7
V90	57.3 ± 4.1**	58.9 ± 3.7	61.0 ± 2.6
Nerve conduction velocities			
MCV	55.8 ± 4.9	54.7 ± 4.1	57.0 ± 2.9
SCV, forearm	55.3 ± 5.1*	54.4 ± 3.3***	59.4 ± 3.2
SCV, palm	40.8 ± 7.8*	40.7 ± 9.0	45.8 ± 5.0

[a] Abbreviations same as in text.

[b] Mean and standard deviation for each group.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$ (student's t test).

forearm segment ($P > 0.05$). None of the CV_{RR}, C-CV_{RSA}, C-CV_{MWSA}, DCV parameters, MCV nor SCV was significantly correlated with the periods of vibration exposure ($P > 0.05$).

DISCUSSION

Central Nervous System Effects of Vibrating Tool Operation

In the 12 chain saw operators, the I–V interpeak and V peak latencies of BAEP were significantly prolonged. In the 8 brush saw operators, the I–V interpeak latency was significantly correlated with working years despite the fact that any latencies of BAEP were not significantly prolonged (the correlation might have been confounded by age because any age-adjusted correlation coefficient was not calculated). Hearing levels at 4 and 8 kHz were significantly lower in the chain saw operators than in the brush saw operators. These findings are consistent with those of Sasaki *et al.* (1987a), who showed prolongation of the I–V and III–V interpeak latencies of BAEP in 17 (85%) of 20 chain saw operators with VWF (the frequency of VWF was higher than ours, 50%). The present result also agrees with the data of Hitora (1977), who showed an abnormal wave pattern with high plateau level in the repetitive evoked electromyogram in 33 of 34 patients with VWF. All these findings suggest that vibrating tool operation affects the auditory pathway from the acoustic nerve to the brain stem.

Attias and Pratt have shown a significant prolongation of the I, III, and V peak latencies of BAEP without significant changes in the interpeak latencies in workers exposed to noise of occupational origin (1984). Degeneration of the cochlear hair cells and myelinated nerve fibers has been shown in four adult patients known to have been exposed to either industrial noise or gunfire (Hawkins and Johnsson, 1976). Similarly, noise-induced degeneration of the auditory pathway from the cochlear nerve fibers to the superior olivary complex and inferior colliculus has been shown in animals (Morest and Bohne, 1983). Therefore, the prolongation of I–V interpeak and V peak latencies in the present study might have been caused by noise of occupational origin.

The BAEP latencies were not significantly prolonged in the brush saw operators of this study despite the significant change of BAEP latencies in the chain saw operators. Two explanations for this finding are possible: (1) In Japan, it has been reported that the vibration acceleration, main frequency, and noise level for chain saw operators are within the range of 144–155 dB (AL), 100–160 Hz, and 105–118 dB (A), respectively, and that those for brush saw operators are within 139–167 dB (AL), 80–160 Hz, and 90–105 dB (A), respectively (Futatsuka *et al.*, 1984). Thus, noise of brush saws at work in Japan seems to be generally lower than that of chain saws while the vibration level of brush saws is as high as that of chain saws. (2) Hearing losses at 4 and 8 kHz were more significant in the chain saw operators than in the brush saw operators.

All peak latencies of the SSEP were significantly prolonged in the 15 chain saw operators (Table 1). This finding coincides with the results by some investigators (Tanabe and Kameda, 1979; Ohta *et al.*, 1985), who have shown prolongation of the peak latencies of somatosensory evoked potential, i.e., delay in nerve conduction from the upper limb to the cerebral cortex. However, no significant prolongation of the interpeak latency of SSEP was found in the same chain saw operators. Therefore, cervicospinobulbar and central conduction of the somato-

sensory pathway may not be significantly affected by vibration. Further studies with larger populations are needed to clarify whether or not local vibration has significant effects on the somatosensory pathway to the cerebral cortex.

Peripheral Nervous System Effects of Vibrating Tool Operation

All parameters of the DCV (V10–V90 velocities) were significantly slowed in the first group of vibrating tool operators (Table 1). Likewise, the faster (V70, V80, and V90) velocities of DCV were significantly slower in the third group of vibrating tool operators (Table 3). In all groups examined in this study, the MCV and SCV were also significantly delayed. These findings are consistent with those in many previous studies on peripheral nerve conduction velocities in workers exposed to local vibration (Seppäläinen, 1972; Araki *et al.*, 1979; Ho and Yu, 1986; Sasaki *et al.*, 1987b). Furthermore, this observation agrees with results from an experimental study using the saphenous nerve of rabbits exposed to vibration, which show that the relatively thick fibers of myelin sheaths are disrupted by exposure to vibration (Ho and Yu, 1989). Thus, among the large myelinated nerve fibers, the faster fibers should be affected by hand–arm vibration more strongly than the slower fibers.

Both the faster and slower nerve fibers were affected in the first group of vibrating tool operators; whereas, only the faster nerve fibers were affected in the third group of vibrating tool operators. The difference probably resulted from a difference in the magnitude of local vibration to which each group was exposed.

Autonomic Nervous System Effects of Vibrating Tool Operation

The CV_{RR} was significantly depressed in both the VWF and non-VWF groups; whereas the C-CV_{RSA} was only depressed in the VWF group. The depression of CV_{RR} is consistent with several observations reported previously (Kobayashi *et al.*, 1987; Sasaki *et al.*, 1987b; Harada *et al.*, 1989). The depression of C-CV_{RSA} also coincides with the data of Heinonen *et al.* (1987), who observed frequency-related power shifts by a spectral analysis of R-R intervals in workers exposed to local vibration. All these findings suggest that vibrating tool operation affects the autonomic nervous system function, especially parasympathetic activity.

Interrelations among Three Nervous System Effects of Vibrating Tool Operation

The C-CV_{MWSA} was positively related to all parameters of the DCV, the MCV, and the SCV in the 24 vibrating tool operators despite the fact that the CV_{RR} and C-CV_{RSA} were not significantly related to the DCV, the MCV, nor the SCV. It appears that the effect of vibrating tool operation on the C-CV_{RSA}, i.e., a parasympathetic activity, is independent of the effects on DCV, MCV, and SCV, i.e., peripheral nerve functions. The sympathetic and peripheral nerves are distributed in the limbs other than in the trunk; on the other hand, the parasympathetic nerves are distributed only in the trunk. This may explain the close correlations of the C-CV_{MWSA} with the DCV, MCV, and SCV.

The exact pathophysiologic mechanism of VWF is not fully understood (NIOSH, 1989). In this study, the C-CV_{RSA} was significantly depressed in the VWF group while, on the other hand, the C-CV_{MWSA} was not. Therefore, VWF may be related to a decreased parasympathetic activity. The CV_{RR} was depressed both in the VWF and non-VWF groups in this study. This observation is not in

line with the data of Harada *et al.* (1989), who have reported that the depression of the CV_{RR} at rest was only found in the VWF group.

Conclusion

It is suggested that vibrating tool operation, i.e., combined stressors of local vibration, noise, cold climate, and heavy work, affects the auditory pathway from the acoustic nerve to the brain stem and the parasympathetic activity as well as faster sensory and motor nerve fibers.

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Effect of Some Factors on Sleep Polygraphic Parameters and Subjective Evaluations of Sleep[1]

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In this study a bedroom in a noisy area and one in a quiet area were used to investigate the effects of road traffic noise on sleep. Subjective sleep and objective sleep polygraphic parameters were obtained from subjects sleeping in each bedroom. Differences in these parameters at the two locations were compared. The experimental environment differed from those formerly used. The authors thus examined the influences on sleep of covariates in terms of subject differences, age differences, sex difference, differences in the order of experimental nights, set differences, and noise differences. From Spearman's rank correlation coefficient and two-way analysis of variance, subject and age differences were found to have greater effects on many sleep parameters than noise differences. Principal factor analysis was done for young and old subjects separately. The sixth factor was related to noise differences. The sleep parameter common to young and old subjects of the sixth factor was %REM.

INTRODUCTION

Road traffic noise is a worldwide problem. There were two ways to study the effects of road traffic noise on sleep, experiments in the laboratory and at the subjects' own homes. In the former, subjects sleeping in the laboratory are subjected to various levels of recorded traffic noise or synthesized noise stimuli, and the effects of noise are determined by analysis of sleep parameters using an EEG (Eberhardt *et al.*, 1987; Griefahn, 1986; Kawada *et al.*, 1989; Ohrstrom and Rylander, 1982; Thiessen and Lapointe, 1978, 1983). In the latter, subjects sleeping in their own homes are exposed to real traffic noise and varied-level noise by opening windows, installing double glazing, or using earplugs. The effects of noises are then examined (Eberhardt and Akselsson, 1987; Griefahn and Gros, 1986; Vallet *et al.*, 1983; Wilkinson and Campbell, 1984).

The laboratory is quite a strange place for subjects although noise can be chosen arbitrarily in experiments. Noise stimuli are recorded or synthesized, and thus are not realistic. At a subject's home, the environment is familiar and adaptation to situations thus is easy. Noise stimuli are real and vary every moment. But noises differ from home to home. In addition, the difference in sound levels obtained by opening the windows, installing double glazing, or using earplugs is not necessarily large enough to detect the effects of noise on sleep.

A third way to study the effects of traffic noise would be to use bedrooms in noisy and quiet areas and then compare the parameters from subjects sleeping in the bedrooms. Such a procedure has unique characteristics. Noise stimuli are real

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road traffic noises, although the problem of adaptation to the environment remains. Noises are not as different as those in a subject's own home. Differences in noise levels in noisy and quiet areas are larger than those in the homes of subjects.

It is important in investigating the effects of road traffic noise on sleep with this new approach to evaluate the degree of influence of covariates such as subject differences and age differences. The extent and interrelationship of the influence on sleep of road traffic noise and covariates were examined in this study.

SUBJECTS AND METHODS

For the noisy area, an apartment was used. It was on the second floor of a reinforced concrete building facing the main road with a traffic volume of about 30,000 vehicles per day. The bed in the room faced the road. For the quiet area, a wooden detached house was used. The room used for housing the instruments such as the EEG was set apart from the bedroom in both places.

The arithmetic means of the equivalent sound level (Leq) in the noisy area was 46.67 dB (A) from 10:00 PM to 7:00 AM and that in the quiet area was 27.72 dB (A) for the same period.

Five young men ages 19–38, a woman age 65, and 2 older men age 66 were the subjects (Table 1). They had no particular health problems or habits of drinking and drug use. They wore electrodes prior to the experiment to become accustomed to the procedure. All subjects continued their usual life during the experimental period. The same bed and clothes were used in both places. Room temperature and clothes were selected according to personal preference. While wearing the electrodes, the subjects went to bed at about their usual hour. In the morning, they filled out sleep questionnaires. The wearing of electrodes, polygraphic recordings, and data processings were described previously by Aoki *et al.* (1989).

The experiment was composed of two parts, the first set and the second set. Each set consisted of three consecutive nights' data in the noisy and the quiet area. The data for the first, second, and third night in the noisy area were paired with those for the quiet area (Table 1). The order of the place was changed in each set. As shown in Table 1, three young men and two older men completed two sets. Two young men and a woman completed only one set owing to their particular circumstances. One young man failed to complete the second set. Thus, 76 nights (38 pairs) were observed in all.

The 17 parameters used in this study were as follows. The sleep period time (SPT) was the time from sleep onset to final awakening from the main sleep period of the day. The time spent getting up to urinate was not counted in the SPT. Total sleep time (TST) was SPT less the time spent awake and in movement (MT) during the sleep period. Sleep latency (SL) was the time from lights-out to sleep onset. Rapid eye movement (REM) latency (RL) was the time from sleep onset to the beginning of the first REM period. %TST was the percentage of SPT spent in TST. %WASO was the percentage of SPT spent on WASO (the time spent awake during SPT). %S1 was the percentage of SPT spent in stage 1 sleep. %S2 was the percentage of SPT spent in stage 2 sleep. %SWS was the percentage of SPT spent in SWS (time spent in both stage 3 and 4). FREM was the average duration between the beginning of one REM period and that of the next REM period. %REM was the percentage of SPT spent in REM. %MT was the percentage of

TABLE 1
SCHEDULE OF EXPERIMENT

Subject	Age (years)	Place	First set			Second set		
			First night	Second night	Third night	First night	Second night	Third night
TK	19							
		Noisy Ap[a]	○	○	○	○	○	○
		Quiet Ho	○	○	○	○	○	○
SA	20							
		Noisy Ap	○	○	○	○	○	○
		Quiet Ho	○	○	○	○	○	—
UY	22							
		Noisy Ap	○	○	○	○	○	○
		Quiet Ho	○	○	○	○	○	○
TM	23							
		Noisy Ap	—	—	—	○	○	○
		Quiet Ho	—	—	—	○	○	○
ST	38							
		Noisy Ap	—	—	—	○	○	○
		Quiet Ho	—	—	—	○	○	○
OH	66							
		Noisy Ap	○	○	○	○	○	○
		Quiet Ho	○	○	○	○	○	○
SH	66							
		Noisy Ap	○	○	○	○	○	○
		Quiet Ho	○	○	○	○	○	○
SK	65							
		Noisy Ap	○	○	○	—	—	—
		Quiet Ho	○	○	○	—	—	—

Note. Eight subjects, 5 young and 3 old; 2 places, noisy and quiet; and 2 sets, each of 3 consecutive nights.

[a] Ap, apartment; Ho, home. ○ indicates the experiment was done, total 77 nights; and — indicates it was not done.

SPT spent in MT. %Change was the rate of the number of sleep-stage changes to the SPT. %Dep was the percentage of SPT spent in Delta epoch, the epoch with delta-waves. %D was the rate of delta-waves to SPT. Mean depth of sleep (MDS) was calculated as follows. WASO, S1, REM, S2, S3, and S4 except for MT were quantified as 0, 1, 1.5, 2, 3, and 4, respectively, and their sum was divided by SPT. Heart rate per minute was abbreviated as HR. The parameters of polygraphic sleep were defined by Miles and Dement (1980) based on the Rechtschaffen and Kales manual (1968) with some modification. The polygraphic parameters of an epoch (the first 20 sec of each minute) were considered as those of the minute.

The sleep questionnaire presented the following six questions and choices for answers. Sub1: Did you sleep well last night? (1) Yes; (2) neither; (3) no. Sub2: Could you get to sleep easily last night? (1) Yes; (2) no. Sub3: Did you awake sometimes last night? (1) No; (2) yes. Sub4: Did you awake early this morning? (1) No; (2) yes. Sub5: Did you have a sound sleep last night? (1) Yes; (2) no. Sub6: Did you have dreams last night? (1) No; (2) a few; (3) often.

Spearman's rank correlation coefficients were calculated to clarify how the 15 objective and the 6 subjective parameters of sleep were related to covariates in terms of age differences, sex difference, place differences, differences in the order

of experimental nights, and set differences. Place differences have been renamed as noise differences hereafter. The two-way analysis of variance was made to clarify the effects of covariates on 17 objective parameters. Principal factor analysis of 14 polygraphic parameters, 6 subjective evaluations, and 4 covariates for young and old subjects was conducted to determine the common factors between sleep parameters and covariates and thus determine sleep parameters reflecting differences in noise levels or covariates hindering the effects of noise.

RESULTS

Age differences were significantly correlated with sleep parameters, according to Spearman's rank correlation coefficients. That is, they were closely correlated with %Dep, %D, and %SWS. Correlations with %TST, %WASO, %MT, %Change, MDS, HR, and Sub6 were also noted. There was also weak correlation with RL and %S1. Differences in the order of experimental nights was weakly correlated with RL and %REM. Noise differences, which were equivalent to place differences, were weakly correlated with %REM. Set differences showed no significant correlation with any parameters (Table 2).

Comparison of sleep polygraphic parameters between those collected at the noisy apartment and those at the quiet house for young subjects showed no significant difference other than %REM by paired *t* test (Table 3). Comparison for older subjects showed no significant difference (Table 4).

The two-way analysis of variance with one factor as subject differences and other factor as noise differences indicated the following (Table 5). The effect of subject differences was significant ($P < 0.05$) for SPT, TST, SL, RL, FREM, %TST, %WASO, %S1, %S2, %SWS, %MT, %Change, %Dep, %D, and MDS. The effects of noise differences were significant ($P < 0.05$) for TST, %WASO, and %REM. The mutual effects of subject and noise differences were significant ($P < 0.05$) for TST, SL, %TST, %WASO, %S2, %Dep, and MDS. The result of the above analysis indicates that subject differences had strong influences on sleep parameters. The issue was same with age differences. Thus, it is reasonable to divide the subjects into two groups by age in the analysis that follows.

The contribution rate of the first six factors was 67.8% for young subjects and 69.1% for older subjects.

The eigenvalue of the first factor was 5.36 for young and 5.75 for older subjects. Variables having large absolute values of factor loading (>0.2) to the first factor for young subjects were SL, %TST, %WASO, %S1, %S2, %SWS, %Dep, %D, and MDS (Table 6). This means that for young subjects the first factor is related to sound sleep: short sleep latency, longer sleep time, smaller wake and shallow sleep, and deep slow wave sleep. For older subjects, the first factor has similar meaning except for delta-wave or slow wave sleep, which is far less for the old.

The eigenvalue of the second factor was 3.43 for young subjects and 3.79 for older subjects. Variables showed that the large absolute values of factor loadings (>0.2) to the second factor for young subjects were age differences, %S2, %SWS, %MT, %Change, %Dep, and %D. The second factor is related to MT and stage shift frequency for the young. However, for the older subjects this factor is related to delta-wave or slow wave sleep with longer REM latency and smaller %REM.

The eigenvalue of the third factor was 2.46 for young subjects and 2.36 for older subjects. Variables showed that the large absolute values of factor loadings (>0.2) to the third factor for young subjects were Sub1, Sub3, Sub4, Sub5, %TST,

TABLE 2
SPEARMAN'S RANK CORRELATION COEFFICIENT OF THE FOUR COVARIATES: AGE, PLACE, SET, AND NIGHT, WITH 15 POLYGRAPHIC PARAMETERS AND SELF-RATINGS OF SLEEP OF THE TOTAL 75 EXPERIMENTAL NIGHTS

	Age	Noise	Set	Night
Percentage in the sleep period				
%TST	−0.559**	−0.031	0.120	0.078
%WASO	0.433**	0.112	−0.145	−0.117
%S1	0.360**	−0.010	−0.169	−0.141
%S2	−0.165	0.031	0.215	0.049
%SWS	−0.853**	0.047	0.134	0.017
%REM	−0.104	−0.265*	0.055	0.230*
%MT	−0.459**	−0.057	0.031	0.056
%Dep	−0.811**	−0.045	0.123	0.032
%D	−0.866**	−0.018	0.141	0.015
%Change	−0.692**	0.084	0.120	−0.084
Latency				
SL	−0.109	−0.001	−0.049	0.019
RL	−0.253*	0.056	0.048	−0.235*
Others				
FREM	0.195	−0.007	−0.060	0.143
HR	0.640**	0.077	0.080	0.026
MDS	−0.522**	−0.019	0.206	0.080
Self-rating of sleep				
Sub1 (not good sleep)	−0.208	0.047	−0.065	−0.159
Sub2 (difficult to sleep)	0.058	0.010	−0.079	−0.181
Sub3 (awakening)	−0.128	0.065	−0.198	−0.106
Sub4 (early awakening)	0.028	−0.119	0.142	0.071
Sub5 (not deep sleep)	−0.092	0.162	−0.212	0.012
Sub6 (much dreaming)	−0.403**	−0.032	−0.006	−0.136

Note. %TST, Percentage of total sleep time (TST) in the sleep period time (SPT); %WASO, percentage of stage wake after the sleep onset (WASO) in SPT; %S1, percentage of stage 1 in SPT; %S2, percentage of stage 2 in SPT; %SWS, percentage of slow wave sleep (S3 + 4) in SPT; %REM, percentage of the stage of rapid eye movement (REM) in SPT; %MT, percentage of movement time (MT) in SPT; %δ ep, percentage of delta wave positive epoch (δ ep) in the total epoch of SPT; %δ, percentage of delta wave time (δ) in SPT; %Change, rate of sleep stage change; SL, sleep latency; RL, REM latency after the sleep onset; FREM, frequency of REM; HR, heart rate per minute; MDS, mean depth of sleep. Sub1: Could you sleep well last night? (1) yes; (2) neither; (3) no. Sub2: Could you get to sleep easily last night? (1) yes; (2) no. Sub3: Did you awake sometimes last night? (1) no; (2) yes. Sub4: Did you awake early this morning? (1) no; (2) yes. Sub5: Did you have a deep sleep last night? (1) yes; (2) no. Sub6: Did you have dreams last night? (1) no; (2) a few; (3) often.

* $P < 0.05$.

** $P < 0.01$.

%WASO, and %REM. This factor is related to subjective sleep ratings both for young and old subjects.

The eigenvalue of the sixth factor was 1.23 for young subjects and 1.11 for older subjects. Variables showed that the large absolute values of factor loadings (>0.2) to this factor for both groups were noise differences and %REM. This factor may thus be related to noise differences. For the young this is supported from the results in Table 3.

DISCUSSION

There are many factors that possibly influence sleep parameters in addition to individual differences, age differences, sex difference, noise differences, differ-

TABLE 3
COMPARISON OF SLEEP POLYGRAPHIC PARAMETERS BETWEEN THOSE COLLECTED AT A NOISY APARTMENT AND THOSE AT A QUIET HOUSE (FOR YOUNG SUBJECTS)

	Noisy Ap (N = 23)		Quiet Ho (N = 23)		P values (paired t test)
	Mean	SD	Mean	SD	
SPT	482.04	42.09	487.26	39.37	0.5071
TST	452.78	61.72	463.70	54.57	0.2698
SL	15.17	14.07	19.65	18.22	0.0518
RL	109.74	42.24	112.22	57.52	0.8648
FREM	92.70	17.79	91.54	21.79	0.8268
%Change	21.00	5.89	19.30	4.41	0.0699
%WASO	6.40	7.57	5.10	4.91	0.3166
%S1	7.82	5.19	8.10	5.93	0.7984
%S2	58.55	7.47	56.81	5.98	0.2921
%SWS	4.48	3.83	3.71	3.32	0.0560
%REM	15.91	4.43	20.04	4.37	0.0046**
%MT	6.84	3.48	6.22	2.62	0.3689
%Dep	31.08	11.01	29.32	9.32	0.1749
%D	3.35	2.05	2.99	1.58	0.0899
MSD	1.68	0.22	1.64	0.20	0.3749
HR	55.67	7.36	54.81	7.37	0.3166

Note. Abbreviations are same as in Table 2.
** $P < 0.01$, applied by paired t test.

ences in the order of experimental nights, and set differences. These factors should be studied before the actual survey on the effects of road traffic noise on sleep. The authors tried to study them by putting all the variables together by multivariate analysis.

TABLE 4
COMPARISON OF SLEEP POLYGRAPHIC PARAMETERS BETWEEN THOSE COLLECTED AT A NOISY APARTMENT AND THOSE AT A QUIET HOUSE (FOR OLDER SUBJECTS)

	Noisy Ap (N = 15)		Quiet Ho (N = 15)		P values (paired t test)
	Mean	SD	Mean	SD	
SPT	430.93	41.48	450.33	57.66	0.1262
TST	341.40	56.80	385.80	43.67	0.0543
SL	20.13	21.53	10.87	8.46	0.1626
RL	87.80	49.41	84.93	40.96	0.8516
FREM	101.11	55.74	103.27	35.16	0.8996
%Change	13.70	4.03	14.87	2.22	0.3702
%WASO	20.63	11.53	13.49	11.00	0.0745
%S1	11.96	8.10	11.58	5.65	0.8760
%S2	48.16	15.86	53.07	14.86	0.4058
%SWS	0.02	0.06	0.28	0.89	0.2662
%REM	15.64	6.31	16.76	3.65	0.5071
%MT	3.60	2.66	4.81	2.82	0.2436
%Dep	11.19	4.36	15.70	10.50	0.0985
%D	0.57	0.25	0.94	0.90	0.1369
MSD	1.30	0.31	1.45	0.26	0.1252
HR	61.56	9.19	59.81	4.04	0.4146

Note. Abbreviations are same as in Table 2.

TABLE 5
RESULTS OF TWO-WAY ANALYSIS OF VARIANCE OF THE SLEEP PARAMETERS WITH A FACTOR OF INDIVIDUAL DIFFERENCE AND THE OTHER FACTOR NOISE DIFFERENCE

Sleep parameter	Individual difference mean sq. *F* value (*df* = 7)	Noise difference mean sq. *F* value (*df* = 1)	Mutual effect mean sq. *F* value (*df* = 7)	Residual mean sq. (*df* = 60)
SPT	13567.5	2222.6	874.1	1312.1
	10.341**	1.694	0.666	
TST	36172.4	11064.3	4817.6	1699.5
	21.284**	6.510*	2.835*	
SL	1328.5	17.1	408.8	131.9
	10.071**	0.129	3.099**	
RL	7466.7	2.6	1104.4	2014.0
	3.707**	0.001	0.548	
FREM	3122.0	0.4	346.3	930.4
	3.355**	0.000	0.372	
%TST	890.6	148.8	189.7	63.7
	13.980**	2.335	2.978**	
%WASO	570.0	247.0	125.2	49.3
	11.562**	5.009*	2.539*	
%S1	146.0	0.0	21.8	30.4
	4.808**	0.000	0.717	
%S2	316.3	15.0	271.7	93.6
	3.379**	0.160	2.903*	
%SWS	100.1	2.5	1.8	2.5
	39.874**	0.988	0.721	
%REM	36.2	164.8	22.3	21.4
	1.696	7.711**	1.041	
%MT	46.2	0.2	9.2	5.9
	7.872**	0.033	1.563	
%Change	219.2	6.0	15.7	8.5
	25.942**	0.708	1.853	
%Dep	1336.7	9.6	110.7	25.5
	52.357**	0.377	4.337**	
%D	31.8	0.1	1.2	0.6
	51.672**	0.164	1.969	
MDS	0.44	0.03	0.10	0.03
	13.181**	1.015	3.025**	
HR	0.09	0.05	0.09	0.04
	1.943	1.181	1.938	

Note. Abbreviations are same as in Table 2.
* $P < 0.05$.
** $P < 0.01$, applied by two-way analysis of variance.

Spearman's rank correlation coefficients of covariates and sleep parameters indicated that age differences were significantly correlated with many sleep parameters. Differences in the order of experimental nights had weak correlation with two sleep parameters. Noise differences, equivalent to place differences, had weak correlation with a sleep parameter. The influence of age differences and order of experimental nights should be minimized to clarify the effects of road traffic noise on sleep. Although individual differences were not included in Spearman's rank correlation coefficients, the between-subjects variance of sleep EEG

TABLE 6

LOADINGS FOR FACTORS DERIVED FROM COVARIATES, SUBJECTIVE, AND OBJECTIVE PARAMETERS OF 46 NIGHT'S FOR YOUNG SUBJECTS (TOP) 30 NIGHTS' SLEEP FOR OLD SUBJECTS (BOTTOM)

	Factor						
	I	II	III	IV	V	VI	VII
Covariate							
Age	−.15	−.84	.30	−.03	−.04	−.05	−.20
	.50	−.41	−.37	.20	.32	.02	
Set	.28	.11	−.12	.01	.02	.08	.50
	.22	−.08	−.13	−.08	.26	−.03	
Noise	.12	.07	.17	−.03	−.05	−.69	.04
	−.15	−.16	−.07	−.18	.03	.60	
Night	.06	.01	−.05	−.02	.00	.10	−.35
	.35	.10	−.14	.00	−.35	.10	
Self-rating of sleep							
Sub1 (not good sleep)	−.12	.06	.69	.07	−.30	−.02	.14
	−.49	.24	.69	−.18	.08	−.07	
Sub2 (difficult to sleep)	−.29	−.07	.21	−.08	−.71	−.09	.26
	−.41	.38	.15	−.20	−.06	−.16	
Sub3 (awakening)	−.06	−.05	.54	.08	.05	−.25	−.05
	−.07	−.03	.76	.06	−.03	−.09	
Sub4 (early awakening)	.33	−.20	.42	−.39	.16	.38	−.12
	.25	−.19	−.64	−.02	.27	−.01	
Sub5 (not deep sleep)	−.10	−.19	.77	−.24	−.24	−.12	−.08
	.18	.33	.60	−.06	.02	.11	
Sub6 (much dreaming)	.15	−.08	−.15	−.12	.63	−.03	.17
	−.42	−.06	.14	.44	.04	−.09	
Objective sleep parameters							
%TST	.82	.26	−.44	−.03	.02	.01	−.01
	.83	.08	−.15	.01	.09	−.32	
%WASO	−.76	−.29	.48	−.16	.03	−.14	.13
	−.91	−.06	.12	−.14	.04	.25	
%S1	−.71	.24	−.14	.43	−.18	−.15	.04
	−.78	−.08	−.04	.41	−.07	−.11	
%S2	.70	−.44	−.15	−.16	.08	−.19	−.04
	.92	.29	.00	−.17	.19	−.01	
%SWS	.65	.46	.18	−.08	.40	.07	.36
	.18	.82	.26	.02	−.15	.04	
%REM	.34	−.09	−.38	−.24	−.08	.60	−.19
	.30	−.57	−.15	−.23	−.48	−.27	
%MT	−.03	.79	.04	.34	−.17	−.09	−.32
	.18	−.25	−.11	.84	.03	−.07	
%Dep	.72	.46	.07	−.33	.26	.03	.08
	.27	.89	−.03	−.20	−.02	−.24	
%D	.67	.48	.15	−.20	.38	.09	.27
	.25	.91	.14	−.15	−.08	−.17	
%Change	.13	.94	−.08	.09	.01	−.19	.09
	−.16	.09	.01	.84	.19	−.11	
SL	−.54	.15	−.07	.49	−.19	.08	−.08
	−.48	.26	.09	−.15	−.47	.44	
RL	−.16	.05	.00	.61	.30	−.01	.26
	−.37	.60	.34	.19	.07	.30	
FREM	−.13	.11	.03	.79	−.18	−.08	−.11
	.17	.02	−.12	.24	.63	.09	
MDS	.95	.14	−.18	−.13	.16	.00	.05
	.96	.10	−.10	.00	.08	−.21	
Eigenvalue	5.36	3.43	2.46	2.08	1.70	1.23	1.02
	5.75	3.79	2.36	2.21	1.36	1.11	
% of variance	22.32	14.29	10.25	8.66	7.10	5.14	4.23
	23.95	15.77	9.83	9.21	5.67	4.63	

Note. Those factor loadings greater than 0.2 are underlined.

parameters may exceed that of the within-subject variance (Kimura *et al.*, 1989; Thiessen, 1988; Thiessen and Lapointe, 1978, 1983; Wilkinson, 1984). The effects of individual differences were significant ($P < 0.05$) for 15 of 17 parameters, and 7 had mutual effects with the two-way analysis (factors: individual differences and noise differences). Only one parameter, %REM, was significantly effected by noise differences. These results are identical with those of Johns (1975). He observed highly significant differences for most variables in analysis of variance for 16 objective and 3 subjective variables among subjects who slept without noise exposure. Coates *et al.* (1979) obtained all-night sleep EEG recordings for 8 subjects who slept for 3 consecutive nights in a standard sleep laboratory and 3 consecutive nights at home. There was less between- and within-subject variability in the laboratory on some variables but other variables were less variable at home. They also found subject sleep in the two locations to be highly correlated.

Changes in sleep parameters with age differences have been noted (Griefahn and Jansen, 1978; Lukas, 1975; Miles and Dement, 1980; Vallet and Mouret, 1984). Miles and Dement (1980) reviewed more than 48 papers on the changes in sleep parameters with age and indicated the following. The mean nocturnal TST was highest in childhood and lowest in elderly subjects. SL changed little before age 70 and showed considerable individual variation. RL after age 30 decreased slightly throughout life in both sexes. But the reduction of RL was not large in the elderly. The duration of WASO increased with age. Sex difference in WASO was quite evident. The mean %S1 increased steadily throughout life. The mean %S2 approximated an inverted U-curve, while that in old age was essentially the same as in early adult life, with little sex difference. The mean %S3 also approximated an inverted U-curve. In elderly females stage 3 sleep was normal or increased differently from that in elderly males. In the aged, absolute and relative reduction was noted in stage 4 sleep. Relative amounts of REM were well maintained up to extreme old age, followed by some decline.

Fourteen of 17 objective sleep parameters for which the effects of age differences were significant from the two-way analysis of variance (factors: age differences and noise differences) were also noted.

Lukas (1975) reviewed papers on response frequency during sleep to simulated sonic booms and summarized his findings for different age groups as follows. The older the individual, the more likely he or she would awaken or the sleep stage would change due to environmental noise. Griefahn and Jansen (1978) confirmed that awakening reactions in 10-year-old children were about 5% and gradually increased to about 30% in 70-year-old people. Vallet *et al.* (1983) found that young subjects were more sensitive than older subjects. The authors found significant differences between the noisy apartment and the quiet house in 1 of 16 objective and 1 of 6 subjective parameters only for young subjects (Sato *et al.*, 1991).

Miles and Dement (1980) summarized papers on sex difference in sleep parameters as follows. Differences in SWS and WASO were the most obvious, but the numbers of subjects were small and the variation was considerable. Dijk *et al.* (1989) found that during non-REM and REM sleep, power densities detected in females were higher than those in males by spectral analysis. However, there were no significant differences in the amounts of SWS and REM by visual scoring of EEGs.

Regarding differences in response to noise stimuli according to sex, Muzet *et al.* (1973) suggests that men may be more responsive than women although not sta-

tistically. Lukas (1975) found that regardless of the sleep stage or type of stimulus, women were more responsive than men. Aircraft noise, much louder than road traffic noises, was used in his study. There were only one female and two males, and thus the significance of sex difference in this investigation may have been too small. First night effects (Agnew *et al.*, 1966) were the same as those in many other studies of differences in the order of experimental nights. However, cases in which the first night effects continued varied from one to several (Schmidt and Kaelbling, 1971) or 20 (Stevenson and McKellar, 1989). Coates *et al.* found no first night effects at any site. In this study also, effects of differences in the order of experimental nights could not be found by two-way analysis of variance (factors: the order of experimental nights and the noise difference). This might result from the subjects' experience of wearing the electrodes.

Effects of set differences were found in only 1 of 17 objective parameters from the two-way analysis of variance.

The effects of individual differences and age differences on sleep parameters were numerous. The order of experimental nights and set differences also influenced some sleep parameters to a certain extent. Noise differences also influenced some sleep parameters, but only slightly. Thus it was important to consider the combinations.

According to principal factor analysis, young subjects resembled older subjects in the factor structures. There were also a few factors which had different structures between young and old. Although the variables adopted differed from those of Johns (1975), the first factor resembled the others and was related to the amount of sleep or time of awakening. However, the first factor of Johns included subjective parameters which received lower factor loadings in the authors' results.

Although factor loadings of sleep parameters other than %REM did not exceed 0.2 for both young and old subjects in the sixth factor, differences in noise levels may reflect sleep parameters such as Sub3, Sub4, SL, RL, %TST, %WASO, %REM, %Dep, and MDS.

CONCLUSIONS

Subjective sleep and objective polygraphic sleep parameters were determined for subjects sleeping in a noisy apartment and those sleeping in a quiet house to investigate the effects of road traffic noise on sleep. The influence of covariates on sleep was examined. Although there may be many factors which influence the sleep parameters, only individual differences, age differences, sex difference, difference in the order of experimental nights, and set differences were used as covariates.

Spearman's rank correlation coefficients of the covariates and sleep parameters indicated that age differences were significantly correlated to many sleep parameters. Differences in the order of experimental nights showed only weak correlations with two sleep parameters. Place differences, equivalent to noise level differences, weakly correlated to a sleep parameter.

The effects of individual differences were significant ($P < 0.05$) for 15 of 17 parameters, although 7 parameters had a mutual effect by two-way analysis (factors: individual differences and place difference). There was only one parameter for which the effect of place differences was significant.

Young subjects resembled older subjects in the factor structures according to

principal factor analysis. However, among these similar factors there were some parameters that had reverse factor loadings between young and old subjects. There were also a few factors that had different structures between young and old subjects. The variables that significantly correlated to the sixth factor for both groups were place differences and %REM.

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Relationship between Subjective Sleep Rating and Objective Sleep Parameters: A Case Study[1]

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The subjective sleep of a male subject was related to 22 objective polygraphic sleep parameters on 39 experimental nights. Subjective sleep was rated by the sleeper himself as "good," "moderate," or "poor" upon arising, for 10, 24, and 5 nights, respectively. Differences in the 22 sleep parameters for these three ratings were examined, and poor sleep showed a significant increase in stage W (waking), an elongation of sleep latency, a decrease in mean sleep depth during the night and 2 hr after going to bed, a decrease in integrated value of sleep depth during the night, an increase in the gradient and decrease in the intercept of regression line of sleep depth against time, and a shortening of total sleep time. The average delta wave percentage per epoch was greater for a subjective rating of "moderate" than for the other groups. Canonical discriminant analysis was conducted using 16 sleep parameters, and the overall correct identification rate of three subjective sleep ratings by the objective sleep parameters was 89.7%. Variables of the first or second axis with large standard coefficients were stages 1, 2, REM (rapid eye movement), integrated voltage of electromyogram, and sleep latency. From the above two sets of analyses, sleep latency was the most useful parameter for predicting subjective sleep.

INTRODUCTION

Subjective sleep is defined as the sleep quality judged by the sleeper himself, and objective sleep as the electrophysiological parameters derived from sleep polygraphy.

Prior to discussing the relationship between subjective and objective sleep, sleep parameters must be selected. Some questionnaires have been recommended (Webb *et al.*, 1976; Herbert *et al.*, 1976; Oguri *et al.*, 1982) for the evaluation of subjective sleep. Quantitative and traceable objective sleep parameters have become available by the development of a sleep electroencephalogram scoring system using a microcomputer (Hiraga *et al.*, 1982; Aoki *et al.*, 1989; Ferri *et al.*, 1989).

Lewis (1969) reports that the relation between subjective and objective sleep is poor in terms of total sleep time, sleep latency, and number of awakenings, indicating difficulty in predicting. A close relation of subjective to objective sleep has been found. Chan et al. (1989) report that subjective good sleepers have a longer sleeping time than poor, based on 108 young females. Oguri *et al.* (1981) recorded the hypnograms on 7 consecutive nights of four university students and found a relation between subjective good sleep and an increase in stages 3 and 4.

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Eberhardt and Akselsson (1987) and Bonnet and Johnson (1978) report increases in stage W upon worsening of subjective sleep. Baekeland and Hoy (1971) found the state of rest after sleep to improve upon decrease in stage W and number of awakenings, but there was no relation between subjective sleep depth and other sleep parameters. Violani and Cagnoli (1985) clarified that sleep comfort and sleep depth have a negative correlation with stage 1 and a positive correlation with stage REM and sleep efficiency. In addition, sleep depth also has a negative correlation with stage W. Tanaka (1975) reports that subjectively poor sleep is related to shortening of total sleep time, decrease in stage 2, and increase in sleep latency and wakening time and not to parameters related to REM.

In the above reports, except for that of Bonnet and Johnson (1978), little attention has been directed to interindividual difference, which is important for analysis (Williams *et al.*, 1964, 1966; Kimura *et al.*, 1989). In this article, the authors took 39 night hypnograms of a young subject, avoiding individual variation.

MATERIALS AND METHODS

The experiment was conducted on a healthy 28-year-old male volunteer. Throughout the experiment, alcoholic beverages and drug intake were prohibited.

EEG (electroencephalogram) electrodes were positioned following the international 10–20 method (C3–A2). EEG, EMG (electromyogram), and EOG (electrooculogram) were recorded using a telemetry system from Nihon Kohden Co., Ltd. Sleep parameters were the percentages of the sleep stages (W (waking) and stages 1, 2, 3, 4, REM (rapid eye movement), and MT (movement time)) against total recording time according to the sleep EEG atlas of Rechtschaffen and Kales (1968); mean sleep depth calculated by stages W, 1, REM, 2, 3, and 4 quantified as 0, 1, 1.5, 2, 3, and 4, respectively; segmented mean sleep depth for 2 hr after going to bed, from 2 to 4 and 4 to 6 hr after falling asleep, and just before arising; integrated value of sleep depth; gradient and intercept of regression line against time; total sleep time (period of stay in bed except waking); time of going to bed; sleep latency (time from lights out until the first appearance of stage 2); number of sleep stage shifts per hour; mean delta and alpha wave percentages per epoch; and integrated voltage of EMG. The reason for segmentation of 2 hr each for calculating of the mean sleep depth was the subject's average sleep cycle of 121 min. Subjective sleep was scored "good", "moderate", and "poor", using a short questionnaire, and quantified as 1, 2, and 3, respectively.

A microcomputer hard disk was used to store digital data of the first 20 sec of every min. Integral voltage of EMG, spindle wave which is typical in stage 2, rapid eye movements, and percentage of alpha and delta (frequencies less than 2 Hz and amplitudes over 75 μV by the EEG atlas (Rechtschaffen and Kales, 1968) wave were then extracted and calculated from the digital data using original software for the sleep stage analysis (Aoki *et al.*, 1989). Sleep stages and most parameters were identified by the computer program with some visual correction. The rate of stage agreement between the automatic analysis system and visual

judgment was 80% or greater. In this system, stage 4 was included in stage 3 because of its rareness.

The subjective went to bed in the experimental room at 1 AM, and the total recording time was 7 to 9 hr. The noise environment was as follows: 13 quiet nights (*L*eq 35 dB(A)); 14 nights of stationary pink noise; 9 of intermittent pink noises of 40, 50, or 60 dB(A), produced by a noise generator (SF-05 Rion Co., Ltd., Tokyo); and 3 of recorded road traffic noises of *L*eq 59 dB(A). Each sleep parameter for eight kinds of environment was compared, but no statistical differences between them could be found. Kruskal–Wallis and Mann–Whitney tests, Spearman's rank correlation coefficient, and canonical discriminant analysis were used for the statistical evaluation.

RESULTS

There were statistical differences in stage W, sleep latency, mean sleep depth during the night, segmented mean sleep depth for 2 hr after going to bed, integrated value of sleep depth, gradient and intercept of regression line against time, and total sleep time between subjectively bad sleep and the other 2 groups. The delta wave percentage of subjectively moderate sleep exceeded that of the other 2 groups (Table 1).

There was a significant correlation between subjective sleep and stage W, sleep latency, mean sleep depth during the night, segmented mean sleep depth for 2 hr after going to bed, that from 4 to 6 hr, integrated value of sleep depth, gradient and intercept of the regression line against time, integrated voltage of EMG, and total sleep time (Table 2).

Applied by canonical discriminant analysis, subjective sleep was predicted by polygraphically recorded objective sleep parameters. Some parameters had higher intercorrelation coefficients over 0.8, causing a problem of multicollinearity. Thus, sleep latency was chosen instead of stage W, mean sleep depth during the night, segmented mean sleep depth for 2 hr after going to bed, or the gradient and intercept of regression line against time. Another parameter was total sleep time, and integrated value of sleep depth was ignored. A total of 16 sleep parameters were used for analysis. Correct judgment of subjectively good sleep was 90.0%, that of moderate sleep was 87.5%, and that of poor sleep, 100%. Overall correct judgment was 89.7% (Table 3). Sleep parameters of the first axis with larger positive standard coefficients were stages 1, 2, REM, and integrated voltage of EMG. Sleep parameters of the second axis with a smaller negative standard coefficients were stages 1, 2, REM, and sleep latency. The canonical correlation coefficient of the first axis was 0.821 and the second one 0.712 (Table 4 and Fig. 1).

DISCUSSION

Examination was made of the effects of noise on human sleep using the mean sleep depth, gradient, and intercept of regression line against time as objective sleep parameters to determine overnight stage change or balance of sleep depth (Kawada *et al.*, 1988a,b; 1989a,b,c). Mean sleep depth has been used elsewhere (Osada *et al.*, 1968). There are some conflicting opinions as to the validity of the

TABLE 1

ARITHMETIC MEANS (WITH STANDARD DEVIATIONS) OF 22 SLEEP PARAMETERS BY THREE GRADES OF SUBJECTIVE SLEEP: "GOOD," "MODERATE," AND "POOR"

Grade of subjective sleep	No. of night	Stage (%)						Sleep latency minute	Mean stage shift/hour	Mean δ wave %	Mean α wave %	Mean sleep depth	Regression line	
		W	1	2	3	REM	MT						Gradient	Intercept
Good	10	3.8**	8.6	60.2	0.29	24.8	2.3	27.6**	52.3	1.20*	22.4	1.71**	0.027**	1.60**
		(1.34)	(3.44)	(4.11)	(0.31)	(3.68)	(0.99)	(10.2)	(10.5)	(0.14)	(1.39)	(0.04)	(0.022)	(0.11)
Moderate	24	5.8**	6.2	60.8	0.44	24.3	2.4	38.4**	44.4	1.40	22.7	1.70**	0.062**	1.47**
		(4.64)	(2.66)	(5.97)	(0.40)	(4.70)	(1.81)	(23.3)	(10.7)	(0.33)	(0.78)	(0.10)	(0.064)	(0.32)
Poor	5	14.2	8.8	55.2	0.26	19.2	2.2	71.4	54.2	1.14*	23.5	1.52	0.159	0.95
		(5.82)	(3.43)	(7.09)	(0.19)	(3.42)	(0.71)	(26.7)	(13.4)	(0.24)	(2.14)	(0.11)	(0.078)	(0.38)

Grade of subjective sleep	No. of night	EMG	Mean sleep depth 1	Mean sleep depth 2	Mean sleep depth 3	Mean sleep depth T	Mean sleep depth E	Mean sleep depth S	Bed time O'clock	TST minute
Good	10	3322.7	1.54**	1.86	1.72	1.87	1.71	783.0**	0.73	439.8
		(341.5)	(0.13)	(0.04)	(0.08)	(0.06)	(0.09)	(71.9)	(0.80)	(41.0)
Moderate	24	3562.3	1.43**	1.84	1.78	1.91	1.74	728.7*	1.04	401.3*[a]
		(612.3)	(0.35)	(0.06)	(0.07)	(0.07)	(0.06)	(71.4)	(0.55)	(42.0)
Poor	5	3895.6	0.85	1.81	1.78	1.83	1.73	646.1	0.98	356.8**[b]
		(471.6)	(0.42)	(0.12)	(0.16)	(0.11)	(0.04)	(64.3)	(1.14)	(34.6)

Note. W, waking; REM, rapid eye movement; MT, movement time; EMG, integral voltage of electromyogram; and TST, total sleep time, not included of sleep latency and intrasleep wakefulness. Mean sleep depth was calculated by stages W, 1, REM, 2, and 3 quantified as 0, 1, 1.5, 2, and 3, respectively. Mean sleep depths 1, 2, and 3 are 2 hr average of sleep depth after going to bed, 2 to 4 hr, and 4 to 6 hr, respectively. Mean sleep depths T and E are 2 hr average of sleep depth after falling asleep and just before getting up. Mean sleep depth S was calculated by integration of sleep depth. Statistical differences of mean by Kruskal–Wallis test and Mann–Whitney test were found in stage W, sleep latency, mean δ wave %, mean sleep depth, gradient and intercept of regression line against time, mean sleep depth 1, S, and TST.

* $P < 0.05$ and ** $P < 0.01$ compared with the underlined value. In addition, [a] is statistically larger than [b] ($P < 0.05$). This experiment was conducted for 39 nights on a male subject.

TABLE 2
SPEARMAN'S RANK CORRELATION COEFFICIENTS OF 23 SLEEP PARAMETERS OF 39 NIGHTS ON A MALE SUBJECT

	Subject	SW	S1	S2	S3	REM	MT	Latency	Shift	Delta	Alpha
SW	0.495**										
S1	−0.111	0.028									
S2	−0.104	−0.541**	−0.214								
S3	0.041	−0.202	0.101	0.094							
REM	−0.302	−0.276	−0.363*	−0.348*	0.132						
MT	−0.030	0.144	−0.121	−0.183	0.092	0.133					
Latency	0.477**	0.904**	0.063	−0.588**	−0.334*	−0.257	0.300				
Shift	−0.118	0.034	0.400*	−0.374*	0.398*	0.152	0.483**	0.041			
Delta	0.116	−0.296	−0.288	0.445**	0.356*	−0.032	0.039	−0.346*	−0.107		
Alpha	0.227	−0.078	−0.010	0.227	0.525**	−0.155	−0.003	−0.210	0.139	0.474**	
Mean	−0.341*	−0.856**	−0.296	0.796**	0.233	0.127	−0.103	−0.843**	−0.205	0.512**	0.210
Gradient	0.540**	0.899**	0.030	−0.529**	−0.288	−0.271	0.136	0.924**	−0.072	−0.299	−0.111
Intercept	−0.482**	−0.914**	−0.109	0.634**	0.289	0.231	−0.148	−0.943**	−0.044	0.392*	0.143
EMG	0.325*	0.520**	−0.211	−0.330*	−0.031	−0.109	0.684**	0.616**	0.192	0.001	0.087
Mean 1	−0.420**	−0.911**	−0.139	0.681**	0.263	0.209	−0.219	−0.950**	−0.132	0.453**	0.209
Mean 2	−0.180	−0.138	−0.243	0.293	−0.022	−0.037	0.278	−0.129	0.021	−0.249	−0.083
Mean 3	0.343*	0.231	−0.110	0.143	−0.009	−0.278	−0.011	0.250	−0.061	0.102	−0.032
Mean T	0.010	−0.198	−0.350*	0.446**	0.060	−0.026	0.282	−0.145	−0.144	0.468**	0.107
Mean E	0.127	−0.112	−0.194	0.159	−0.191	−0.018	0.045	0.073	−0.204	0.051	−0.061
Mean S	−0.472**	−0.517**	0.082	0.369*	−0.012	0.149	−0.098	−0.468**	0.172	−0.025	−0.033
Bedtime	0.178	0.004	−0.175	0.013	0.091	−0.066	−0.136	−0.070	−0.190	0.389*	0.147
TST	−0.532**	−0.453**	0.230	0.196	0.092	0.197	−0.062	−0.424**	0.303	−0.127	−0.046

	Mean	Gradient	Intercept	EMG	Mean 1	Mean 2	Mean 3	Mean T	Mean E	Mean S	Bedtime
Gradient	−0.826**										
Intercept	0.907**	−0.975**									
EMG	−0.395*	0.558**	−0.515**								
Mean 1	0.931**	−0.913**	0.965**	−0.514**							
Mean 2	0.174	−0.202	0.214	0.153	0.128						
Mean 3	−0.005	0.176	−0.156	0.068	−0.204	−0.238					
Mean T	0.456**	−0.319*	0.362*	0.057	0.349*	0.213	−0.010				
Mean E	0.124	0.184	−0.093	0.176	0.046	0.029	−0.117	0.047			
Mean S	0.451**	−0.540**	0.468**	−0.551**	0.438**	0.132	0.029	0.138	0.079		
Bedtime	0.054	0.051	0.007	0.152	0.090	−0.148	−0.188	0.106	0.073	−0.554**	
TST	0.305	−0.503**	0.398*	−0.552**	0.337*	0.072	−0.027	0.006	−0.056	0.950**	−0.596**

Note. See Table 1 for abbreviations used. Latency, sleep latency; Shift, mean stage shift per hour; Delta, mean δ wave %; Alpha, mean α wave %; Mean, mean sleep depth; and Subject, subjective sleep. Mean 1, 2, 3, T, E, and S is mean sleep depth 1, 2, 3, T, E, and S, respectively. A correlation coefficient greater than 0.31 or 0.41 was statistically significant at $P < 0.05$ or 0.01, respectively.

quantification of each stage, particularly stage REM, and approximation of overnight stage change to the linear regression line.

The above three parameters were found to have the same meaning as sleep latency by correlation coefficients. Stage W was closely related to sleep latency

TABLE 3
PREDICTION RESULTS OF CANONICAL DISCRIMINANT ANALYSIS

Reported grades	Predicted grades			Total
	Good	Moderate	Poor	
Good	9	1	0	10
(%)	(90.0)	(10.0)	(0.0)	(100.0)
Moderate	3	21	0	24
(%)	(12.5)	(87.5)	(0.0)	(100.0)
Poor	0	0	5	5
(%)	(0.0)	(0.0)	(100.0)	(100.0)

Note. Overall correct rate = 89.74%.

TABLE 4
STANDARDIZED COEFFICIENT OF 16 SLEEP VARIABLES BY CANONICAL DISCRIMINANT ANALYSIS WITH CANONICAL CORRELATION COEFFICIENTS (CC)

	Axis 1	Axis 2
S1	1.660[a]	−1.955[a]
S2	3.042[a]	−3.950[a]
S3	0.707	−0.828
REM	2.608[a]	−3.331[a]
MT	−0.969	−0.069
Latency	1.092	−3.759[a]
Shift	−1.166	0.182
Delta	0.027	−0.206
Alpha	−0.403	−0.510
EMG	1.577[a]	0.407
Mean 2	−0.447	−0.079
Mean 3	−0.125	−0.523
Mean T	0.050	−0.491
Mean E	−0.480	−0.148
Bedtime	−0.151	0.019
TST	0.698	0.797
Canonical C.C.	0.821	0.712

Note. See Tables 1 and 2 for abbreviations used.
[a] Larger absolute values.

since there was little intrasleep or early morning wakefulness. Once the subject fell asleep, the subsequent mean sleep depth for some intervals did not change very often, independent of subjective sleep. These are findings for one case and do not deny the usefulness of mean sleep depth, gradient, and intercept of regression line against time on other subjects.

The relation between subjectively good sleep and increase in deep sleep, reported by Oguri *et al.* (1981), corresponds to the delta wave percentage of our

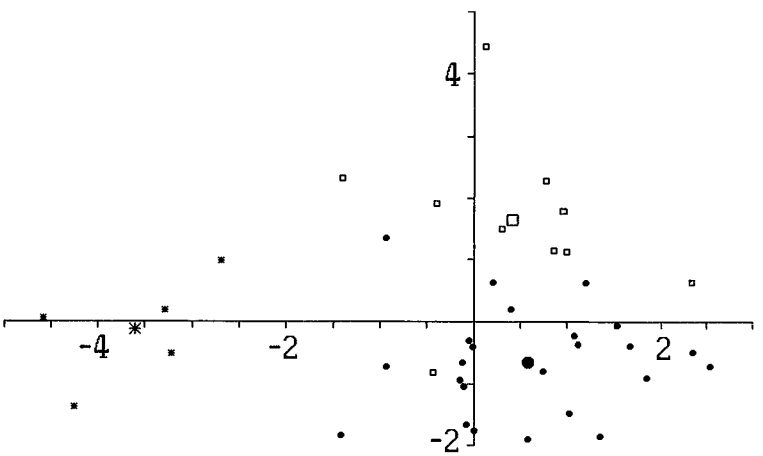

FIG. 1. Distribution of 39 nights of a male subject to predict the three grades, good (□), moderate (●), poor (*), of subjective sleep by canonical discriminant analysis.

results expressed by a nonlinear dose–effect relationship with the highest delta wave percentage in subjectively "moderate" sleep nights.

Eberhardt and Akselsson (1987), Bonnet and Johnson (1978), Violani and Cagnoli (1985), and Tanaka (1975) all found worsening of subjective sleep to correspond to an increase in stage W, as also noted in the present study. Violani and Cagnoli (1985) noted an increase in stage 1 by worsening of subjective sleep because stage 1 is shallow sleep that may shift to stage W. Decrease in stage 2 by poor subjective sleep was found by Tanaka (1975). The change in direction of stages 1 and 2 is the same as that in this study, partly because young subjects were used. There is controversy on REM sleep, and the stage REM appeared associated with subjective sleep in this study by canonical discriminant analysis.

Johns (1975) measured the sleep polygraphy of four male subjects for 11–12 nights and conducted a factor analysis of the sleep parameters. The factors for each of the four subjects were essentially the same and consisted of four factors: 1, sleep fragmentation and REM sleep; 2, length of sleep and stages 2; 3, sleep latency; and 4, delta wave sleep. Subjective quality of sleep was included in factor 1. Statistically, canonical discriminant analysis is an expansion of factor analysis. Subjective sleep was predicted using two axes, in which effective sleep parameters were stages 1, 2, REM, integrated voltage of EMG, and sleep latency. These parameters correspond to Johns' factors 1, 2, and 3.

Lesser slow wave sleep was noted in this subject, and stage 2 includes the element of deep sleep not judged as stage 3 or 4 and assessed by the delta wave percentage. Recently, Eberhardt and Akselsson (1987) proposed a division of stage 2 into three categories, stages 21, 22, and 23, in deepening order by delta wave percentage, which would increase parameter sensitivity. This subdivision corresponds to the delta wave percentage, and subjectively "moderate" sleep was higher than the other groups. This finding shows poor sleep to be related to deep sleep, but good sleep is not influenced by deep sleep.

From 39 nights' analysis of a male subject, worsening of subjective sleep was related to an increase in stage W, which emerged at sleep latency period, elongation of sleep latency, decrease in total sleep time, and delta wave percentage.

We substituted sleep latency for five parameters, the average sleep depth during the night and those of 2 hr after going to bed, gradient and intercept of regression line of sleep depth against time, and stage W. Substitution of total sleep time for the integrated value of sleep depth was also done for canonical discriminant analysis. Selected parameters of large standard coefficients were stages 1, 2, REM, integrated voltage of EMG, and sleep latency.

The above two sets of analyses show sleep latency to be the most useful parameter for predicting subjective sleep in this case, although the number of poor sleep nights may not be sufficient for the analysis. The meaning of the other sleep parameters selected by multivariable analysis should be clarified by analysis of other subjects with sufficient data.

CONCLUSIONS

Sleep quality reported by the sleeper himself was related to the polygraphic sleep parameters. The findings are as follows:

(1) Poor subjective sleep was related to an increase of stage W, elongation of

sleep latency, decrease of mean sleep depth associated parameters, and shortening of total sleep time. Slow wave sleep, which is the indicator of deep sleep, increased by moderate subjective sleep, not by good sleep.

(2) Stages 1, 2, REM, integrated voltage of electromyogram, and sleep latency were related to subjective sleep by multivariable analysis.

From the above results, sleep latency seems to be a useful and reliable parameter for predicting subjective sleep.

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Evaluation of the Critical Value of Driving Fatigue Based on the Fuzzy Sets Theory[1]

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Applying the newly developed multistage evaluation method based on the fuzzy sets theory, we have evaluated the sense of driving fatigue of 8 male drivers who covered 400 km in 8 hr in the daytime, empirically revealing the distribution of the degree of driving fatigue. The critical values from "no" to "a bit" and "a bit" to "fatigued" are 1 and 5.5 hr, respectively. Finally, a tentative study of the psychophysical relations between driving time and driving fatigue has been made, leading to a curve of driving fatigue under the conditions of this experiment.

INTRODUCTION

Driving fatigue is an important factor in traffic accidents. As estimated by Hulbert, traffic accidents on rural highways caused by driving fatigue constitute 35–50% of highway fatalities (McKenna, 1982). In China traffic accidents caused by driving fatigue occur occasionally, and most are grave in nature.

Factors affecting driving fatigue are of different types: somatic property, driving experiences, driving time, amount of sleep, emotional state of the driver, etc. As we know, long driving time and continuous long-distance driving tend to be the most important causes of driving fatigue leading to traffic accidents. Studies by Harris (1977) and Mackie and Miller (1978) showed that the number of hours driven may have a significant effect on the occurrence of accidents. They found that the number of accidents that occur in the second half of a 10-hr trip is twice that occurring in the first half. According to the results of a study by Hamelin, the accident risk for drivers behind the wheel for 14 or more hours was 2.5–3 times higher than that for drivers driving 10 hr or less (McDonald 1984). Therefore, many countries have strict restrictions on the driving time of drivers. Very few studies have been conducted in China, and still fewer studies have addressed the fatigue caused by long-distance driving. The purpose of this paper has been to evaluate the critical value of driving fatigue based on the fuzzy sets theory, with a view to ascertaining the relationship between the driving fatigue of a long-distance passenger car driver and the driving time under the present Chinese road conditions. It is hoped that these will serve as scientific bases for traffic safety management in their formation of policy and regulations regarding driving time.

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MATERIALS AND METHODS

Subjects

Eight male long-distance passenger car drivers with more than 3 years of working experience were studied. Their ages ranged from 23 to 48 and they were in good health.

Evaluation Scale

The evaluation scale is based on the fuzzy sets model of category judgment (Ma and Cao, 1983; Ma and Wang, 1985) and multistage evaluation categories of the sense of driving fatigue have been worked out. Two dimensions are contained in the scale: one is an ordered sense category scale and the other is the degree of response positivity. The categories of the sense of fatigue are "no," "a bit," "fatigued," "very," and "extremely." They are marked as 0, 0.25, 0.50, 0.75, and 1.00. Each category is defined as follows:

No fatigue: Feeling no fatigue, quite vigorous.

A bit fatigued: Feeling a bit fatigued, but still vigorous.

Fatigued: Decreasing in physical strength with poor concentration, but still able to hold on.

Very fatigued: Weak in physical strength and deficient in energy, unable to hold on.

Extremely: Utterly exhausted, immediate rest needed.

The degrees of response positivity are resolutely, basically, and slightly in both positive and negative attitudes. In evaluating the sense of driving fatigue during a certain duration of driving time, the driver is asked to select a certain category as well as to adopt a suitable degree of response positivity. The practical evaluation is performed with the test form that appears in Table 1.

The main points in Table 1 are the choice of the category that best describes one's sense of driving fatigue and the choice of the suitable degree of response positivity. By making a judgment on the two neighboring categories, one can surely make a positive or negative degree of positivity. Similarly, by marking another "√" in the corresponding column, one can go on to evaluate all the category levels of the sense of driving fatigue.

TABLE 1
SHEET FOR MULTISTAGE EVALUATION OF CATEGORY SCALE OF DRIVING FATIGUE

		No	A bit	Fatigued	Very	Extremely
	Resolutely			√		
Positive	Basically					
	Slightly				√	
	Slightly		√			
Negative	Basically					
	Resolutely	√				√

Generally speaking, the regulations for evaluating the scale are set so that every category is evaluated only once, without neglecting a single category or repeating a certain category.

Testing Procedure

To ensure consistency in conditions (such as the distance of travel and highways), we made the route from Shanghai to Yecheng our experimental route. Our working staff performed the experiment on this route. The experiment lasted from October 26 to November 4, 1989. The weather was fine during the entire period. The car set forth at 0630 in the morning and arrived at the destination at about 1600 in the afternoon, covering a distance of 400 km. It took about 8 hr, including time for meals and rest, with an average speed of about 50 km per hour. An evaluation of the sense of driving fatigue was made both before setting forth and after reaching the destination. During the trip an evaluation was made every 2 hr, for a total of five evaluations. JT-663 type passenger cars produced by Yangzhou Passenger Car Factory were used.

RESULTS

In Different Fatigue Categories Different Hours of Driving Resulted in Distribution of Response Positivity

Figure 1 demonstrates the subjective response of the sense of driving fatigue during a certain test time. The particular responses are characterized by different degrees of positivity in fatigue categories, i.e., a set of values, and not a definite single value. The fact shows that the response of a driver to driving fatigue is of positivity and not of probability in nature.

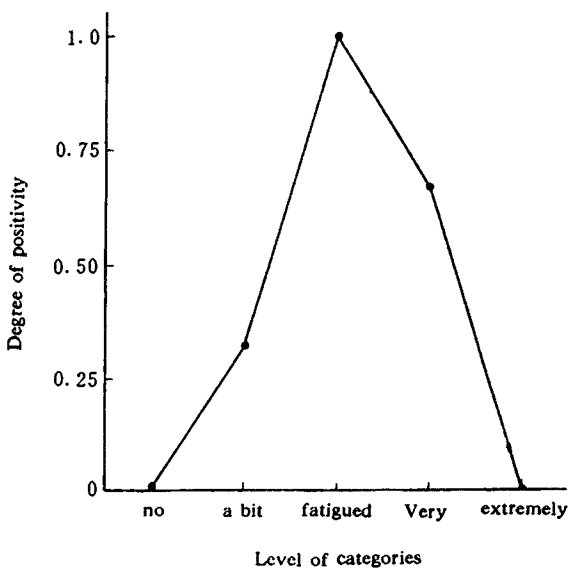

FIG. 1. Distribution of positivity of response of a certain driver after 8 hr of driving.

Owing to disparities in the sense of driving fatigue among drivers, the set values expressed by their responses are also different. Statistical analysis should be conducted on the responses of all subjects. All statistics conducted on set values are called set-valued statistics. Table 2 shows the set-valued statistic results acquired from the eight drivers in this experiment.

Critical Values of Different Categories of Driving Fatigue

Based on the data in Table 2, curves of response positivity representing the number of hours driven in different categories of driving fatigue can be drawn, as shown in Fig. 2.

Many intersecting points can be seen clearly from the direction of the curves in Fig. 2. However, some of the intersecting points are meaningful, while others are not. According to the rules of the interpolative threshold of the multistage evaluation method, even if the intersecting value of two neighboring category curves is greater than 0.5, a vertical line is drawn from that point on the abscissa. The stimuli value obtained from the interpolation can thus be regarded as the critical value or threshold value passing from one category to another. Therefore, from Fig. 2 we can interpolate the critical value from "no" to "a bit" as 1 hr, while the critical value from "a bit" to "fatigued" is 5.5 hr.

Psychophysical Relation between Number of Hours Driven and Degree of Driving Fatigue

The formula for calculating the degree of driving fatigue on the basis of empirical data using the multistage evaluation method is

$$R = \sum_{i=1}^{n} C_i S_i \Big/ \sum_{i=1}^{n} C_i,$$

where C_i represents the average degree of positivity of different kinds of senses of driving fatigue, acquired through experiment, and S_i represents the assignment of various kinds of senses of driving fatigue. According to the data of semantic measurement, five categories of sense of driving fatigue are designated as 0, 0.25, 0.50, 0.75, and 1.00, respectively. If we take the degree of driving fatigue derived from the above-mentioned formula as the ordinate value, with the corresponding

TABLE 2
DISTRIBUTION OF RESPONSE POSITIVITY OF DRIVING FATIGUE

Hours driven	No	A bit	Fatigued	Very	Extremely
Before driving	6.00 (1.00)	1.25 (0.21)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
2	2.25 (0.38)	5.88 (0.98)	1.50 (0.25)	0.00 (0.00)	0.00 (0.00)
4	1.25 (0.21)	5.63 (0.94)	2.88 (0.48)	0.50 (0.08)	0.00 (0.00)
6	0.13 (0.02)	3.63 (0.60)	4.50 (0.75)	0.50 (0.08)	0.00 (0.00)
8	0.00 (0.00)	1.75 (0.29)	6.00 (1.00)	2.75 (0.46)	0.00 (0.00)

Note. Data in parentheses are standardized.

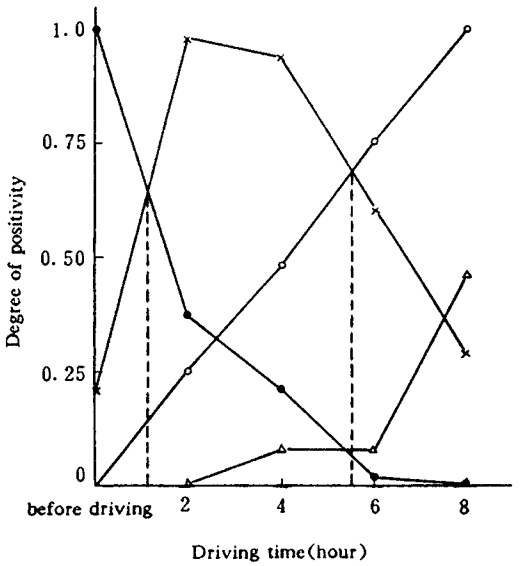

FIG. 2. Curves of positivity of categories of driving fatigue. Symbols used: ●, no; x, a bit; ○, fatigued; △, very.

time as abscissa, then we can draw a figure, thus deriving the curves of the degree of driving fatigue as shown in Fig. 3, i.e., the psychophysical functional relation between driving time and driving fatigue. It indicates that the degree of driving fatigue increases gradually with the continuation of driving time. Analysis of variance of the value of the degree of driving fatigue shows that the difference in

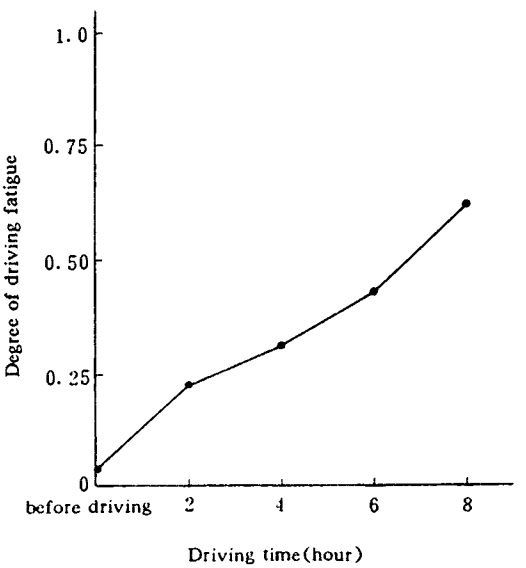

FIG. 3. Curve of the degree of driving fatigue.

driving fatigue between different durations of driving time is statistically significant ($P < 0.01$), as shown in Table 3. The results of an additional post hoc test, Tukey's least significant difference (LSD), demonstrate that the degree of driving fatigue between different durations of driving time was also significantly different ($P < 0.05$ or $P < 0.01$), as shown in Table 4. This fact indicates that the degree of the sense of driving fatigue between different durations of driving time differs greatly, with the degree of fatigue in the second half greater than that in the first half. Hence, driving fatigue is a process of gradual accumulation and development.

DISCUSSION

In the past, many evaluations of driving fatigue have been made with the help of the classical category scale method, but few studies on the critical threshold of driving fatigue have been conducted. This paper describes a study in which the newly developed multistage evaluation method based on the fuzzy sets theory was used to evaluate the sense of driving fatigue and the critical value on the part of long-distance passenger drivers. The results of this study are very similar to those of Kobayashi (He and Xin, 1989). The classical category scale method, although easy to handle and producing results of practical value, is not ideal from a methodological viewpoint. As pointed out in a review: "a well-known shortcoming of the traditional category scales lies in their incapacity to reflect the real process of sensory changes" (Morgan, 1983). We believe that driving fatigue is a rather fuzzy phenomenon; it is of positivity and not of probability in nature, which serves to be the foundation of the classical category scale. It is therefore more suitable to evaluate the sense of driving fatigue with the help of the multistage evaluation method based on the fuzzy sets theory.

In this study, by applying the multistage evaluation method, we have empirically obtained a distribution of positivity of driving fatigue as well as the critical value among the categories of driving fatigue. The critical values from "no" to "a bit" and "a bit" to "fatigued" have been obtained. However, we failed to obtain the critical values from "fatigued" to "very" or from "very" to extremely." This may be due to the time limitation (8 hr of driving with rest breaks). Therefore, further study is necessary.

Driving fatigue is conditioned by many factors. Highway conditions differ greatly in different countries. Therefore, the critical values of driving fatigue will also be disparate. Judging from the results of our study, we deem it appropriate to have driving time restricted to 8 hr per day in certain countries.

TABLE 3
ANALYSIS OF VARIANCE OF THE DEGREE OF DRIVING FATIGUE DURING DRIVING TIME

Source of variance	*df*	SS	MS	*F*	*P*
Between groups	4	1.081	0.270	47.73	<0.01
Within groups	35	0.198	0.0057		
Total	39	1.297			

TABLE 4
MEAN DEGREE OF DRIVING FATIGUE DURING DRIVING TIME

		Hours		
Hours	Initial value	2	4	6
2	0.187**			
4	0.270**	0.083*		
6	0.360**	0.173**	0.090*	
8	0.479**	0.292**	0.209**	0.114**

Note. Initial value, 0.04.
* $P < 0.05$.
** $P < 0.01$.

Driving fatigue is a gradual, accumulative process. The results of the research demonstrate that the degree of driving fatigue differs greatly according to the duration of driving, with the second half of an 8-hr trip producing a greater degree of driving fatigue than the first half. Thus, to prevent driving fatigue, the driver ought to take rest breaks after every 2 hr of driving. In this way, safety in driving can be improved and the number of traffic accidents caused by driving fatigue can be greatly reduced.

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Brain Imaging Techniques Applied to Chronically Solvent-Exposed Workers: Current Results and Clinical Evaluation[1]

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The application of neuroimaging techniques such as cerebral blood flow (CBF), single photon emission tomography (SPECT), X-ray computed tomography (CAT), and magnetic resonance imaging (MRI) on solvent-exposed workers and patients with toxic encephalopathy results in different and somewhat inconclusive pictures. The aim of this paper is to therefore critically review the current knowledge on chronic neurotoxicity of solvent exposures with respect to neuroimaging technique. CAT measurements of 86 house or construction painters, 82 spray painters, and 81 nonpainters showed no abnormal diffuse brain atrophy due to chronic solvent exposure after controlling for confounding variables such as age, alcohol consumption, or former disease. Correlation analyses did not show any consistent, biologically plausible exposure–effect relationship. Neuropsychologic test results did not correlate significantly with CAT parameters, whereas a strong age dependency exists. It is concluded that long-term exposure to solvent concentrations not exceeding permissible occupational limit values does not cause increased brain atrophy.

INTRODUCTION

During recent years, there has been an exponential increase in the number of publications relating to modern brain imaging techniques in studying central nervous system (CNS) disorders. Recently published reviews cite more than 200 original articles regarding brain and neuroimaging methods such as X-ray computed tomography (CAT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single photon emission tomography (SPECT) (Lang *et al.*, 1990; Alavi and Hirsch, 1991).

These aforementioned techniques differ in several important aspects, such as form of applicated energy, diagnostic value, and cost of examination. In the following some brief characteristics of these techniques are given. For detailed or specific technical information we refer to recently published monographs (Radü *et al.*, 1987; Huk *et al.*, 1988; Hartmann and Hoyer, 1985).

Other methods such as computerized EEG and evoked potentials—also described as brain imaging—are not considered further.

Computed tomography (CAT) of the brain is the result of digital reproduction of

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computer-analyzed x-ray refraction in biological materials, such as bone, brain tissue, liquid fluid. Because x-radiation is used, there is a direct relation between the Hounsfield unit (HU) upon which the image is based and the CT density of the substance imaged (range, −1000 to +1000 HU: −1000 for air, 0 for water, +1000 for bone). Present-day scanners have within-plane resolution of 4 to 5 mm when regions differ by less than 5 HUs in density, but less than 1 mm when the contrast (percentage difference between the object of interest and surrounding tissue) is maximum. Limitations caused by bone-hardening artifact, the effect of adjacent tissues of different density, window settings, and partial volume averaging of tissue within the width of the CT slice, have been reviewed extensively (for review, see DeCarli *et al.*, 1990).

Nuclear magnetic resonance (NMR or MRI) is based on the absorption of radio-frequency energy by the magnetic moments of atomic nuclei in samples placed in a strong magnetic field (Moonen *et al.*, 1990). It employs the reconstruction of data obtained when mobile protons of a tissue are excited by the application of an oscillating magnetic field in the radio-frequency range, to display an image of those data. In summary, the ensemble of nuclei is characterized by five parameters: (1) magnitude of the longitudinal magnetization, (2) phase and magnitude of the transverse magnetization, (3) resonance frequency, (4) longitudinal relaxation time T_1, and (5) transverse relaxation time T_2. A change in one of these parameters may be caused by a function, such as blood flow, and may thus be used to visually quantify that particular function (Moonen *et al.*, 1990). So-called "T_2-weighted" spin echoes provide information of the gray matter of the brain superior to CAT scan.

Positron emission tomography (PET) provides a method of measuring the metabolism, perfusion, and pharmacology of human organs *in vivo* (Brooks, 1991). A tracer tagged with a short-lived positron-emitting isotope is generated by a cyclotron and is administered intravenously, or by inhalation, to the subject. Quantitative tomographic images of regional cerebral function can be generated using established mathematical models from the scans of regional cerebral uptake with the knowledge of the arterial plasma tracer activity.

Measuring the oxygen and glucose metabolism has been widely used to investigate cerebral function and disease during the 10 years that PET has been used in humans. The brain uses these substrates as the sole providers of metabolic energy, and hence the consumption of glucose or oxygen, or both, reflects total neuronal function (Frackowiak and Jones, 1989).

PET technique is difficult (short-lived radionuclides), expensive because it needs a cyclotron or positron emitter generator system, and demands multidisciplinary teams (Frackowiak and Jones, 1989). Thus, at present the widespread clinical application of PET is limited (Jagust *et al.*, 1987).

Single photon emission computed tomography (SPECT), however, utilizes radionuclides that are capable of measuring cerebral function and are commercially available (Jagust *et al.*, 1987). One such tracer, *N*-isopropyl-*p*-iodoamphetamine (IMP), labeled with iodine-123, is distributed in brain in proportion to blood flow, and when scanned tomographically can provide three-dimensional information about rCBF (Jagust *et al.*, 1987; Podreka *et al.*, 1984; Sharp *et al.*, 1986). Another

tracer is hexamethylpropyleneaminoxin (HMPAO) marked with technetium-99m, which demonstrates a rapid and intensive binding to substrates (Podreka *et al.*, 1987). Despite of some advantages such as application, costs, and feasibility, SPECT has an important limitation, namely the measured activities are relative and not absolute values (English and Brown, 1986; Rootwelt *et al.*, 1986). The overall results depend therefore on the blood flow in the region of interest (ROI) and do not necessarily indicate disturbed brain metabolism, rather than perfusion (Deisenhammer *et al.*, 1989; Heiss *et al.*, 1988).

Despite this great amount of scientific literature, there is relatively little and additionally inhomogeneous knowledge about brain-imaging results in workers with long-term exposures to organic solvents.

Our purpose is to critically review the literature and

(1) to discuss our own experiences in studying solvent-exposed workers with CAT,

(2) to evaluate current knowledge according to chronic neurotoxicity in occupationally exposed populations, and

(3) to discuss feasibility of the neuroimaging methods in further studies on neurotoxicity of solvents to human brain.

Other areas of solvent neurotoxicity such as alcoholism, solvent abuse, or acute intoxications, which are also important, are not further considered.

SUBJECTS AND METHODS

In two multidisciplinary retrospective studies we examined

- 105 construction and house painters,
- 105 spray painters, and
- 111 control persons

to evaluate neurotoxic effects due to a chronic exposure to organic solvents. In addition to clinical, neurological, and neuropsychological as well as neurophysiological examinations, a CAT scan without application of contrast fluid was performed on a Siretom 2000 E or Somatom DR 2 (Siemens, Erlangen).

According to data given in literature, the following CAT parameters were determined (Gosling, 1955; Meese and Grumme, 1980; Claus and Aschoff, 1982; Nagata *et al.*, 1987; Tavaras and Wood, 1976).

1. Cella media index (CMI): The CMI is the quotient of the smallest cross-sectional diameter of the lateral ventricles and the maximal transverse diameter of the cranial fossa in the same section. CMI quantifies alterations in the region of the corpus ventriculi (Meese and Grumme, 1980).

2. Greatest transverse diameter of the third ventricle: This parameter is a direct measure of the width of the third ventricle in its middle section (Gosling, 1955; Tavaras and Wood, 1976).

3. The sum of the transverse diameters of three cerebral sulci (pre-, post-, and central), so-called "Sigma": These parameters are a measure of the atrophy of the cerebral cortex. The width of individual sulci reflects the local intensity of the process. The sum of the cross-sectional diameters indicates the extent of atrophy.

4. Number of vermis sulci: This parameter gives the degree of atrophy in the medial cerebellum.

5. Number of cerebellar sulci: This parameter detects atrophy of the cerebellar cortex.

To determine the distances, an evaluascope was used. The rating was blinded. A pooling of the data and an overall evaluation was for methodologic reasons not possible because the rating of painters and spray painters was not done by the same person.

With respect to the aim of these studies, several confounding factors were controlled, such as head injuries, alcohol abuse, and hypertension. Subjects with those nonoccupational risk factors were excluded from further analyses.

According to the methods of the studies it was possible to analyze for possible associations between CAT findings and neuropsychologic test results (Triebig, 1989). This was done only for spray painters. In case of CAT parameters the CMI, the diameter of the third ventricle and "sigma" (sum of transverse diameter of pre-, post-, and central cerebral sulci) was chosen. Neuropsychological variables were short-term memory (KAI-TR), speed of information processing (KAI-CK), quotient of fluid intelligence (KAI-IQ), quotient of memory (KAI-GQ), crystallized intelligence (WUT-B), speed of information (d2-Test), and choice reaction time (Lehrl and Weidenhammer, 1989; Lehrl *et al.*, 1980).

Statistical Analysis

For statistical analysis of the data and for comparison of the two-tailed Student's test or the U test according to Wilcoxon, Mann, and Whitney, and the H test according to Kruskal and Wallis were used, respectively. To test for the presence of statistical correlations, rank correlation and regression analyses were applied. For all tests, P value < 0.05 was considered as the level of statistical significance.

RESULTS

The results of CAT-scan measurements in painters are given in Table 1. An evaluation of abnormal findings revealed no statistical significant differences, although the number of increased CMI is higher in painters (see Table 2). Normal values were defined as mean value plus/minus twice standard deviation in nonpainters. In case of number of cerebellar and vermis sulci, references from literature were used (Claus and Aschoff, 1982). In painters correlation analysis does not reveal any consistent and plausible "dose/effect" relationships using as

TABLE 1
CAT PARAMETERS IN PAINTERS AND CONTROLS

	Painters[a] (N = 86)		Controls[a] (N = 39)	
Parameter	Mean	SD	Mean	SD
Cella media index	187	(39)	177	(33)
Diameter of third ventricle (mm)	4.8	(2.0)	4.8	(2.2)
Sigma (mm)	8.3	(2.4)	8.8	(2.5)

[a] All differences are not statistically significant ($P > 0.05$).

TABLE 2
NUMBER AND PERCENTAGE OF ABNORMAL FINDINGS IN CAT

Parameter	Normal value[a]	Painters[b] (N = 86)	Nonpainters[b] (N = 39)
Cella media index	243	10/11%	2/5%
Diameter of third ventricle (mm)	9	4/4%	2/5%
Sigma (mm)	14	4/4%	2/5%
No. of vermis sulci	$N > 1$	29/33%	20/53%
No. of cerebellar sulci	$N > 2$	3/3%	3/8%

[a] Defined as mean value plus/minus twice standard deviation in nonpainters.
[b] All differences are not statistically significant ($P > 0.05$).

"dose" the duration and level of exposure and "chronic exposure index (CEI)," and as "effect" the "cella media index" (Triebig *et al.*, 1988).

In Table 3 the results of CAT scan for spray painters are shown. A comparison demonstrates a significant difference only for the parameter "cell media index (CMI)."

Spray painters have a higher mean value and thus a higher atrophy index. There are no significant differences for the other CAT parameters. Number and percentage of abnormal findings are given in Table 4. Although the CMI was exceeded in 11 spray painters compared to 2 nonpainters, the difference is statistically not significant. For the other CAT parameters no significant differences are obvious.

Further statistical analyses did not demonstrate any significant association between CAT parameters and parameters of exposure such as years working as spray painter or various cumulative exposure indices (Lang and Erbguth, 1989).

On the other hand the relationships between age and CMI ($r = 0.457$, $P < 0.001$) as well as between age and diameter of the third ventricle ($r = 0.266$, $P < 0.01$) are significant. Because of the fact that the duration of exposure correlates significantly with age ($r = 0.790$, $P < 0.001$), the above-mentioned associations must be seen in this context.

Correlation analyses between three CAT parameters and various neuropsychological variables, which are regarded as sensitive to organic brain dysfunction, demonstrate only a few significant results over and above age (see Table 5).

TABLE 3
CAT PARAMETERS IN SPRAY PAINTERS AND CONTROLS

	Spray painters (N = 82)		Nonpainters (N = 42)	
CAT parameter	Mean	SD	Mean	SD
Cella media index (CMI)	227	30[a]	119	34[a]
Diameter of third ventricle (mm)	4.5	1.7	4.0	1.7
Sigma (mm)	8.1	2.6	8.2	2.5

[a] Difference statistically significant (t value, 4.7).

TABLE 4
NUMBER AND PERCENTAGE OF ABNORMAL FINDINGS IN CAT SCAN

CAT parameter	Normal value[a]	Spray painters[b] (N = 82)	Nonpainters[b] (N = 42)
Cella media index	260	11/13%	2/5%
Diameter of third ventricle (mm)	7.5	12/14%	5/12%
Sigma (mm)	13	4/5%	2/5%
No. of vermis sulci	$N > 1$	49/60%	26/62%
No. of cerebellar sulci	$N > 2$	7/9%	8/19%

[a] Defined as mean value plus/minus twice standard deviation in nonpainters.
[b] All differences are not statistically significant ($P > 0.05$).

After controlling for age, the overall analyses do not show any relevant associations between these variables (Weidenhammer *et al.*, 1989).

DISCUSSION

The CAT of the brain images cerebral structures according to the density. The CAT produces accurate neuroanatomic images sensitive to changes in brain anatomy (Lee and Krishna, 1987). Previously there have been several studies indicating that CAT findings correspond to clinical overt neurologic conditions such

TABLE 5
CAT PARAMETERS AND NEUROPSYCHOLOGICAL VARIABLES IN 72 SPRAY PAINTERS (SP) AND 41 NONPAINTERS (NP)

Neuropsychological variable	CAT parameter					
	CMI		Diameter of third ventricle		Sigma	
	SP	NP	SP	NP	SP	NP
Short-term memory (KAI-TR)	−0.18	−0.07	−0.05	−0.14	−0.06	−0.20
Speed of information processing (KAI-CR)	−0.07	−0.23	−0.23[a]	−0.14	0.08	−0.18
Fluid intelligence (KAI-IQ)	0.12	−0.23	−0.17	−0.21	0.03	−0.29[a]
Quotient of memory (KAI-GQ)	−0.13	−0.11	−0.05	−0.03	0.01	0.06
Crystallized intelligence (MWT-B-IQ)	−0.10	0.21	−0.04	0.05	0.30[b]	0.20
Speed of information (d2-GZ-F)	−0.07	−0.20	0.00	−0.22	0.03	−0.32[a]
Choice reaction time	0.11	−0.04	−0.15	0.16	0.04	0.10
Age	0.07	0.40[b]	0.27[a]	0.42[b]	0.46[b]	0.69[b]

Note. The analyses were performed separately for 72 spray painters (SP) and 41 nonpainters (NP) (For abbreviations see text).
[a] $P < 0.05$ (one-sided).
[b] $P < 0.01$ (one sided).

as primary degenerative diseases of the brain or those in alcoholics (Cala *et al.*, 1983; Cala 1987; Eslinger *et al.*, 1984; De Leon *et al.*, 1979; Diener *et al.*, 1986). However, the brain functions, as measured by neuropsychological tests, do not or only weakly correlate with their morphological aspects in healthy and not demented persons, as well as in alcoholics (Bigler *et al.*, 1989; Moore *et al.*, 1989; Pfeiffer, 1985).

The brain is subject to physiological age atrophy (Nagata *et al.*, 1987). This fact became obvious in our study. In contrast to nonpainters, the CMI was not subject to a measurable age influence in spray painters. On the other hand, the CMI is the only variable that differs significantly between spray painters and nonpainters. In painters, however, only a tendency was seen. Correlation analysis revealed no dose–effect relationship, which is in general a valid toxicological aspect (Gossel and Bricker, 1990; Mayer, 1989; Strubelt, 1990). Therefore, the hypothesis of a neurotoxic effect is not supported.

In the current literature the results of neuroradiologic examination in solvent-exposed workers are inhomogeneous. In Table 6 a survey for the time period from 1979 to 1990 is given. With respect to chemicals, persons studied and neuroradiologic methods, the given results are different and not directly comparable.

Pneumoencephalography (PEG) was done before CAT was available to assess for brain atrophy. The PEG allows imaging mainly of the inner ventricular system. In comparison to CAT the diagnostic sensitivity is lower. Therefore, PEG results are not further discussed.

Although some authors found in clinical cases with suspected or proven chronic toxic encephalopathy a mild to moderate brain atrophy in the CAT scan (Gregersen *et al.*, 1987), this could not be confirmed in a recent Swedish study (Orbaek *et al.*, 1987). It is commonly agreed that solvent exposure levels in the workplaces do not result in abnormal brain atrophy (WHO, 1985; Cranmer and Golberg, 1985). In accordance with our results a former Swedish study in solvent-exposed painters and spray painters did not demonstrate any significant brain atrophy (Elofsson *et al.*, 1980; Triebig *et al.*, 1988; Lang and Erbguth, 1989). This seems to be also in accordance with the study of Danish painters (Mikkelsen *et al.*, 1988). The results were interpreted as follows: "Solvent exposure was associated with both cortical and central CT variables and the results suggest that a diffuse cerebral atrophy may be caused by occupational solvent exposure, high alcohol consumption, and age" (Mikkelsen *et al.*, 1988). Although a dose–effect relationship was described, the number and frequency of "abnormal" CAT findings in relation to exposure was not presented.

In contrast to our results, in the Danish study "the degree of dementia, dyscoordination, and cerebral atrophy increased significantly with the degree of solvent exposure" (Mikkelsen *et al.*, 1988). This conclusion is, however, based on a correlation analysis of a subsample of the total cohort including 46 painters and 34 bricklayers without any differentiation between solvent-exposed painters and unexposed controls.

Actual results of NMR examinations seem to confirm the CAT findings, assuming an association between chronic solvent intoxication and mild brain atrophy (Lorenz *et al.*, 1990; Aaserud *et al.*, 1990; Myint, 1990). With respect to the

TABLE 6
SURVEY OF NEURORADIOLOGIC FINDINGS IN SOLVENT-EXPOSED WORKERS AND IN PATIENTS WITH TOXIC ENCEPHALOPATHY

Year	Authors	Study population	Method	Main results
1979	Arlien-Søborg *et al.* (Denmark)	50 painters with suspected solvent intoxication	PEG (N = 12) CT (N = 38)	Brain atrophy in N = 25 (PEG = 12, CT = 13)
1980	Juntunen *et al.* (Finland)	37 patients with suspected solvent intoxication	PEG	Cerebellar atrophy, N = 5 Cerebral atrophy, N = 23
1980	Elofsson *et al.* (Sweden)	73 car painters	CAT	No significant brain atrophy
1982	Arlien-Søborg *et al.* (Denmark)	9 painters	CAT CBF	No or minimal cerebral atrophy No differences in CBF in painters and controls
1986	Triebig *et al.* (Germany)	105 painters	CAT	No cerebral atrophy
1987	Orbaek *et al.* (Sweden)	32 Patients with toxic encephalopathy	CAT	No cerebral atrophy
1987	Gregersen *et al.* (Denmark)	4 patients with toxic encephalopathy	CAT PEG	Mild atrophy in 3 cases
1988	Mikkelsen *et al.* (Denmark)	46 painters	CAT	Positive correlations between solvent exposure and cortical and central CT variables
1989	Lang and Erbguth	105 spray painters	CAT	No significant association between solvent exposure and CAT variables
1990	Lorenz *et al.* (Germany)	13 patients suspected tetrachloroethene intoxication	NMR	Mild to moderate cerebral atrophy in 12 patients
1990	Aaserud *et al.* (Norway)	16 rayon viscose workers	CAT SPECT	Brain atrophy in 13 workers No pathologic findings
1990	Myint (USA)	14 patients	NMR	?

other neuroimaging methods, CBF/SPECT and PET, respectively, at present only a few studies in small numbers of solvent-exposed workers have been published (Arlien-Søborg *et al.*, 1982; Aaserud *et al.*, 1990; Risberg and Hagstadius, 1983).

The overall impression is that the results may indicate some evidence of disturbances of brain blood flow related to influences of organic solvents. Regarding the relatively wide range of CBF in healthy persons between 45 and 54 ml/100 g/min (Shirahata *et al.*, 1985), the results in the Swedish study are considered to be "normal" (Risberg and Hagstadius, 1983). From the viewpoint of neurotoxicology it is unclear if the CBF demonstrates effects of acute or chronic solvent exposures. Furthermore, it is of interest whether solvents alter brain matter, blood–brain barrier, or blood vessels. To our knowledge at present no studies using PET in solvent-exposed workers or in patients with suspected solvent intoxication have been published.

CONCLUSION

In summary the following conclusions may be drawn:

(1) On the basis of the evaluation of the CAT scan in 210 solvent-exposed workers no increased frequency of abnormal brain atrophy exceeding age-related effects was seen. No statistical association between solvent exposure and CAT parameters was found.

(2) Data from literature are somewhat inhomogeneous with respect to type of chemical structure of the solvents, examined persons or groups, and neuroimaging technique.

(3) Comparisons with other fields of application of neuroimaging techniques should be helpful for interpretation of solvent and related results (Burns, 1990; Besson, 1990; Bench *et al.*, 1990; Geaney and Abou-Saleh, 1990).

(4) For further investigations it seems necessary to examine

- the prognosis of abnormal findings in neuroimaging methods,
- the correlations between neuroimaging findings and clinical as well as neuropsychological parameters,
- possible associations between morphological and functional results after application of neuroimaging techniques in patients with suspected solvent-induced encephalopathy (Alavi *et al.*, 1985). At present these questions cannot be answered; however, this will provide further aspects in the puzzling field of solvent neurotoxicity in humans.

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Three-Dimensional Brain Metabolic Imaging in Patients with Toxic Encephalopathy[1]

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Thirty-three workers, ages 24 to 63, developed clinical toxic encephalopathy after exposure to neurotoxins and were studied by SPECT brain scans. Five were exposed to pesticides, 13 were acutely exposed to mixtures of solvents, 8 were chronically exposed to mixtures of hazardous wastes that contained organic solvents, 2 were acutely exposed to phosgene and other toxins, and 5 had exposures to hydrogen sulfide. Twenty-nine had neuropsychological testing and all had a medical history and physical. Of the workers who had a clinical diagnosis of toxic encephalopathy, 31 (93.9%) had abnormal SPECT brain scans with the most frequent areas of abnormality being temporal lobes (67.7%), frontal lobes (61.3%), basal ganglia (45.2%), thalamus (29.0%), parietal lobes (12.9%), motorstrip (9.68%), cerebral hemisphere (6.45%), occipital lobes (3.23%), and caudate nucleus (3.23%). Twenty-three out of 29 (79.3%) neuropsychological evaluations were abnormal. Other modalities when performed included the following percentages of abnormals: NCV, 33.3%; CPT sensory nerve testing, 91.3%; vestibular function testing, 71.4%; olfactory testing, 89.2%; sleep EEG analysis, 85.7%; EEG, 8.33%; CT, 7.14%; and MRI brain scans, 28.6%. The complex of symptoms seen in toxic encephalopathy implies dysfunction involving several CNS regions. This series of patients adds to the previous experience of brain metabolic imaging and demonstrates that certain areas of the brain are typically affected despite differences in toxin structure, that these lesions can be globally defined by SPECT/PET brain scans, that these lesions correlate well with clinical and neuropsychological testing, and that such testing is a useful adjunct to previous methods. EEG and structural brain imaging such as CT and MRI are observed to have poor sensitivity in this type of patient. Additional metabolic imaging studies need to be done to explore dose, time, and specific toxin effects as well as mechanisms of toxicity and olfactory migration.

INTRODUCTION

It has been estimated that in the United States alone, over 9 million individuals are exposed to neurotoxins in the workplace (Current Intelligence Bulletin, 1990). Because of their special affinity for lipid-rich tissues, including brain tissue, neurotoxins such as organic solvents have been implicated in psychiatric, cognitive, and somatic disorders (Baker *et al.*, 1985; Husman, 1980; Juntunen *et al.*, 1980; Struwe and Wennberg, 1983). A topology describing encephalopathy has been proposed (Baker and Fine, 1988; Cranmer and Goldberg, 1986). In the mildest

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form, Type 1, symptoms consist of fatigue, impaired concentration, and loss of initiative and are believed to be reversible if exposure is terminated. At the next level (Type 2A/2B), decreased intellectual function and changes in mood and affect are noted. Subtle neurological signs may be present and reversibility is questionable. The most severe disorder, Type 3, typically follows years of occupational exposure or voluntary abuse and results in irreversible neuropsychiatric and neuroradiological abnormalities (Baker *et al.*, 1985; Cranmer and Goldberg, 1986).

Attributing physical and cognitive deficits to neurotoxin exposure has been complicated by several factors. First, there currently exists no well-established biological markers of most neurotoxins. Accurate measurements, i.e., "body burden," are difficult to obtain because of the small quantities involved and the short half-life of many toxins. When a measurable quantity of toxin is observed, clinical relevance is often difficult to determine. Second, many workers reporting to an occupational medicine clinic are involved in litigation and, therefore, the possibility of symptom exaggeration exists. Finally, epidemiologic studies have not demonstrated consistent neuropsychiatric impairment following toxic exposure (Eskanazi and Maizlish, 1988). Many health professionals maintain that the effects of solvents are reversible; when symptoms persist they are often regarded as being psychogenic in origin. New technologies are needed to shed light on these issues. One technology that shows promise is neurofunctional imaging which has been demonstrated in several cases including organic solvents, MPTP, manganese, and the halogenated solvent, tetrabromoethane.

In cases of intoxication resulting from occupational exposures via inhalation of manganese dust, with resulting clinical signs and symptoms of extrapyramidal lesions, PET scans have demonstrated decreased glucose uptake in cortical structures (Wolters *et al.*, 1989). Patients with a diagnosis of toxic encephalopathy associated with exposure to organic solvents have been evaluated with xenon cerebral regional brain blood flow techniques and were shown to have decreased blood flow to the frontal temporal areas (Hagstadius *et al.*, 1989).

Studies of patients exposed to MPTP, via PET, demonstrate lesions to the caudate nucleus/nigrostriatal nerve endings (Perlmutter *et al.*, 1987; Calne *et al.*, 1985).

A patient exposed to tetrabromoethane was evaluated with PET using F-2-deoxyglucose (FDG) and was found to have decreased glucose uptake to both cortical and subcortical areas. In this patient, both computerized tomography (CT) and magnetic resonance imaging (MRI) scans were negative (Morrow *et al.*, 1990).

These previous attempts indicate that imaging of brain metabolic functions, i.e., functional imaging, is informative and provides useful clinical information. In contrast, CNS structural imaging studies such as MRI or CT are frequently negative in neurotoxic cases (Arezzi *et al.*, 1989) In fact, structural imaging such as CT and MRI has often proven to be negative in contrast to positive functional imaging, i.e., PET, in cases with a clear mechanism of nervous system damage and demonstrable neuropsychological deficits, i.e., head trauma (Ruff *et al.*, 1989). MRI creates an image of the central nervous system by allowing mag-

netic and electrical fields to interact with the protons (hydrogen atoms) in tissue water and the resultant MRI image reflects differences in proton or water density (H_2O) throughout the tissue being studied (Bradley *et al.*, 1985). Computerized tomography illuminates a target tissue from different angles with beams of X rays to produce a 3-D representation of tissue structure via X-ray attenuation density throughout the tissue understudy. Unlike PET, SPECT imaging is an imaging method developed and improved over the last 25 years which is currently available in most clinical nuclear medicine departments. Current methods use an Anger scintillation camera and tomographic software to provide cross-sectional imaging via movement of the camera about the patient to detect X rays emanating from biotracers tagged with the single photon emitters, iodine-123, or technetium-99m. The overall effect of this imaging system is to provide a direct measure of microscopic, regional brain function via the perfusion of the radiopharmaceuticals across the blood–brain barrier and via cerebral distribution that is proportional to regional brain blood flow. At present, SPECT technology approaches that of PET in resolution and has been proven to have good clinical correlation with neurological conditions such as cerebral infarction, Alzheimer's disease, and in describing epileptic foci (Holman and Tumeh, 1990). The success of SPECT is not dependent upon a specific disease process but is dependent upon SPECT's ability to measure the disruption of regional brain blood flow by any disease process.

Most SPECT systems at present do not provide quantitative measurements; thus, the scans must be read by trained physicians according to principles applicable to any such interpretative method, e.g., symmetry and expected patterns of intensities. The patterns of intensities are very similar to those expected from basic neuroanatomy making interpretation relatively easy. In the data presented in this article, only two false negatives and no false positives were found. Therefore, our experience suggests a tendency for such qualitative readings to underreport abnormalities on SPECT scans. Quantitative analysis of SPECT scans by future devices would serve to supplement qualitative interpretations and would increase the sensitivity of the instrument since abnormalities could then be determined by subtle changes in regional intensity, as compared to a reference group. Current qualitative methods of interpretation use to using gross changes in intensity to demarcate abnormality.

MATERIALS AND METHODS

Definition of Study Group

The subjects in this study presented to the Med-Health, Ltd. Occupational Medicine Clinic for the first time between March 1988 and March 1992 with central nervous system complaints after exposure to chemicals in the workplace. Except for one of the subjects, none had an alcohol intake exceeding, on average, two drinks per day. All patients met the criteria for Type 2A/2B toxic encephalopathy (i.e., sustained personality or cognitive changes). All patients (29 men, 4 women) were white, native English speakers with a mean age of 41.9 years with a range of 19 to 63 years at the time of study. The range of ages at exposure was 18 to 61 years with a mean of 38.7 years. Estimates of exposure were assessed by

a detailed lifetime job history questionnaire. All patients had a complete medical history and physical examination with a review of their medical records. All were diagnosed as having toxic encephalopathy based upon the following criteria:

(1) A history of an exposure to neurotoxic agents with the proper temporal relationship to other pertinent clinical facts as below.

(2) A history of an absence of any significant confounding factors or significant illnesses.

(3) A lack of significant preexposure signs and symptoms.

(4) By having significant postexposure signs and symptoms.

(5) A physical examination most compatible with neurotoxicity and least compatible with any other diagnoses.

(6) Test results compatible with diffuse neurological dysfunction and consistent with patient symptoms.

All 33 patients had SPECT brain scans and 2 had both SPECT and PET. Other testing relevant to their evaluation was done when possible or the data was taken from the patients' medical records. Those tests include ENG (electronystagmography), ABR (auditory brain stem response), fine color discrimination by the 15 hue Lanthony test (Mergler *et al.*, 1990), neuropsychological assessment, olfactory discrimination as measured by the University of Pennsylvania smell identification test (Doty *et al.*, 1984), comprehensive (two nights) sleep analysis, EEG (electroencephalograph), and peripheral quantitative nerve sensory function by electrical current perception threshold. The PET method using 18-F-deoxyglucose has been previously described in detail (Morrow *et al.*, 1990).

Sleep analysis was performed by hospital-based sleep labs and was analyzed by a neurologist. It consisted of monitored sleep with EEG, respiratory effort, oxygen saturation, EKG, respiratory rate, and respiratory flow.

The SPECT method used Amersham Corporation technetium-99m hexanemethylpropylene amine oxime (^{99m}Tc HMPAO) and a rotating General Electric Starcam 400 AC/T digitally integrated camera/computer system with 360° acquisition in a circular orbit at 128 stops of 10 sec each for a total of 3–5 million counts. The cine display of the tomographic images was assessed for patient motion. In case of significant patient motion, the study was repeated. Small motion artifacts of less than a pixel were corrected using special software. The tracer was reconstituted by diluting 25–30 mCi ^{99m} sodium pertechnetate from ^{99}Mo/^{99m}TC generator with 5 cc of 0.9% normal saline solution. The patient was injected within 30 min of the dose preparation. Hexamethylpropylene amine oxime (HMPAO) is a neutral, lipid soluble compound with a molecular weight of 384 Da. ^{99m}Tc HMPAO acts as a "chemical microsphere," crossing the blood–brain barrier and remaining trapped in brain tissue, with a distribution proportional to blood flow. Approximately 5% of the injected dose of radiotracer localizes in the brain. ^{99m}Tc HMPAO does not exhibit intracellular redistribution patterns over time. More than 70% of the initial brain uptake remains fixed in the brain up to 24 hr postinjection.

Imaging was done 30 to 60 min after iv injection of the radiotracer. Coronal, sagittal, and transaxial images were reconstructed using a Butterworth prefilter, ramp back projection filter and an attenuation coefficient of 0.11. Each image was

analyzed by visual inspection for regional blood flow distribution patterns by two board-certified nuclear medicine physicians, one of whom read the scan without any clinical history. If the independent readings varied, a consensus was reached by mutual discussion.

NCV, EEG, and ABR were obtained through various medical clinics and hospitals and were performed according to standard clinical techniques.

Peripheral sensory threshold testing was done by CPT (current perception threshold) using a device called a Neurometer. Various levels of electrical current at 5, 250, and 2000 Hz were applied to eight locations corresponding to the of the trigeminal, ulnar, median, and peroneal nerves. The current was applied in such a manner as to determine the threshold of current required to produce a sensation. Placebo trials were provided by the Neurometer with the patient blind to the current settings. The definition of a threshold level required at least three current readings of the same value (Bleeker, 1985). Any measurable hypoesthesia or hyperesthesia was counted as abnormal if the final graded score was 5 or more on one or more sites.

Neuropsychological Assessment

Neuropsychological examinations were conducted on 24 patients at the Med-Health clinic using the Pittsburgh Occupational Exposures Test (POET) battery and five patients had neuropsychological test results available via their medical records. The POET battery consists of a number of well-known learning, memory, visuospatial, attention, and motor skills tests that have been shown to be sensitive in detecting impairments in groups of brain-damaged individuals (Lezak, 1983). On the basis of a factor analysis of 182 nonexposed male blue-collar workers, POET component tests were clustered into five functional categories: general intelligence, visuospatial processes, learning and memory, attention and mental flexibility, and psychomotor speed/manual dexterity. Based on the healthy blue-collar normative sample, means and standard deviations for each neurobehavioral test have been established for four age groups (21–30, 31–40, 41–50, 51+). Therefore, t scores can be calculated (mean = 50; standard deviation = 10) for each subject and an average obtained for the five general categories. Detailed descriptions of the normative data and administration procedures for the individual tests are provided elsewhere (Ryan *et al.*, 1987).

Clinical Summary and Exposure Data for 33 Case Studies

The following chronic symptoms were present in essentially all patients: Confusion, numbness, depression, memory loss, headaches, imbalance, fatigue, decreased attention, decreased concentration, difficulty making decisions, mood swings, disorientation, slow mental functions, increased anxiety, increased perspiration, personality changes, dizziness, weakness, malaise, night vision decreased, frustrated easily, incoherent thoughts, abnormal eye movements, heat intolerance, taste decreased, decreased exercise tolerance, visual disturbances such as blurry vision and decreased peripheral vision, emotionally labile, tingling sensation or numbness, loss of libido, bad temper, easily startled, difficulty thinking, bizarre ideation, vertigo, flu-like symptoms, insomnia, and tinnitis.

These chronic symptoms were present in many, but not all, of the patients: Increased thirst, myalgia, sense of smell decreased, impotence, hot flashes, syncope, diplopia, photophobia, weight gain, palpitations, seeing flashing lights, sleep apnea, nystagmus, paresthesia, tight muscles, bladder spasms, muscle cramps, visual, tactile or auditory hallucinations, euphoria, nausea, emesis, hypertension, feverish, chills, salivation increased, ataxia, tremors, myoclonus, stuttering, seizures, tachycardia, arrhythmias, dysphagia, arthralgia, alcohol intolerance, muscle fasiculations, low body temperature, suicidal ideation, voice loss, paranoia, poor calculation ability, hoarseness, dyspnea, transiently paralyzed upon awakening, and nightmares.

The following is a description of exposure, acute and chronic symptoms unique to each patient, physical exam and confounding factors, if applicable.

Patient 1, DOB 02/04/33, was exposed 11/1976 and was first examined on 06/29/87, 138 months postexposure.

Creosote was used to paint the inside of room with poor ventilation. The patient was exposed to strong creosote fumes for about 8 hr.

Acute symptoms: First- and second-degree skin burns, hoarseness, and respiratory distress requiring hospitalization.

Unique chronic symptoms: Hair brittle, arthritis, intolerance to odors, rash, respiratory distress due to bronchospasm.

Physical exam: Decreased strength in hands, depressed affect, wheezes in lung, slight tremors, increased sway on Romberg.

Confounding factor: Motor vehicle accident with neck strain and headaches, 1987.

Patient 2, DOB 09/08/37, was last exposed 03/01/88; she was examined on 05/30/90, 27 months postexposure.

She was exposed to varsol used to clean floors since the 1950s and became symptomatic in the late 1960s. Her symptoms reached their maximum in 1988 after being exposed at work to a pesticide used to control book mites at which time she had an extreme exacerbation.

Unique chronic symptoms: Bronchial asthma, syncope.

Physical exam: Normal.

Patient 3, DOB 05/21/47, was last exposed 03/01/90, and was examined on 09/08/90, 6.3 months postexposure. Total exposure 44 months.

Skin contact 50%, inhalation 100% of the work time.

Toluene, xylene, benzene, lead, tin, dioxin, ethylene oxide, SO_2, HF, HCl, Cl_2, phenol, naphthalene, epoxy resins, styrene, malathion, other pesticides, hydrazine, methylene chloride, chlorinated hydrocarbons, ethylene glycol, propylene glycol dinitrate, 2-nitrodiphenylamine, dibutyl sebacate, cyanide, formaldehyde, methyl ethyl ketones, various aromatic and nonaromatic hydrocarbons, phosgene, phenol chlorine, PCB, acetone, propyl alcohol, hexane, and rocket fuel.

Cleaned tanks containing mixtures of chemicals and would often wade in sludge to knees.

Acute symptoms: Eye irritation and blurry vision, cough, coryza, incoordination, poor concentration, ataxia, dizziness, nausea, severe headaches, muscle

cramps, hallucinations, disorientation, hoarseness, nasal and sinus burning, and congestion.

Unique chronic symptoms: Arthritis, myalgia, emotional problems, poor memory, tremors, hot flashes, chills, insomnia, withdrawn, muscle fasiculations, abdominal pain, and suicidal.

Physical exam: Nasal mucosa edematous and inflamed with decreased superficial blood vessels. Increased sway on Romberg, poor depth perception.

Patient 4, was last exposed 03/01/88 and was examined on 08/01/90, 29 months postexposure. Exposed for 84 months.

He was exposed to tetramethyl tin, sulfuric acid, malic acid, urea, fluid used for rocket gyroscopes, carbon monoxide, hydrofluoric acid, ammonia, acrylonitrile. He had contact with the chemicals cleaning the chemicals spills, he occasionally wore a carbon filter, acid-type respirator, and a porous work suit.

Exposure: Intimate skin contact and breathing respirations.

Acute symptoms: Crushing pain in his chest, cough, severe headache, dizziness, confusion, syncope.

Physical exam: Inappropriate affect, stutters, body movement slow, somewhat awkward. Imbalance on tandem walk, finger-to-nose awkward, coarse positional tremor, decreased right patella deep tendon reflex.

Patient 5, DOB 01/13/52, was last exposed on 05/01/88 and was examined on 09/07/90, 28 months postexposure.

He worked at hazardous waste incinerator as a supervisor, and was exposed to multiple hydrocarbons from 1985 to 05/01/88. Had intimate skin contact and exposure to vapors without safety equipment. See chemicals of Patient 3.

Physical exam: Pale nasal mucosa with wheezes and rhonci, decreased breath sounds, wide gait and stance, positive Romberg positional tremor, poor finger-to-nose, decreased pain, vibratory, and temperature sense in lower extremities, hyperpigmented scars on dorsum of feet.

Confounding factors: Used marijuana.

Patient 6, DOB 12/25/49, was last exposed 04/30/89 and was examined on 10/10/90, 17.4 months postexposure.

He worked at a hazardous waste disposal facility without safety equipment and was asymptomatic until unloading a barge of hazardous waste, when he and several other men were incapacitated within 3 hr by the fumes, and required hospitalization. The materials contained benzene, xylene, toluene, ethylbenzene, and possibly other substances.

Acute symptoms: Imbalance, headaches, dizziness, immediate chest pain, eye and throat irritation, and shortness of breath.

Unique chronic symptoms: Extremely erratic heart beat.

Physical exam: Positive Romberg, poor grip strength, slightly decreased pulmonary functions (obstructive airway).

Patient 7, DOB 01/27/53, was last exposed 01/14/89 and was examined 01/14/88, 22.5 months postexposure.

Exposed to malathion- and xylene-laden dust and fumes while loading grain on to a ship for approximately 88 hr during January 1988. No other exposures. A co-worker had a similar exposure and resultant problems.

Acute symptoms: Feverish feeling, sweating, headaches, chills, weakness, coughing, spitting up blood, blurry vision, increased mucous and salivation, chest pains, numbness of hands and feet, shortness of breath, sharp pain throughout the body.

Physical exam: Pale boggy nasal mucosa, decreased sensations in feet and hands, decreased pinprick, dry skin, lateral nystagmus.

Patient 8, DOB 05/17/47, was last exposed on 03/02/89 and was examined on 11/30/89, 8.97 months postexposure.

Exposed for approximately 20 to 30 min to hydrogen sulfide from an industrial accident.

Acute symptoms: Immediate headache, staggering, slurred speech, imbalance, nausea, confusion and disorientation.

Chronic symptoms as above.

Confounding factors: Automobile accident in 1969 with a fractured jaw and possible brief loss of consciousness, in 1971 had vertigo that resolved a few months later. No symptoms. Physically active, excellent function and health until 18 years later.

Physical exam: Slurred speech, wide stance, poor finger-to-nose, poor alternating movements, poor heel-to-shin, nystagmus left eye, dresses with difficulty, positive Romberg, wide gait, difficulty standing with feet together, poor abstractions, rambling speech.

Patient 9, DOB 07/07/38, was last exposed on 01/14/88 and was examined on 01/05/90, 24.2 months after exposure.

Exposure approximately 40 hr.

See Patient 7 for a listing of chemicals.

Acute symptoms: Nausea, emesis, dizziness, watery eyes, increased salivation, dyspnea, sinus pain, disorientation, ataxia, hallucinations, abdominal cramps, brief syncope, congested chest, chest pain, and nightmares.

Unique chronic symptoms: Frequent paroxysmal coughing.

Patient 10, DOB 07/24/39, was last exposed on 08/30/86 and was examined on 12/15/86, 3.52 months postexposure.

One year exposure to two levels of formaldehyde and phenol from particle board; 1986, acute high-level occupational exposure for several workdays to strong fumes of formaldehyde, phenol, and glue containing tetrachlorophenol, dichlorophenol, ammonia, pentachlorophenol, methanol, petroleum distillates, ethylbenzene, methylene chloride, xylene, methyl ethyl ketone, toluene.

This exposure occurred in a small room without ventilation.

Acute symptoms: Irritation of the eyes, ears, nose, and throat.

Confounding factors: Brief situational depression about 20 years prior to the onset of his illness.

Physical exam: Depressed affect, blood pressure 158/90, hand weakness, tremors on left hand, imbalance.

Patient 11, DOB 08/29/51, was last exposed on 06/01/87 and was examined on 10/15/89, 28.5 months postexposure.

Six hours exposure to an unknown pesticide used to kill mites in a paper doc-

ument archive, possibly Dursban or Diazanon. Patient was seen acutely in ER for organophosphate poisoning.

Acute symptoms: Dyspnea, headache, dizziness, hoarseness, imbalance, dysphagia, hypersalivation, nausea, eye irritation.

Unique chronic symptoms: Right-sided muscle tremors, hemiballistic movement of the right arm, voice loss for 2 years postexposure, severe weakness with inability to stand.

Confounding factors: Situational depression in 1975, smoked marijuana occasionally.

Physical exam: Positive Romberg, myoclonic movements, hemiballistic movement of right arm, positional tremors in arms less than legs, poor motor control, wide gait, unable to stand without assistance, right foot turns inward with efforts to walk, Babinski present on right, hyperactive reflexes with clonus of right platella and Achilles' reflex, wide stance, and decreased sensation in lower extremities.

Patient 12, DOB 07/04/49, was last exposed on 06/11/89 and was examined on 05/22/90, 11.3 months postexposure.

Exposed for 2 to 2.5 hr to fumes from a waste pit for an entire petrochemical complex while in a boat when attempting to remove a sludge floating on a pit. The pit contained phenols, beryllium, benzene, hydrogen sulfide as well as a mixture of other volatile hydrocarbons.

Acute symptoms: Nausea, headaches, dizziness, severe chest pain, dyspnea, fatigue, and near syncope.

Unique chronic symptoms: Paroxysmal cough, nasal sores that do not heal, legs collapse without warning.

Physical examination: Pale, boggy nasal mucous membranes, rales, wheezes, ronchi to ascultation, increased bronchial sounds, ulcerated lesions of nasal bridge, decreased short-term memory, poor tandem walk, slight tremors, clonus of left Achilles' deep tendon reflex.

Patient 13, DOB 12/16/62, was last exposed on 08/01/87 and was examined on 09/07/90, 37.3 months postexposure.

He worked for 16 months at hazardous waste incinerator. He had direct skin contact with volatile liquids and extreme amounts of vapor, accidentally swallowed the liquids on several occasions.

See Patient 3 for a listing of chemicals.

Acute symptoms: Headaches, dizziness, vomiting, lightheadedness, burns on the body, heavy sweating, and ataxia.

Unique chronic symptoms: Uncontrolled unilateral tremors, heart palpitations, involuntary crying, urticaria, extensive vision loss, diplopia, stutter, twitching eyelids, and severe progressive arthritis.

Physical exam: Pale, cobblestone nasal mucosa, decreased deep tendon reflexes, right Achilles', coarse, large amplitude tremors, arms greater than legs, positive Romberg, abnormal heel-to-shin and heel-to-toe walk, at rest and positional tremors, intention tremors, nystagmus, decreased temperature and vibratory sensation, diffuse motor weakness, wide gait and stance, abnormal finger-

to-nose, abnormal alternating movements, falls with feet together, multiple whelps partially healed on back and feet with urticaria of axilla and biceps, poor dexterity of hands, and decreased grip strength.

Patient 14, DOB 04/13/57, was last exposed on 05/23/89, and was examined on 07/03/90, 13.4 months postexposure.

He was using a protective hood while sandblasting when 15 min after breathing air from his air compression system, he noticed symptoms and was forced by his supervisor to continue working for 4 hr. His air compression system was contaminated with transmission fluid and silicone glue (alkyloxysilanes, (e.g., methyltriancetoxysilane and polydimethylsiloxane) two trade-secret components, acetic acid, methanol (amyl, vinyl, and glycidoxy derivatives), fluorinated hydrocarbons, silicone, methyl methacrylate. The air in the mask had a strong glue-like smell.

Acute symptoms: Dizziness, headaches, nausea, syncope, numbness of body, extreme chest pain, tearing eyes, diplopia, and shortness of breath.

Unique chronic symptoms: Auditory, episodic chest pain, visual and tactile hallucinations, transiently paralysis upon awakening, severe progressive deforming arthritis of extremities.

Confounding factors: Alcohol abuse for 7 years with 6–7 drinks per day and marijuana use twice per week until 02/86. Patient entered a detoxification program and was discharged in good health with a normal neurological examination on March 1986. No alcohol or drug use since March 1986.

Physical exam: Abnormal drawing skills, poor tandem walk, inability to extend fingers due to arthritic deformities, swollen knees, positive Romberg, decreased sensation in lower extremities, anisocoria with spontaneously fluctuating pupil size.

Patient 15, DOB 07/04/57, was last exposed on 02/01/87 and was examined on 02/01/89, 24 months postexposure.

Used tetrabromoethane (TBE) in a contaminated work area for several months, causing frequent headaches. On one occasion a peak exposure occurred when the TBE accidentally splashed onto the patient's face and chest causing instant headache and disorientation. In an attempt to assist the patient, his coworkers placed his head over a sink and covered him with hot towels. The sink was also contaminated with TBE and therefore, for over 2 hr he breathed concentrated TBE fumes. Neither his skin nor his clothing was decontaminated until the patient was brought to a hospital approximately 3 hr later.

Acute symptoms: Dizziness, floating sensation, emesis, severe headache, dry mouth, difficulty thinking, confusion, and shortness of breath.

Chronic symptoms: As acute, numbness in wrists and feet, rage episodes, auditory, visual and tactile hallucinations, suicidal ideation, amnesia, cacosmia, and parosmia.

Confounding factors: Patient had three episodes of driving while intoxicated due to episodic intoxication related to marital problems 2 years prior to his accident. He had a normal preemployment physical at the time of his accident and had not drunk heavily in 2 years. He had a high-paying job that required him to be disciplined, mentally alert, and functional.

Physical exam: Anxious, terrorized facies, increased imbalance on Romberg,

flushing of face, hypertension, decreased sensation in lower extremities, and fine tremors.

Patient 16, DOB 07/15/35, was last exposed on 06/18/87 and was examined on 04/30/89, 22.4 months postexposure.

Worked around petrochemical plants for about 20 years without any untoward effects. Patient had an acute exposure when a pump that was accidentally opened while pressurized with a heat transfer mixture, i.e., ethylbenzene, isopropylbenzene, diethylbenzene, sec butylbenzene, triethylbenzene, cumene. The force of the discharged liquid knocked the patient down and he cut his forearms on a cat walk during the fall. He was covered with the above chemicals and had to wait approximately 30 min before he could decontaminate his skin but he did not remove his clothing until 4–6 hr later.

Acute symptoms: Immediate burning in forearms, headaches, nausea, dizziness, chills, coordination problems, vomiting, feverish for several hours.

Unique chronic symptoms: Severe incoordination, chronic rashes on forearms, ankles, and feet with friable peeling skin with subdermal hemorrhaging and draining sores, odd gait and stance.

Physical exam: Stuttering, severe intentional and rest tremors in hands, hyperpigmentation with friable skin and subdermal hemorrhaging of forearm and ankles, slight decreased sensation in lower extremities, weak upper extremities, poor finger-to-nose, intermittent myoclonus, lateral nystagmus.

Patient 17, DOB 01/10/54, was last exposed on 11/08/87 and was examined on 05/07/90, 30 months postexposure.

He works as a truck driver hauling hazardous waste. When symptoms developed he had considerable vapor exposure. Often the chemicals would leak and he would repair the leak himself. His clothes would become saturated with the hazardous chemicals and it would be hours before he could change and bathe. When he parked the truck on his property, the residuals would drip onto the ground and kill the grass and induced symptoms in family members.

He had exposure to organic solvents, hydrogen cyanide, PCP, PCB, styrene, benzene, and many unknown hazardous chemicals.

Acute symptoms: Excessive sweating, severe headaches, tachycardia, shortness of breath, and nausea.

Unique chronic symptoms: Uncontrollable right hand tremors and jerked movement.

Physical exam: Intentional tremors, myoclonus, and right arm hemiballistic movements.

Patient 18, DOB 10/07/38, was last exposed on 01/01/90 and was examined on 04/06/90, 3.12 months postexposure.

From 1980–1990 the patient was exposed to herbicides, including (sulfomeuron methyl) Oust, Paraquat, Roundup, Velpar, 2,4-D-MSMA. He worked spraying herbicides on roadways during spring and summer when spray would typically blow into the unprotected cab of his vehicle during work hours. His main exposure was to 2,4-D via the skin.

From 1976–1978 the patient was exposed to ethylbenzene, isopropylbenzene, diethylbenzene, butylbenzene, triethylbenzene, cumene, formaldehyde.

He also painted several cars as hobby, used Varsol, carburetor cleaner, and, rarely, carbon tetrachloride.

From 1957–1964 the patient was exposed to organic solvents used frequently in military as mechanic.

Fine intentional tremors started 1959 with solvent exposure in service. From 1956 to 1957 he worked packaging detergents, shoe polish, lubricants with minimal exposure.

In 1955 his summer work was on a golf course using lead and arsenic fungicides without acute symptoms and minimal exposure.

During his childhood his mother used DDT around the home. (His father had mild tremors.)

Chronic symptoms: Slight intentional tremors started in 1959 and were intermittent and mild until January 1989 when they became severe with pain, poor coordination, sleep apnea, and hypoglycemic episodes. He developed grand mal-like movements without loss of consciousness.

Confounding factors: Long history of similar but mild symptoms and multiple exposures over many years; father had mild tremors.

Physical exam: Poor tandem walk, increased sway on Romberg, severe positional tremors with rest tremors, decreased deep tandem reflexes, decreased sensation in feet, vibratory sense and sensory discrimination decreased in lower extremities in stocking pattern, coarse 10 Herz tremors of upper body that were worse with intention and with episodes of severe bilateral myoclonus activity of upper body when under mental stress or when attempting physical exercise.

Patient 19, DOB 09/05/51 was last exposed on 04/07/88 and was examined on 04/12/90, 24.2 months postexposure.

Five year exposure (40 hr per week) to multiple solvents and pesticides fumes and liquids that leaked out of containers in a warehouse storage area.

Acute symptoms: Depression, anxiety, dizziness, gastrointestinal disturbances, disorientation, bizarre ideation, and visual disturbances.

Unique chronic symptoms: Bizarre ideation, pounding heart.

Confounding factors: He had a history of mild, chronic depression symptoms since childhood that predated his exposures, were related to specific social problems, and that responded well to counseling. These symptoms were minimal and stable compared to his postexposure severe disabling symptoms.

Physical exam: Depressed affect (mental status), slight increase on Romberg.

Patient 20, DOB 09/27/51, was last exposed on 06/15/90 and was examined on 09/28/90, 3.45 months postexposure.

Chloppyfas or Diazanon was sprayed into work area twice over several days resulting in acute symptoms within 5 min. Total recognized exposure time was 1 hr, however, intermittent previous and lesser exposure probably did occur in a similar manner over 10 weeks.

Acute symptoms: Immediate irritated eyes, sinus congestion, headaches, nausea, tightness in chest, numbness of arms and legs, weakness, dizziness, vertigo, disorientation, erratic pulse, muscle aches, flu-like syndrome that reoccurred upon reentering work area.

Unique chronic symptoms: Muscle aches, stiffness in neck, and myoclonic movement that wakes him from sleep.

Physical exam: Congested nasal mucosa, rash, cool hands and feet, increased sway on Romberg, fasiculations of muscles in forearms, erythematous rash on right leg.

Patient 21, DOB 01/22/35, was last exposed on 04/04/86 and was examined on 05/22/90, 49.6 months postexposure.

Exposure was 8–12 hr per day to direct skin contact and heavy inhalation with several chemicals and the combustion products of those chemicals. The exposure usually took place in a confined work area that was oxygen deprived to improve quality of welds, and the source of combustion was an acetylene cutting torch used to burn off the chemical coatings or to cut metal or welding torches used to patch cracks in metal.

Chemicals were difluoromethane, trifluoromethane, fumes (HF, HCl, carbonyl halides, phosgene, NOx, CO, CN, halogenated hydrocarbons), phenyl isocyanate, xylene, silicone, catalysts, flame retardants, triorthocresyl phosphate, diphenylmethane diisocyanate, burning epoxy resins, toluene diisocyanate, coal tar, organic solvents, burning urethane foam, creosote, paints and paint primers, phenol, fumes of iron, manganese, chromium, amine resins, and methanol.

Acute effect: Chest pain, shortness of breath, trembling, dizziness, confusion, hemoptysis, and syncope.

Unique chronic symptoms: Episodic chest pain.

Physical exam: Pale nasal mucosa, few rales and increased expiratory phase, dry skin, red scaly rash at hairline and on face, noticeable sway on Romberg, severe heel-to-toe walk, imbalance with eyes closed, abnormal finger-to-nose, muscle fasciculation, slight dysconjugate vision, longitudinal ridging of fingernails, positional tremor, startles easily, difficulty remembering three items, and emotionally labile.

Patient 22, DOB 09/13/52, was last exposed on 10/01/87 and was examined on 09/08/90, 35.3 months postexposure.

Worked at a hazardous chemical waste plant taking samples of waste stream. Intimate, daily exposure to heavy fumes and skin contact. Direct contact with waste stream 30% of 8- to 10-hr working day.

See Patient 3 for chemical list.

Acute symptoms: Nasal and eye irritation and burning, shortness of breath, nausea, headaches, vertigo, confusion, disorientation.

Unique chronic symptoms: Personality change, explosive temper, and heat intolerance.

Physical exam: Pale nasal mucosal membranes, stutters with speech, erythematous rash on ankles, longitudinal ridges of fingernails, profuse sweating, decreased grip strength and dexterity, positive Romberg, wide gait and stance, decreased sensation to pinprick in feet, intentional tremors with arms worse than legs, droopy left eyelid, and poor stereognosis.

Patient 23, DOB 07/05/56, was last exposed on 09/01/88 and was examined on 03/16/90, 18.4 months postexposure.

Worked in a small store directly across from a chemical company. Fumes leaked into the air causing the patient and a co-worker to become incoherent and to collapsed, then she had a seizure. Both were rushed to the emergency room of a nearby hospital for treatment.

Phosgene, hydrogen chloride, monochlorobenzene, possibly toluene diisocyanate.

Acute symptoms: Eye, nose, throat, chest burned, chest pain with inspiration, dizziness, imbalance, nausea, emesis, syncope, stomach pain, seizure, and incoherence.

Unique chronic symptoms: Grand mal and complex visual seizures.

Physical exam: Depressed affect and congested lungs.

Patient 24, DOB 05/25/61, was last exposed on 03/08/90 and was examined on 05/10/90, 2.07 months postexposure.

Exposed to condensate from natural gas holding tanks each day and would inhale the fumes frequently. Exposure was 30 min per day over 2½ years. A peak exposure occurred due to an increased amount of fumes that precipitated immediate symptoms and was incapacitated for several hours.

Benzene, toluene, xylene, stoddard solvent, ethylbenzene, and other volatile hydrocarbons.

Acute symptoms: Immediate shortness of breath, upper respiratory irritation, headaches, nausea. He developed spasms within several hours and severe lower back pain and chills. Development of jerking movements throughout his entire body which occurred several times a day. These gradually resolved over the next few months.

Unique chronic symptoms: Scaly red rash and rapid onset of fatigue with physical exertion.

Physical exam: Periodic jumping movement of trunk at one to two times per minute, faint red rash on face, generalized mild motor weakness.

Patient 25, DOB 08/29/56, was last exposed on 01/02/90 and was examined on 07/05/90, 6.05 months postexposure.

Chemicals were methyl ethyl ketone, 2-butanone ketone, toluene, dimethyl ketone, methanol, mixed hydrocarbons, and kerosene.

Patient cleaned equipment in mixture of solvents as above without safety equipment of any kind. Hands to elbows were frequently immersed in the solvents for several hours per day and skin was always coated with some solvents. Frequently worked 70 hr per week.

Acute symptoms: Dry, shiny hands, sinus and chest congestion, nausea, syncope, flu-like symptoms, drunkenness, imbalance, pain and numbness from hands to elbows.

Unique chronic symptoms: Stutter, bad temper, difficulty finding words when speaking, and muscle fasiculations.

Physical exam: Hoarse voice, decreased grip strength, slight sway on Romberg, tender wrists to palpation.

Patient 26, DOB 07/30/43, was last exposed on 06/15/90 and was examined on 06/22/90, .23 months postexposure.

Exposed to *n*-hexane, ethylbenzene, toluene, xylene, benzene, mercaptan, slight amounts of hydrogen sulfide, various volatile hydrocarbons as condensate from natural gas. Exposure was chronic over several years with an acute, peak exposure over several minutes that caused syncope.

Acute symptoms: Syncope, nausea, headaches, sudden chills, panic attacks,

burning sinuses, slowed mental function, confusion, disorientation, groggy, and burning sensation in the back of his neck.

Unique chronic symptoms: Tingling feelings in neck.

Confounding factors: During work-up bilateral carotid artery disease was found with 70–80% stenosis found 1½ years after onset of illness.

Patient 27, DOB 05/21/47, was last exposed on 12/31/89 and was examined on 09/07/90, 8.22 months postexposure.

Worker at a hazardous chemical waste plant taking samples of waste stream. Intimate, daily exposure of heavy fumes and skin contact. Direct contact with waste stream 30% of 8- to 10-hr working day.

See Patient 3 for chemical list.

Acute symptoms: Nausea, dizziness, emesis, severe headaches, imbalance, disorientation, and ataxia.

Unique chronic symptoms: (6–7 months later) Easy bruising, poor body temperature regulation, sees flashing lights, and had chronic bladder discomfort.

Physical exam: Pale nasal mucous membranes, positive Romberg, fine intentional tremors, and longitudinal ridges.

Patient 28, DOB 01/29/54, was last exposed on 06/14/90 and was examined on 11/07/90, 4.80 months postexposure.

Worked for 13 years around fumes and liquids for over 90% of his workday. He worked around hot caustics, melting plastics, polypropylene, polyethylene, lead fumes, various catalysts, solvents, burning plastics, welding of metals, carbon monoxide, formaldehyde, hydrogen sulfide, smoke, fiberglass, aluminum vapors, cadmium vapors, chromium vapors, manganese, tin vapors, naphthalene, petrochemical distillates, epoxy resins, and methyl ethyl ketone.

Acute symptoms: Nausea, dizziness, emesis, severe headaches, imbalance, disorientation, and ataxia.

Unique chronic symptoms: Decreased peripheral vision, sleep apnea without hypoxia, reactive airway disease, and easy bruising.

Physical exam: Positive Romberg, spasm of abdominal, and bilaterally decreased patellar deep tendon reflexes. No loss of consciousness. Symptoms as on page 299. Physical examination revealed increased sway on Romberg.

Patient 29, DOB 10/21/70, was last exposed on 09/07/90 and was examined on 06/05/91, 8.91 months postexposure.

Frequently exposed to hydrogen sulfide at paper mill and often had to run to avoid pockets of H_2S. On one occasion he was exposed to high amounts of odor and became instantly and chronically symptomatic.

Patient 30, DOB 10/22/59, was last exposed on 09/01/89 and was examined on 01/03/91, 16.1 months postexposure.

Exposed to three percent hydrogen sulfide and mixture of volatile hydrocarbons.

Acute symptoms: Patient was breathing an atmosphere of H_2S and fumes from crude petroleum for 1 to 2.5 hr with the development of symptoms. No rotten egg odor was reported.

Acute symptoms: Headache, nausea, abdominal cramps, diaphoresis, tremors, sweating, shortness of breath, nervousness, chest pain, and dizziness.

Unique chronic symptoms: Lateral nystagmus, severe muscle spasms.

Confounding factors: Three drinks per day average before accident, exposure to cleaning solvents 86-88 without symptoms and with normal physical examination after solvent exposure and before H_2S exposure.

Physical exam: Was done postexposure, cool lower extremities with good pulses, slight increase in sway, poor heel-to-toe balance.

Patient 31, DOB 09/29/33, was last exposed on 03/19/82 and was examined on 04/12/91, 109 months postexposure.

Chemicals were phosgene, tetrachloroethylene, carbon tetrachloride, toluene diisocyanate, dichlorobenzene. A pressurized line cracked open and leaked phosgene and a mixture of chemicals onto the patient's face.

Acute symptoms: Cough, emesis, imbalance, disorientation, nose and throat burned, shortness of breath, severe hoarseness, eye irritation, hives, and syncope for 30 min.

Unique chronic symptoms: Stiffness and pain in joints with deformities, nystagmus, muscle twitching, nose bleeds, dysphagia, epigastric burning, esophageal reflux, esophageal myopathy, asthma, and transient paralysis upon awakening.

Physical exam: Hypertension 158/100, increased sway, tremors of left hand, deformity of proximal joints in hands, abnormal blink and snout reflex, abnormal memory test, abnormal esophageal muscle function, and abnormal sensory nerve function.

Patient 32, DOB 03/30/44, was last exposed on 05/01/91 and was examined on 05/20/91, 0.62 months postexposure.

Exposed to hydrogen sulfide frequently every day with intermittent peak exposures at a paper mill for 12 years, also around terpinene, turpentine and Toll oil. He also worked around pitch, methanol, toluene, isopropanol, ether, acetone, polychlorinated phenol, xylene, benzene in a lab 15 years prior to hydrogen sulfide exposure with symptoms in last 4 years.

Acute symptoms: None, all were gradual.

Unique chronic symptoms: Startled by noises.

Confounding factors: Exposed to multiple solvents over 15 years ago, without symptoms.

Physical exam: Pale nasal mucosa, slight increased sway on Romberg slight imbalance on tandem walk.

Patient 33, DOB 04/08/56, was last exposed on 01/01/78 and was examined on 04/22/91, 160 months postexposure.

Hydrogen sulfide, solvent fumes, pesticides and other vapors at a hazardous waste site 1978. Exposure to maximum intensity fumes was approximately for 30 min and had to be hospitalized. Rotten egg smell was present. A co-worker died from same event.

Acute symptoms: Burning eyes, general weakness, headaches, shortness of breath, nausea, and emesis.

Unique chronic symptoms: Dysphagia, clumsiness, and heightened anxiety.

Confounding factor: Diabetes mellitus diagnosed 3 months prior to evaluation.

Physical exam: Increased sway on Romberg, depressed affect.

RESULTS

Table 1 presents the results of the neuroimaging techniques of SPECT, MRI, and CT. The neuropsychological test results are given in Table 2. Table 3 demonstrates SPECT results by area of the brain affected. Table 4 gives the comparative results of color, odor, EEG, ABR, ENG, and CPT. Table 5 demonstrates MRI results by area of the brain affected.

The total number of abnormal neuropsychological test data available was 21/29 (72%). Neuropsychological tests results on five patients were only available via review of their medical records. Neuropsychological test results utilizing the POET were available for 24 patients. Of these, the majority (67%) performed

TABLE 1
NEUROIMAGING

ID	SPECT	Months postexposure	MRI	Months postexposure	CT	Months postexposure
1	N	171				
2	A	29.7			N	<1
3	A	6.25	N	1.12		
4	A	35.5				
5	A	28.2	A	9.01	A	27.74
6	A	16.7				
7	A	28.5	N	28.11		
8	A	12.4	A	0.66	N	0.85
9	A	28.2	N	41.42		
10	A	42.9				
11	A	33.7	N	33.66		
12	N	11.6	A	12.16		
13	A	27.3	N	1.35	N	1.35
14	A	16.7	A	0.66	N	0.03
15	A	27.7	N		N	2.24
16	A	33.4	A	10.85	N	7.76
17	A	30.0	N	16.6	N	0.03
18	A	21.5	N	2.53		
19	A	26.7			N	0.66
20	A	3.68	N	4.77		
21	A	51.3				
22	A	35.2	A	38.46	N	4.04
23	A	18.6	N	1.97	N	0.16
24	A	1.84	N	1.58		
25	A	6.48				
26	N	17.7	N	17.69		
27	A	8.19	N	5.79	N	5.62
28	A	2.56				
29	A	9.00				
30	A	8.88			N	8.45
31	A	109			N	93.52
32	A	0.70	N	0.69		
33	A	168	N	169.63		

Note. A, abnormal; N, normal; blank, not done.

TABLE 2
NEUROPSYCHOLOGICAL TEST RESULTS

ID	Months postexposure	General intelligence	Learning and memory	Attention and mental flexibility	Motor	Visuospatial	Composite interpretation
1	171.4	N (42.3)	A (33.5)	N (43.8)	N (41.6)	A (35.5)	A
2	29.95	N (62.5)	N (41.5)	N (41.6)	N (47.3)	N (51.3)	N
3	2.83	N	A	A	A	A	A
4	28.99	N (56.7)	A (35.9)	A (34.4)	A (11.1)	A (37.2)	A
5	8.94	N	N	A	N	N	A
6	23.37	A (32.7)	A (39.0)	N (42.4)	A (39.3)	N (40.5)	A
7	22.75	N (49.2)	N (43.2)	A (40.0)	A (36.7)	N (52.3)	A
8	13.15	N (58.2)	N (55.0)	A (37.8)	A (21.7)	N (47.0)	A
9	26.66	N (42.7)	A (32.4)	A (37.1)	N (41.1)	A (39.2)	A
10	34.22	N (46.0)	N (45.4)	A (32.9)	A (37.6)	N (42.8)	A
11	30.28	N (44.6)	N (56.2)	A (40.0)	A (20.4)	A (38.0)	A
12	11.41	N (47.1)	N (46.4)	N (44.0)	A (31.2)	A (32.6)	A
13	1.25	N	A	A	N	A	A
14	16.9	A (34.8)	N (44.9)	A (34.1)	A (32.5)	A (33.8)	A
15	25.97	N (46.2)	A (33.5)	A (31.9)	A (20.5)	A (37.0)	A
16	34.02	A (35.3)	A (35.9)	A (13.5)	A (1.4)	A (35.4)	A
17	30.90	N (43.7)	A (38.3)	N (43.4)	A (33.0)	N (41.1)	A
18	3.98	N (51.9)	N (48.6)	N (48.9)	A (37.6)	N (56.2)	N
19	27.42	N (65.1)	N (53.0)	N (60.5)	N (45.1)	N (54.6)	N
20	3.45	N (59.3)	N (55.2)	N (49.6)	N (45.1)	N (51.4)	N
21	45.76	N (57.4)	N (53.4)	N (47.6)	N (44.2)	N (53.8)	N
22	15.02	A	A	A	A	A	A
23	18.57	N (46.2)	N (50.0)	N (44.3)	A (28.8)	N (45.6)	A
24	2.07	N (45.0)	A (37.9)	A (39.3)	N (48.9)	N (53.6)	A
25	4.37	N (49.0)	N (50.4)	N (51.7)	N (40.5)	N (53.3)	N
26	0.89	N (46.3)	N (49.2)	N (53.7)	N (46.6)	N (44.6)	N
27	7.50	N	N	A	N	N	A
28	2.53	N (50.0)	A (34.2)	A (35.2)	N (44.9)	N (54.4)	A
29							
30	16.07	N (43.7)	N (45.9)	N (44.9)	A (31.2)	N (52.3)	N
31							
32							
33							

Note. A, abnormal; N, normal; blank, pending (*t* scores shown when available).

significantly below average on the neuropsychological measures (i.e., *t* scores on at least one category by two or more standard deviations below the mean or *t* scores on two or more categories by one or more standard deviations below the mean).

CPT tests were abnormal in 27/30 cases, i.e., 90%. See Table 4.

DISCUSSION

These results suggest that symptomatic patients with a history of exposure to a variety of toxins, i.e., organic solvents, pesticides, hydrogen sulfide, phosgene, isocyanates, and others, have an increased risk of manifesting CNS abnormalities as measured by SPECT and/or PET. In addition, a high percentage of patients have deficits on neuropsychological, color discrimination, olfactory discrimination, and vestibular testing. A comparison of SPECT results in different CNS tissues for different exposure groups demonstrates a tendency for dysfunction of frontal and temporal lobes, thalamus, basal ganglia, and cerebellum. With expo-

TABLE 3
SPECT SCAN FINDINGS

ID	Months postex-posure	Cere-bellum	Caudate nucleus	Frontal lobe	Motor strip	Occipital lobe	Parietal lobe	Tem-poral lobe	Thala-mus	Cere-brum	Basal ganglia
1	171										
2	29.7								A		
3	6.25							A			A
4	35.5		A					A	A		
5	28.2			A				A	A		A
6	16.7							A			
7	28.5			A			A	A			A
8	12.4	A		A				A			A
9	28.2			A				A	A		
10	42.9			A				A	A		A
11	33.7							A			A
12	11.6										
13	27.3			A		A		A			
14	16.7			A	A			A			
15	27.7			A				A	A		A
16	33.4	A		A			A	A			
17	30.0			A	A			A	A		A
18	21.5			A			A	A		A	
19	26.7			A							
20	3.68										A
21	51.3			A				A			
22	35.2			A				A	A		
23	18.6										A
24	1.84										A
25	6.48						A				
26	17.7			A					A		A
27	8.19			A	A			A			
28	2.56			A				A			
29	9.00							A		A	
30	8.88			A							A
31	109			A				A			
32	0.70	A									
33	168										A

Note. A, abnormal region of activity.

sure to a systemic neurotoxin, we would expect diffuse abnormalities throughout the nervous system. This expectation is confirmed by the other test modalities used. In this patient group there is a high incidence of abnormal CPT tests. CPTs have been shown to be highly effective predictors of neuropathies. However, neurotoxic patients frequently have normal nerve conduction velocity tests and in metabolic derangements, such as diabetes and uremia, peripheral sensory function may be altered long before axonal degeneration can be measured by nerve conduction velocity tests (Rendell *et al.*, 1989). Considering the thalamic abnormalities seen on SPECT, it is possible that neurotoxin-induced sensory dysfunction is at least in part central and not peripheral in origin. Furthermore, there seems to be a correspondence between neuropsychological impairment and neurofunctional deficits in this group. If the SPECT was positive, then the POET was positive in 74% of the cases. If the POET was positive, then the SPECT was positive in 83% of the cases. The POET was negative in five cases with four positive SPECTS. There was one case with a negative POET and negative SPECT. This would seem to indicate that for patients with toxic exposure and

TABLE 4
NEUROPHYSIOLOGICAL TESTING

ID	Color	Odor	EEG	ABR	ENG	CPT
1	A	A		A	A	A
2	N	A	A	N	N	A
3	A	N		A	A	A
4	A	A		A	A	A
5	A	A	N			A
6	A	A		N	A	A
7	A	A	N	A	A	A
8	A	A	N	A	N	A
9	A	A		N	N	A
10					N	
11	N	A		N	A	
12		A		N	A	A
13	A	A	N			A
14	A	A	N			A
15	A	A				A
16	N	A			A	A
17	A	A	N			A
18						
19	N	A		A	N	A
20	A	A		N	A	A
21	A	A	N		A	N
22	A	A				A
23	A	A	N		A	A
24	N	N	N	N	N	A
25						A
26	A	A	N	A	A	A
27	A	A			A	A
28	A	A		A	A	A
29	A	N				N
30	A	N	N	A		A
31	A	A		N	A	A
32	A	A				N
33						A

Note. A, abnormal; N, normal; blank, not done.

typical chronic encephalopathic symptoms, SPECT or PET is a more sensitive measure of CNS than neuropsychological testing via POET. The cooccurrence of a neurotoxin exposure and neurofunctional abnormalities lends objective support to the hypothesis that these workers have suffered CNS damage. The exact mechanism and time course by which neurotoxins act on the CNS is unknown and there may be synergistic effects between agents. The relationship between the occurrence of SPECT, neuropsychological disturbances, and specific exposure characteristics (e.g., duration of exposure, type of solvent) remains poorly understood due to the random and often delayed presentation of patients for evaluation. From the patients' historical description of their symptoms and by extrapolating from relevant measures of neurotoxicity (e.g., SPECT), it can be implied that in some cases substantial and chronic CNS changes can take place with exposures lasting

TABLE 5
MRI FINDINGS

ID	Months postexposure	MRI abnormal	Cerebellum
3	1.12		
5	9.01	A	Prominance of lateral and third ventricles, cysterns of posterior fossa, skull-based Sylvian fissures, and sulci over cerebral convexities. All consistent with mild atrophy.
7	28.11		
8	0.66	A	A questionable, small region of abnormal increased signal in the posterior aspect of the right thalamus.
9	41.42		
11	33.66		
12	12.16	A	Multiple foci. Possibly benign.
13	1.35		
14	0.66	A	Increased signal in the anterior right temporal lobe.
16	10.85	A	Moderate diffuse cortical atrophy.
17	16.6		
18	2.53		
20	4.77		
22	38.46	A	Minimal prominence of the subarachnoid spaces about vermis, compatible with hypoplasia or atrophy.
23	1.97		
24	1.58		
26	17.69		
27	5.79		
32	0.69		
33	169.63		

Note. A, abnormal region of activity; blank, normal.

just minutes to hours. Using these techniques in this group of patients, lesions of specific areas of the central nervous system are seen more frequently, i.e., the brain stem (based upon ABR/ENG) (see Table 3), frontal lobes, temporal lobes, and basal ganglia dysfunction (based upon SPECT and PET). These findings are consistent with the current body of data available that indicates such diverse chemicals as carbon monoxide, MPTP, manganese, and carbon tetrachloride (Klassen *et al.*, 1986) can cause damage to the basal ganglia and that organic solvents in general affect the frontal and temporal lobes. (Hagstadius *et al.*, 1989; Morrow *et al.*, 1990). It may be either that these tissues are more susceptible to the effects of a chemical stressor or that the damage is more likely to occur in specific areas due to route of entry (such as olfactory nerve penetration), differences in regional blood–brain barrier properties, increased regional blood flow or differences in regional metabolism, enzymatic activity, or membrane properties. It has been shown that a physical pathway for transport of materials exists between the CSF and the cribiform plate (Erlich *et al.*, 1986). Considering the lipophilic nature of most neurotoxins, their volatility, the high incidence of frontal and temporal lobe abnormalities (see Table 3), and the high incidence of olfactory dysfunction (see Table 4), it is possible that at least part of the mechanism in-

volved in the pattern of abnormalities seen in Table 3 could be an olfactory nerve-to-temporal and frontal lobe physical migration pathway.

It was noted that chronic fatigue and sleep disturbance symptoms were universal in this group of patients with toxic encephalopathy. Sleep analysis revealed a high incidence of abnormal sleep patterns (i.e., central apneas, fractured brain wave patterns, myoclonus). These complaints could be due to secondary damage to the pons area of the brain, also an area of high regional blood flow. Note that abnormal ABR and ENG tests results frequently suggest dysfunction in brainstem tissue. The possibility of neurotoxic effects on the pons is also suggested by data showing that rats and humans exposed to xylene or toluene have an increased incidence of sleep disturbances (Erlich *et al.,* 1986; Kilburn *et al.,* 1985).

Two patients were studied with both PET and SPECT. In one patient, both modalities showed identical findings involving the temporal lobe. In the other patient, SPECT showed frontal and temporal lobe abnormalities, while PET imaging indicated additional abnormalities in the hypothalamus, amygdala, thalamus, and hippocampus. Although an extensive comparison of SPECT and PET technologies is beyond the scope of this article, it is pertinent to point out that PET, due to its superior resolution, sensitivity, and availability of such positron-emitting radiotracers as [^{11}C]-glucose, [^{15}O]-carbon dioxide, and [^{18}F]-fluorodeoxyglucose, lends itself to extensive absolute quantification of metabolic activities in the brain. However, PET is less available and much more expensive. With new innovations in SPECT technology, such as multiple head cameras, SPECT is expected to remain a viable alternative to PET in clinical practice.

One patient with a severe movement disorder (tremors, ataxia, hemiballistic movement) had a repeat SPECT after her clinical status improved dramatically on the anti-Parkinson's medications Eldepryl (selegiline) and Symmetrel (amantadine). Her follow-up SPECT showed significant improvement of blood flow to the basal ganglia. This case suggests that metabolic imaging can be useful not only in diagnosis but also in predicting degree of disability and effectiveness of treatment regimens. In this study, not only did the structural CNS studies (MRI and CT) have a low incidence of abnormalities (Table 1), but also the abnormalities seen with MRI represented a very small fraction of the CNS tissue volume described as abnormal by SPECT or PET (Table 5). Fourteen cranial CT head scans were done and only one was positive indicating cerebellar and cerebral atrophy (Patient 5) MRI was positive in 5 out of 21 cases (see Table 1). In this study, several patients were tested years after a very brief exposure and had objective measurements of alterations of their CNS. However, the SPECT scans were usually not done close to exposure and long-term SPECT/PET follow-up data is not available on most of the patients. Several studies have addressed prognosis following a diagnosis of solvent encephalopathy. Individuals who develop neurological and cognitive impairments following chronic solvent exposure do not necessarily show significant clinical improvement following removal from the exposure source (Kilburn *et al.,* 1985; Bruhn *et al.,* 1981). Moreover, these impairments may prevent a return to work or seriously limit employment and social functioning, especially in older individuals with a long exposure history (Juntunen *et al.,* 1982; Gregerson *et al.,* 1987; Morrow *et al.,* 1990; Gade *et al.,* 1988).

CONCLUSION

The complex of symptoms seen in toxic encephalopathy such as fatigue, headaches, poor memory, depression, anxiety, dizziness, heat intolerance, ataxia, tremors, insomnia, etc. imply diffuse dysfunction involving several regions of the central nervous system.

Previous studies of brain regional blood flow by xenon methods in solvent patients have shown chronic frontotemporal effects. Additional support for the utility of metabolic imaging studies in neurotoxicology has been evident in PET imaging of patients exposed to manganese, MPTP, and tetrabromoethane (Morrow *et al.*, 1990). This series of patients adds to the previous data of brain metabolic imaging. Furthermore, this study confirms previous observations that certain areas of the brain are typically affected despite differences in toxin structure and that the areas of abnormal tissue can be globally defined by SPECT/PET brain scans. In addition, these lesions are associated with positive clinical signs and observations, neuropsychological, olfactory discrimination, and color discrimination tests. Standard EEG and structural brain imaging techniques such as CT and MRI have poor sensitivity in this type of patient. PET and SPECT technology does not seem to be as sensitive for brainstem dysfunction as neurophysiological methods such as ABR and ENG. This may be inherent in the limits of resolution of PET/SPECT as compared with the size of the structures being examined, or may mean that new imaging methods need to be devised for brainstem evaluations. The emerging data from metabolic brain studies suggests that neurological dysfunction due to toxic exposures disrupts various susceptible neural systems. This damage results in a combination of symptoms and dysfunction depending on which specific tissues were damaged and to what degree. These susceptible neural systems are likely to include all cranial nerves and their brainstem-associated structures, the frontal lobes, temporal lobes, limbic system, hypothalamus, thalamus, basal ganglia, and the pons. The data presented suggest that further research is indicated using metabolic imaging techniques (especially PET) in conjunction with cranial nerve function, neuroelectrophysiological, and neuropsychological testing. Pharmaceutical challenges to stress nervous system tissue during testing may further improve sensitivity. Despite overwhelming evidence of clinical disease in individuals with a history of significant solvent exposure (Baker *et al.*, 1985), doubts as to the presence of central nervous system damage still exist (Gade *et al.*, 1988). Therefore, it becomes increasingly important to establish sensitive indicators that will distinguish between normal and abnormal disease states. There is evidence from our study as well as other studies which indicates that various sensory systems, specifically color vision and olfaction, are also impaired in solvent-exposed individuals (Mergler *et al.*, 1990). It is possible that the disruption of these various sensory systems interferes with the individual's ability to integrate and process information quickly and accurately. SPECT or PET cognitive measures appear to be useful in the early detection of central nervous system abnormalities following neurotoxic exposures. These technologies may also be useful in the prevention of more serious or permanent damage by providing insight into the mechanisms of neurotoxicity.

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Magnetic Resonance Imaging (MRI), Neurobehavioral Testing, and Toxic Encephalopathy: Two Cases[1]

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The objective of this investigation was to examine cerebral magnetic resonance imaging (MRI) pathology and functional deficits demonstrated by neuropsychological testing in cases of toxic encephalopathy. Two subjects, occupationally exposed to toxic chemicals, were studied. As part of their neurological assessment, MRI was done and each underwent a neuropsychological battery for patients with toxic exposures (White *et al. Clin. Neuropharmacol.* 13(5), 392–412, 1990). In Case 1, who was exposed to inorganic mercury, MRI showed mild central and cortical atrophy. Punctiform foci (T2) were noted in both frontal regions underlying the precentral gyri and in the subcortical myelin. Neuropsychological testing showed problems in cognitive flexibility, cognitive tracking, inhibiting perseveration, fine manual motor coordination, visuospatial analysis and organization, memory, and affect and personality. In Case 2, who was exposed to 2,6-dimethyl-4-heptanone, MRI showed multiple small foci in the white matter and pons. Neuropsychological testing indicated affective changes, deficits in manual motor speed, verbal fluency, visuospatial organization, and short-term memory. Lack of aphasia in patients with toxic encephalopathy indicates that neurotoxins probably affect subcortical and mesial temporal structures more than cortical gray matter. These MRI studies show subcortical sites of pathology.

INTRODUCTION

Studies on the behavioral effects of neurotoxicants have consistently shown that exposure may be associated with functional deficits in complex motor skills, executive system function, problem solving, visuospatial abilities, and short-term memory, with relative preservation of verbal abilities. The studies which have incorporated investigation of personality and affective change into the study of cognitive dysfunction also consistently report mood disturbances as part of the general pattern of behavioral change seen with such exposures (White *et al.*, 1991). Findings of behavioral impairments within one or more of these general domains have been reported for a wide range of neurotoxicants, including metals such as lead (Baker *et al.*, 1984, 1985; Grandjean *et al.*, 1978; Hanninen, 1982; Valciukas *et al.*, 1978; Jeyartnan *et al.*, 1986) and mercury (Miller *et al.*, 1975;

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Langolf *et al.*, 1978; Angotzi *et al.*, 1980; Forzi *et al.*, 1976; Piikivi and Hanninen, 1981; Vroom and Greer, 1972), and industrial solvents such as trichloroethylene (Salvini *et al.*, 1971; Feldman *et al.*, 1985), carbon disulfide (Vigliani, 1954; Hanninen, 1974; Hanninen *et al.*, 1978; Tuttle *et al.*, 1973, 1976; Lilis, 1974), and toluene (Bor and Hurtig, 1977; Grabski, 1961; Rosenberg *et al.*, 1988a; Cherry *et al.*, 1985; Filley *et al.*, 1990).

Because specific patterns of functional deficits among these domains do vary among toxins, localization of cerebral damage appears to be toxicant specific. However, the consistent pattern of retained verbal abilities occurring with such exposures may place them in a class of neuropsychological syndromes which are specifically characterized by dysfunctions in nonverbal processing. It is hypothesized that cerebral white matter is a site for neurotoxic effects since many neurotoxins, particularly solvents, are lipid soluble. Studies of MRI in cases of chronic toluene exposure have shown increased periventricular white matter signal intensity on T2-weighted images and a loss of differentiation between gray and white matter throughout the central nervous system (Rosenberg *et al.*, 1988a, 1991).

This paper is a summary of two case studies of patients with toxic exposures evaluated in our Environmental and Occupational Neurology Program at Boston University Medical Center.

SUBJECTS AND METHODS

Case 1. This was a 48-year-old Hispanic male who was exposed to inorganic mercury at his job in a thermometer factory. He was right-handed, married with two children, and had an eighth grade education. He began work in the factory in January 1981. His first job was to sweep mercury off the floors using a vacuum cleaner or hose blower and to repair and clean machines. Then, he disassembled machines containing mercury and, in his last job, operated a crusher machine which crushed the instruments so he could then separate the mercury from other materials for reuse.

He left work in July 1984 initially because he injured his elbow. However, from January 1981 to June 1984 he experienced a number of symptoms including blurred vision, ocular pain, rash, strange taste in the mouth, weakness, memory loss, rage, and irrational behavior. In August 1984 he was sent to a psychiatric facility because of his behavioral problems, and a physician upon hearing his occupational history ordered that a mercury level be done. Urine mercury level was 690 mcg/liter. The occupational recommended standard is 150 mcg/liter (USEPA, 1984). He was given penicillamine as a chelation treatment to eliminate body mercury in September and October 1984. The urine mercury level was 480 mcg/liter in September, 240 mcg/liter in October before the second chelation, and 184 mcg/liter after chelation. In December 1984, his urine mercury level was 17 mcg/liter.

He was seen by us in April 1986 for evaluation of continuing neurological and behavioral problems. Neurological examination showed nystagmus on upward gaze, bilateral rapid manual tremor, diminished reflexes, diminished sensation to pain, and peripheral neuropathy. He also showed abnormalities in nerve conduc-

tion and EMG studies. He had no history of hypertension, closed head injury, or prior neurological disease.

Case 2. This was a 60-year-old male who was exposed to a long-chain hydrocarbon 2,6-dimethyl-4-heptanone in his job as a senior laboratory technician. He was right-handed, married with three children, and had a college education.

In his job performing hydraulic stripping experiments on iron ore, he was exposed to heptanone vapors. The chemical was heated to temperatures around 700°F and placed under 2300 pounds of pressure. He had been exposed for approximately 1 month (May to June 1985) when he developed a severe headache and a 20-min loss of vision.

He was seen for neurological and neuropsychological evaluation in late 1985 and again in early 1986 on referral from his occupational doctors because of continuing headaches. Neurological examination revealed a back problem and headache as well as peripheral neuropathy. A CT scan done in November 1985 was read as normal.

Each case underwent formal neuropsychological testing and magnetic resonance imaging. The battery of neuropsychological tests (Table 1) was drawn from the battery described in White *et al.* (1990), to be used for clinical purposes, e.g., designed to detect dysfunction attributable to toxic exposure and to allow differential diagnosis of toxic encephalopathy vs other long-standing or acquired disorders. (For a discussion of the differences between neuropsychological test battery selection for clinical and epidemiologic study purposes, see Proctor and White, 1990; White and Proctor, 1992.)

Magnetic resonance imaging was performed using a FONAR MR (0.5 Tesla magnet) in a routine manner at Somerset Laboratory, Boston, Massachusetts.

TABLE 1
CLINICAL BEHAVIORAL NEUROTOXICOLOGY BATTERY[a]

Wechsler Adult Intelligence Scale—Revised
Wechsler Memory Scale, WMS—Revised
Controlled Word Fluency (FAS)
Boston Naming Test
Writing Sample
Reading Comprehension (BDAE)
Wide Range Achievement Test—R^2
Boston Visuospatial Drawings
Santa Ana Formboard
Milner Facial Recognition Test
Benton Visual Recognition Test
Difficult Paired Associate Learning Test
Delayed Recognition Span Test
Multiple Loops, Recurrent Series Writing
Trails
Wisconsin Card Sorting Test
Minnesota Multiphasic Personality Inventory
Profile of Mood States

[a] For test description and references, see White *et al.*, 1990.

RESULTS

Case 1. The MRI showed mild central and cortical atrophy. Punctiform foci of T2 were noted in both frontal regions, especially underlying the precentral gyri and in the subcortical white matter and in the white matter of the gyri. The MRI was interpreted as being consistent with diffuse and focal white matter disease. The lesions did not resemble those seen in multiple sclerosis plaques or in micro-infarcts.

Neuropsychological testing was given to this patient in both English and Spanish. He demonstrated significant deficits in attention and executive function on all tasks administered including digit spans, mental control, alternating sequences, and card sorting. Performance was marked by perseveration and problems with set. Mild problems were seen on verbal concept formation tasks. Free speech was within normal limits. Visuospatial and visual–motor testing showed problems on most of the tasks administered in this functional domain. Bilateral motor weakness was observed and he had difficulty with visuospatial analysis, coding, sequencing, and visual organization. Difficulties were also observed in facial matching.

In addition, he had difficulty with memory tests, particularly those involving memory for visuospatial materials, such as visual reproductions of the Wechsler Memory Scale and all conditions of the delayed recognition span test.

On personality and mood testing, he reported significant symptoms of irritability, fatigue, and confusion on the Profile of Mood States with reports of low levels of vigor. His family and the patient himself reported irritability, anger, aggressiveness, paranoia, suicidal ideation, hallucinations, anxiety, fatigue, and social withdrawal.

In sum, neuropsychological test results for Case 1 suggest specific problems with cognitive flexibility, cognitive tracking, inhibiting perseveration, fine manual motor coordination, visuospatial analysis and organization, memory for visuospatial information, affect, and personality.

Case 2. The MRI completed in January 1986, at the time of initial evaluation, showed numerous small, discrete foci scattered throughout the white matter and bilaterally in the upper pons. In August 1986, multiple focal areas of high signal intensity were seen in the white matter on MRI, some in the periventricular white matter but some midway between the ventricles and the cortex. There was a question of one or two areas of increased signal in the brain stem. In May 1987, repeat MRI showed multiple small foci deep in the white matter of both cerebral hemispheres and the pons. It was thought that there was some improvement in the MRI relative to August 1986. No more lesions (no progression of disease) was noted at this MRI.

Neuropsychological testing was completed in February 1986. He performed normally on most tests in the area of attention and executive function, but had difficulty with recurrent series writing of *mn,* on which he perseverated bilaterally. On verbal and language testing, he performed somewhat below expectation when asked to generate words beginning with F, A, and S, and his writing sample was marked by perseveration and sequencing errors.

Visuospatial and visuomotor functioning were notable for slowing with both hands on the Santa Ana Form Board test. In addition, he had some difficulties in drawing spontaneously on the Wechsler Memory Scale. Memory testing showed a tendency to forget newly learned information on delay and he had difficulty with multiple choice memory on the Benton Visual Recognition Test, performing below expectation given other abilities.

On personality testing, somatic preoccupation was suggested by his responses to the Minnesota Multiphasic Personality Inventory. His wife reported that he was irritable and apathetic, but the patient himself denied changes in mood.

Overall, results on this patient suggest some affective changes with deficits in manual motor speed, verbal fluency, visuospatial organization, and short-term memory.

DISCUSSION

In these case studies, the neuropsychological test results are consistent with the localization of pathology to the subcortical regions, as seen on MRI. Our cases illustrate the value of using neuropsychological measures in toxic encephalopathy to quantify deficits and confirm dysfunction localizable to subcortical areas of the brain. Both the MRI and testing showed changes in white matter structure and function which cannot be explained except as effects of toxic exposure. These changes were noted using a 0.5 Tesla magnet MR. As Rosenberg *et al.* (1991) showed, a stronger 1.5 Tesla MR was able to demonstrate even more delineation of pathology in solvent encephalopathy due to tolerance. Future studies of clinical neuropsychological tests and brain imaging with strong MR, SPECT, and PET scanning will be important in neurotoxicology.

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Proton Magnetic Resonance Imaging and Phosphorus-31 NMR Studies on the Rat Brain Intoxicated with Methyl Mercury[1]

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Model rats of methyl mercury intoxication were made by orally administering 5 mg mercury/kg methyl mercury daily for 12 days. Proton magnetic resonance imaging and phosphorus-31 nuclear magnetic resonance spectroscopy measurements were performed on the brain of these model animals *in vivo* under anesthesia. Proton images contrasted with the longitudinal or transverse relaxation times of protons on water and lipid molecules exhibited an internal structure of the poisoned brain. No particular difference was, however, observed between the poisoned and normal control rats in either image. On the other hand phosphorus-31 NMR spectra showed a 17% decrease in phosphocreatine and a corresponding increase in inorganic phosphate in the methyl mercury-poisoned brain. It was also shown that the ATP concentration and the intracellular pH were maintained at a normal level even in the poisoned brain.

INTRODUCTION

The toxic property of mercury has been known for a long time. Minamata disease, which broke out in the 1950s in Japan, was one of the worst cases of methyl mercury poisoning in man. Since then extensive efforts have been made to elucidate the mechanism of intoxication of alkyl mercury compounds (WHO, 1990; Chang, 1977; Clarkson, 1987). It has now been established that the major target of methyl mercury toxicity is the nervous system. Pathological studies have shown that methyl mercury causes lesions in various parts of the cerebrum and cerebellum. Damage in the granular layer of the cerebellum is especially common in certain animals including man (Hunter and Russell, 1954; Takeuchi and Eto, 1975). Biochemical studies have shown that methyl mercury poisoning causes an inhibition of protein synthesis (Yoshino *et al.*, 1966; Omata and Sugano, 1985; Cheung and Verity, 1985) and the decreased activity of several sulfhydryl enzymes such as succinic dehydrogenase and fructose-1,6-bisphosphate aldolase (Yoshino *et al.*, 1966). Disorder in the integrity of microtubules has also been reported (Abe *et al.*, 1975; Miura *et al.*, 1978; Vogel *et al.*, 1985).

Despite numerous findings related to the toxicity of methyl mercury, the cellular and molecular mechanisms of the toxic action of methyl mercury in the nervous systems are still unclear. This is partly due to the lack of methods available for directly accessing and investigating the brain *in vivo* on a molecular basis. In the present work nuclear magnetic resonance (NMR) methods are applied to elucidate the neurotoxicity of methyl mercury. We demonstrate some proton MRI and ^{31}P NMR spectra obtained with the experimental animal model of methyl mercury poisoning.

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Of the various techniques aimed at noninvasive analysis of living systems, NMR is one of the most potent tools, along with X-ray tomography (X-ray CT) and positron emission tomography (PET). NMR spectroscopy is based on the behavior of nuclear spins contained in various metabolites in living systems when they are placed in a highly homogeneous magnetic field. It provided biochemical information such as identification, reaction, and mobility of metabolites through the physical parameters, chemical shift, spin coupling, longitudinal and transverse relaxation times (T_1 and T_2), and spin density (Budinger and Margulis, 1988). In principle each of these parameters can be used to construct an image. Routine NMR imaging (MRI) utilizes only T_1, T_2, and spin density for an image, ignoring other parameters. The images obtained by routine MRI are anatomical ones in which we can distinguish gray matter, white matter, and other detailed structures. Abnormalities such as tumors are diagnosed through these images in a noninvasive manner in the field of clinical medicine.

If further biochemical information is required, we must introduce the parameter of chemical-shift value, with which various metabolites could be identified without any separation or purification procedures. Great efforts are being made to accommodate chemical-shift information into the imaging technique. One of these techniques is a spectrocopic localization method which enables us to obtain a spectrum from a localized area of interest (Ordidge *et al.*, 1986; Frahm *et al.*, 1987; Blackledge *et al.*, 1987) and another is a spectroscopic imaging method which produces a metabolite mapping (Brown *et al.*, 1982). Unfortunately, spectroscopic localization or imaging methods are not yet satisfactory for use with small experimental animals. Thus, we used ^{31}P NMR spectroscopy, performed with the help of surgery, to eliminate ^{31}P signals from tissues other than brain in the present work. Compared with the popular use of MRI and NMR spectroscopy in the field of clinical medicine, few attempts have been made to apply these methods to the problems in the field of environmental health. We are sure that NMR, a noninvasive analytical method, can help elucidate the intoxicating mechanisms of methyl mercury.

MATERIALS AND METHODS

Animal model preparation of methyl mercury intoxication. Male Wistar rats (12 weeks old) were orally administered methyl mercury complexed with equimolecular cysteine (5 mg Hg/kg body wt) daily for 12 days. All the methyl mercury-treated rats dragged their hindlimbs when walking in the later stages of the administration. By the day on which NMR measurements were carried out some of the animals showed a crossing phenomenon of hindlimbs when they were held upside down by their tails (Klein *et al.*, 1972). After NMR measurements were finished, animals were sacrificed for the determination of mercurial concentration in the brain. The excised brain (cerebrum and cerebellum) was hydrolyzed by heating at 70°C for 30 min after 1 ml of NaOH (10 *N*) and 1 ml of cysteine (1%) solution were added. Total mercury was determined in an aliquot of the above solution using atomic absorption spectroscopy (Magos and Clarkson, 1972). Since no perfusion of the brain was carried out before excision, the obtained mercurial concentration of the brain could be slightly overestimated due to contamination from residual blood in the brain.

NMR imaging measurements. During the period of 14 and 16 days after the start of methyl mercury administration NMR measurements were carried out.

Anesthesia was induced with sodium pentobarbital (25 mg/kg body wt) and maintained with 1% halothane carried in 50% N_2O and 50% O_2 through a nose cone. NMR measurements were performed at 100.3 MHz with a Bruker Biospec 24/30 spectrometer equipped with a 30-cm horizontal bore magnet operating at 2.35 T. Proton images were taken with a bird cage-type resonator (inner diameter, 15 cm) using a routine multislice multiecho spin-echo sequence. Four coronal slices were measured at the fore-, mid-, and hindcerebrum and at the cerebellum with a slice thickness of 2 mm. T_2-weighted images were obtained with echo times (TE) of 34, 68, 102, and 136 msec and a pulse repetition time (TR) of 5136 msec. A T_1-weighted image was obtained with TE of 34 msec and TR of 334 msec. Each image was reconstructed from a data set of 256 × 256 data points with 256 steps of phase encoding.

^{31}P NMR measurements. Experimental animals were anesthetized in the same manner as those in the imaging measurements. Surgical operations were performed to avoid signal contamination from the skin and muscles surrounding the skull of the rat. The skin over the skull was pulled back laterally, and the masseter muscles on both sides were removed using electrocautery. The spinal muscles at the base of the skull were divided, and these muscles were pulled back. Each prepared rat was placed on a cradle made of Perspex and styrofoam. The skull was fixed on the cradle with adhesive vinyl tapes. A 2-cm-diameter surface coil doubly tuned at phosphorus and proton resonance frequencies was positioned over the skull. ^{31}P NMR spectra were measured at 40.6 MHz by a pulse and collect sequence. A total of 256 free induction decay signals were accumulated with a pulse length of 25 μsec and a pulse recycling time of 20 sec. Before Fourier transformation the obtained signals were deconvoluted by profile correction to remove the unwanted broad components derived from the skull and membranes (Gordon *et al.*, 1982).

RESULTS

The mercurial concentration in the poisoned brain was high at 195 ± 21 μmol/kg wet wt (mean ± SD, n = 5), compared with the value of 0.039 ± 0.004 μmol/kg wet wt (n = 5) in the control brain. This result indicated the successful accumulation of methyl mercury in the brain of the model animal of intoxication.

Figure 1 shows the four proton image slices obtained for the rat head poisoned by methyl mercury. They were T_2-weighted images taken with a TE of 34 msec and TR of 5136 msec. Internal structures of brain such as cerebral cortex, striatum, and ventricles are easily distinguished. No difference, however, was observed between the poisoned and the normal brain. Although we measured further T_2-weighted images with longer TEs up to 136 msec and T_1-weighted images with a TE of 34 msec and TR of 334 msec, neither showed any differences between the poisoned and normal brain (data not shown).

Figure 2 illustrates ^{31}P NMR spectra obtained from the methyl mercury-poisoned and normal brain. Both spectra show resonances derived from phosphomonoester (PME), inorganic phosphate (P_i), phosphodiester (PDE), phosphocreatine (PCr), and three phosphate groups of ATP (α-, β-, γ-ATP).

The quantities of PCr, P_i and ATP were evaluated by relative peak areas of PCr/(PCr + P_i) and β-ATP/(PCr + P_i) in ^{31}P NMR spectra. Figures 3 and 4 show these relative PCr and ATP concentrations in the normal and methyl mercury-poisoned rat brain. The PCr/(PCr + Pi) ratio of 0.69 ± 0.05 (mean ± SD, n = 5)

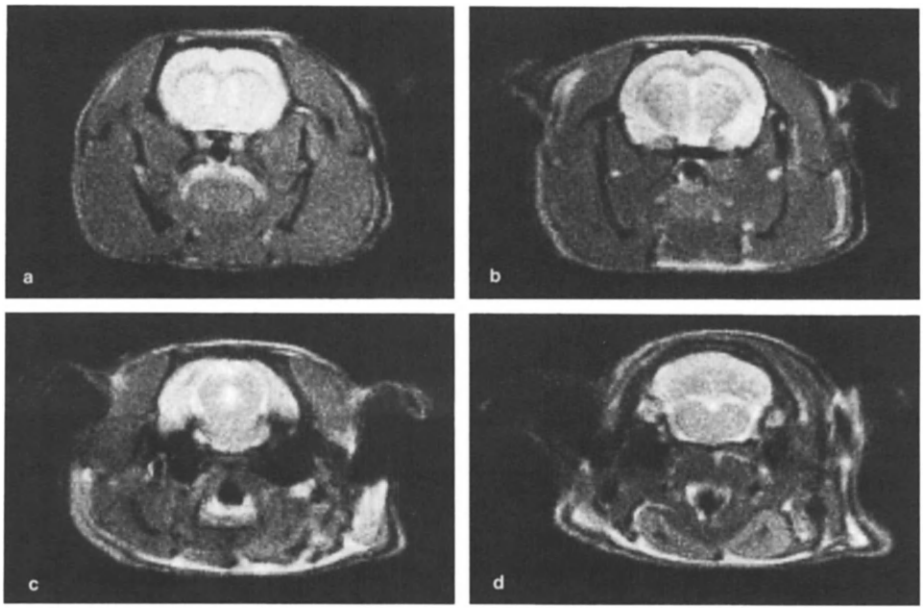

FIG. 1. Proton magnetic resonance images of the brain coronally sliced at the (a) fore-, (b) mid-, and (c) hindcerebrum and at the (d) cerebellum (slice thickness, 2 mm) obtained from rat head poisoned by methyl mercury. Images were measured using a multislice multiecho sequence with a TE of 34 msec and TR of 5136 msec.

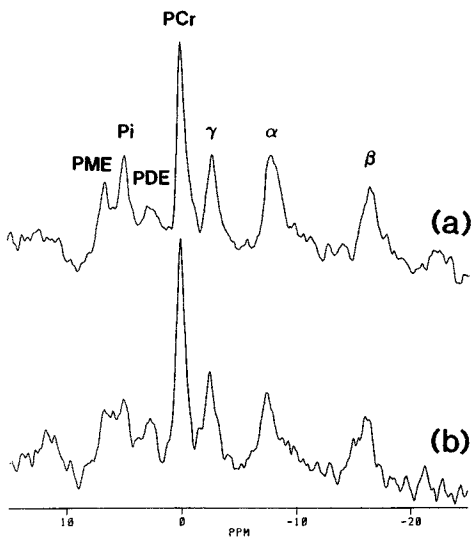

FIG. 2. ^{31}P NMR spectra of (a) methyl mercury-poisoned and (b) normal brain. A total of 256 transients were accumulated with a pulse length of 25 μsec and a pulse recycling time of 20 sec. Profiling (500 Hz, factor 20) and exponential multiplication (line broadening, 15 Hz) were applied on the accumulated signal. Spectral assignments are PME, phosphomonoester; P_i, inorganic phosphate; PDE, phosphodiester; PCr, phosphocreatine; α, β, γ, α-, β-, γ-phosphate groups of ATP. Chemical-shift values are in parts per million from the resonance of PCr.

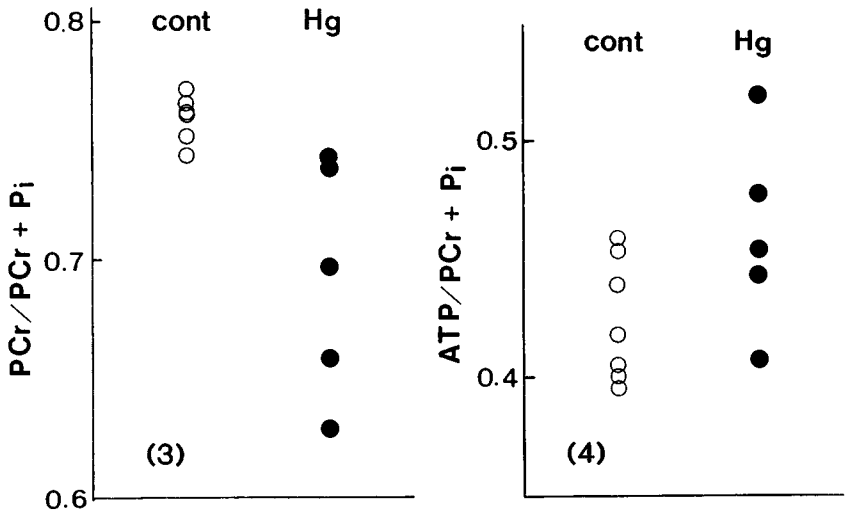

FIG. 3. Individual plots of the relative concentration of PCr/(PCr + P_i) in the normal (○) and methyl mercury-poisoned (●) brain.

FIG. 4. Individual plots of the relative concentration of β-ATP/(PCr + P_i) in the normal (○) and methyl mercury-poisoned (●) brain.

in the poisoned brain was significantly ($P < 0.01$) lower than the normal ratio of 0.76 ± 0.01 ($n = 6$). On the other hand, the β-ATP/(PCr + P_i) ratio of 0.46 ± 0.04 ($n = 5$) in the poisoned brain was slightly higher than but not significantly different from the normal ratio of 0.42 ± 0.03 ($n = 7$). Assuming that the ATP concentration in both groups was 3.00 μmol/g wet wt (Chapman *et al.*, 1981), the PCr concentration in the poisoned and normal brain was calculated to be 4.55 ± 0.34 and 5.45 ± 0.33 μmol/g wet wt, respectively. Thus the PCr was approximately 17% decreased in the poisoned brain.

The intracellular pH was estimated to be 7.1 in both the poisoned and the control brain using the chemical-shift value of the P_i resonance based on the relation between chemical-shift value and pH reported by Taylor *et al.* (1983).

DISCUSSION

Proton MRI did not show any changes in the image of cerebrum or cerebellum of the rat brain poisoned by methyl mercury. Our results for the cerebrum are consistent with the pathological findings of others who observed no particular change in the cerebrum of the poisoned rat model prepared by the same procedure as that in the present study (Kajiwara, personal communication). Proton MRI could not detect the degenerative changes in the form of pyknosis and karyorrexhis in the granular layer of the cerebellum established in the various animal models (Klein *et al.*, 1972; Chang, 1977). This result might suggest that the damages are localized to minute areas smaller than the spatial resolution of the present MRI measurement.

Kuwabara *et al.* (1989) performed proton MRI measurements on the methyl mercury-poisoned rat brain in the presence and absence of a contrast agent and calculated the T_1 and T_2 values at four locations in the brain. They reported prolonged T_1 (1.285 → 1.347 sec) in the cerebral white matter and prolonged T_2 (22.56 → 24.15 msec) in the cerebellar cortex even in the absence of the contrast

agent. It is, however, difficult to distinguish the reported changes of several percent in T_1 and T_2 by visual inspection. Since we have measured four images with different TEs, it could have been possible to calculate T_2 values using intensity changes in these images. However, considering that our minimum TE is still longer than the expected T_2 value, the calculated T_2 value should be erroneous. Therefore, we have not attempted to calculate the absolute T_2 value from our images.

^{31}P NMR showed a 17% decrease in PCr and a corresponding increase in P_i with the ATP maintained at the normal level in the methyl mercury-poisoned brain. This result indicates an increased ADP concentration, assuming equilibrium in the creatine kinase reaction.

Paterson *et al.* (1971) observed acute effects of methyl mercury on adenine nucleotides along with the glycolytic intermediates in rat brain. By using conventional biochemical methods they found an increase of ADP or AMP with no significant change in ATP at around 2.9 μmol/g wet wt in the brain 1 hr after a single dose of 5.0 or 0.5 mg/kg methyl mercury. On the contrary, Salvaterra *et al.* (1973) reported a dose-dependent increase in PCr and ATP along with decreased ADP and AMP in the brain of mouse injected with 1~10 mg Hg/kg.

Our NMR result is consistent with the result of Paterson *et al.*, but not with the result of Salvaterra *et al.* The concentrations of ATP and PCr reported by Salvaterra *et al.* were as low as 1.45 and 1.22 μmol/g wet wt, respectively, with high ADP and AMP concentrations even in the normal brain.

There are two possibilities which may account for the decreased PCr in the poisoned brain shown in the present work. One is the decreased capacity of ATP production, and the other is the increased consumption of ATP in the poisoned brain. It is not possible to conclude which is really the case before we measure the ATP turnover rate in the poisoned brain. Nevertheless the latter case is unlikely because various biochemical studies to date have shown that methyl mercury is rather inhibitory to the ATP-consuming processes such as ion transport by Na^+,K^+-ATPase (Ahammadsahib *et al.*, 1987; Unnikumar *et al.*, 1987) or protein synthesis (Yoshino *et al.*, 1966; Cheung and Verity, 1985).

On the other hand, there are a considerable body of findings suggesting dysfunction in mitochondria in the methyl mercury-poisoned brain. O'Kusky (1983) found ultrastructural changes in mitochondria in the cortical neurons in the methyl mercury-treated rat using electron microscopy. An acute mitochondrial degeneration was also reported in the developing rat brain (Geelen *et al.*, 1990).

Yoshino *et al.* (1966) found a significant decrease in succinic dehydrogenase activity in the brain of methyl mercury-treated rat at the stage when the rat shows neurological symptoms. A linear inhibition of succinic dehydrogenase activity was also reported with increasing duration of dose (10 mg methyl mercury chloride/kg daily) from 1 to 15 days (Unnikumar and Sood, 1987). All of these findings suggest a deficiency in the oxidative metabolism in mitochondria, thus a reduced capacity in the ATP synthetic reaction. The present work is, however, the first report showing directly a damage in the energetics *in vivo* in the methyl mercury-poisoned brain utilizing the noninvasive analytical method of NMR.

There have been several studies investigating ATP concentration in the cultures of brain cells in relation to the inhibitory mechanism of protein synthesis (Cheung and Verity, 1981; Sarafian *et al.*, 1984; Grundt and Bakken, 1986). Most of them reported a dose-dependent decline in ATP in the cell in the presence of 10 to 50

μM methyl mercury. Although PCr decreased, the ATP concentration was not affected by the presence of 195 μM total mercury in our *in vivo* measurement. Thus, the above *in vitro* results were found not to mimic the *in vivo* situation. The present result is consistent with the recent result of Sarafian and Verity (1990), which suggests the irrelevance of the ATP level to the inhibition of protein synthesis.

In conclusion the NMR method gave us a means to investigate methyl mercury intoxication in brain from both anatomical and metabolical aspects. Although proton MRI failed to distinguish the morphological damage, ^{31}P NMR spectra showed a decrease in PCr with no change in ATP and intracellular pH in the poisoned brain. This result demonstrates the damage in energetics in the methyl mercury-poisoned brain.

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Neurochemical Approaches to Developing Biochemical Markers of Neurotoxicity: Review of Current Status and Evaluation of Future Prospects[1]

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INTRODUCTION

The goal of public health is to prevent disease and injury in populations. In environmental and occupational health, this means identifying toxic substances or hazardous conditions and controlling exposures of populations to levels below which no discernible unacceptable risk of disease or injury is likely to occur. A range of regulatory approaches has been developed in most countries toward meeting these goals. Critical to these approaches is an appropriate definition of both exposure and disease. Increasingly, it is recognized that these definitions should support the goal of prevention by defining both exposure and disease in such a manner as to *prevent* disease. In the case of exposure, this has been implemented by setting exposure levels at very low levels—in some cases, at levels determined by control technology or by analytic sensitivity rather than by an overt health-based objective. In the case of disease, over the past 5 years there has been considerable movement toward defining outcomes not in terms of an increase in rates of clinically defined diseases repeats but in terms of an increased incidence of preclinical manifestations, or physiological effects measurable in exposed persons long before or well below the induction of frank disease. This new approach in public health is based upon the following assumptions: (1) toxicant-induced diseases (like most diseases) progress from early subcellular events to severe disease (including death); (2) this progression is in a series of biologically linked steps such that the probability of each step is increased by the occurrence of the step preceding it; (3) prevention of toxicant-induced disease requires identification and reduction of exposures before irreversible damage has been induced in a target organ system; (4) monitoring of exposures for purposes of reducing risk is best done by monitoring early biological responses of exposed persons. These assumptions underlie such measures as the U.S. occupational lead standard (Silbergeld *et al.*, 1991).

The new methodology of biological markers has been developed to meet these goals. Biological markers have been defined by the U.S. National Research Council Committee on Biological Markers as "indicators signalling events in biologic systems or samples. It is useful to classify biologic markers into three types—exposure, effect, and susceptibility—and to describe the events particular to each type. A biologic marker of *exposure* is an exogenous substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target

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molecule or cell that is measured in a compartment within an organism. A biologic marker of *effect* is a measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease. A biologic marker of *susceptibility* is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance" (National Research Council, 1989).

Biological markers include signals that denote exposure only (or mainly), response, or susceptibility (which may precede or be induced or altered by exposure). They may be considered schematically as shown in Fig. 1 (National Research Council, 1987). As may be obvious, a marker or biological signal may denote more than one event. For instance, as shown in Fig. 2, elevations in erythrocyte protoporphyrin (EP) are widely used as markers for increased lead *exposure* in children and workers; however, EP represents a cellular *response* to the presence of lead in the erythrocyte, and it may also signal *susceptibility* due to preexisting iron deficiency or genetics (Silbergeld, 1985). Thus the most appropriate definition of a marker probably depends upon its use, rather than any absolute nature. If EP is used as a marker of exposure, then it is an independent variable in studies of exposure:effect; if EP is an outcome variable, then it is a dependent variable; if it is a marker of susceptibility, then it may be classified as a confounder in certain studies.

In general, biochemical events are considered to be a potentially rich source of biological markers. If it is assumed that biochemical alterations precede structural damage, then detection of biochemical changes may provide opportunities for early identification of excess exposure and intervention to prevent irreversible damage and frank disease. This strategy is based on the identification of *early* and *reversible* biochemical events that are sensitive and specific indicators of exposure and early organ system response. The extent to which such biological markers are *predictive* of later responses (including disease or risk of disease) requires considerably more research, integrated with epidemiological surveillance and prospective studies, as discussed below. In general, the earlier the marker in the progression of biological response, the less strongly it predicts later outcome (see Fig. 1), however, the more useful it may be for purposes of prevention.

This paper does not discuss biologic markers of exposure to neurotoxicants, since these do not usually involve special neurochemical methods. Concerns for

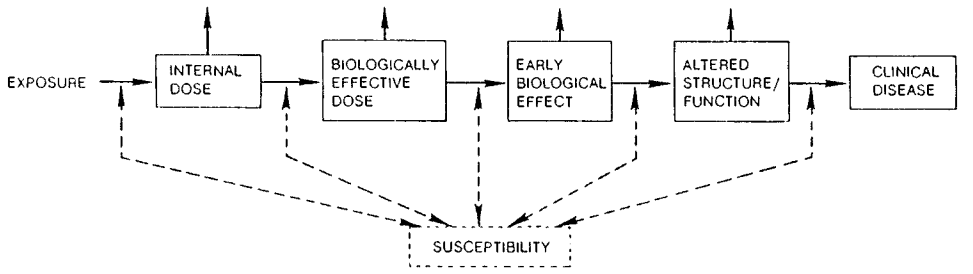

FIG. 1. Schematic diagram of biological markers, indicating a theoretical progression from external exposure, through absorption and distribution, to early biological effect through to clinical disease. Susceptibility factors may influence all steps in this progression. From the National Research Council (1989).

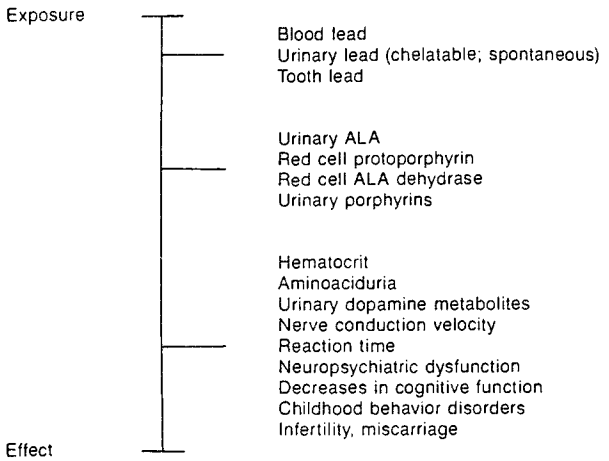

FIG. 2. Schematic diagram of markers for lead, from exposure to severe intoxication. Responses of the heme biosynthetic pathway are considered to represent both exposure and response, depending on their utilization. From Silbergeld (1985).

neurotoxicity may require very sensitive measures of exposure, based upon the experience that neurotoxicity is frequently a consequence of relatively low-level exposures (Office of Technology Assessment, 1990; National Research Council, 1991).

In this paper, I discuss mainly biological markers of response in the context of neurochemistry. While the focus of this paper is on neurochemical markers of neurotoxicity in humans, it is appropriate to note that there is considerable interest in developing biological markers in ecotoxicology, and some of these concepts may be relevant to utilizing markers for the protection of species other than humans. I discuss only briefly biologic markers of susceptibility with respect to neurotoxicity. These markers may reflect preexisting disease, genetic heterogeneity in target proteins, or coexisting intoxication due to other exposures (such as drug use).

GENERAL BACKGROUND ON NEUROCHEMISTRY AND NEUROTOXICOLOGY

The nervous system can be defined as a large and complex set of interacting cells whose communications are transduced by molecular signals that initiate, modulate, or terminate chemical reactions (Snyder, 1986). These signals may be ions, amino acids, proteins, or peptides. The chemical reactions they control may be quite complex, involving a cascade of associated chemical events (Berridge, 1986; Hollenberg, 1991). The fundamental function of the nervous system—to process, store, retrieve, and send information—is accomplished by biochemical reactions that may occur as quickly as a microsecond to as long as several days.

Neurochemistry is the field of biochemistry that deals with biochemical pathways and processes in the nervous system that support these functions. As such, it encompasses much of physiological chemistry that occurs in all cells as well as those biochemical pathways that are unique to or particularly important to cells of the nervous system. The nervous system, central and peripheral, consists of many cell types in addition to those excitable cells called neurons. Because of the close

interactions between neurons and other cells, the biochemistry of nonneural cells can often influence the functional status of neurons. The close physical association between neurons and supporting cells, such as glia in the CNS, may also be important in toxicology. For example, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is processed by astrocytes in the CNS, which then release the pyridinium metabolite 1-methyl-4-phenylpyridinium (MPP+); MPP+ is taken up selectively by monoaminergic neurons to which it is cytotoxic (reviewed by Johannessen, 1991). Thus the neurotoxicity of this agent is dependent on the spatial proximity of astrocytes and neurons and the sequential biochemical processing of the protoxin into a toxic metabolite and its subsequent availability for high-affinity uptake (Fig. 3).

Neurochemical pathways may also be differentially dependent upon precursors owing to the physiological compartmentation of the nervous system, particularly the brain. The CNS must obtain glucose, choline, tyrosine, tryptophan, and other precursors materials from the periphery. Thus brain chemistry is influenced by plasma concentrations of these essential precursors and, hence, potentially by dietary intake of amino acids although the extent of this dietary influence is not clear (Wurtman and Wurtman, 1983). These molecules are transported across specific structures into brain by specific transporters which may be important biochemical targets for neurotoxins.

Even for those biochemical pathways which nervous tissue shares with other organ systems—such as oxidative phosphorylation and heme biosynthesis—these pathways may be more sensitive to depletion or interruption in neurons because of high demands for functional output. Anoxic stress, for instance, is well known to result in more profound damage to the CNS than to other organ systems (Windle, 1983).

Much of neurochemistry research is focused upon those biochemical pathways involved in signal transduction through the regulation of the synthesis, storage, release, recognition, and catabolism of neurotransmitters and other types of neuromodulators. This provides several types of measurable markers: precursor amino acids, amino acid transporters, synthesizing enzymes, concentrations of transmitter in intracellular compartments, membrane-bound synaptic receptors,

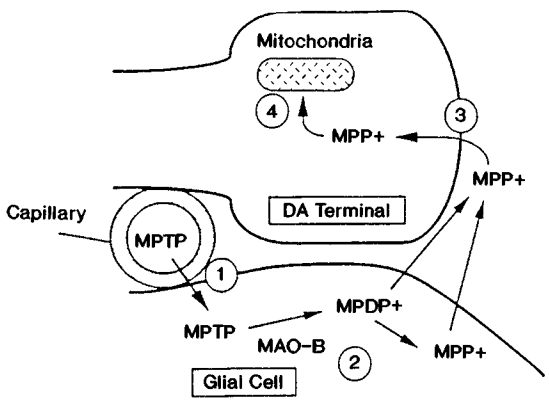

FIG. 3. Current model of the activation of the neurotoxin MPTP by glial cells and the uptake of the toxic metabolite MPP+ by dopaminergic neurons. From J. N. Johanessen (1991). A model of chronic neurotoxicity: Long term retention of the neurotoxin 1-methyl-4-phenylpyridinium (MPP+) within catecholaminergic neurons. *Neurotoxicology*, Volume 12, pp. 285–302.

catabolizing enzymes, high-affinity reuptake transporters, and metabolites. These neurochemical markers can be measured as concentrations in nervous tissue, or in terms of rates of reaction. Many of these events occur within nervous tissue, and this restriction gives rise to one of the biggest obstacles to developing biochemical markers of neurotoxicity: the relative compartmentalization of the nervous system and its inaccessibility to *in vivo* measurement without extraordinary invasive techniques. Some approaches to dealing with this problem are discussed below.

Many neurochemical events are interactive because of the high degree of feedback circuitry within the nervous system. The nervous system is highly adaptive at the biochemical level, perhaps to compensate for a lack of cell replaceability in the CNS. For instance, a reduction in the amount of neurotransmitter released (as a consequence of cell loss, inhibition of neurotransmitter synthesis, or blockade of presynaptic release) results in upregulation of postsynaptic receptors, to compensate for decreased signal input in the receiving cell. Conversely, an increase in the rate of presynaptic release or an inhibition in the rate of catabolism (or reuptake, another clearance mechanism to terminate cell:cell communication characteristic of aminergic neurotransmission) results in decreased release and/or downregulation of postsynaptic receptors to reduce signal input.

Both types of neurochemical change—decreased *or* increased neurotransmission—can result in neurotoxicity. Neurotransmission *deficits* underlie such neuropathological conditions as lead-induced peripheral neuropathy (Schwartz *et al.*, 1988); neurotransmission *excess* is thought to produce excitotoxic damage and may underlie conditions such as dementia and parkinsonism (Weiss, 1990; Langston, 1989).

In addition to information processing in the mature nervous system, neurochemical modulators shape the morphological development of the CNS. During neurodevelopment, transmitters act as trophic agents to guide the cytoarchitectural formation of the brain. The trophic effects of the neurotransmitters acetylcholine, dopamine (DA), γ-aminobutyric acid, glutamate, norepinephrine, and serotonin are shown in Table 1 (Lipton and Kater, 1989). Thus, as with many aspects of neurotoxicity, alterations of neurochemical events have different functional implications depending on developmental stage.

Finally, for a full appreciation of the complexity of the nervous system as a set of biochemical reactions, it is important to keep in mind that many peptides originally identified in peripheral nonneural tissue are now recognized to possess significant neuroactivity—for instance, bradykinin, cholecystokinin, and bombesin (Snyder, 1986). Moreover, the nervous system interacts with, and controls in some instances, the functions of other systems. For instance, given the controlling role of the hypothalamus in regulating pituitary factors, it is not surprising that molecules such as the gonadotropins, estrogen, testosterone, progesterone, and other steroid hormones have specific binding sites in neural tissue to exert feedback control on the CNS (Snyder, 1986). Similar commonality in terms of receptors has been reported for lymphocytes and nerve cells. The ability of human immunodeficiency virus (HIV) to infect neurons also indicates the existence of membrane receptors common to neurons and immune cells (discussed in Silbergeld, 1990a). These complexities may open up opportunities for investigating the nervous system indirectly, as discussed below.

TABLE 1
TROPHIC EFFECTS OF NEUROTRANSMITTERS AND NEUROPEPTIDES ON GROWTH, PLASTICITY, AND SURVIVAL OF NEURONS

Neurotransmitter	Neuronal preparation	Effect
Acetylcholine	Rat retinal ganglion cells	Inhibits neurite outgrowth
	Chick retina	Inhibits neurite outgrowth
	Hippocampus	Inhibits dendrite outgrowth
	Adult *Helisoma*	Prevents inhibition of outgrowth by serotonin
Dopamine	Chick retina	Inhibits neurite outgrowth
	Helisoma	Inhibits neurite outgrowth
	Rat striatum	Prerequisite for ischemic injury
GABA	Rat hippocampus	Prevents glutamate-induced dendritic regression
Glutamate	*Helisoma*	Promotes neurite sprouting
	Rat hippocampus	Promotes dendritic regression, low dose (kainate, quisqualate)
	Rat hippocampus	Promotes neurite sprouting (NMDA)
	Rat hippocampus	Promotes neurite sprouting
	Rat hippocampus	Produces cell death, high dose
	Mouse cortex	Produces cell death, high dose
	Rat retinal ganglion cells	Produces cell death, high dose
	Tadpole optic tectum	Stabilizes coactive visual synapses (NMDA)
Norepinephrine	Rat cortex	Produces cell death
Serotonin	*Helisoma*	Inhibits neurite outgrowth
Somatostatin	Adult *Helisoma*	Promotes neurite sprouting
Vasoactive intestinal peptide	Mouse spinal cord	Prevents cell death produced by electrical blockade
Vasoactive intestinal peptide	Rat retinal ganglion cells	Prevents death produced by electrical blockade

Note. From Lipton and Kater (1989).

PROBLEMS IN DEVELOPING NEUROCHEMICAL MARKERS FOR NEUROTOXICOLOGY

Despite the richness of neurochemical phenomena and the extensive although far from complete knowledge of neurochemical events in the nervous system, biologic markers for neurotoxicity are underdeveloped (National Research Council, 1989). There are several important obstacles to developing neurochemical markers for use in neurotoxicology, basic and applied. First, at the fundamental level, there are still large gaps in our knowledge of neurobiology such that we do not know the biochemical substrates of much of the nervous systems's cellular or system-level function, nor do we know enough to evaluate the cellular or system-level consequences of neurochemical alterations in most cases. While there are some well-investigated examples where studies have demonstrated the correlations among neurochemical changes and behavioral outcomes (e.g., Gibb *et al.*, 1990), these are relatively few. With the exception of the hexacarbons they do not relate to neurotoxins of importance to environmental or occupational medicine. Second, we know little about the mechanisms of action of most neurotoxins so that it is difficult to identify appropriate biologic markers.

Even if these fundamental limitations were surmounted, major problems still exist in the development of neurochemical markers. The nervous system is generally isolated from those compartments of the body which can be readily sampled for measurement of biological markers of exposure or response, such as blood, exhaled breath, skin, or urine. The cerebrospinal fluid (CSF) is the immediate proximate compartment to the CNS, but it cannot be easily or routinely sampled. The choroid plexus exerts a great deal of regulation on the entry of xenobiotics into CSF. Not all neurochemical signals reach CSF; many are rapidly cleared or metabolized and conserved to the synapse.

Several molecules have been identified as important molecular markers of nervous system function, as listed in Table 2 (O'Callaghan and Miller, 1983). None of these have been found in blood or urine. Of course, these markers may be very useful tools in experimental studies in neurotoxicology, but this is outside the scope of this paper.

In most cases, sampling for neurochemical markers must be limited to blood or urine. By the time endogenous compounds from the nervous system, such as neurotransmitter metabolites, reach blood or urine, their relationship to the individual's neurobiological function is uncertain for two reasons. The rate of appearance of metabolites in urine may not be related to neuronal function. Also, tissues other than the CNS may contribute to the pool of urinary and plasma metabolites (Karoum, *et al.*, 1984).

Another problem is developing and interpreting neurochemical markers is the rapidity of biochemical events in the nervous system, including response to the stress of sampling, and the impact of diurnal rhythms on neurochemical function. These factors make it difficult to interpret results of samples taken at one particular time, rather than periodic sampling of blood or complete collections of daily urinary output. Other factors such as age and diet may also change the levels or concentrations of measurable neurochemical markers, such as plasma tryptophan. Of course, individual use of drugs (including alcohol) will significantly affect neurochemical markers. Finally, there is little information on "normal ranges" of these markers so that all current studies must utilize carefully matched subjects with accurate information on differences in exposure.

TABLE 2
BIOCHEMICAL MARKERS OF CNS CELLS

Biochemical Marker	Indicator For
Central Spinal Fluid Marker	
Protein I	Status of synaptic membranes of CNS neurons
D2 (neural tube)	Status of synaptic membranes of CNS neurons
B50	Status of synaptic membranes of CNS neurons
P5D 95	Postsynaptic receptors
Myelin basic protein (MS)	Status of oligodendroglia and myelin sheath
Myelin-associated glycoprotein	Oligodendroglia
GFAP	Astrocytes (gliomas)
Brain Tissue Markers	
Protein III	Cell loss (nerve terminals)
Synapsin I	Cell loss (nerve terminals)

Note. From O'Callaghan and Miller (1984).

CURRENT METHODS IN NEUROCHEMICAL MARKERS

With all these problems, a few studies have been conducted using neurochemical markers in studies of populations exposed to neurotoxins. These studies have utilized different methods for measuring as well as different exposure conditions. Two are discussed below as they exemplify the potential for and present limitations of neurochemical markers in neurotoxicology.

Lead Neurotoxicity

A great deal of experimental research has been conducted on the effects of lead on neurochemistry (see Silbergeld, 1991, for review). Many studies have reported that lead exposure affects aminergic, cholinergic, and GABAergic pathways in the CNS. Of these systems, the catecholaminergic pathways utilizing dopamine and norepinephrine yield metabolites that can be measured in plasma and urine. Relatively high-level lead exposure has been reported to increase the concentrations of the dopamine metabolite homovanillic acid and the norepinephrine metabolite vanillylmandelic acid in 24-hr urine collections taken from children (Fig. 4) (Silbergeld and Chisolm, 1976). Intervention—removing children from leaded environments and administering chelation drugs—was associated with reductions in

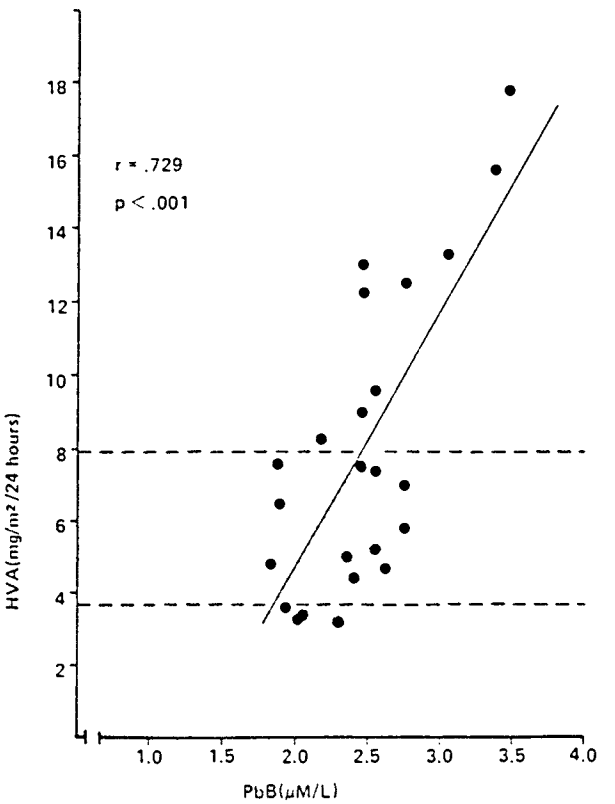

FIG. 4. Relationship between blood lead concentrations and urinary excretion of the catecholaminergic metabolite HVA in children. From E. K. Silbergeld and J. J. Chisolm (1976). Lead poisoning: Altered urinary catecholaminergic metabolites as indicators of intoxication in mice and children. *Science* Volume 192, pp. 152–153. Copyright © AAAS.

both blood lead levels and urinary catecholamine metabolites, further supporting an association between lead exposure and this neurochemical marker. These results have been replicated in populations in Poland and the U.S. but not in Yugoslavia (Graziano, personal communication; Hu, personal communication).

MPTP Intoxication

Studies have also been done on MPTP-intoxicated persons, using positron emission tomography (PET) as the method for assessing brain chemistry *in vivo*. Calne *et al.* (1986) showed that dopaminergic neurons in nigrostriatal regions are damaged in persons with MPTP exposure, manifested as decreased uptake of the precursor fluorodopa that can be visualized by computer-assisted tomography scanning techniques.

Both of these studies demonstrate the potential for neurochemical markers in studies of persons exposed to identified neurotoxicants. However, they also demonstrate some of the current limitations on the application of such approaches in neurotoxicology. First, these studies were quite complex to carry out. In the lead studies quantitatively complete 24-hr urine samples were collected from children in hospital for EDTA chelation therapy; the ability to ensure reliable samples on an outpatient basis is uncertain. In the MPTP study, subjects had to be administered a specific stable isotope tracer to label dopaminergic neurons and rather expensive technology was used to detect neurochemical change. It is therefore probably not surprising that relatively few similar studies have been conducted.

Moreover, these studies did not detect neurotoxic damage that was not evident by other means. It is well known that children with blood lead levels in the range studied by us manifest significant decrements in peripheral nerve conduction velocity, reaction time, and cognitive performance (Needleman, 1988). Measuring urinary neurotransmitter metabolites, while it confirms the mechanistic studies done in animals, does not add information critical to the clinical evaluation of individual children or to groups with similar exposures to lead. In the MPTP case, it is somewhat more arguable that the PET scanning added information on the extent of morphologic damage induced by MPTP in human basal ganglia *prior* to the manifestation of parkinsonism-like signs. However, MPTP was already known to be cytotoxic to these dopaminergic neurons from animal studies (Langston, 1989; Johanssen, 1991). It is certainly the case that investigations that measure such markers in the *absence* of clear exposure information are unlikely to be of use in determining either what exposures may have occurred or what long-term consequences may develop in individuals. A similar critique can be made of neurochemical investigations conducted in patients with psychiatric or neurologic diseases. In some of these cases, studies of neurochemical markers in plasma and urine have reported significant changes as compared to controls, but in no cases did these studies identify "presymptomatic" cases, aid in devising pharmacologic interventions, or elucidate neuropathological correlates of disease. (Karoum *et al.*, 1984; Muscettola *et al.*, 1984; Deutsch and Campbell, 1984).

NEUROCHEMICAL MARKERS OF EXPOSURE AND SUSCEPTIBILITY

As noted earlier, markers of exposure are not usually specific to the target organ since in most cases it is inaccessible to measurement. Theoretically, magnetic resonance imaging (MRI) technology may permit visualization of some neurotoxins in brain. This has not been done in humans; however, studies on man-

ganese have utilized MRI to demonstrate its accumulation in brain in experimental animals (Weiss, 1991). MRI studies in humans have followed the uptake and retention of manganese in fetuses (Mattison *et al.*, 1988).

Markers of susceptibility provide information on inherent or acquired factors that influence response. Such factors may modulate absorption and retention of the toxicant, its relative distribution to the nervous system, and the status of the target site(s). Although genetic determinants are known to influence neurobiology and neurotoxicity in rodents and other organisms (for instance, susceptibility to neonatal ethanol exposure in rats (Goodlett *et al.*, 1989)), little is known of the distribution of genetic heterogeneity of CNS function in humans or even the incidence of genetically determined neurologic disease (Gusella *et al.*, 1984). The subject of genetic factors in complex behaviors such as intelligence remains quite controversial. There is no evidence that persons with lowered IQ (as measured by conventional methods) are more susceptible to neurotoxicants. Studies of lead indicate that the neurobehavioral and cognitive deficits induced by lead affect children across the range of psychometric performance (Needleman, 1988). Social class and economic status, not genetics, confer advantages in terms of apparent reversibility of lead-induced neurotoxicity (Bellinger *et al.*, 1989).

The only identified interactions between a gene defect and a neurotoxin relate to lead and acute intermittent porphyria (AIP); persons with AIP, a hereditary disorder of heme biosynthesis, are reportedly susceptible to lead (Wetterberg, 1966). Nothing is known of the interactions between neurotoxins and other genetic mutations related to altered neurobiological function and disease, such as trisomy 21 or Huntington's (Gusella *et al.*, 1984). The possibility of gene:environment interactions cannot be excluded and may be an important determinant of the incompletely understood neurodegenerative disorders of the Western Pacific, some of which may involve dietary neurotoxins (Calne *et al.*, 1986).

Nongenetic factors may be very important determinants in neurotoxic response: nutrition, age, and coexposure to other toxicants may influence outcome. These are important variables to evaluate in clinical studies; markers for nutritional status (e.g., serum calcium and total iron-binding capacity) are important correlates in studies of lead toxicity (Silbergeld, 1990b).

PROSPECTS FOR ADVANCES IN NEUROCHEMICAL MARKERS

The general limitations on measuring neurochemical markers, discussed above, will not be overcome in the near term. There are perhaps three directions in which research may be fruitful in this field over the longer term. Studies combining neurochemical techniques with other methods of assessing neurobiological status—neurobehavioral tests and electrophysiology—are likely to be important.

First, the known interactions between the nervous system and other systems may provide *secondary* markers of nervous system function, that is, markers of systems that are responding to changes in neurobiological control signals. For example, measurement of pituitary peptides such as growth hormone and prolactin have been used in studies of neuroendocrine disorders such as acromegaly; FSH is reported to be altered in lead-exposed workers. While such signals may also reflect toxic effects on pituitary and target organs (such as gonads), they indicate an involvement of the nervous system in overall toxicity.

For instance, these studies could be expanded to investigate the utility of using neuroimmunological interactions to provide additional secondary markers. As

Saunders has found (V. Saunders, personal communication), changes in noradrenergic output affect B-lymphocyte secretion of immunoglobulin through α-2 and β-2 noradrenergic receptors; with further definition of neuromodulation of such response, immunoglobulins or other markers of B-lymphocyte function might serve as secondary markers of neurochemical change. Of course, the more remote the marker is from the putative site of toxic action, the more likely other factors will influence the expression of the marker unless the two (primary and secondary) markers are very tightly coupled. Similar strategies might be used to explore interactions between gut peptides and the CNS, or between steroid hormones and the hypothalamus (Snyder, 1986).

Another strategy is to investigate *parallel* systems for markers. One such system, which has been used in neuropharmacology, is the blood platelet system (Pletscher, 1986). Blood platelets express some of the same neurochemical functions as neurons, including high-affinity amine uptake, storage of amines in intracellular vesicles, and membrane binding of neuroactive drugs and other receptor ligands (Rotman, 1983). A number of studies have reported changes in platelet serotonergic markers in patients with migraine and affective disorders (Rotman, 1983). Blood platelets also reflect MPTP damage (Johanssen, 1991). In studies of workers exposed to aliphatic and aromatic hydrocarbon solvents, some effects on serotonin concentration and uptake have been reported (Lam *et al.*, 1985). The red cell has also been utilized as a parallel marker for neurochemistry, in studies of cholinesterase (Levine *et al.*, 1985), which is widely used as a marker for exposure to organophosphate insecticides. Blood choline concentrations may also be useful indicators of CNS cholinergic function in connection with assessing responses to low-level pesticide exposure (Boyd *et al.*, 1990).

Exploiting these and other parallel systems for markers must be based on understanding the biochemistry of these nonneural systems as well as their responsiveness to neurotoxic compounds. They may be more useful as sources for markers of exposure rather than effect, just as lymphocyte and hemoglobin adducts of DNA electrophiles are used as parallel markers for DNA adducts in studies of biological markers of chemical carcinogens (Perera and Weinstein, 1982).

More attention might be paid to two other accessible compartments for sampling and analysis that have not been exploited in the development of biologic markers. These are tears and saliva. Tears are in contact with the eye and hence with certain neural structures; saliva is affected by such neurological diseases as parkinsonism and acromegaly (Kandel, 1990). Steroid hormones and many drugs, including psychoactive drugs of abuse, are also found in saliva (Kandel, 1990). It is not known if neurotransmitter metabolites, found in plasma, are also recoverable in saliva, or if peptides common to mandibular gland and CNS might be detectable in this compartment.

In the longer term, technological advances in PET and MRI may expand opportunities for using neurochemical markers and assessment of nervous system function in general. PET techniques can now be used to measure local blood flow in the CNS, blood volume, oxygen consumption, blood:brain barrier permeability, receptor binding, and neurotransmitter metabolism (Druckman and Lacey, 1989). Further developments may reduce the costs of applying PET, as well as expand the range of events that can be detected. Similarly, MRI may be refined to detect subtle changes in phosphorylation of critical proteins, such as changes in

phosphorylation associated with the second messengers of the primary signals in neurotransmission, cyclic AMP, and inositol triphosphate (Berridge, 1986).

CONCLUSIONS

The need for sensitive and specific methods to assess early events in neurotoxicity is particularly important for achieving the goal of preventing neurotoxic disease (Office of Technology Assessment, 1990; National Research Council, 1992). Neurochemical methods are theoretically ideal sources for identifying biologic markers for this purpose; however, the obstacles to developing neurochemical markers are considerable. While technological advances may improve our ability to monitor neurochemical events in the CNS, these advances must be coupled with increases in our knowledge of the mechanisms of specific neurotoxicants and the neurochemical substrates of alterations in other neurobiological phenomena, such as electrophysiology and behavior. Thus, further advances in neurochemical methods will probably be accomplished by studies that combine several approaches to the assessment of neurotoxins.

Validation of neurochemical markers requires understanding of the role of specific neurochemical events in the pathogenesis of neurotoxic damage. Experimental research is required to develop this knowledge. Eventual clinical validation will involve long-term prospective studies of well-defined populations whose status is periodically assessed to correlate progressive changes in specific markers with eventual outcome. These studies are difficult to design and execute; international collaborative approaches—similar to those undertaken in studies of environmental carcinogens—may be necessary to develop the necessary database and long-term followup.

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Nerve-Specific Marker Proteins as Indicators of Organic Solvent Neurotoxicity[1]

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Effects of chronic exposure to *n*-hexane and toluene on some nerve-specific marker proteins in rat central nervous system (CNS) and peripheral nervous system (PNS) were assessed and compared. The rats were exposed to 2000 ppm *n*-hexane, 12 hr/day, 6 days/week, for 24 weeks, and to 1000 ppm toluene, 8 hr/day, 6 days/week, for 16 weeks. The level of neuron-specific enolase (NSE), creatine kinase-B (CK-B), and β-S100 protein in cortex, cerebellum, spinal cord, and proximal and distal sciatic nerve was determined by enzyme immunoassay method. In *n*-hexane-exposed rats, the level of NSE, CK-B, and β-S100 decreased significantly in the distal segment of the sciatic nerve, while the marker proteins in CNS and proximal sciatic nerve remained unchanged. In contrast, chronic exposure to toluene mostly affected these marker proteins in CNS tissues, displaying the increase of NSE, CK-B, β-S100 in cerebellum, as well as the increase of β-S100 in spinal cord. No quantitative changes of the three proteins in distal sciatic nerve were observed after exposure to toluene. *n*-Hexane-induced peripheral distal neuropathy and toluene-induced brain gliosis appeared to be responsible for this different pattern of biochemical changes. The present study suggests the usefulness of using these nerve-specific marker proteins to assess the solvent-related CNS and PNS neurotoxicity.

INTRODUCTION

It is well recognized that organic solvents represent one group of neurotoxicants and can product neurobehavioral dysfunction and peripheral neuropathy in both humans and animals (Spencer and Schaumburg, 1985). However, the biochemical basis of the solvent-related neurotoxicity is poorly defined. The sensitive and specific indicators to assess the effects are generally lacking. Recent progress in neurochemistry and immunohistology has confirmed that some specific isoenzymes or isoproteins, e.g., neuron-specific enolase (γ-enolase, NSE), creatine kinase-B (CK-B), and β-S100 protein are specifically distributed in central neuron (NSE) and glial cells (CK-B, β-S100), as well as in peripheral axons as the components of axoplasmic transport (Isobe *et al.*, 1990). Various clinical investigations have demonstrated the feasibility of using these nerve-specific marker proteins for evaluating the neurological disorders (Royds *et al.*, 1981; Michetti *et al.*, 1980; Pfeiffer *et al.*, 1983).

To clarify the usefulness of these nerve-specific marker proteins as indicators of solvent neurotoxicity, we initiated the present study to examine (1) whether long-term solvent exposure induces any changes in these biochemical markers in var-

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ious nervous tissues, and (2) if so, whether the solvents having central nervous system (CNS)- and peripheral nervous system (PNS)-predominant neurotoxic effects result in different patterns of the marker proteins in CNS and PNS tissues. For these purposes, *n*-hexane and toluene, two kinds of the most commonly used solvents in industry, were selected as model solvents with peripheral and central neurotoxicity after long-term exposure, respectively (O'Donoghue, 1985).

MATERIALS AND METHODS

Animals and Solvent Exposure

Wistar male rats weighing about 300 g were separated into four groups (eight rats per group) to conduct two inhalation experiments. In the first experiment, one group of animals was exposed to 2000 ppm *n*-hexane vapor (99% pure, Katayama Chemical Co.) for 24 weeks (12 hr/day, 6 days/week, 1600–0400 hr) in an inhalation chamber. Another group of rats served as control and was housed in the identical chamber ventilated with fresh air only. In the second experiment, one group of rats was exposed to 1000 ppm toluene for 16 weeks (8 hr/day, 6 days/week, 1600–2400 hr). The control rats were only exposed to fresh air for the same period. The environment was kept on a 12-hr alternation light/dark cycle and held at 20–21°C and 57–60% humidity. Food and water were freely available.

The body weight gain of the rats was measured every 2 weeks. In the *n*-hexane experiment, peripheral motor nerve conduction velocity (MCV) and distal latency (DL) were measured in the tail nerves before and after 4, 8, 12, 16, 20, and 24 weeks of exposure.

The computerized inhalation exposure system was used for solvent exposure. The details of this system have been described previously elsewhere (Takeuchi *et al.*, 1989; Huang *et al.*, 1990). The time-weighted average concentrations of *n*-hexane and toluene during the whole exposure period were 1954 ppm (SD, 86 ppm) and 1003 ppm (SD, 24 ppm), respectively.

Enzyme Immunoassay of the Marker Proteins

The rats were killed by decapitation about 48 hr after the last exposure. Then, the cerebral cortex, cerebellum, brain stem, spinal cord (at the level of S_5–S_7), and left sciatic nerve (from sciatic notch to knee) were immediately isolated, weighed, frozen, and kept at −30°C to be analyzed a few days later. Sciatic nerve was cut into a proximal and distal segment at the level of the triceps surae nerve.

In enzyme immunoassay, various tissues were first homogenated at 0°C in a Teflon-pestled glass homogenizer with 50 mM Tris–HCl buffer (pH 7.5). The homogenates were centrifuged at 15,000*g* for 20 min at 4°C, and the supernatants were used for enzyme immunoassays and for the determination of soluble protein concentration.

NSE, CK-B, and β-S100 proteins were determined by means of the sandwich-type enzyme immunoassay systems developed by Kato *et al.* (1981, 1982, 1986). The system is composed of a solid phase (polystyrene ball) with immobilized rabbit antibodies monospecific to the respective subunits of enolase (γ-enolase), CK (CK-B), and S100 protein (β-S100) and the same antibodies labeled with β-D-galactosidase from *Escherichia coli*.

The marker substances were quantified by using purified rat γγ-enolase, CK-BB, and human ββ-S100, respectively, as standards, and the results were ex-

pressed as micrograms of the respective homodimeric isoprotein equivalent per milligram soluble protein. Soluble protein concentrations of the crude extracts were determined by the dye-binding method of Bradford (1976).

Statistical Analysis of Data

Control and exposure groups were compared by the Student *t* test. *P*-values of less than 0.05 were considered significant.

RESULTS

Clinical State

n-Hexane experiment. The body weight gain of the rats exposed to *n*-hexane was progressively depressed from the 4th week. From the 12th week, marked decrease in grip strength was observed. From the 16th week, three exposed rats displayed unsteady and slight waddling gait. The electrophysiological study demonstrated the significant decline of MCV and prolongation of DL in exposed rats when compared with those of controls (data not shown).

Toluene experiment. No effects of exposure to toluene on body weight gain and brain weight were seen. Neither the signs of peripheral neuropathy nor definite behavioral abnormality were observed during the whole exposure period.

Nerve-Specific Marker Proteins in CNS

Table 1 shows the content of NSE, CK-B, and β-S100 in cortex, cerebellum, and spinal cord. In the *n*-hexane experiment, the content of the three markers in cortex and cerebellum did not significantly differ between exposure and control groups. In spinal cord, only NSE in the rats exposed to *n*-hexane displayed a

TABLE 1
EFFECTS OF CHRONIC EXPOSURE TO *n*-HEXANE AND TOLUENE ON SOME NERVE-SPECIFIC MARKER PROTEINS IN THE CNS OF RATS (μG/MG SOLUBLE PROTEIN)[a,b]

	n-Hexane experiment		Toluene experiment	
	Control	Exposure	Control	Exposure
Cerebral cortex				
NSE	14.4 ± 2.1	12.7 ± 1.3	10.0 ± 0.8	10.6 ± 0.8
CK-B	26.3 ± 3.5	23.0 ± 2.9	19.5 ± 1.7	20.4 ± 1.8
β-S100	4.31 ± 0.28	3.90 ± 0.50	3.29 ± 0.47	3.67 ± 0.78
Cerebellum				
NSE	13.6 ± 1.6	15.1 ± 1.9	9.6 ± 1.0	11.0 ± 0.7*
CK-B	33.5 ± 4.8	37.5 ± 3.5	26.9 ± 2.0	32.4 ± 2.1**
β-S100	4.47 ± 0.51	4.57 ± 0.61	3.10 ± 0.34	4.79 ± 0.94**
Spinal cord				
NSE	11.5 ± 0.7	9.9 ± 0.9**	14.6 ± 1.9	13.9 ± 1.4
CK-B	25.9 ± 2.3	25.8 ± 1.6	35.3 ± 3.1	31.0 ± 2.2**
β-S100	6.92 ± 0.82	7.78 ± 0.89	4.25 ± 0.68	5.74 ± 0.70**

[a] *n*-Hexane, 2000 ppm, 12 hr/day, 6 days/week, for 24 weeks; Toluene, 1000 ppm, 8 hr/day, 6 days/week, for 16 weeks.

[b] Each value represents means ± SD of determinations made on eight rats.

* $P < 0.05$.

** $P < 0.01$.

significant reduction when compared to that of the control rats, whereas the other two proteins remained unchanged.

In contrast to this, 16 weeks' exposure to toluene produced marked changes in the proteins in cerebellum and spinal cord. In cerebellum, the amount of NSE, CK-B, and β-S100 were elevated by 15, 20, and 55%, respectively. In spinal cord, CK-B was reduced by 13%, while β-S100 increased by 35%.

Nerve-Specific Marker Proteins in PNS

The response pattern of the marker proteins examined in peripheral nerves varied depending upon both the longitudinal location of the nerves and the solvents employed (Table 2). In the *n*-hexane experiment, the significant changes were observed only in the distal segment of the sciatic nerve, manifesting the decrease in NSE, CK-B, and β-S100 by 36, 29, and 23%, respectively. The amount of these markers in the proximal segment, however, was not significantly altered.

On the other hand, chronic toluene exposure did not change the amount of any markers in distal sciatic nerve. In proximal sciatic nerve, however, NSE and CK-B showed a small but significant increase.

DISCUSSION

The adverse effect induced by *n*-hexane is characterized clinically by polyneuropathy and pathologically by distal and retrograde axonal degeneration occurring in long and wide nerve fiber tracts (Spencer and Schaumburg, 1977). On the other hand, toluene is known to exert its main toxic effects on CNS, manifesting as impairment of memory, cerebellar ataxia, personality change, and intellectual decline (Spencer and Schaumburg, 1985). To clarify the mechanism and biochemical basis of these kinds of CNS and PNS toxic responses, we used NSE, CK-B, and β-S100 as indicators to evaluate solvent-related neurotoxicity. The results indicate that *n*-hexane treatment seems to selectively affect the nerve-specific

TABLE 2
EFFECTS OF CHRONIC EXPOSURE TO *n*-HEXANE AND TOLUENE ON SOME NERVE-SPECIFIC MARKER PROTEINS IN RAT PERIPHERAL NERVE TISSUES (μG/MG SOLUBLE PROTEIN)[a,b]

	n-Hexane experiment		Toluene experiment	
	Control	Exposure	Control	Exposure
Proximal sciatic nerve				
NSE	2.63 ± 0.31	2.33 ± 0.39	3.18 ± 0.43	3.70 ± 0.35*
CK-B	4.61 ± 0.40	4.57 ± 0.65	4.56 ± 0.36	5.56 ± 0.47*
β-S100	1.28 ± 0.24	1.13 ± 0.16	1.95 ± 0.21	2.12 ± 0.45
Distal sciatic nerve				
NSE	2.22 ± 0.33	1.42 ± 0.31**	2.13 ± 0.34	2.12 ± 0.95
CK-B	3.99 ± 0.72	2.83 ± 0.49**	3.92 ± 0.38	3.70 ± 0.88
β-S100	0.82 ± 0.17	0.65 ± 0.13*	0.85 ± 0.28	0.85 ± 0.49

[a] *n*-Hexane, 2000 ppm, 12 hr/day, 6 days/week, for 24 weeks; Toluene, 1000 ppm, 8 hr/day, 6 days/week, for 16 weeks. The sciatic nerve from the sciatic notch to the knee was removed and cut into a proximal and distal section at the level of the triceps surae nerve.

[b] Each value represents means ± SD of determinations made on eight rats.

* $P < 0.05$.

** $P < 0.01$.

marker proteins in distal sciatic nerves. In contrast, toluene-induced biochemical changes are preferentially concentrated in CNS tissues. These findings agree with the clinical and neuropathological manifestations characterized by intoxication by the two solvents.

Some hypotheses have been proposed for the mechanism of toxic action of *n*-hexane, including defects in energy metabolism, neurofilament cross-linking, and pyrrole adduct formation (Couri and Milks, 1985). NSE, CK-B, and β-S100 are known to exist in peripheral nerve as constituents of axonally transported proteins (Brady and Lasek, 1981; Miani *et al.*, 1972). Moreover, NSE and CK-B are glycolytic enzymes which play important roles in the energy supply in nervous system. The quantitative decline of the three proteins in distal peripheral nerves may be, therefore, due to (1) the pathological leakage of the proteins from the distal degenerated fiber tracts, and (2) the disturbance of energy metabolism and axonal transport induced by *n*-hexane (Sabri *et al.*, 1979).

Worthy of note is the fact that chronic exposure to *n*-hexane did not change the amount of markers in brain regions. This is quite different from the observation in toluene exposure. In our previous experiment, we found that NSE, CK-B, and β-S100 exhibited a dose-dependent elevation in various brain regions after 2 weeks' toluene inhalation (Huang *et al.*, 1990). Rosengren *et al.* (1986; 1989) have reported that chronic exposure to dichloromethane and styrene could enhance the amount of two kinds of astroglial markers, S100 protein and glial fibrillary acidic protein (GFA). Bjornaes and Unninaalsund (1988) reported that 1000 ppm subchronic exposure to toluene significantly increased the activity of one glial enzyme, glutamine synthetase, in the cerebellar hemisphere. Since CK-B and β-S100 are mainly distributed in glial cells in brain (Thompson *et al.*, 1980; Eng *et al.*, 1971), the increase of these glial cell marker proteins may suggest the development of gliosis (glial proliferation and reactivity) which is regarded as a common and early finding of CNS damage. With regard to the behavior of a neuronal marker, NSE, a significant increase rather than decrease was found in cerebellum. This would mean that the obvious neuronal loss had not yet occurred by the time of sacrifice under the treatment schedule employed. The reactive astrocytes which also can produce neuron-specific enolase (Vinores, 1984) may be responsible for the increase of this neuron marker in cerebellum.

We also noted that the most pronounced biochemical changes following chronic toluene exposure occurred in cerebellum, whereas the cerebral cortex did not reveal any alteration. Many case reports of toluene abuse demonstrate that cerebellar signs are the most commonly and convincingly described disorders among the persistent neurological deficits induced by toluene intoxication (Ron, 1986). The results of our experiment support this clinical observation and suggest that cerebellum may be a vulnerable target for detecting toluene-induced CNS effects.

In conclusion, the present study indicates that *n*-hexane-induced peripheral neuropathy and a toluene-induced CNS effect are accompanied by specific biochemical changes in rat PNS and CNS tissues. Measurement of the nerve-specific marker proteins seems to be useful to assess the solvent-related neurotoxicity.

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Neurologic Diseases Associated with Use of Plant Components with Toxic Potential[1]

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Epidemics of neurotoxic disease in developing regions of the world are often associated with dietary dependence on plant components with inherent toxic potential or which have spoiled and become contaminated with mycotoxins. Diseases triggered by plant toxins include lathyrism and cassavism, types of irreversible spastic parapareses associated with staple diets of grass pea and bitter cassava root, respectively. Mildewed sugarcane poisoning, an encephalopathy and tardive dystonia, illustrates the neurotoxic effects of a widely distributed plant and fungal toxin. Food and medicinal use of the neurotoxic cycad plant is thought to have a role in the etiology of western Pacific amyotrophic lateral sclerosis and parkinsonism-dementia. Plant-associated neurotoxicity is a significant and preventable cause of morbidity in certain regions of Africa, Asia, and Oceania.

INTRODUCTION

The explosion of interest and concern in developed countries over the toxic potential of drugs and synthetic chemicals has tended to obscure the importance of plant and fungal toxins as a cause of morbidity in other regions of the world. Numerous plants used for food harbor chemicals that are potentially neurotoxic to humans (Vennesland *et al.*, 1981; Keeler and Tu, 1983), but their presence in the varied diet of well-fed subjects is not recognized to elicit adverse health effects. However, in settings of food deprivation and malnutrition, where single plant products with toxic potential occupy the principal dietary component, the toxic threshold may be exceeded, resulting in acute and chronic neurologic illnesses leading to widespread morbidity. Lathyrism and cassavism, types of spastic parapareses triggered by heavy intake of grass pea and bitter cassava root, respectively (Spencer *et al.*, 1983; Haimanot *et al.*, 1990; Howlett *et al.*, 1990), illustrate this principle. Additionally, in places where storage and transportation are not optimal, food products may become contaminated by fungal products with neurotoxic activity. For example, consumption of mildewed sugarcane has been related to the induction of dystonia in Chinese children (He *et al.*, 1990; Ludolph *et al.*, in press). The neurotoxic potential of botanic agents also appears to be relevant to an understanding of the etiology of western Pacific amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (P-D), a prototypical neurodegenerative disease linked to dietary and medicinal exposure to seed of the neurotoxic cycad plant (Whiting, 1963; Spencer, 1987). What sets this disorder apart from established neurotoxic diseases is the long latent period separating exposure and clinical onset. This paper examines briefly the evidence linking human neurodegeneration and associated diseases to plant and fungal toxins.

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LATHYRISM

Lathyrism is a form of spastic paraparesis associated with continuous dietary intake of the seed of *Lathyrus sativus* (grass pea) or related species (e.g. *cicera, clymenum*) (Spencer *et al.*, 1983). The grass pea is an important and inexpensive food component in parts of Bangladesh, Ethiopia, India, and Nepal (Spencer, 1989). As a minor component of the regular diet, the grass pea is of little consequence, but its real value to an economically deprived community is its ability to thrive on untilled ground and to survive in harsh environmental conditions, including dry and water-logged soils. The legume fixes soil nitrogen, requires no pesticide treatment, and has seed with a high protein content and caloric value. During drought or flood, when other plants perish and food stores consequently diminish, there is increasing reliance on the hardy grass pea. After several weeks as a staple, lathyrism ensues. Commonly, this takes the form of an epidemic, with postepidemic prevalence rates of spastic paraparesis in affected communities rising as high as 3% of the population (Haimanot *et al.*, 1990). Few neurotoxic diseases display such a remarkable frequency.

The agent primarily responsible for the induction of neuronal degeneration in lathyrism is a non-protein amino acid, β-*N*-oxalylamino-L-alanine (BOAA), which is present in *L. sativus* seed in concentrations approaching 1%. BOAA is a stereospecific excitant and excitotoxic amino acid that acts as a competitive agonist at the 3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or ionotropic quisqualate receptor (Ross *et al.*, 1989), one of the postsynaptic neuronal receptors that binds the putative excitatory neurotransmitter glutamate. BOAA also facilitates the spontaneous and stimulus-evoked release of glutamate from presynaptic elements (Gannon and Terrian, 1989). Single large doses of BOAA induce convulsive behavior in mice and an excitotoxic pattern of neuronal degeneration (postsynaptic vacuolation and dark, shrunken cells) in mouse cortical explants; these behavioral and neuropathological changes are attenuated by prior treatment with an antagonist for AMPA- and kainate-preferring glutamate receptors (Ross *et al.*, 1987, Ross and Spencer, 1987). Taken together, available data suggest that BOAA acts directly at both pre- and postsynaptic sites, causing maintained depolarization of the target neuron, failure of energy-dependent cellular homeostasis, and neuronal death.

The clinical presentation of disease may be acute (common) or more insidious, with calf-muscle spasm and weakened and heavy legs comprising the most common initial complaints (Ludolph *et al.*, 1987). Seizures are not reported, although early and reversible clinical features, such as myoclonus, global muscle spasms, and urinary frequency suggest diffuse and transitory BOAA-induced CNS excitation of somatic motor and autonomic neurons. Young males are most commonly and seriously affected, and the disease often begins in a setting of physical exertion and malnutrition. The constant clinical features of lathyrism include a pyramidal pattern of leg weakness and a greatly increased tone of thigh extensor, thigh adductor, and gastrocnemius muscles which forces individuals to walk on the balls of their feet with a lurching cross-legged gait. Electrophysiological and neuropathological studies of established cases demonstrate the heavy involvement of the central portion (pyramidal tracts) of the corticomotoneuronal pathway, presumably as a result of neuronal degeneration in the motor cortex. The sensory

examination is usually unremarkable, although perverse sensations in the extremities are reported at disease onset.

Experimental studies aimed at reproducing the disease in laboratory animals have met with mixed success. The primate alone has succeeded in mimicking the clinical picture of human lathyrism, as a result either of continuous daily intake of a fortified grass pea diet or of synthetic BOAA (Spencer *et al.*, 1987). After months of grass pea diet or weeks of oral daily BOAA treatment, animals develop behavioral (extensor hindlimb posturing, myoclonus) and electrophysiological evidence of pyramidal involvement (Hugon *et al.*, 1988). However, corresponding structural changes are sparse or absent, suggesting the well-nourished primate is relatively refractory to BOAA and only able to reproduce the early, reversible phase of human lathyrism. The limitations imposed by the primate and rodent responses to BOAA have led researchers in Ethiopia and the United States to consider experimentation with the horse, a species that seems to be especially susceptible to the neurotoxic effects of *Lathyrus* spp. Establishment of a reliable animal model of lathyrism is of great importance to determine the safety of low-BOAA strains of the grass pea. These are currently under development as part of a global, cross-disciplinary scientific effort to harness the useful properties of the grass pea to control and eradicate lathyrism and to provide a nourishing food and fodder for drought-prone, nonirrigated areas of the world (Spencer *et al.*, 1990).

CASSAVISM

Cassavism (also known by local names, such as *konzo* and *mantakassa*) is another form of acute spastic paraparesis associated with heavy reliance on a potentially toxic plant, namely the bitter varieties of *Manihot esculenta* (cassava) (Howlett *et al.*, 1990). Disease is endemic in poverty-bound African populations that employ the cassava root as a staple. A native of South America where cassavism is essentially unknown, the cassava plant was aggressively introduced into Africa and other regions as an environmentally tolerant and readily cultivated plant with a high energy yield (Cock, 1982). Both the leaves and tuber are eaten, the latter after various types of processing (washing, sun drying) which attempt to remove cyanide liberated from cyanogenic glucosides stored in the plant. Inevitably, in times of water shortage from drought, processing times are shortened, detoxication is incomplete, and dietary reliance on cassava root increases. These conditions set the stage for epidemic spastic paraparesis (cassavism), an acute-onset disease found among poor peasant families in Mozambique, Tanzania (Howlett *et al.*, 1990) and elsewhere in southern Africa.

The clinical manifestations of cassavism are remarkably similar to those reported for lathyrism. Difficulty walking begins abruptly, with heavy, weakened, and trembling legs. These subjects develop symmetrical spastic paraparesis, with increased muscle tone, hyperreflexia, and bilateral extensor plantar responses. Pyramidal signs may also be present in the upper extremities, a feature also seen in extremely severe cases of lathyrism. Victims of cassava intoxication sometimes report leg numbness, difficulty speaking, visual impairment, and hearing loss at onset. These are often reversible, and patients show no evidence of distal sensory loss in the face of spasticity. However, optic atrophy, dysarthria, and hearing impairment are reported. As in lathyrism, the walking deficit is permanent. Families may experience repeated outbreaks of disease during annual periods of food

shortage. Children and young adults are often most heavily affected, and male cases may predominate over females (Howlett *et al.*, 1990). It is unclear (as in lathyrism) whether this is simply a reflection of differential cassava intake or a true gender susceptibility.

Because there is no experimental animal model of cassavism, the causal agent has not been identified with certainty. However, disease outbreaks are constantly associated with a high cyanide and low sulfur intake from a diet dominated by insufficiently processed toxic cassava roots and lacking protein-rich supplementary foods to supply the sulfur needed for cyanide detoxification (Howlett *et al.*, 1990). One possibility is that cyanide reacts with cysteine residues to form 2-iminothiazolidine-4-carboxylic acid; this agent is proposed to have excitotoxic properties comparable to those of BOAA (Lundquist *et al.*, 1985), but the subject is unstudied. Another untested scenario is that nerve cell degeneration is linked to cyanide-induced energy dysfunction, the compromised nerve cell displaying an increased susceptibility to the excitotoxic effects of endogenous neurotransmitter levels of glutamate (Henneberry *et al.*, 1989). Whatever the explanation for the presumed motor cortical neuronal degeneration of cassavism, it seems likely that the molecular etiopathogenesis is closely linked with that of lathyrism. The possibility of determining the basis for selective CNS neuronal degeneration in human neurological diseases is one reason why cassavism merits close study.

Another is the human suffering associated with this preventable but untreatable disorder. While the prevalence of cassavism has not been estimated, it seems clear the disease should be expected among poor, cassava-reliant African communities that lack protein-rich supplementary foods. As with lathyrism, clinically apparent cases of cassavism probably make up a small fraction of the total number of subjects with neural damage induced by the respective plant components. The possibility that subclinical neurotoxic damage may combine with age-related neuronal attrition to express disease in later life would be a major concern except for the fact that life expectancy in these communities rarely exceeds 50 years.

TOXIC DYSTONIA

Ingestion of sugarcane contaminated with a toxin-generating fungus (*Arthrinium* spp.) has been held responsible for encephalopathy and late-onset dystonia in Chinese children (He *et al.*, 1990). Sugarcane is harvested in late summer in the southern parts of the People's Republic of China, shipped northward, stored (where fungal contamination develops), and consumed throughout the country in January and February around the Chinese New Year. The mildewed sugarcane is sold on the side of the road and consumed by adults and children alike. Within hours of exposure, there is sudden-onset nausea, vomiting, abdominal pain, and diarrhea. Children are especially vulnerable and may develop double vision, somnolence, nystagmus, convulsions, decerebrate rigidity, and coma. Nearly 900 cases of mildewed sugarcane encephalopathy, including 88 who died, were recognized between 1972–1989. Those that regain consciousness are mute and incontinent. Some develop delayed dystonia 7–40 days later. The clinical picture includes facial grimacing, sustained athetosis of hands and fingers, torsion spasm, spasmodic torticollis, hemiballismus, and painful spasms of the extremities. Computerized tomography of these totally disabled children reveals bilateral hypodensity of the putamen and, to a lesser extent, the globus pallidum. The caudate and

claustrum are occasionally involved. Like the spastic parapareses of lathyrism and cassavism, the clinical features of toxic dystonia are permanent.

The proximate cause of mildewed sugarcane poisoning appears to be the *Arthrinium* mycotoxin 3-nitropropionic acid (NPA). NPA is a suicide inhibitor of succinic dehydrogenase (SDH) (Alston *et al.*, 1977), and SDH activity is greatly depressed throughout the brain of rodents receiving the agent (Gould and Gustine, 1982). However, the neuronal damage is restricted to the basal ganglia in mice dosed intraperitoneally with NPA. Treated animals display bilaterally symmetrical degeneration of the caudate–putamen, globus pallidus, enteropeduncular nucleus, and the pars reticulata of the anterior substantia nigra. There is marked postsynaptic dendritic swelling, with nuclear pyknosis and chromatin clumping of nerve cell bodies, a pattern reminiscent of excitotoxic damage. Mouse cortical explants treated with NPA show decreased energy levels and develop comparable patterns of neuronal pathology; these changes are attenuated by prior treatment with glutamate antagonists (MK-801, kynurinic acid (Ludolph *et al.*, unpublished data)). These findings strongly suggest that NPA blocks ATP production and thereby renders nerve cells susceptible to the excitotoxic effects of glutamate neurotransmitter, as described by Henneberry and colleagues (1989). While this might provide an attractive explanation for the convulsive features of mildewed sugarcane poisoning, it does not explain the peculiar vulnerability of the basal ganglia to NPA, as well as to other agents (such as carbon monoxide) that markedly perturb energy metabolism. Furthermore, there is an obvious and unexplained contrast between the distribution of neuronal vulnerability in cyanide-associated cassavism (presumably corticomotoneurone) and NPA toxicity (basal ganglia). Experimental studies are underway using NPA to explore the differential susceptibility of neurons to energy deprivation.

WESTERN PACIFIC ALS/P-D

The combination of amyotrophic lateral sclerosis (motor neuron disease), parkinsonism, and dementia has been common in three disparate populations of the western Pacific region: the Chamorros of Guam and Rota in the Mariana Islands, the Auyu and Jaqai linguistic groups of the western half of the island of New Guinea (Irian Jaya, Indonesia), and Japanese residents of the Kii peninsula of Honshu Island, Japan (Garruto and Yase, 1986). The widespread presence of Alzheimer-like neurofibrillary tangles in clinically unremarkable Guam subjects who died from other causes indicated the disease in a subclinical form was phenomenally prevalent in the Chamorro population (Anderson *et al.*, 1975). Moreover, the observation that Chamorros develop ALS/P-D decades after migration from Guam and that immigrants to Guam who adopt the Chamorro lifestyle become candidates for disease, strongly suggests a slowly evolving pathogenetic process quite distinct from rapid-onset toxic disorders such as lathyrism or cassavism. Nevertheless, there is widespread agreement that ALS/P-D likely involves the operation of a disappearing nonviral environmental factor, and plants with toxic potential are high on the list of suspect agents (Spencer, 1987). Of the three plants formerly used by Chamorros for food—cassava, yams, and cycad—the latter is of greatest interest because it induces hindlimb weakness, muscle atrophy, and long-tract degeneration in ruminants (Whiting, 1963; Spencer *et al.*, 1990). Flour prepared Chamorro-style by water-soaking the seed of *Cycas circi-*

nalis contains two potential neurotoxins, in contrast to the absence of these compounds in the fermented food products prepared from *Cycas revoluta* in the Ryukyu Islands of southern Japan where ALS/P-D is unknown (Kobayashi, 1972; Kisby *et al.*, unpublished data). *Cycas* spp. are not used for food in the two other high-incidence disease foci of ALS/P-D, but recent studies have demonstrated its use as a Japanese folk medicine in the Kii peninsula and as a topical poultice for large open wounds on the extremities of residents of Irian Jaya who later develop ALS (Spencer *et al.*, 1990). While these observations demonstrate a common cycad exposure of all three disease-prone groups, they do not constitute proof of a cause-and-effect relationship.

Two compounds with neurotoxic potential have been isolated from *Cycas* spp. seed. The minor component is an excitotoxic nonprotein amino acid, β-*N*-methylamino-L-alanine (BMAA), which displays neurotoxic activity associated with both NMDA and non-NMDA receptors (Ross and Spencer, 1987; Ross *et al.*, 1987; Weiss and Choi, 1988; Weiss *et al.*, 1989; Smith and Meldrum, 1990). The excitotoxic potential of BMAA is enhanced in physiological concentrations of bicarbonate (Weiss and Choi, 1988). In addition to its apparent action on plasma-membrane receptors of neurons, BMAA (unlike BOAA) is transported into neural cells where it has unknown effects (Kisby *et al.*, unpublished data). These may be relevant to the cerebellar degeneration seen in chronically dosed rats (Seawright *et al.*, 1990) and the motor neuronal, extrapyramidal, and behavioral changes seen in comparably treated primates (Spencer *et al.*, 1987a). But for its short latency, lack of progression, and absence of Alzheimer-like neurofibrillary tangles, the BMAA-induced primate disease shows remarkable similarities to the cycad-associated human disorder.

Another important but unconfirmed link to the human disease is the induction of motor neuron degeneration in a single primate fed Chamorro-style cycad flour (Dastur, 1964). This contains small concentrations of BMAA and larger amounts of cycasin, the major toxic component of *Cycas* spp. (Duncan *et al.*, 1990; Kisby *et al.*, 1990, and unpublished data). Cycasin, the glucopyranoside of methylazoxymethanol (MAM), also induced a neuromuscular disorder similar to cycadism in goats (Shimizu *et al.*, 1986). Administered in micromolar concentrations to the nutrient fluid of mouse cortical explants, cycasin induces neuronal degeneration (Kisby *et al.*, unpublished data). The potent genotoxin MAM forms DNA adducts (7-methylguanine) that are thought to be related to the experimental carcinogenic, mutagenic, and developmental toxic properties of this agent. MAM disrupts cerebellar development in rodents, with the production of ectopic and multinucleated neurons reminiscent of those seen in the cerebellum of Guamanian and Japanese victims of ALS/P-D (see Spencer *et al.*, 1990). These abnormalities may represent biological indicators of cycad exposure during the postnatal developmental period. Whether they also mark the onset of a neuropathological process which culminates in ALS/P-D is the subject of intensive study. One idea is that cycasin forms DNA adducts in nerve cells which are not repaired and which form the backdrop to a permanent change in genomic expression that culminates in slowly evolving neuronal degeneration (Spencer *et al.*, 1991). A long-latency toxic phenomenon of this type is known to occur in cycasin-induced tumorigenesis in rodents, but the concept of a slow toxin is new to, and unproven in, neurotoxicology.

IMPLICATIONS FOR HUMAN HEALTH

This brief review raises some important issues regarding the relationship between chemical agents and neurodegeneration:

- certain botanic chemicals have potent neurotoxic properties capable of inducing neurological disorders in humans and animals;
- human diseases associated with plant and fungal neurotoxins cause widespread morbidity in some rural populations of certain developing countries;
- the culpable agents are useful experimental tools with which to probe selective neuronal vulnerability; and
- environmental substances with chemical relationships to plant and fungal neurotoxins may play a role in the etiology of clinically similar disorders in other parts of the world.

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ENVIRONMENTAL RESEARCH 60, 248–253 (1993)

Significance of Subclinical Entrapment of Nerves in Lead Neuropathy[1]

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We examined a left-handed 25-year-old man engaged for 2 years at a vinyl chloride resin factory where he had been exposed to lead stearate, a stabilizer of resin. Neurological examination revealed atrophy of small hand muscles, especially in the left dorsal interossei. Sensation of all modalities was intact. A nerve conduction study showed conduction block at the elbow, indicating possible cubital tunnel syndrome. Following CaEDTA therapy, continued recovery of conduction velocities, amplitude of compound muscle action potential, and diminution of conduction block at elbow were observed within a few months. In lead intoxication, nerves may incur mechanical damage. Subclinical entrapment may thus be an important factor leading to vulnerability of nerves in lead neuropathy.

INTRODUCTION

Lead poisoning has become increasingly rare owing to improvement in occupational health. Although much study has been done on experimental lead neuropathy, there is rather limited comprehensive electrophysiological investigation in human beings. We report here a 25-year-old man with typical lead neuropathy. Comprehensive nerve conduction studies were carried out during treatment with CaEDTA, and the results indicated significant contribution of subclinical entrapment syndrome in causing vulnerability of nerves in lead neuropathy.

CASE REPORT

The patient was a left handed 25-year-old man. He started working in July 1987 at a vinyl chloride resin factory. His daily work was to mix two kinds of powder, vinyl chloride polymer resin and stabilizer which contained lead stearate. He seldom wore a mask to protect himself from dust in the atmosphere. During the 2 years, he had been treated three times for colicky abdominal pain with constipation. Unexplained anemia and proteinuria were pointed out. He first experienced hand weakness in January 1989. Because of progressive muscle atrophy of the hands, he was admitted to our neurologic clinic on October 2, 1989.

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Examination

The patient was alert and well nourished. Blood pressure was 122/66 mm Hg, and his pulse was 66/min. There was no lead line on the gums. Neurological examination revealed severe atrophy of both hand muscles, especially dorsal interossei. The left hand was predominantly affected (Fig. 1). He was unable to bring his fifth finger into contact with his fourth finger. Fromment sign was observed on the left hand. He was unable to straighten the left fingers. Left and right grip power were 5 and 18 kg, respectively. Dorsal flexion of the ankles was also slightly weak. There was no sensory disturbance of any modality. All deep tendon reflexes had decreased and no pathological reflex could be produced.

Laboratory Findings

The hematocrit value was 29.7% and the hemoglobin value was 9.7 g/dl. Serum

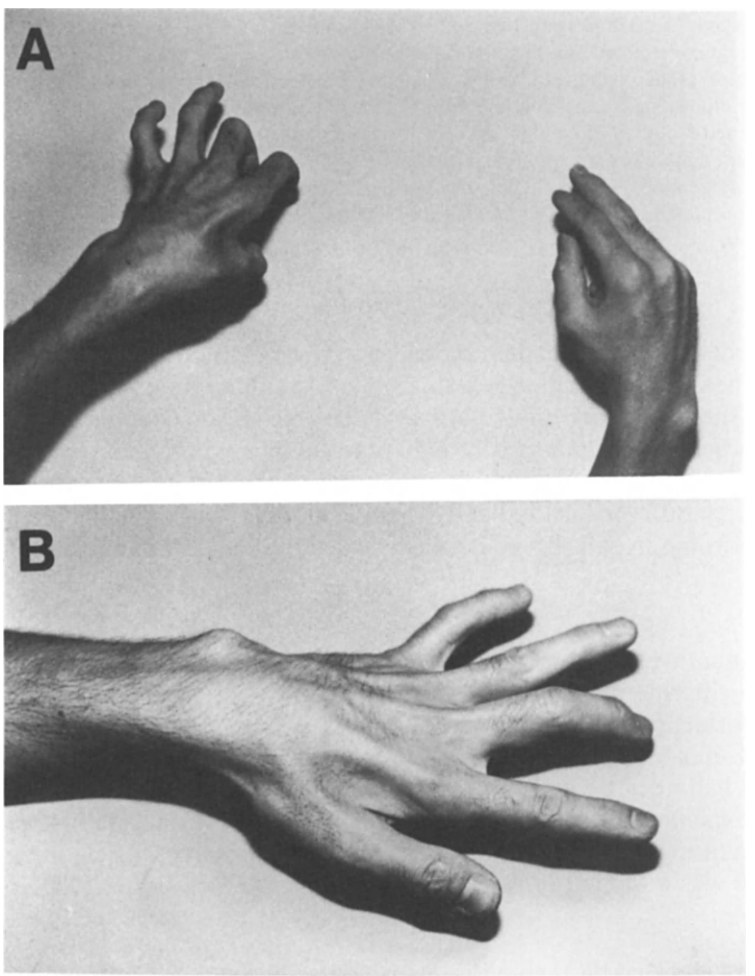

FIGURE 1

iron and transferin concentration were normal. A blood smear failed to show basophilic stippling. Transient proteinuria was observed. Blood lead concentration was 100 μg/dl. Urinary coproporphyrin concentration was 4503 μg/liter and urinary ALA concentration was 138 mg/liter. The urinary lead excretion following 1 g CaEDTA infusion was 3938 μg/day. Enzyme activity of uroporphyrinogen-1-synthase and ALA dehydrase was 38 nmol/ml/RBC/hr (normal 38–74) and 0.01 μmole PBG/ml RBC/hr (normal >0.8).

Electrophysiological Studies

A nerve conduction study of the left median, left ulnar, and left radial nerves was conducted by the inching method before (except for radial nerve) and during chelating therapy. The patient was examined in an air-conditioned room and skin temperature was kept around 32°C. The motor nerve conduction velocity (MCV) of the median nerve and sensory nerve conduction velocity (SCV) of the ulnar nerve had mildly decreased. Compound action potentials (CMAPs) markedly decreased for all nerves examined (Tables 1 and 2). In median and radial nerves, there were no conduction block from wrist to axilla. But in the ulnar nerve, apparent conduction block was observed at the elbow. CMAP recorded on abductor digiti minimi decreased amplitude and was temporally dispersed when the stimulating electrode crossed over the cubital tunnel. Mean motor conduction velocity across the elbow was 20 m/sec. Sensory nerve action potential (SNAP) could not be detected when the ulnar nerve was stimulated proximal to the elbow.

Electromyography of left extensor carpi radialis, flexor carpi ulnaris, and opponens showed increased polyphasic motor unit potentials of long duration. Electromyography of the left dorsal interossei showed fibrillation.

F wave conduction velocities of left ulnar and median nerves were normal.

Treatment and Course

Treatment, begun on October 7, consisted of 1 g CaEDTA infusion once a

TABLE 1
MOTOR NERVE CONDUCTION STUDY

L. Median		89/9	/11	90/3	/6
	MCV (E → W)	43	48	54	52
	Amp	6.3	6.5	7	7.9
	RL	2.5	2.5	2.4	2.3
L. Ulnar		89/9	/12	90/2	/5
	MCV (E′ → W)	50	55	54	61
	Amp	3.7	4.5	5.1	5.1
	RL	2.1	1.9	1.9	2
L. Radial		—	89/11	90/1	/5
	MCV (E → W)	—	48	53	54
	RL	—	1.5	1.5	1

Note. MCV, motor nerve conduction velosity (m/sec); Amp, Amplitude (mV); RL, Residual latency (msec); E, elbow; E′, below elbow; W, wrist; F, finger.

TABLE 2
SENSORY NERVE CONDUCTION STUDY

L. Median		89/9	—	90/3	/6
	SCV (W → F)	50	—	55	52
	SCV (E → W)	60	—	65	67
L. Ulnar		89/9	—	90/2	/5
	SCV (W → F)	44	—	47	46
L. Radial		—	—	90/1	/5
	SCV (E → W)	—	—	53	56

Note. SCV, sensory nerve conduction velosity (m/sec); E, elbow; W, wrist; F, finger.

week. Steady improvement of anemia and muscular weakness was observed (Fig. 2) and, after 1 year of treatment, grip power was almost normal and muscular atrophy was hardly evident. Urinary ALA and coproporphyrin concentration decreased exponentially, becoming normal within about 2 months (Fig. 3, right). Blood lead concentration and daily urinary lead excretion decreased more gradually (Fig. 3, left). Serial nerve conduction study indicated significant recovery of NCV and amplitude of CMAP of the left median and ulnar nerves (Tables 1 and 2). Temporal dispersion of CMAP of the ulnar nerve diminished within a few months (Fig. 4), although SCV and MCV across the elbow remained low even after therapy.

DISCUSSION

In this era, overt cases of lead neuropathy are rare. There are only a few case reports with comprehensive electrophysiological and pathological investigations. With conventional electrophysiological techniques, normal or slightly decreased

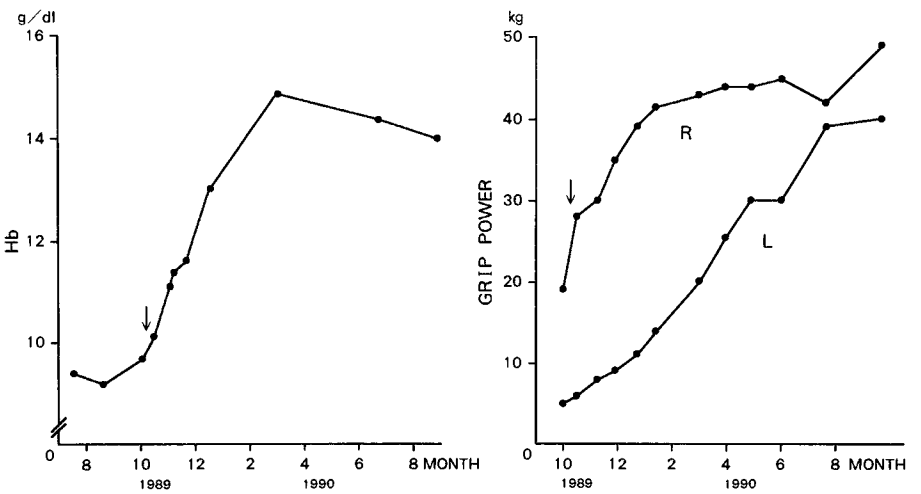

FIGURE 2

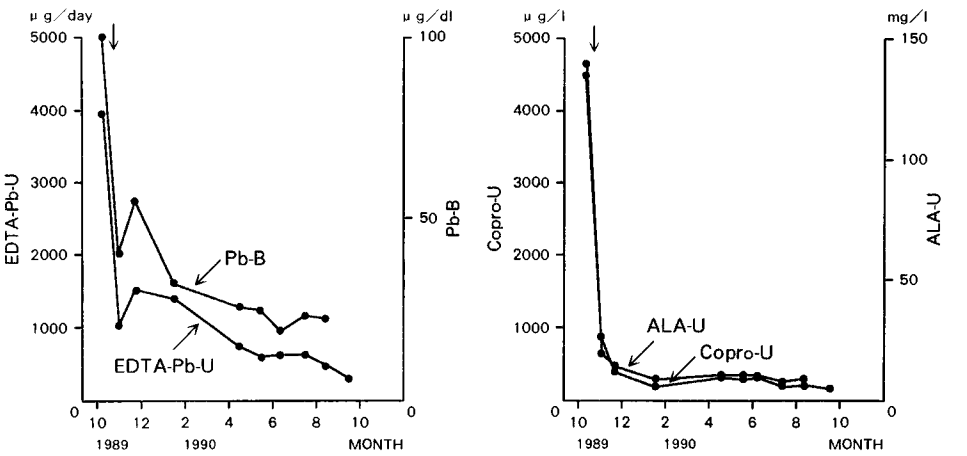

FIGURE 3

nerve conduction velocities and diminished amplitude of nerve action potentials are reported. The main feature of the histological change was reported to be loss of large fibers. These data are indicative of peripheral neuropathy with predominant axonal degeneration.

However, the clinical manifestation of lead neuropathy has a peculiar distribution of involved muscles. The upper extremities are more frequently affected than the lower extremities and the dominant hand is primarily involved. "Wrist drop," involvement of the extensors of the wrists and fingers, is widely accepted as a typical and first manifestation of lead neuropathy. These patterns of involvement resemble more closely that of a mononeuritis multiplex than that of a polyneuropathy.

Oh (1975) demonstrated severely decreased nerve conduction velocity in the radial nerve of a patient with lead neuropathy which is sharply contrasted by mild decrease of conduction velocities in other nerves. In this case, an additional focal mechanism of nerve injury might be postulated. Buchthal and Behse (1979) ex-

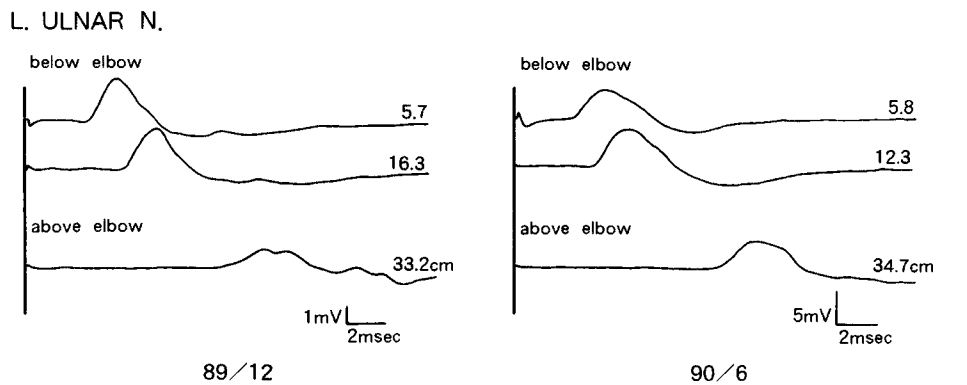

FIGURE 4

amined men exposed to lead and demonstrated that the only relevant abnormality was the prolonged latency from the ankle to the extensor digitorum brevis muscles, which they considered to be caused by local compression of the deep peroneal nerve by safety shoes.

In our case, left-hand muscles innervated by the ulnar nerve were predominantly affected. Nerve conduction study showed conduction block of the ulnar nerve at the elbow. After chelating therapy, the temporal dispersion of CMAP disappeared, although SCV and MCV across the elbow remained low even after therapy. The subclinical cubital tunnel syndrome thus appeared to be present in this patient and it seems to have been aggravated by lead intoxication.

In experimental lead neuropathy, Ohnishi *et al.* (1977) showed markedly increased endoneurial space, suggesting accumulation of water. They also showed Schwann cell division and axonal regrowth to be retarded following nerve damage in experimental lead neuropathy. Nerves in lead intoxication, particularly entrapped nerves, thus appear to be vulnerable to mechanical damage.

Subclinical entrapment may be an important factor causing vulnerability of lead neuropathy. Detailed electrophysiological studies such as the inching study should be conducted for more detailed clarification of lead neuropathy.

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Neurobehavioral Effects of Intrauterine Mercury Exposure: Potential Sources of Bias[1]

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Sources of bias were examined in a study of neurobehavioral effects of intrauterine exposure to methyl mercury in the Faroe Islands. The cohort of 1000 children was born during a 21-month period and did not differ from Faroese births in general as regards major obstetrical parameters. However, high mercury concentrations in the cord blood was associated with increased birth weight, presumably because other constituents of marine fish may cause a prolongation of the gestation period. Thus, children with high mercury exposures were somewhat protected against low birth weight and its associated neurobehavioral risks. Less than 25% of the women indicated occasional alcohol drinking during pregnancy, thus suggesting a limited fetal exposure to this neurobehavioral risk factor. However, maternal alcohol drinking caused a decrease in mercury concentrations in cord blood, probably because of a toxicokinetic interaction between ethanol and mercury. Any alcohol-related effect on neurobehavioral development would then be associated with lower levels of mercury exposures. The effects of these confounders would tend to bias the results of the study toward the null hypothesis.

INTRODUCTION

Intrauterine exposure to methyl mercury is a documented cause of severe neurobehavioral dysfunctions in children; most of the evidence in this area originates from pollution episodes and allows only approximate risk estimations (WHO, 1990). A high intake of seafood, including meat from marine mammals, causes considerable methyl mercury exposures, and a detailed dose–response relationship is therefore highly desirable for an improved risk assessment.

In the Faroe Islands, a questionnaire study showed that adults consume an average of 72 g fish, 12 g whale muscle, and 7 g blubber per day; fish and pilot whale constituted 44 and 9.5% of Faroese dinner meals, respectively (Vestergaard and Zachariassen, 1987). Cod is the main fish consumed, with the average mercury concentration being 0.07 μg/g (0.35 nmole/g) (Hygienic Institute, Tòrshavn, personal communication). Meat from pilot whales contained a much higher average mercury concentration of 3.3 μg/g (16 nmole/g), about half of which was methyl mercury (Juhlshamn *et al.*, 1987). Thus, especially if whale meat is included in the diet, methyl mercury exposures could be considerable in this population.

We have established a cohort of about 1000 Faroese children whose intrauterine methyl mercury exposure was determined by analysis of umbilical cord blood and

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maternal hair. In the absence of frank methyl mercury poisoning, as seen in serious episodes in Japan and Iraq (WHO, 1990), the objective is to identify and characterize any neurobehavioral changes and their relationship to mercury exposure.

Studies in environmental epidemiology are particularly sensitive to bias because environmental exposures tend to be variable and long term, thus being difficult to characterize. The effects are frequently nonspecific or develop insidiously. Despite such difficulties, a recent study from New Zealand (Kjellström *et al.*, 1986) suggested that slight neurobehavioral changes in 4-year-old children were associated with intrauterine exposure to mercury originating from shark meat.

SUBJECTS AND METHODS

The cohort included children born at the three Faroese hospitals from 1 March 1986 to the end of 1987, provided that a cord blood sample, a sample of maternal hair, and a questionnaire were obtained by the midwives. Although delayed delivery of blood vials prevented sampling during one month (summer of 1987), the goal of including at least 1000 children was reached. Details on the analytical techniques and quality assurance are reported elsewhere (Grandjean *et al.*, 1991).

The midwife asked the mother questions concerning the course of the pregnancy, nutritional habits (frequency of dinners with fish or pilot whale), and use of alcohol (never, occasionally, or frequently) and tobacco (none, less than or more than 10 cigarettes per day, or other tobacco) during the pregnancy; the answers were entered on a questionnaire that also included information on the course of the parturition and data on the infant. Routine obstetric parameters were obtained from the medical birth registry and from the patient charts. This information was available for all births during the sampling period.

A total of 997 cases had complete questionnaire information and blood analyses (mercury analysis could not be performed in 27 cases). The median mercury concentration (B-Hg) in the cord-blood was 121 nmole/liter (24.2 μg/liter), and a total of 250 samples (25.1%) had a B-Hg higher than 200 nmole/liter (40 μg/liter), and 20 samples (2.0%) were higher than 500 nmole/liter (100 μg/liter) (Grandjean *et al.*, 1991). These results confirmed the prediction that high intrauterine exposures occurred.

RESULTS

Biases in Cohort Selection

Incomplete sampling and subsequent attrition may severely hamper the validity of epidemiological studies, as the study cohort could differ in important respects from the background population. Through intensive cooperation with the Faroese health care system, we have intended to minimize this problem. During the active sampling period, a total of 1367 children were born at single births, and complete or almost complete data were obtained from 1024 children (74.9%) (Table 1).

Incomplete sampling particularly occurred at the small hospitals 2 and 3 where fewer births took place, mainly those that were not associated with any antici-

TABLE 1
ATTRITION AND CORD BLOOD MERCURY CONCENTRATIONS (nmole/liter) IN RELATION TO PREGNANCY OUTCOME AND OBSTETRICAL PROCEDURES OR COMPLICATIONS

	Total number	Within cohort number (percentage)	P^a	Blood–mercury	
				Median	P^b
Total	1367	1024 (74.9)	—	121	—
Hospital 1	1061	902 (85.0)		114	
Hospital 2	166	76 (45.8)	<0.0001	142	<0.0001
Hospital 3	128	42 (32.8)		275	
Breech presentation	126	80 (63.5)	0.003	140	0.2
Placental insufficiency	13	9 (69.2)	0.9	92	0.6
Preeclampsia	32	19 (59.4)	0.06	110	0.5
Serious complications	82	52 (63.4)	0.02	123	0.9
Other complications	323	225 (69.7)	0.02	114	0.7
Cesarian section	249	137 (55.0)	<0.0001	115	0.7
Other obstetric procedures	730	567 (77.7)	0.01	113	0.02
Gestation <38 weeks	55	37 (67.3)	0.2	131	0.9
Birth weight <2500 g	32	19 (59.4)	0.06	138	0.9

[a] Comparison within background population by χ^2 test.
[b] Comparison within cohort by Mann–Whitney test.

pated difficulties that would mandate referral to hospital 1. The B-Hg was considerably higher at the small hospitals, particularly at hospital 3 (Table 1); pilot whale was eaten significantly more frequently during pregnancy by the women who gave birth at this hospital. As attrition was larger at the two smaller hospitals that also exhibited higher B-Hg levels, the overall average mercury level is likely to be lower than the true average for the background population.

Attrition was examined in relation to relevant obstetrical parameters available for all births. Because scheduled cesarean sections and other parturitions with anticipated complications are routinely referred to hospital 1, the more efficient sampling at this hospital could result in an increased proportion of such births in the cohort. Also, even without anticipated complications, obstetrical procedures appear to be used more often at this hospital. However, these factors appear only to cause an increased sampling of births where minor obstetrical procedures, such as vacuum delivery or episiotomy, were used (Table 1). With other complications or procedures, a proportion lower than average was included in the cohort, probably because cord blood sampling and questionnaire interview were not always possible or convenient under these circumstances. However, except for the minor obstetrical procedures that showed a B-Hg below the general average, these parameters were not associated with mercury exposure.

Bias Related to Birth Weight

Low birth weight is of interest in this study, because the risk of delayed or deficient neurobehavioral development is increased, particularly when the birth weight is below 2500 g (Zachau-Christiansen, 1972). A recent publication (Foldspang and Hansen, 1990) has suggested that high methyl mercury exposure from

seafood may be associated with a decrease in birth weight. Although this suggestion may be based on an incomplete analysis of the data, a toxic effect cannot be excluded. However, the findings of a previous study (Eyssen *et al.*, 1983) had suggested that higher birth weights may actually be related to maternal ingestion of mercury-contaminated fish. In addition to mercury, fish also contains (*n*-3)-polyunsaturated fatty acids that, according to a recently proposed hypothesis (Olsen *et al.*, 1986), could delay parturition and thereby cause an increased birth weight.

Inspection of Table 1 reveals that birth weight below 2500 g is surprisingly rare in the Faroe Islands. Also, major complications, such as placental insufficiency and preeclampsia, were infrequent and not at all associated with an increased mercury concentration in cord blood. A high birth weight could perhaps be due to the fact that few Faroese women smoke, i.e., in the present study 408 (39.6%), with only 126 (12.2%) smoking more than 10 cigarettes per day. As smoking is the major risk factor in relation to birth weight (Kramer, 1987), we examined in more detail the predictors for birth weights for nonsmoking and smoking mothers separately.

Table 2 shows that both birth weight and cord blood mercury tend to increase with the frequency of fish dinners during pregnancy for nonsmoking mothers. The lowest averages occurred when the mother had not eaten fish at all during pregnancy. In a multiple regression analysis, a B-Hg in the upper quartile (above 200 nmole/liter) was associated with an increase in birth weight of 125 g ($P = 0.04$), while no difference could be detected between the lower three quartiles. If birth weight was adjusted for maternal fish intake, the increase for the upper quartile of the B-Hg was only diminished to 113 g ($P = 0.07$).

As high mercury exposure alone would be expected to cause a decrease, rather than an increase, of birth weight (Foldspang and Hansen, 1990), the association observed in the present study is unlikely to be causally related to the mercury exposure. Instead, with an incomplete assessment of maternal fish intake, this parameter may be acting as a proxy variable for active compounds present in fish, perhaps polyunsaturated fatty acids, as suggested by Olsen *et al.* (1986).

Regression analyses were also carried out for mothers who had smoked during

TABLE 2

MEDIANS (AND 25th–75th PERCENTILES IN PARENTHESES) FOR BIRTH WEIGHT (g) AND CORD BLOOD MERCURY CONCENTRATION (nmole/liter) IN RELATION TO NUMBER OF FISH DINNERS PER WEEK DURING PREGNANCY FOR NONSMOKING MOTHERS

Number of fish dinners per week	Number	Birth weight[a]	Mercury[b]
0	13	3400 (3250–3950)	20 (6–98)
1	83	3600 (3400–4000)	118 (45–163)
2	220	3850 (3500–4100)	105 (65–174)
3	183	3800 (3500–4150)	133 (64–212)
≥4	114	3750 (3500–4100)	138 (91–189)

[a] $r_s = 0.09$, $P = 0.06$.
[b] $r_s = 0.19$, $P < 0.001$.

pregnancy, but fish intake and B-Hg showed a less clear relationship with birth weight. A similar relationship in nonsmokers, but not in smokers, was seen in a recent study of birth weight in relation to fish intake in Denmark (Olsen *et al.*, 1990). This difference between smokers and nonsmokers could be due to masking effects of different degrees of smoking, but compounds from tobacco smoke could perhaps also inhibit the effect caused by the active compounds in fish.

Bias Related to Alcohol Habits

In studies of neurobehavioral changes in children, maternal alcohol drinking during pregnancy is a risk factor of considerable importance (Streissguth *et al.*, 1990). In the present cohort, alcohol intake was limited; 771 women (75.4%) were abstainers during pregnancy, and all other women drank alcoholic beverages only occasionally. Alcohol will increase the formation of mercury vapor (Hg^0) from ionic mercury (Hg^{2+}) in the blood, and some of the Hg^0 is exhaled (Dunn *et al.*, 1981). Accordingly, mercury-exposed human volunteers tend to show a decreased concentration of inorganic mercury in blood after alcohol ingestion (Hursh *et al.*, 1980). However, it is unclear if transplacental passage of mercury is changed due to the presence of alcohol in the blood. Also, only inorganic mercury has thus far been studied in this regard. However, some demethylation may occur in the body, and at least some of the mercury from pilot whale will be in an inorganic form (Juhlshamn *et al.*, 1987). Thus, as alcohol intake may affect both mercury retention and neurobehavioral functions, this parameter could be an important confounding factor.

In the Faroese cohort, the mercury concentration in cord blood was significantly lower when the mother had ingested alcoholic beverages during pregnancy (Fig. 1). The median B-Hg levels were 126 and 103 nmole/liter in abstainers and alcohol drinkers, respectively (Mann–Whitney U test, $P = 0.005$). A regression

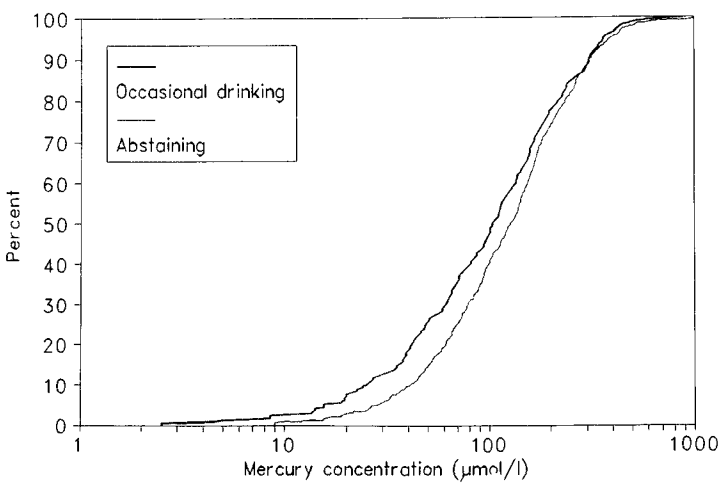

FIG. 1. Cumulated distribution of the mercury concentrations in cord blood in relation to maternal alcohol-drinking habits during pregnancy.

analysis using the log-transformed B-Hg as the dependent variable showed that alcohol ingestion decreased B-Hg by 17% ($P = 0.02$).

The interaction observed could, at least in part, be due to differences in diet between abstaining and nonabstaining women. As shown in the left panel of Fig. 2, women who drank alcohol during pregnancy constituted more than half of the women who did not eat fish. However, the mercury concentration in cord blood is primarily influenced by the whale meat consumption. The right panel of Fig. 2 shows that whale meat ingestion varies much less in relation to alcohol habits. In a multiple regression analysis, adjustment for both fish and whale meat intake decreased the effect related to alcohol drinking only by one-half. Thus, as differences in dietary intake seem to be an incomplete explanation for the difference in B-Hg seen in Fig. 1, a toxicokinetic interaction seems likely.

DISCUSSION

With methyl mercury being recognized as a documented neurobehavioral toxicant, especially under intrauterine exposure conditions, detailed dose–response relationships are badly needed to evaluate the safety of exposures in populations with a high intake of seafood. However, epidemiological studies in this area are susceptible to several sources of bias that must be taken into account in the design and analysis of the data. We have examined potential biases due to the selection of the cohort or due to two possible confounders.

The data shown in Table 1 indicate only one source of major selection bias in this study. As a result of incomplete sampling, particularly at hospital 3 that showed very high mercury exposures, the overall average mercury concentration is probably lower than in the background population. However, except perhaps

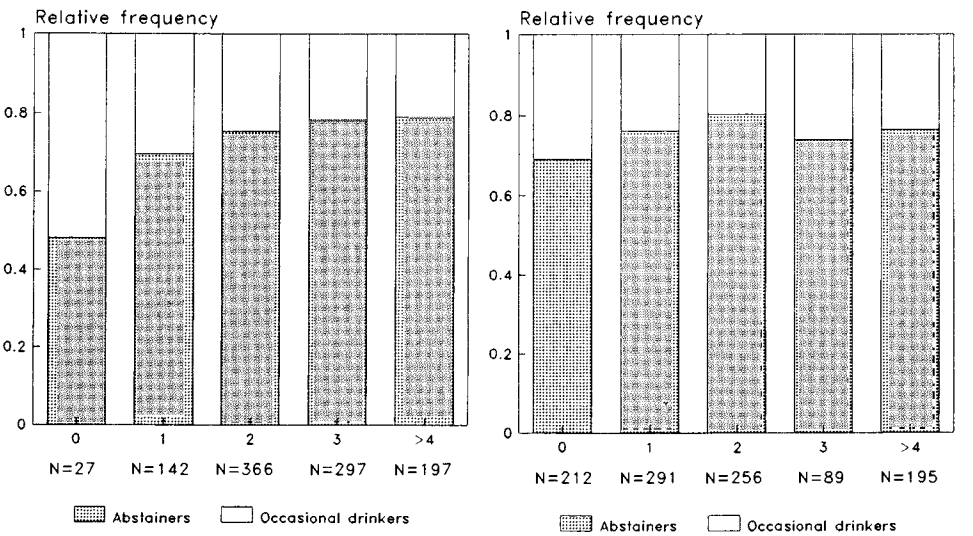

FIG. 2. Alcohol-drinking habits and diet: Number of fish dinners per week (left) and number of whale meat dinners per month (right) during pregnancy in abstaining and alcohol-drinking mothers.

from decreasing the power of the study, this bias is not likely to influence the relation between mercury exposure and neurobehavioral dysfunction.

As emphasized by Lyngbye *et al.* (1989), neurobehavioral risk factors other than the exposure under study must be assessed to determine the extent of possible confounding. In this study, the mercury originates from seafood, and other biologically active components in marine food could either prevent expression of mercury toxicity, or, alternatively, mimic such effect. In this regard, the effects on birth weight provide a useful illustration (Table 2). Thus, when the mother had refrained from smoking during pregnancy, the seafood diet appeared to cause an increased birth weight. Although an increased birth weight may not necessarily be beneficial, this tendency may offer some protection against low birth weight and its associated neurobehavioral risks. Although it is unclear whether high mercury exposure alone may result in a decreased birth weight, children with low birth weight would be more likely to exhibit neurobehavioral abnormalities. By leading to an increase in birth weight, the seafood diet therefore causes a confounding effect that could bias a study of mercury-associated neurobehavioral effects toward the null hypothesis.

Another potential relates to, e.g., chlorinated hydrocarbons, because these compounds may occur in seafood and some are suspected to cause adverse effects on the nervous system. This possibility was not examined in the present study.

With alcohol, another type of confounding may occur. Thus, maternal alcohol drinking during pregnancy seems to lower the mercury concentration in the blood (Fig. 1), but, at the same time, the intrauterine exposure to alcohol also carries an increased risk of subsequent neurobehavioral dysfunction in the child (Streissguth *et al.*, 1990). This factor, as well, will tend to bias the study outcome toward the null hypothesis.

The availability of detailed obstetrical information and extensive questionnaire data therefore allowed exploration of potential sources of bias in a prospective study of neurobehavioral effects related to intrauterine mercury exposure. This experience will be utilized in the design and analysis of further data collected, and it may also be useful in the interpretation of the evidence already available in this area.

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Profile of Subjective Complaints and Activities of Daily Living among Current Patients with Minamata Disease after 3 Decades[1]

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We surveyed 1144 current patients with Minamata disease (MD) aged 40 or over in the Minamata area and the same number of neighbor controls matched with age and sex by questionnaire interview with regard to subjective complaints and activities of daily living (ADL). From analysis of subjective complaints, it was found that MD patients had significantly higher rates of all complaints than controls ($P < 0.05$). Multivariate analysis showed that subjective complaints in controls were clearly separated into the following two categories: sensory disturbances and movement nerve disturbances, but all complaints in MD patients formed one cluster. Such variation seemed to be due to methylmercury exposure to the central nervous system. ADL analysis revealed that the difference in the ADL disability between MD patients and controls significantly increased with age ($P < 0.05$) and that ADL disability in MD patients was aggravated by aging.

INTRODUCTION

In 1953, the first Minamata disease outbreak occurred among the people living around the Minamata Bay, in Kumamoto, Japan, who ingested fish and shellfish contaminated with environmental methylmercury discharged from a chemical plant in Minamata City (Irukayama *et al.*, 1962). Major signs and symptoms of the early stage were ataxia, impairment of speech, and constriction of visual fields, often accompanied by hearing impairment and sensory disturbances (Takeuchi *et al.*, 1959).

By the end of 1990, over 2000 inhabitants were certified as having Minamata disease (MD) and qualified for compensation by a Japanese government committee. Three decades have passed since the first outbreak of the disease, and over 1000 MD patients have survived and advanced in years. It is, however, unknown whether past methylmercury exposure affects directly and/or indirectly the morbidity of current MD patients. There is little available information about the health conditions and physical activity of present MD patients.

In response to this lack of data, we surveyed all MD patients to clarify the current status of their complaints and activities of daily living (ADL). We compared these data with those from controls in the neighboring area where there was not any specific methylmercury pollution.

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SUBJECTS AND METHODS

In 1985, we conducted a questionnaire interview in cooperation with three administrative health centers (Minamata, Hondo, and Izumi), which cover most MD patients in the Minamata area. This study was based on a home-care program for MD patients started in 1983 by public health nurses belonging to these health centers. The program was well carried out in cooperation with MD patients and their families, and cooperation in the interviews was very good. As shown in Fig. 1, the survey area consisted of two cities (Minamata and Izumi) and eight towns (Tanoura, Ashikita, Tsunagi, Gosyonoura, Takaono, Noda, Nagashima, and Azuma). A number of 1144 non-fetal MD patients aged 40 or over (586 males and 558 females) were interviewed. They accounted for nearly 90% of all MD patients living in the above area. In 1990, we also surveyed 3212 inhabitants (1424 males and 1788 females), which made up about 70% of all inhabitants aged 40 or over in Nejime Town (Fig. 1). Control selection was made by an age (5-year intervals) and sex matching procedure. A total of 1144 controls were matched completely.

Questionnaire items in the above surveys largely concentrated on subjective complaints and ADL as shown in Table 1. The subjective complaint questionnaire covered 18 items, and the response categories were "yes," "no," or "uncertain." Katz's ADL questionnaire (Katz *et al.*, 1963) was used to evaluate functional capability of daily living activity. The ADL questionnaire consisted of five items (i.e., Qa–Qe in Table 1) with regard to eating, bathing, dressing, washing one's face, and use of toilet. The capability of activity was classified into the

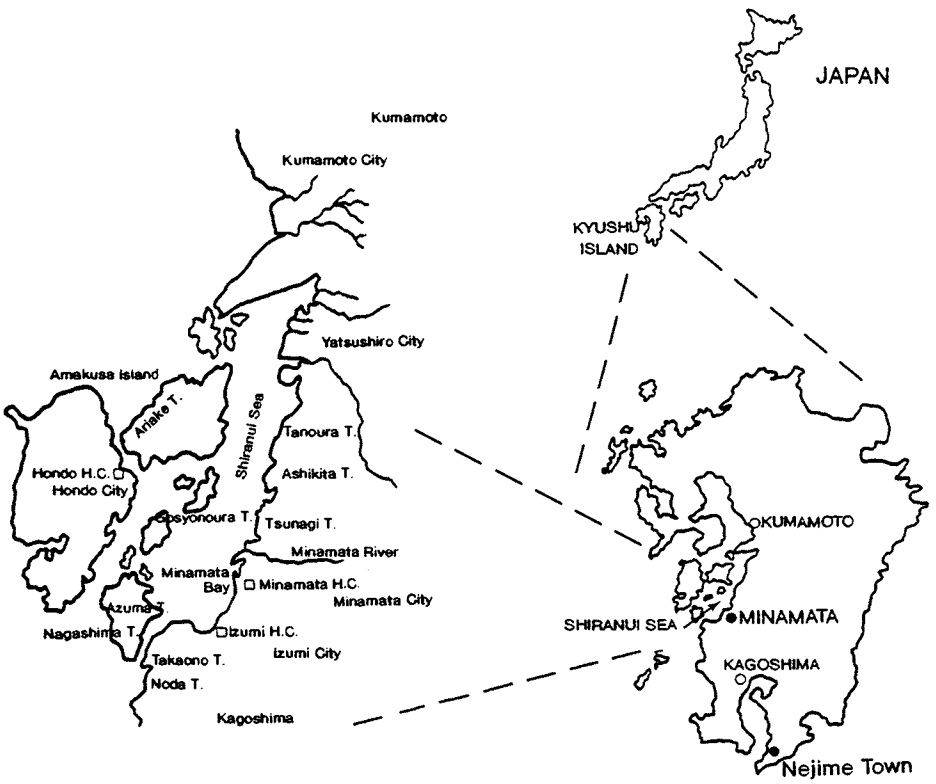

FIG. 1. Map of Minamata area and location of Nejime Town.

TABLE 1
QUESTIONNAIRE ON SUBJECTIVE COMPLAINTS[a] AND ADL[b]

Subjective complaint
- Q1. Do you have incontinence? (incontinence)
- Q2. Do you have ptyalism? (ptyalism)
- Q3. Do you have difficulty speaking? (difficulty speaking)
- Q4. Do you knock your head against the door frames or furniture easily? (constriction of visual field)
- Q5. Do your fingers tremble when using chopsticks? (tremor)
- Q6. Do you find it hard to button your clothing? (difficulty buttoning)
- Q7. Do you stumble easily? (stumbling)
- Q8. Is it hard to hear people or television? (difficulty hearing)
- Q9. Have you become forgetful? (forgetfulness)
- Q10. Do you get tired easily? (fatigability)
- Q11. Do you have hypoesthesia around your mouth? (hypoesthesia of mouth)
- Q12. Do you have hypoesthesia of the limbs? (hypoesthesia of limbs)
- Q13. Do you have dysesthesia of the limbs? (dysesthesia of limbs)
- Q14. Do you feel weak in the limbs? (weakness)
- Q15. Do you feel dizzy rather often? (dizziness)
- Q16. Do you have tinnitus? (tinnitus)
- Q17. Do you get cramps in your limbs? (cramp)
- Q18. Do you have neck–shoulder discomfort or low back pain? (low back pain)

ADL
- Qa. Can you feed yourself without any help? (eating)
- Qb. Can you bathe yourself without any help? (bathing)
- Qc. Can you wash your face yourself without any help? (washing face)
- Qd. Can you dress yourself without any help? (dressing)
- Qe. Can you get to and from the toilet without any help? (use of toilet)

[a] Answers to Q1 to Q18 are "Yes," "No," or "Unknown."
[b] Answers to Qa to Qe are "Yes (no assistance)" or "No (some or full assistance)."

following two categories: subjects in no need of assistance and those needing some or full assistance.

We calculated the prevalence rate and odds ratio following the McNemar procedure (McNemar, 1947) to compare subjective complaints and ADL between the MD patient group and the control group. Difference in prevalence rate between MD patients and controls for subjective complaints and ADL was also calculated by age group. A relationship between age group and difference in prevalence rate was statistically tested by the Cochran–Armitage procedure (Cochran, 1954; Armitage, 1955). Subjective complaints were also assessed by multivariate analysis for categorical data, according to the quantitation method of the third type which was developed by Hayashi (1952, 1979). We performed this type of analysis to grasp the mutual relationship of subjective complaints, since this statistical procedure is able to arrange categorical data by maximizing the correlation coefficient between subjects and categories. Details of this analysis were introduced by Tanaka (1979).

RESULTS

Frequency distributions of subjective complaints in both MD patients and controls are shown in Table 2. The MD patients had a significantly higher prevalence rate for every subjective complaint than controls ($P < 0.05$). In the MD patients, prevalence rates of subjective complaints, such as difficulty speaking, tremor,

TABLE 2
COMPARISON OF PREVALENCE OF SUBJECTIVE COMPLAINTS (%) BY AGE AND MINAMATA DISEASE

Subjective complaint by group	Age 40–49 (111)	50–59 (266)	60–69 (285)	70–79 (322)	80+ (160)	Total (1144)[a]
MD patients						
Incontinence	0.9	4.5	6.7	11.8	18.1	8.9
Ptyalism	7.2	7.5	11.9	17.4	18.1	13.7
Difficulty speaking	28.8	33.5	35.4	42.5	42.5	38.2
Constriction of visual field	12.6	12.0	18.9	27.6	25.0	19.3
Tremor	35.1	32.3	37.5	46.6	49.4	39.5
Difficulty buttoning	44.1	38.0	51.2	66.1	66.9	52.8
Stumbling	59.5	57.5	72.3	84.2	77.5	69.3
Difficulty hearing	31.5	46.6	49.8	67.4	78.8	54.0
Forgetfulness	80.2	88.3	93.0	96.0	92.5	88.4
Fatigability	91.9	88.7	87.4	83.2	73.1	82.9
Hypoesthesia of mouth	25.2	27.8	28.1	29.2	20.6	25.4
Hypoesthesia of limbs	72.1	66.5	65.6	74.2	66.9	67.1
Dysesthesia of limbs	87.4	93.6	93.7	90.7	83.1	88.4
Weakness	75.7	78.6	78.2	84.8	83.1	79.1
Dizziness	46.8	47.4	46.0	44.7	36.9	43.0
Tinnitus	59.5	51.9	58.6	55.6	48.1	52.6
Cramp	75.7	85.3	85.3	82.6	68.8	80.0
Low back pain	86.5	92.9	90.5	89.8	86.3	87.3
Controls						
Incontinence	—	0.8	2.1	7.5	23.8	6.1
Ptyalism	0.9	0.8	2.8	5.9	8.8	3.8
Difficulty speaking	3.6	3.8	2.8	7.5	13.1	5.9
Constriction of visual field	0.9	3.8	4.2	7.1	8.8	5.2
Tremor	0.9	1.9	3.5	8.7	16.9	6.2
Difficulty buttoning	1.8	4.5	6.3	15.5	32.5	11.7
Stumbling	0.9	9.4	13.7	28.9	47.5	20.5
Difficulty hearing	5.4	6.0	8.8	23.6	36.9	15.9
Forgetfulness	19.8	25.2	31.9	41.9	49.4	34.4
Fatigability	25.2	27.8	28.1	47.5	50.0	36.3
Hypoesthesia of mouth	—	1.9	2.1	3.1	3.1	2.3
Hypoesthesia of limbs	3.6	13.5	10.5	15.5	23.8	13.6
Dysesthesia of limbs	18.9	27.1	29.5	29.8	36.3	28.9
Weakness	7.2	6.4	11.2	23.0	38.8	16.9
Dizziness	3.6	7.1	6.7	8.1	11.3	7.5
Tinnitus	9.0	12.4	11.6	18.0	18.8	14.3
Cramp	8.1	8.6	10.5	20.8	16.3	13.5
Low back pain	59.5	65.8	66.7	70.2	64.4	66.2

[a] Numbers in parentheses denote number of subjects belonging to respective group.

stumbling, and difficulty buttoning or hearing, increased with age. On the other hand, subjective complaints related to sensory disturbance, such as hypoesthesia of mouth and limbs, dysesthesia of limbs, and weakness did not show any age-dependency. It was also clear that prevalence rates of all subjective complaints increased with age in the control group.

Table 3 shows the odds ratios and 95% confidence intervals by subjective complaint. Each odds ratio of a subjective complaint significantly exceeded 1.0 (*P*

TABLE 3
ODDS RATIOS AND 95% CONFIDENCE INTERVALS BY SUBJECTIVE COMPLAINT

Subjective complaint	OR (95% CI)
Incontinence	1.5 (1.1–2.1)
Ptyalism	3.7 (2.6–5.4)
Difficulty speaking	10.7 (7.6–15.3)
Constriction of visual field	5.2 (3.7–7.4)
Tremor	9.5 (6.9–13.0)
Difficulty buttoning	10.5 (7.8–14.1)
Stumbling	11.5 (8.7–15.2)
Difficulty hearing	8.5 (6.4–11.1)
Forgetfulness	26.1 (17.4–39.4)
Fatigability	8.4 (6.6–10.8)
Hypoesthesia of mouth	14.5 (9.1–23.1)
Hypoesthesia of limbs	14.2 (10.5–19.2)
Dysesthesia of limbs	19.6 (14.0–27.6)
Weakness	29.0 (19.4–43.9)
Dizziness	9.7 (7.2–13.2)
Tinnitus	6.7 (5.3–8.5)
Cramp	22.5 (16.0–31.9)
Low back pain	4.6 (3.5–5.9)

Note. OR, odds ratio; CI, confidence interval.

< 0.05). We confirmed that the MD patients had higher prevalence rates than the controls with regard to all 18 subjective complaints investigated in this study.

The difference in prevalence rate of subjective complaints between MD patients and controls is depicted in Fig. 2. These complaints were mainly categorized into the following two groups: an age-specific group (expressed by a solid line) related to sensory disturbance and a non-age-specific group (expressed by a dotted line) related to motor nerve disturbance. In the age-specific group, the difference in prevalence rate decreased with age ($P < 0.05$). On the contrary, the difference in prevalence rate in a non-age-specific group showed a flat pattern with age.

Results by multivariate analysis for quantification data are given in Fig. 3. The maximum eigen value except for 1.0 was given as vertical axis (MD patients, 0.241; controls, 0.308). The second largest eigen value was given as horizontal axis (MD patients, 0.113; controls, 0.106). The total of the two axes accounted for 31.6% of total association in MD patients and 36.5% of that in controls. Since the third largest eigen value (MD patients, 0.070; controls, 0.066) made little contribution to the total association (MD patients, 6.3%; controls, 5.9%), it was not used in the present analysis. The answer category of "yes" for each subjective complaint in MD patients is indicated by a blank circle (○). Solid triangles (▲) indicate controls. All subjective complaints in the controls were clearly separated into the following two clusters: a sensory disorder group (circle A) and a movement nerve disorder group (circle B). All complaints in the MD patients formed one cluster.

Results of the comparison for ADL ability are shown by age and group in Table 4. The number of patients responding that they required no assistance decreased with age after 60 years of age or more in every item of ADL in both MD patients and controls.

Table 5 shows odds ratios and 95% confidence intervals by age and ADL item.

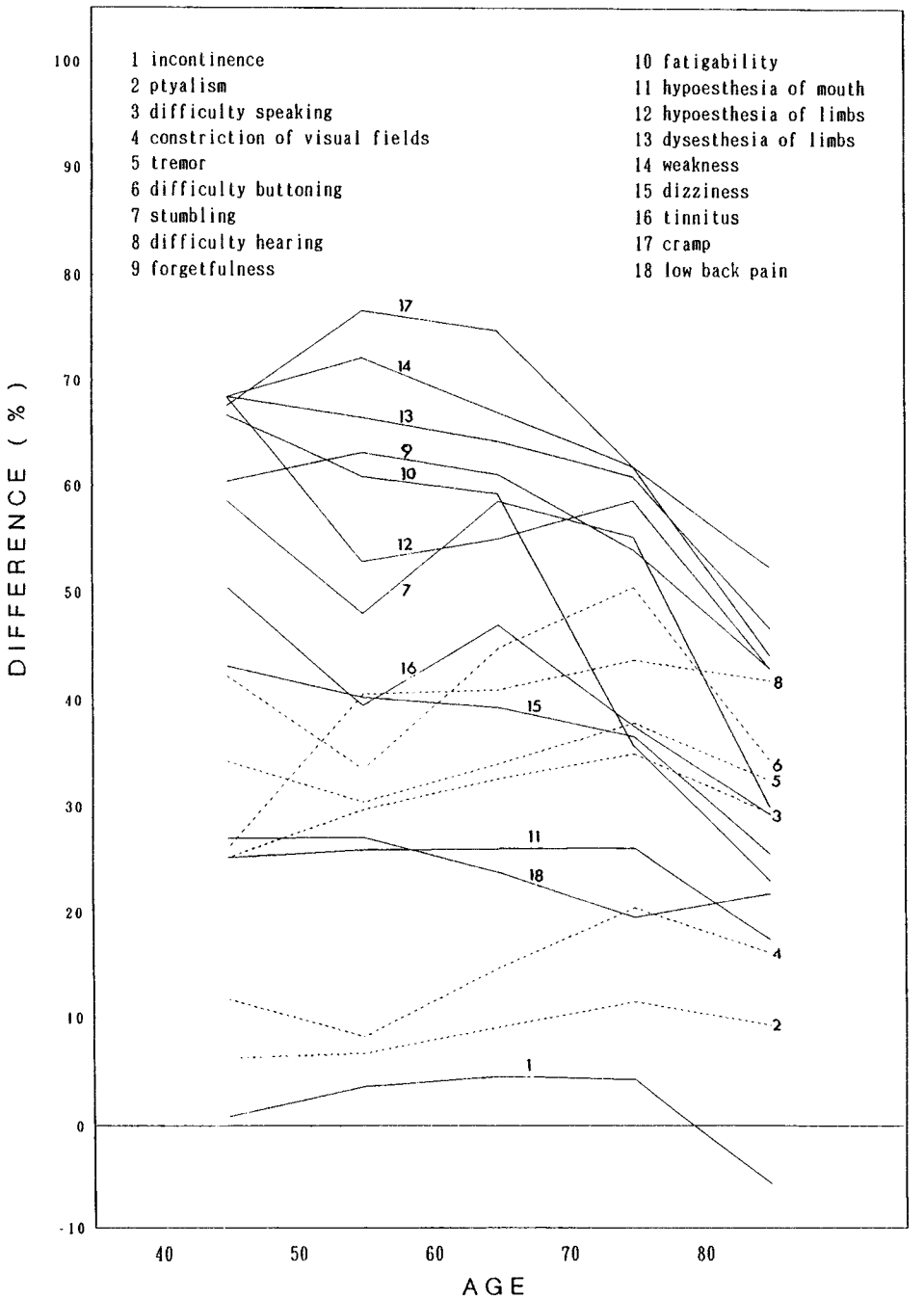

FIG. 2. Comparison of difference in prevalence rate of subjective complaints between MD patients and controls by age. Vertical axis, the difference in prevalence rate (MD patients (%) minus controls (%)). Relationship between age and the difference in prevalence rate was determined through the Cochran–Armitage test (solid line, $P < 0.05$; dotted line, not significant).

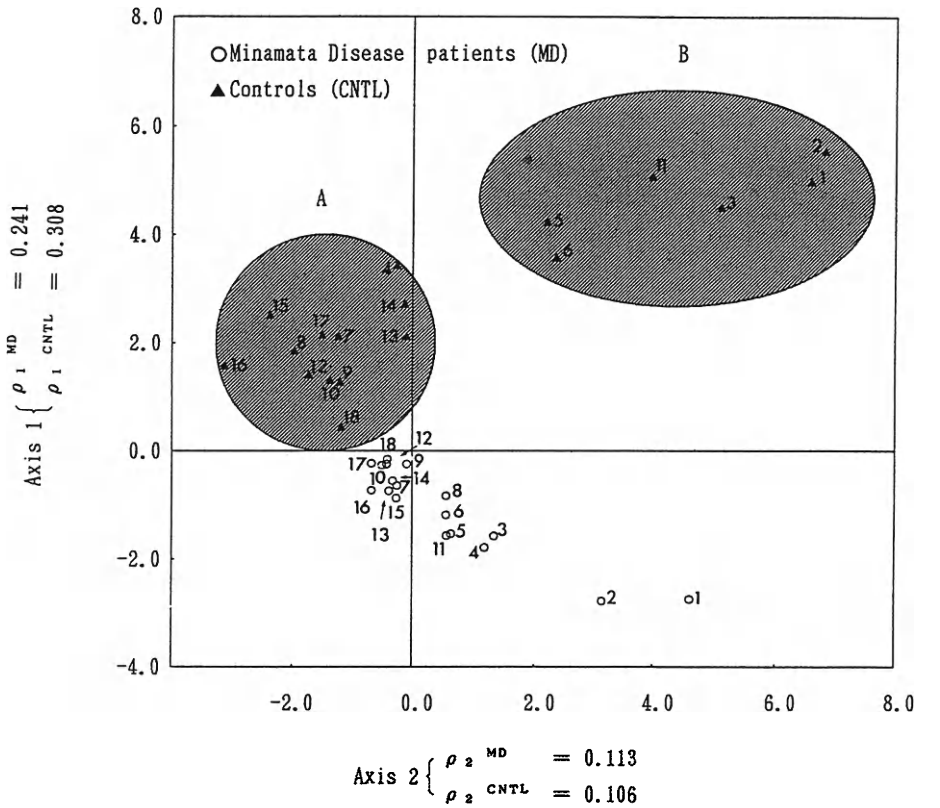

FIG. 3. Result of multivariate analysis of subjective complaints by quantification method of third type. Vertical axis indicates value of the positive response category in each subjective complaint based on the maximum eigen value (MD patients, 0.241; controls, 0.308). The horizontal axis also represents the second largest eigen value (MD patients, 0.113; controls, 0.106). 1, incontinence; 2, ptyalism; 3, difficulty speaking; 4, constriction of visual field; 5, tremor; 6, difficulty buttoning; 7, stumbling; 8, difficulty hearing; 9, forgetfulness; 10, fatigability; 11, hypoesthesia of mouth; 12, hypoesthesia of limbs; 13, dysesthesa of limbs; 14, weakness; 15, dizziness; 16, tinnitus; 17, cramp; 18, low back pain.

There was no statistical difference in ADL ability between MD patients under 60 years of age and the matched controls ($P > 0.05$). On the contrary, MD patients aged 60 or over had a significantly lower ADL ability than the corresponding controls ($P < 0.05$). In terms of overall age, MD patients were significantly inferior to controls in all ADL capacities ($P < 0.05$).

The difference in ADL ability between MD patients and controls (MD patients (%) minus controls (%)) by age class is depicted in Fig. 4. The absolute value of the differences in ADL ability significantly increased with age in all ADL items ($P < 0.05$).

DISCUSSION

In our present investigation, we researched the current health status of MD patients from two points of view: subjective complaints and ADL. In the following sections, we discuss those, respectively.

Previous studies demonstrated that there are both qualitative and quantitative differences in the target effects and dose–response relationships between adults

TABLE 4
COMPARISON FOR ADL ABILITY[a] BETWEEN MINAMATA DISEASE PATIENTS AND CONTROLS

Item of ADL by group	Age 40–49 (111)	50–59 (266)	60–69 (285)	70–79 (322)	80+ (160)	Total (1144)[b]
MD patients						
Eating	99.1	97.4	90.9	90.7	76.9	91.2
Bathing	97.3	94.7	88.4	78.6	63.8	84.5
Face washing	99.1	97.0	90.9	89.1	75.0	90.4
Dressing	98.2	96.6	90.5	86.0	73.1	89.0
Use of toilet	98.2	97.0	93.0	89.4	75.6	91.0
Controls						
Eating	98.2	99.2	98.9	96.3	91.9	97.2
Bathing	97.3	98.1	97.5	92.2	78.8	93.5
Face washing	97.3	98.9	98.6	93.8	85.6	95.4
Dressing	97.3	98.1	97.2	93.5	83.1	94.4
Use of toilet	98.2	98.9	97.9	93.5	83.8	94.9

[a] Subjects in no need of assistance (%).
[b] Numbers in parentheses denote number of subjects belonging to respective group.

and fetuses (Marsh *et al.*, 1980, 1981, 1987; Mckewon-Essen *et al.*, 1983; Kjellstrom *et al.*, 1989; Cox *et al.*, 1989). In a Minamata outbreak, 23 children were reported as fetal MD patients who were severely exposed to methylmercury via the placenta (Takeuchi, 1977). As there seems to be some difference in the aging effects between MD patients with postnatal and prenatal exposure, we excluded the above fetal MD patients from the present study.

Matsushita *et al.* (1972) surveyed inhabitants of Ariake Town, Gosyonoura Town, and Minamata City and compared the prevalence of various subjective complaints. The prevalence of subjective complaints in the controls in the present study did not differ from that of inhabitants at Ariake Town, which was free from methylmercury pollution. Araki *et al.* (1990) investigated 345 inhabitants aged 60 or over in a non-methylmercury-polluted district near Kumamoto City to elucidate the prevalence of neurological complaints, signs, and symptoms among the elderly. From the comparison of subjective complaints between the elderly and the controls aged 60 or over in the present study, we found similar prevalence rates in subjective complaints such as tinnitus, dizziness, difficulty hearing, hypoesthesia of limbs, and low back pain. It was also ascertained that the prevalence

TABLE 5
ODDS RATIOS AND 95% CONFIDENCE INTERVALS BY AGE AND ADL ITEM

ADL item	Age group: Less than 60 OR (95% CI)	60 or over OR (95% CI)	Total OR (95% CI)
Eating	0.50 (0.12–1.82)	0.27 (0.16–0.43)	0.29 (0.18–0.44)
Bathing	0.44 (0.16–1.12)	0.32 (0.22–0.45)	0.34 (0.24–0.46)
Face washing	0.67 (0.21–2.04)	0.42 (0.28–0.61)	0.44 (0.30–0.63)
Dressing	0.73 (0.26–1.94)	0.41 (0.28–0.59)	0.44 (0.31–0.62)
Use of toilet	0.50 (0.14–1.58)	0.51 (0.34–0.75)	0.51 (0.35–0.73)

Note. OR, odds ratio; CI, confidence intervals.

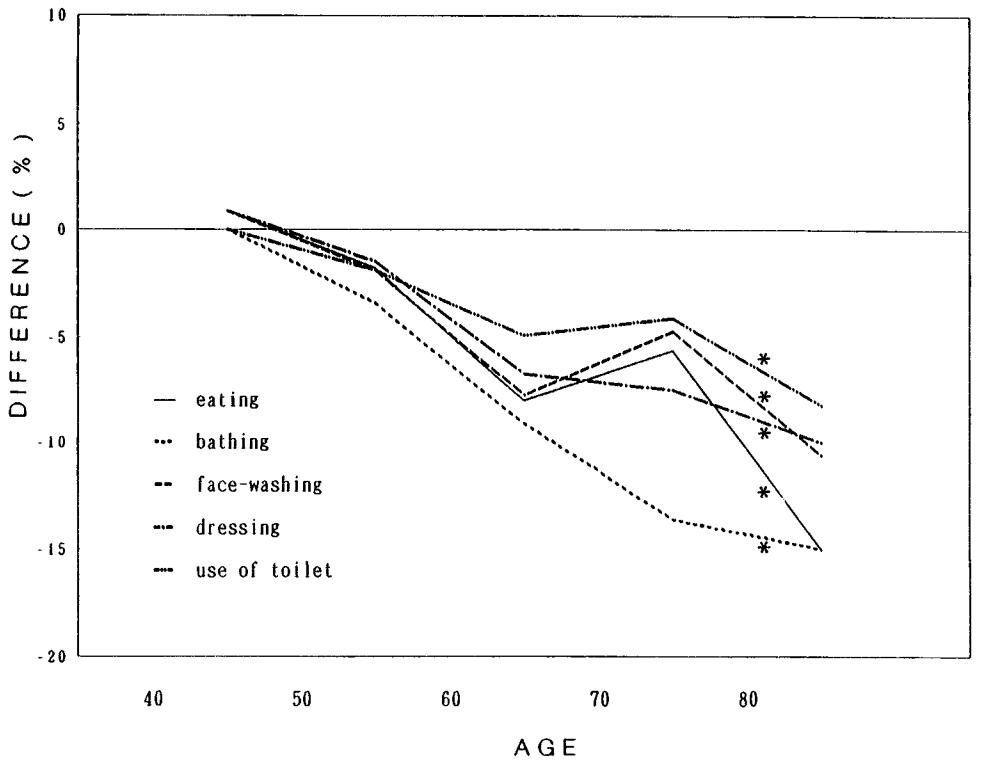

FIG. 4. Comparison of difference in ADL ability between MD patients and controls by age. Vertical axis, the difference in ADL ability (subjects in no need of assistance (%); MD patients (%) minus controls (%)). Relationship between age and the difference in ADL ability was determined through the Cochran–Armitage test ($*P < 0.05$).

of subjective complaints in the MD patients in the present study corresponded to the prevalence of neurological signs and symptoms of MD patients in previous clinical surveys (Harada, 1972; Okajima *et al.*, 1976). The above comparisons corroborate reliability of the data obtained from our questionnaire interview.

The present study shows that subjective complaints of MD patients are more prevalent than those among the controls (Table 3). This result also agrees with earlier findings reported at the time of a Niigata outbreak of Minamata disease (Shirakawa *et al.*, 1972).

Emphasis was placed on seeking differences in age distribution of subjective complaints between MD patients and controls. The subjective complaints of the MD patients can be classified into two groups: those where frequency increased with age and those mainly related to sensory disturbance, which although high, remained unchanged with age (Table 2). Among controls, all complaints showed obvious age-dependency. It should be mentioned here that a subjective complaint of paraesthesia was transient in most patients in an Iraqi outbreak (IPCS, 1990). The reason for the high prevalence rate of sensory disturbance among current MD patients is not clear.

The present result from multivariate analysis revealed that the aging effects of subjective complaints in controls are clearly different between motor nerve function and sensory function (Fig. 3). On the other hand, all subjective complaints in

MD patients made one cluster, which suggests damage to broader CNS areas by methylmercury exposure.

We used ADL to estimate functional capability in the elderly (Warren and Knight, 1982; Asberg, 1986). Fujita and Hatano (1989) estimated that prevalence rates of ADL disability in the Japanese population of young–old (60–64) and the old–old (85–89) were 2% and 32%, respectively. Our results for prevalence rates of controls aged 60 to 69 (Table 4) were quite similar to those of the Japanese young–old mentioned above. It is therefore considered that our data of ADL is reliable.

In the present study, ADL indexes are age-dependent in both MD patients and controls. There is significantly lower ADL in MD patients aged 60 or over than in controls (Table 5). These results coincide with the trend which is presented in Fig. 4. Thus the difference in ADL disability between MD patients and controls increased with age in every item of ADL. It is, therefore, suggested that ADL disability in MD patients is accelerated by aging. It should be noted here that the difference in prevalence rate of subjective complaints between MD patients and controls did not increase with age. This result is contrary to the present results for ADL. As the ADL is able to consider the evaluation level of all complaints by an individual, it is clear that a total level of subjective complaints in each MD patient is very much lower than that of controls.

As described above, the present case–control study obviously indicates that differences in prevalence rates of subjective complaints, in mutual relationships of subjective complaints, and in ADL disabilities between MD patients and controls depend on methylmercury exposure. Further epidemiological studies using neurological and clinical tests are necessary to elucidate acceleration of degenerative changes in the human nervous system, which is thought to be a later effect of methylmercury exposure in the present study.

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Dose-Dependent Increase in Subjective Symptoms among Toluene-Exposed Workers[1,2]

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A factory survey on dose–response relationship in toluene toxicity was conducted in 1985–1989 in four cities in China. The examination items consisted of personal diffusive sampling for TWA exposure measurement, questionnaires on subjective symptoms, hematology and serum biochemistry, and clinical examination including simple neurology tests. Hippuric acid was also determined in urine samples collected at the end of the shift. With selection criteria that (1) complete results were available on all study items and (2) valid toluene exposure data (i.e., toluene shared 90% or more of the exposure) were obtained for the exposed, 452 toluene-exposed workers (206 men and 246 women; toluene exposure at 24.7 ppm as GM) and 517 nonexposed controls (246 men and 271 women) were selected. The subjective symptoms increased in close association with the intensity of exposure to toluene; the threshold concentration appeared to exist at 100 ppm in the case of symptoms during work, and it might be at 50–100 ppm when symptoms off work were evaluated. During the work with exposure at higher concentrations, various symptoms possibly related to CNS or local effects (e.g., eyes, nose, and throat) were complained, and dizziness and floating sensations were identified as typical symptoms with significant dose–response relationship. Several symptoms persisted off work, most of which were apparently related but not necessarily limited to CNS effects. Hematology and serum biochemistry were essentially negative.

INTRODUCTION

Toluene is a solvent very widely applied in various solvent products such as thinners, paints, and adhesives (Inoue *et al.*, 1983; Kumai *et al.*, 1983; Saito and Ikeda, 1988) and is also present at high concentrations in automobile gasoline, especially when unleaded (Ikeda *et al.*, 1984a), as well as petroleum distillates for solvent use (Kasahara *et al.*, 1987). It is known that toluene is toxic to the central nervous system (CNS) and may increase prevalence of subjective symp-

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toms such as headache among exposed workers (e.g., Browning, 1965; Sandmeyer, 1981; Antti-Poika *et al.*, 1987). In addition, intentional inhalation of the dense toluene vapor will induce dependency to this CNS effects. There is, however, a paucity of information on the quantitative relation of the symptoms and the exposure intensity.

The present study was initiated to elucidate the possible dose–response relationship in toluene toxicity in terms of subjective symptom prevalence and, if this is the case, to identify any symptoms that would be reliable indicators of toluene exposure.

A preliminary study on this solvent has been published (Lee *et al.*, 1988). Similar subjective symptom analyses, although of a smaller scale, have also been conducted on trichloroethylene (Liu *et al.*, 1988) and tetrachloroethylene (Cai *et al.*, 1991).

MATERIALS AND METHODS

Subjects studied. The survey was conducted in 1985–1989. In total, 1316 solvent-exposed workers and 769 nonexposed control subjects were examined. The exposed workers had served in large-scale printing, painting, surface-coating, paint-producing, or shoe-making plants in the four cities of Beijing, Shanghai, Wuxi, and Hefei in China. The control subjects were recruited either from workshops of the same factory or from neighboring factories where no solvents were used. Selection criteria were set to identify (from the groups of examinees) those who were considered fit for further statistical analysis for the effects of toluene exposure. Namely, the criteria were (1) exposed and nonexposed subjects for whom complete test results are available on each and all study items (for details of the items, see below) and (2) the exposed workers with valid toluene exposure data [i.e., toluene shared 90% or more (in terms of ppm) of the solvent vapors as confirmed by the diffusive air sampling method] and shift-end urine samples for toluene metabolite.

Examination protocol. The occupational health examination took 2 days. On the first day, each exposed worker was equipped with a diffusive sampler on his/her lapel from the beginning of the workshift of a study day until the end of the shift (for about 8 hr). They were asked to pass urine a few hours before the shift was finished, and then urine samples were collected at the end of the shift. On the second day, both exposed and nonexposed workers were invited (during a shift) to the health examination room, usually in a clinic in the factory studied, for blood sampling from cubital vein for hematology and serum biochemistry, neurological examination (i.e., patellar tendon reflex and tandem walk), and self-completion-type questionnaires.

Examination methods. Hematology and serum biochemistry were by conventional methods under strict quality control. The questionnaires employed were developed by Inoue (1968) originally in Japanese and translated into Chinese; an English version has been previously published (Yin *et al.*, 1987). The questionnaires consist of two parts; 12 questions on the current subjective symptoms during work in Part 1, and 57 questions on the subjective symptoms (while out of a shift) in the past 3-month period in Part 2. Positive answers of the respondents

were confirmed in a clinical interview by a medical doctor. The prevalence of the subjective symptoms is calculated as

$$\frac{\text{the number of affirmative answers by the group}}{\text{(the number of the people in the group)} \times \text{(number of questions)}} \times 100\ (\%).$$

Diffusive sampling with carbon cloth KF-1500 (Toyobo, Osaka, Japan) was as previously described (Hirayama and Ikeda, 1979; Ikeda *et al.*, 1984b; Kasahara and Ikeda, 1987).

Criteria for the evaluation of hematology and serum biochemistry. The evaluation of hematology and serum biochemistry results were conducted as previously described (Cai *et al.*, 1991). In brief, log-normal distribution was assumed for enzymic parameters taking the clinically established normal range as a 4 × GSD (geometric standard deviation) range, whereas normal distribution was considered for nonenzymic ones taking the normal range as 4 × ASD (arithmetic standard deviation) range. Thus, the lower and upper limits of the borderline range (i.e., the line between the borderline and abnormal values) were set at GM/(GSD)[3] and GM × (GSD)[3] respectively for the formers, and at AM-4ASD and AM + 4ASD respectively for the latters (except for WBC counts). The values thus set agree well with the evaluation in clinical practice. In the case of WBC counts, 3000 cells/mm^3 was selected as the lower borderline limit based on the clinical experience.

When ASAT and ALAT, or ALP and LAP were evaluated in pairs, the case was classified as *normal* when both enzyme activities (i.e., ASAT and ALAT, for example) stayed in normal ranges, and classified as *abnormal* when one of the two parameters was in the borderline or abnormal range and the other was in the abnormal range. Other cases were classified as *borderline*. The criteria thus established are summarized in Table 1.

Statistical analysis. Significance of the difference in prevalence was examined by χ^2 test and t test, as indicated.

RESULTS

Selection of Study Population

When the selection criteria (for details, see Materials and Methods) were applied to the exposed and control workers, 452 toluene-exposed workers [206 men (average age; 31.4 years) and 246 women (32.1 years)] and 517 nonexposed controls [246 men (36.8 years) and 271 women (31.2 years)] cleared the criteria and were identified as the study populations fit for evaluations. The ages ranged from 16 to over 60 years. The geometric mean intensity of exposure to toluene among the 453 exposed workers was 24.7 ppm (20.3 ppm for 206 men and 29.1 ppm for 246 women) with a geometric standard deviation of 4.43 (4.60 for men and 4.22 for women).

[3] Abbreviations used: AM, arithmetic mean; ASD, arithmetic standard deviation; GM, geometric mean; GSD, geometric standard deviation; γ-GTP, γ-glutamyl transpeptidase (EC 2.3.2.1); ASAT (or GOT), aspartate aminotransferase (EC 2.6.1.1); ALAT (or GPT), alanine aminotransferase (EC 2.6.1.2); ALP, alkaline phosphatase (EC 3.1.3.1); LAP, leucine aminopeptidase (EC 3.4.1.1).

TABLE 1
CRITERIA FOR HEMATOLOGY AND SERUM BIOCHEMISTRY EVALUATION

Item	(unit)	Sex	Normal range	Limit[a] for borderline case
Leukocytes	($\times 10^3/mm^3$)	Men	4.1–6.1	<3.0
		Women	3.9–6.3	<3.0
Hemoglobin	(g/100 ml)	Men	13.7–17.4	<11.9
		Women	11.3–14.9	<9.5
Total bilirubin	(mg/100 ml)		0.3–1.1	>1.5
Total protein	(g/100 ml)		6.7–8.3	<5.9
Creatinine	(mg/100 ml)		0.6–1.2	>1.5
γ-GTP	(IU/liter)		3–45	>72
ASAT	(IU/liter)		13–36	>46
ALAT	(IU/liter)		5–33	>53
ALP	(IU/liter)		101–356	>488
LAP	(IU/liter)		30–53	>61

[a] Those with < or > are the limits below or above which, respectively, the value is considered abnormal.

Increased Subjective Symptoms among Toluene-Exposed Workers

Prevalence of subjective symptoms as studied by the questionnaires in the toluene-exposed workers is summarized in the top half of Table 2 in comparison with that in the nonexposed control subjects. The symptom prevalence was calculated separately for Parts 1 and 2, and also for the two sexes as well as men and women in combination.

It is evident from the table that the prevalence of Part 1 symptoms (i.e., symptoms during work) among the exposed workers was several times higher in the exposed than in the controls ($P < 0.01$ in all cases) in men (3.1 times), in women (9.6 times), and therefore in men and women in combination (5.3 times). Thus, toluene exposure appeared to be associated with an increase in subjective symptoms during the work. Increase in the prevalence of Part 2 symptoms among the exposed in comparison with that in the nonexposed is also significant in women ($P < 0.01$) and the two sexes combined ($P < 0.01$) but not in men. The rate of the exposed over the nonexposed was 1.01 for men, 1.51 for women, and 1.27 for the two sexes in combination. In other words, there is also a toluene-associated increase in the subjective symptoms off the work, but the increment over the control levels is not as large as that during work.

Dose Dependency of the Increase in Subjective Symptom Prevalence

To examine further the association between the observed increase in subjective symptom prevalence and toluene exposure, the exposed workers were subgrouped depending on the exposure intensity into those with 1 to 20, 21 to 50, 51 to 100, and ≥101 ppm exposure (the top half of Table 3). Comparison of the subgroups in terms of subjective symptom prevalence showed that there was a statistically significant increase in the prevalence of Part 1 symptoms as a function

TABLE 2
PREVALENCE OF SUBJECTIVE SYMPTOMS AMONG EXPOSED WORKERS IN COMPARISON WITH THAT AMONG NONEXPOSED WORKERS

Questions, all or selected	Sex	Controls	Exposed
All[a]			
Part 1	Men	102 (246): 3.5%	270 (206): 10.9%**
	Women	48 (271): 1.5%	425 (246): 14.4%**
	Men + women	150 (517): 2.4%	695 (452): 12.8%**
Part 2	Men	1107 (246): 7.9%	934 (206): 8.0%
	Women	1165 (271): 7.5%	1580 (246): 11.3%**
	Men + women	2272 (517): 7.7%	2514 (452): 9.8%**
Selected[b]			
Part 1	Men	99 (246): 4.0%	264 (206): 12.8%**
	Women	43 (271): 1.6%	415 (246): 16.9%**
	Men + women	142 (517): 2.7%	679 (452): 15.0%**
Part 2	Men	372 (246): 7.2%	492 (206): 11.4%**
	Women	426 (271): 7.5%	788 (246): 15.3%**
	Men + women	798 (517): 7.4%	1280 (452): 13.5%**

Note. The values in the table are number of affirmative answers (number of the respondents): the prevalence. The prevalence is defined as

$$\text{Prevalence (\%)} = \frac{\text{Number of affirmative answers}}{\text{Number of responders} \times \text{Number of questions}} \times 100.$$

The asterisks indicate that the difference in the prevalence is statistically significant (** for $P < 0.01$).

[a] With all 12 questions for Part 1 symptoms (symptoms during work, and 57 questions for Part 2 symptoms (symptoms off the work).

[b] With selected 10 questions for Part 1 symptoms and 21 questions for Part 2 symptoms. For basis of selection, see text.

of exposure intensity in men ($P < 0.01$), in women ($P < 0.05$), and in the combination ($P < 0.01$). Such was also the the case with the increase in Part 2 symptoms ($P < 0.01$ for all cases). Further perusal of changes in the prevalence (Fig. 1) indicates that the prevalence of Part 1 questions stays rather unchanged up to 100 ppm exposure, above which there was an increase in the prevalence. When statistically examined (by t test), the increase in the prevalence as compared with that in the 1- to 20-ppm group was significant ($P < 0.05$) in the $\geq$101 ppm group but not in the 51- to 100-ppm group ($P > 0.10$). The findings were essentially the same in the case of Part 2 symptoms, but the increment at $\leq$101 ppm appeared to be more marked. Statistical evaluation (by t test) showed that the increase was significant at 51–100 ppm ($P < 0.05$) and further so at $\geq$101 ppm ($P < 0.01$) although insignificant ($P > 0.10$) at 21–50 ppm.

It should also be noted that the symptom prevalence among the controls were generally lower than that in the lowest exposed group (i.e., 1–20 ppm group) in Part 1 symptoms (Tables 2 and 3), e.g., 2.4% for controls and 12.0% for the exposed at 1–20 ppm in the case of the combination of two sexes. Such a difference was not remarkable in Part 2 symptoms.

TABLE 3
DOSE-DEPENDENT INCREASE IN SUBJECTIVE SYMPTOM PREVALENCE AMONG EXPOSED WORKERS

Questions, all or selected	Sex	Exposure intensity				*P* for difference
		1–20 ppm	21–50 ppm	51–100 ppm	≥101 ppm	
All[a]						
Part 1	Men	116 (91):10.6%	52 (55): 7.9%	55 (40):11.5%	46 (20):19.2%‡	**
	Women	157 (98):13.4%	101 (60):14.0%	81 (44):15.3%	87 (44):16.5%	*
	Men + women	273 (189):12.0%	153 (115):11.1%	136 (84):13.5%	133 (64):17.3%†	**
Part 2	Men	367 (91): 7.1%	192 (55): 6.1%	210 (40): 9.2%‡	165 (20):14.5%‡	**
	Women	551 (98): 9.9%	333 (60): 9.7%	251 (44):10.0%	445 (44):17.7%‡	**
	Men + women	918 (189): 8.5%	525 (115): 8.0%	461 (84): 9.6%†	610 (64):16.5%‡	**
Selected[b]						
Part 1	Men	113 (91):12.4%	52 (55): 9.5%	55 (40):13.5%	44 (20):22.0%‡	**
	Women	149 (98):15.2%	101 (60):16.8%	81 (44):18.4%	84 (44):19.1%†	*
	Men + women	262 (189):13.9%	153 (115):13.3%	136 (84):16.2%	128 (64):20.0%‡	**
Part 2	Men	187 (91): 9.8%	107 (55): 9.3%	121 (40):14.4%‡	77 (20):18.3%‡	**
	Women	281 (98):13.7%	178 (60):14.1%	137 (44):14.8%	192 (44):20.8%‡	**
	Men + women	468 (189):11.8%	286 (115):11.8%	258 (84):14.6%‡	269 (64):20.0%‡	**

Note. *P* values are for significant difference in distribution (** and * for $P < 0.01$ and 0.05, respectively). Daggers indicate that the difference from the prevalence at 1 to 20 ppm is statistically significant (‡ and † for $P < 0.01$ and 0.05, respectively). For other notes to the table, see Table 2.

Evaluation of Individual Symptoms

Possible increase in the prevalence was examined between the control and exposed workers (Comparison A) and also among the subgroups of different exposure intensities (Comparison B) for each of the 12 symptoms questioned in Part 1 of the questionnaires. The results are summarized in Table 4. The results of a similar comparison for the 57 symptoms in Part 2 are given in Table 5.

Comparison in Table 4 shows that the prevalence of 10 symptoms out of 12 Part 1 symptoms is significantly ($P < 0.01$ in the cases of 9 symptoms and 0.05 for remaining one) different between the controls and the exposed when men and women in combination were analyzed. The increase in association with increasing exposure intensity was significant ($P < 0.01$) in Symptoms 8 (dizziness) and 9 (floating sensation), and might be present ($P < 0.10$) in Symptom 11 (heavy feeling in the head). In the case of Symptom 12 (headache), the dose dependency was significant ($P < 0.05$) in men but not in women ($P > 0.10$) or the combination ($P > 0.10$). The dose–response relationship of Part 1 symptoms with significant dose–response relationship is depicted in Fig. 2. The prevalence of headache is shown separately in Fig. 3 because the dose dependency was different in two sexes.

In the case of Part 2 symptoms (Table 4), significant ($P < 0.01$) difference in the prevalence between the controls and the exposed (Comparison A) was detected in 12 symptoms when the two sexes in combination were evaluated. They are Symptoms 6 (difficulty in sleep), 13 (forgetfulness), 22 (general dullness), 24 (poor appetite), 26 (dry mouth), 39 (loss in hearing capacity), 42 (reduced sense of taste), 48 (reduced grasping power), 49 (reduced muscle power in extremities), 51 (rough skin), 52 (unusual feeling in throat), and 54 (frequent bleeding from gums). The symptoms that were significantly ($P < 0.01$) dependent to toluene dose (Comparison B) were Symptoms 4 (nausea), 14 (inability to concentrate), 26 (body weight

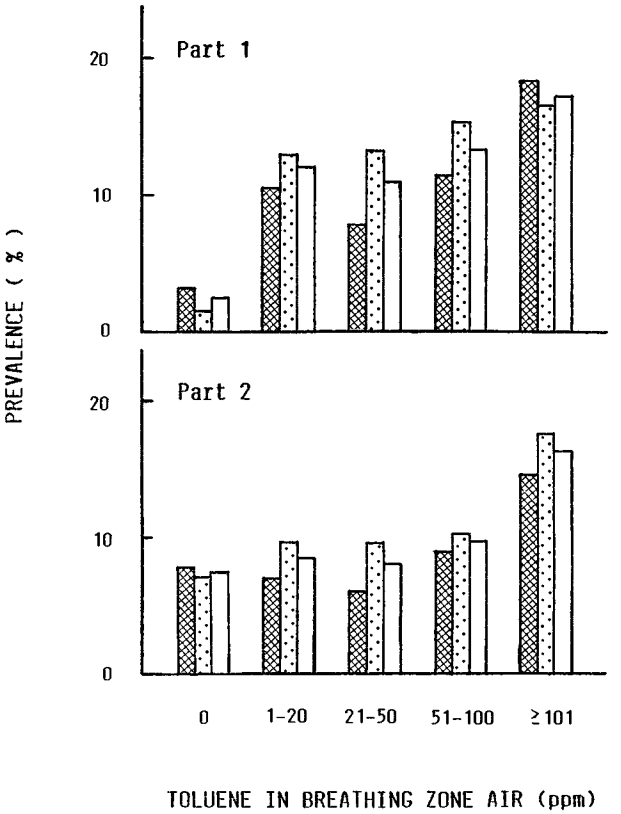

FIG. 1. Prevalence of Part 1 and Part 2 symptoms, by intensity of exposure to toluene. For definition of prevalence, see Materials and Methods. Cross-hatched columns are for men, dotted ones are for women, and open ones are for men and women in combination.

loss), 39 (loss in hearing capacity), 40 (difficulty in speech), 41 (reduced sense of smell), 49 (reduced muscle power in extremities), and 51 (rough skin). The relations of the prevalences of these symptoms with toluene exposure intensity are shown in Fig. 4 for visual presentation.

In accordance with the observation in Tables 2 and 3, the prevalence of Part 1 symptoms tended to be twofold or more higher in the exposed at 1–20 ppm than in the controls.

Further Evaluation of Increase in Symptom Prevalence

The evaluation of individual symptoms indicated that the difference in the prevalence was significant ($P < 0.10$ or less) only in 10 symptoms in Part 1 and 21 symptoms in Part 2. Accordingly, the evaluation on the group of symptoms basis was repeated limiting 10 and 21 symptoms in Parts 1 and 2 symptoms, respectively, with expectation that the difference in prevalence will be more sensitively detected when the sensitive symptoms were selected. The results of recalculation are presented in the bottom half of Tables 2 and 3. The comparison between the

TABLE 4
PART 1 SYMPTOMS WITH SIGNIFICANT DIFFERENCE IN PREVALENCE

Symptom[a]	Comparison A[b]			Comparison B[c] (ppm)				
	Control	Exposed	P^d	1–20	21–50	51–100	>101	P^e
1. Irritation in eyes	3.3	8.0	**	7.9	6.1	14.3	3.1	
2. Dimmed vision	2.7	11.3	**	12.2	11.3	9.5	10.9	
3. Nasal irritation	4.1	9.7	**	10.6	7.0	13.1	7.7	
4. Unusual smell	0.6	2.2	*	1.6	1.7	3.6	3.1	
5. Sore throat	2.9	25.0	**	28.0	21.7	20.2	28.1	
6. Unusual taste	0.6	4.9	**	4.2	4.3	3.6	9.4	
8. Dizziness	2.9	8.4	**	6.9	4.3	6.0	23.4	**
9. Floating sensation	5.2	49.8	**	38.6	52.2	57.1	68.8	**
11. Heavy feeling in the head	1.4	6.9	**	5.3	5.2	7.1	14.1	†
12. Headache	3.9	24.1	**	23.2	19.1	27.4	31.3	

Note. Values are prevalence in percentages for men and women combined. *P* values are for significant difference in distribution (**, *, and † for $P < 0.01$, 0.05 and 0.10, respectively).

[a] Only those with a significant difference are shown.

[b] Comparison between the exposed (452 subjects) and nonexposed controls (517 subjects).

[c] Comparison among 1–20 ppm (189 subjects), 21–50 ppm (115 subjects), 51–100 ppm (84 subjects), and ≥101 ppm (64 subjects) groups.

[d] *P* for the difference between the two groups.

[e] *P* for the difference among the four exposed groups.

exposed and controls (the bottom half in Table 2) shows that the prevalence for the exposed tended to be elevated by the selection of questions whereas that for the controls stayed essentially unchanged. Thus, the difference between the two groups became larger so that the prevalence of Part 2 symptoms among the ex-

TABLE 5
PART 2 SYMPTOMS WITH SIGNIFICANT DIFFERENCE IN PREVALENCE

Symptom[a]	Comparison A[b]			Comparison B[c] (ppm)				
	Control	Exposed	P^d	1–20	21–50	51–100	>101	P^e
4. Nausea	10.6	15.3	*	11.6	12.2	19.1	26.6	**
6. Difficulty in sleep	30.2	41.1	**	38.6	41.7	45.2	42.2	
7. Nightmare	19.0	23.7	†	21.7	18.3	31.0	29.7	
13. Forgetfulness	16.8	29.2	**	32.8	18.3	31.0	35.9	
14. Inability to concentrate	3.9	7.1	*	3.2	6.1	8.3	18.8	**
22. General dullness	13.4	25.2	**	20.6	33.0	22.6	28.1	
24. Poor appetite	5.0	11.1	**	5.8	12.2	16.7	17.2	*
26. Dry mouth	7.4	24.8	**	28.0	19.1	16.7	35.9	*
30. Body weight loss	1.2	2.9	†	1.6	0.0	6.0	7.8	**
35. Reduced sexual desire	0.6	2.0	*	1.1	0.9	2.4	6.3	†
39. Loss in hearing capacity	4.1	8.4	**	5.8	7.0	8.3	18.8	*
40. Difficulty in speech	0.4	1.6	†	0.5	0.9	0.0	7.8	**
41. Reduced sense of smell	1.4	3.5	*	0.5	1.7	8.3	9.4	**
42. Reduced sense of taste	0.0	2.0	**	1.6	1.7	0.0	6.3	*
47. Abnormal feeling of skin in extremities	11.4	15.5	†	12.7	17.4	15.5	20.3	
48. Reduced grasping power	1.0	4.6	**	4.2	2.6	3.6	10.9	†
49. Reduced muscle power in extremities	3.5	8.0	**	5.3	3.5	8.3	23.4	**
51. Rough skin	3.1	14.6	**	11.1	10.4	16.7	29.7	**
52. Unusual feeling in throat	0.4	2.4	**	1.6	0.9	2.4	7.8	*
54. Frequent bleeding from gums	19.0	36.1	**	36.5	34.8	40.5	31.3	
55. Frequent nasal bleeding	2.3	4.2	†	2.6	5.2	4.8	6.3	

[a–e] See Table 4 notes.

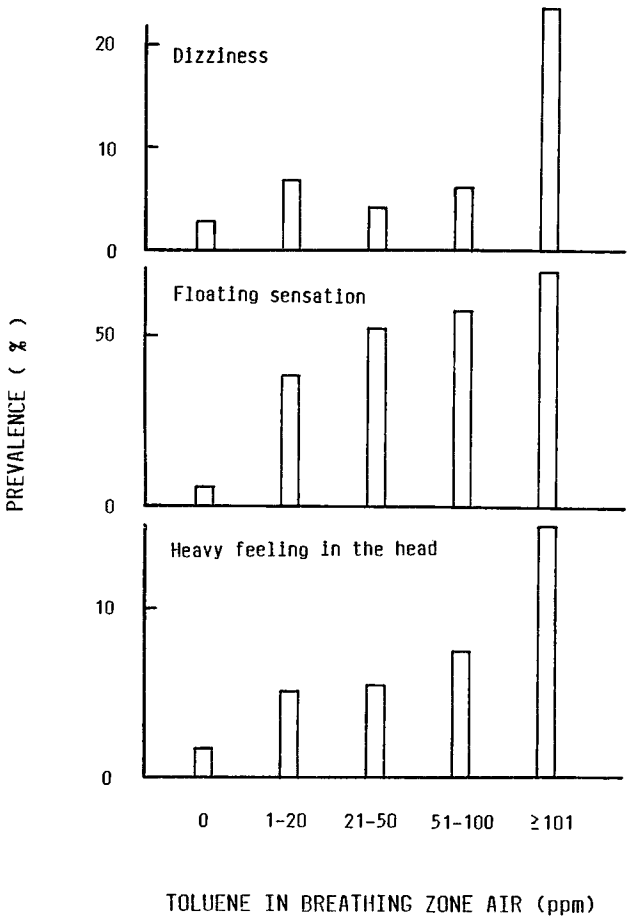

FIG. 2. Dose–response relationship of three Part 1 symptoms.

posed men was also significantly ($P < 0.01$) higher than that in controls after selection (the bottom half in Table 2), although the difference before selection (i.e., with all questions) was insignificant (the top half in Table 2).

The effect of question selection on the evaluation of dose–response relationship was not remarkable (the bottom half of Table 3). The only significant effect of selection was that the prevalence of Part 2 symptoms among women exposed at $\geq$101 ppm became significantly ($P < 0.05$) higher than that for the women exposed at 1–20 ppm and, resultingly, the difference of Part 2 symptom prevalence in men and women at $\geq$101 ppm from the counterpart prevalence at 1–20 ppm became more significant (i.e., from $P < 0.05$ before selection to $P < 0.01$ after selection).

Absence of Toluene-Induced Changes in Hematology and Serum Biochemistry

Distributions of abnormal and borderline cases in hematology and serum biochemistry are compared between the exposed and the controls (Table 6). The statistical examination with χ^2 test showed that there was no bias in the distribu-

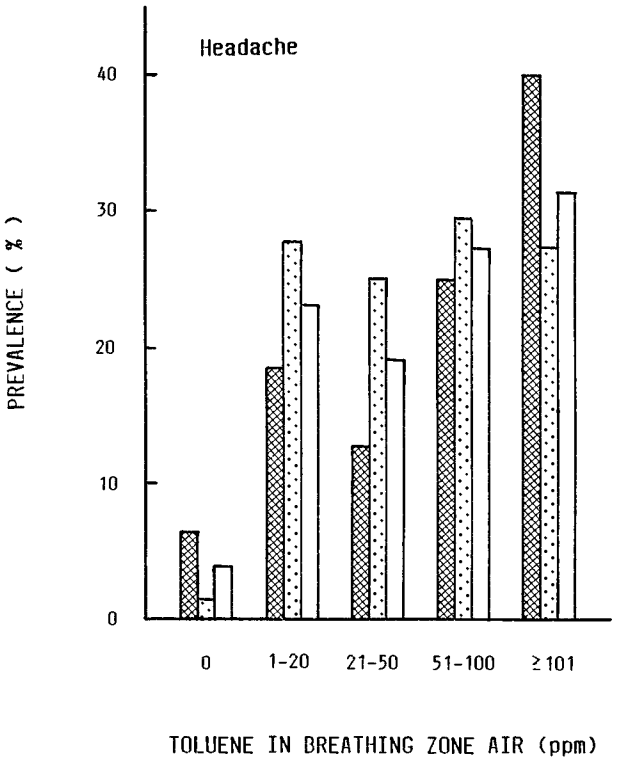

FIG. 3. Increase in prevalence of "headache" as a Part 1 symptom in response to increasing intensity of toluene exposure. For details, see legend for Fig. 1.

tion of borderline and abnormal cases in leukocyte counts or hemoglobin concentration between the two groups. In serum biochemistry, also, the findings were essentially negative. When distributions in both men and women were combined for evaluation, there was a significant difference in distribution in ASAT/ALAT ($P < 0.05$) and creatinine ($P < 0.05$). However, both were due to higher prevalence in the controls than in the exposed.

DISCUSSION

The present study made it clear that the subjective symptoms will increase in close association with the intensity of exposure to toluene; the threshold concentration appears to be 100 ppm in the case of symptoms during work (Table 2), and it may be 50–100 ppm when symptoms off work are evaluated (Table 3). During the work with exposure at higher concentrations, various symptoms possibly related to CNS or local effects (e.g., eyes, nose, and throat) were complained, and dizziness and floating sensation were identified as typical symptoms with significant dose–response relationship (Table 4). When the symptoms off work were evaluated, several symptoms showed dose–response relationship; some of them (e.g., inability to concentrate) are apparently related but not necessarily limited to CNS (Table 5). The observation as a whole is a good confirmation of the findings

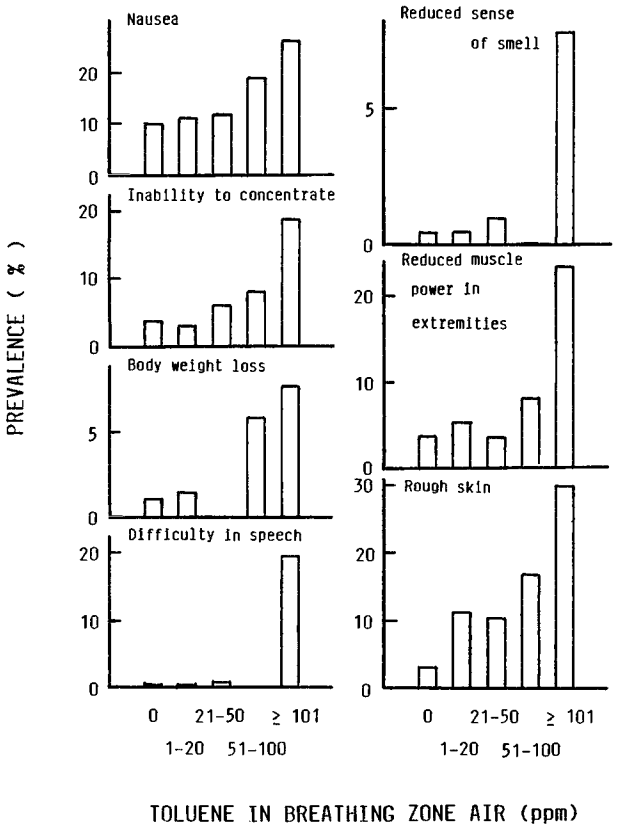

FIG. 4. Dose–response relationship of seven Part 2 symptoms.

in preceding small-scale studies from this group (Yin *et al.*, 1987; Lee *et al.*, 1988). Namely, Yin *et al.* (1987) observed that sore throat, dizziness, and headache are the most frequently complained symptoms among shoemakers exposed to toluene up to 123 ppm, and Lee *et al.* (1988) found that a heavy feeling in the head and headache are among the most common symptoms during work and nervousness and rough skin are often complained of off work in female shoemakers with toluene exposure up to >150 ppm.

Subjective symptoms have attracted the attention of researchers of solvent toxicity since early days. For example, Wilson (1943) summarized his experience on toluene-induced subjective symptoms in such a way as mild and psychogenic symptoms at 50 to 200 ppm, symptoms including definite impairment of coordination and momentary loss of memory at 200 to 500 ppm, pronounced loss of coordination and impaired reaction time among others at 500 to 1500 ppm. Bearing the fact in mind that the technology to measure toluene concentration had not been well developed in 1940s, his summary apparently needs to be reevaluated. Dose–response relationship in toluene-induced subjective symptoms has not been extensively studied in the recent years, however, possibly because only limited numbers of toluene-exposed workers were available for each study, and the ob-

TABLE 6
DISTRIBUTION OF NORMAL, BORDERLINE, AND ABNORMAL HEMATOLOGY AND SERUM BIOCHEMISTRY IN CONTROL AND EXPOSED WORKERS

Item	Sex	Controls		Exposed		*P* for difference
		Borderline	Abnormal	Borderline	Abnormal	
Leukocytes	M:W:S	6:17:23	0: 2: 2	2:12:14	0: 1: 1	−:−:−
Hemoglobin	M:W:S	26:27:53	3: 5: 8	19:12:31	0: 3: 3	−:−:−
Total bilirubin	M:W:S	6: 6:12	4: 3: 7	1: 1: 2	5: 1: 6	−:−:−
Total protein	M:W:S	7: 6:13	0: 0: 0	5: 5:10	0: 0: 0	−:−:−
γ-GTP	M:W:S	5: 1: 6	1: 0: 1	3: 0: 3	0: 1: 1	−:−:−
ASAT/ALAT	M:W:S	46:26:72	15: 6:21	27:14:41	9: 5:14	−:−:*
ALP/LAP	M:W:S	59:32:91	5: 1: 6	57:18:75	1: 2: 4	−:−:−
Creatinine	M:W:S	3: 3: 6	5: 3: 8	5: 0: 5	0: 0: 0	−:−:*

Note. The control and exposed groups consisted of 517 subjects (245 men and 272 women) and 453 subjects (208 men and 245 women), respectively. The values in the table indicate the number of borderline or abnormal cases (in the order of men:women:the sum of men and women). The remaining were normal cases. The *P* values are for the significant difference (* for <0.05, and − for >0.10) in distribution between the control and exposed groups.

servations in workers exposed to toluene at a given range of concentration are often compared with the findings among the nonexposed controls.

Whereas some authors (e.g., Antti-Poika *et al.*, 1985; Foo *et al.*, 1990) did not find significant CNS effects in the workers exposed to toluene at 50 to 185 ppm, CNS effects were evident in the intoxicated patients after heavy, intensive exposures (Goldbloom and Chouinard, 1985; Meulenbelt *et al.*, 1990). At lower doses, Matsushita *et al.* (1975) reported anxiety as a symptom among 38 women exposed to toluene at 60–100 ppm, and also Mørk *et al.* (1988) as well as Larsen and Leira (1988) observed various subjective symptoms among workers exposed at 50–80 or 50–100 ppm, respectively. In addition, quite a variety of symptoms were reported by Øbaek and Nise (1989) for rotogravure printers with toluene exposure at 11–42 ppm. Such discrepancy might be due to the difficulty in obtaining a good control group for the evaluation of the prevalence observed in the exposed group. In fact, there was a marked elevation in the prevalence among those with exposure of as low as 1–20 ppm as compared with the controls who were either from the same factories or the factories from the same region (Tables 2 and 3). This observation may suggest that confounding factors other than toluene exposure might have led to the misevaluation of the results.

Of particular interest are such symptoms as memory disturbance and difficulty to concentrate. These symptoms had been described by several authors who examined workers exposed to toluene at 11–42 ppm (Øbaek and Nise, 1989), at 50–80 ppm (Larsen and Leira, 1988), at 50–100 ppm (Mørk *et al.*, 1988), or at 78 ppm as a mean (Juntunen *et al.*, 1985). The same symptoms were also complained by workers exposed to solvent products the constituents of which were only poorly described (Arlien-Søborg *et al.*, 1979; Cherry *et al.*, 1985). In the present study, the off-work prevalence of the subjective symptom of "inability to concentrate" was not only elevated significantly in the exposed as compared with

that in the controls, but also in a manner linearly related to the toluene dose (Table 5). In contrast, the prevalence of "forgetfulness" was higher in the exposed than in the controls, but the dose–response relationship could not be established (Table 5).

The exposure to toluene was not associated with any hematological changes (Table 6) in the present study. Such observation is in agreement with the previous study results (Yin *et al.*, 1987) and also on the line of the general opinion that toluene with little benzene impurity has no hematotoxicity (Sandmeyer *et al.*, 1981; Antti-Poika *et al.*, 1987), although there are still sporadic reports to suggest the contrary (e.g., Bergés, 1972; Bosch *et al.*, 1989).

Findings were also negative on the possible hepatorenal toxicity of toluene in the present study (Table 6) in accordance with the results of previous studies (Szadkowski *et al.*, 1976; Waldron *et al.*, 1982; Seiji *et al.*, 1987). Recently, Boewer *et al.* (1988) made a cross-sectional study of 181 male printers who had been exposed to toluene at well over 50 ppm to find 55 subjects (about 30%) with pathological liver screening values in ASAT, ALAT, γ-GTP, or liver size. Regarding the etiology, they observed in 51 subjects out of the 55 an association of the pathology with alcohol intake and to a lesser extent overweight. Guzeliam *et al.* (1988) examined the liver functions of 289 printing factory employees (either pressmen or those involved in cylinder preparation) and found mild elevation (less than 2 to 3 times the upper normal limits) in ASAT and ALAT in 8 men; liver biopsy revealed mild pericentral fatty liver change in all 8 cases. None of them were obese or diabetic. Guzeliam *et al.* (1988) considered it unlikely that alcohol consumption (e.g., two patients admitted consumption of 10 to 15 drinks per week) alone accounts for the observed liver condition. Brugnone and Perbellini (1985) found only very mild ASAT and ALAT elevation (up to two times the upper normal limits) in two cases of toluene-induced coma after acute heavy (occupational) exposure.

On the nephrotoxicity, Franchini *et al.* (1983) studied possible renal damage of workers exposed to a complex mixture of solvents and concluded that the lesions were mild and tubular rather than glomerular. Askergren *et al.* (1981) also examined urine samples from 134 workers exposed to various solvents (including 42 printers who were exposed mainly to toluene at about 100 ppm) and found that urinary albumin levels were elevated in the exposed workers with no significant changes in β_2-macroglobulin excretion as compared with 48 nonexposed controls. Subsequently, Ng *et al.* (1990) studied kidney function of 45 paint workers exposed principally to toluene (less than 100 ppm) in comparison with controls matched with sex and age. No significant changes were observed in urinary albumin concentration, whereas retinol-binding protein levels were elevated in a manner dependent to urinary *o*-cresol concentration but not to that of hippuric acid. No information was given on individual intensity of exposure to toluene, unfortunately. In this connection, it should be considered that hippuric acid correlates better with toluene exposure than *o*-cresol (e.g., Hasegawa *et al.*, 1983; De Rosa *et al.*, 1987). BUN and creatinine values stayed normal in the two toluene coma cases reported by Brugnone and Perbellini (1985). Thus, it is possible to summarize that toluene under mild exposure conditions (e.g., below 100 ppm) will

not induce marked liver damage nor kidney dysfunction as examined by serum biochemistry, although the possibility remains that the slight changes in kidney function might be detectable with more sophisticated methods.

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n-Hexane Polyneuropathy in Japan: A Review of *n*-Hexane Poisoning and Its Preventive Measures[1]

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n-Hexane is used in industry as a solvent for adhesive, dry cleaning, and vegetable oil extraction. In 1963, the first case of severe polyneuropathy suspected to be caused by *n*-hexane was referred to us. Case studies, animal experiments, and field surveys on *n*-hexane poisoning were conducted, and preventive measures like threshold limit value revision and biological monitoring were also studied. I review a brief history of our investigations on *n*-hexane poisoning and its preventive measures in Japan. *n*-Hexane could cause overt polyneuropathy in workers exposed to more than 100 ppm time-weighted average concentrations [TWA]. The present threshold limit value of 40 ppm in Japan is considered low enough to prevent subclinical impairment of peripheral nerve caused by *n*-hexane. Urinary 2,5-hexanedione could be a good indicator for biological monitoring of *n*-hexane exposure. About 2.2 mg/liter of 2,5-hexanedione measured by our improved method corresponds to exposure of 40 ppm (TWA) of *n*-hexane.

INTRODUCTION

A large amount of *n*-hexane is used in industry as a solvent for adhesives, dry cleaning, and vegetable oil extraction. *n*-Hexane is a natural component of petroleum. Therefore, many petroleum solvents contain *n*-hexane as a component. Although petroleum solvents have been used for a long time, polyneuropathy due to them has rarely been reported. However, many cases of polyneuropathy due to *n*-hexane occurred in the late 1960s, when the petroleum refinery industry began to develop rapidly, providing a large amount of rather pure and cheap *n*-hexane not only in Japan but all over the world. *n*-Hexane came to be used in place of benzene, which was already known to be severely toxic to the hematopoietic system. Since then, many cases of polyneuropathy due to *n*-hexane have been reported, and the mechanism and the dose–response relationship of *n*-hexane polyneuropathy have been intensively studied. In Japan, the threshold limit value of *n*-hexane in the workplace was revised from 500 to 100 ppm in 1967 and from 100 to 40 ppm in 1986. Monitoring of biological levels of urinary 2,5-hexanedione in workers exposed to *n*-hexane has been required by law since 1989 in Japan. I review a brief history of our investigations of *n*-hexane poisoning and its preventive measures.

CASE STUDIES

The first case of severe polyneuropathy was referred to our department from a doctor of neurology in a hospital in 1963. The patient was suspected to suffer from some kind of poisoning. Further, three other cases with symptoms and signs similar to those of the first case were referred one after another to our department

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during the same year (Ohishi *et al.*, 1964; Wada *et al.*, 1965; Yamada, 1967). The working conditions of these patients were investigated. All four patients were engaged in laminating polyethylene on cellophane for candy bags, etc., in two factories. They used mainly two kinds of chemicals, one was a solvent which contained about 65% *n*-hexane and the other was an organic titanium mixture which is easily oxidized to titanium oxide in air. Therefore, *n*-hexane and titanium oxide were suspected as the causative agents at first. However, polyneuropathy was not found in workers using trichloroethylene instead of *n*-hexane in a similar polyethylene-laminating process. Consequently *n*-hexane was suspected as the main causative agent of the polyneuropathy (Yamada, 1967).

ANIMAL EXPERIMENTS

There were no reports at that time that *n*-hexane could cause polyneuropathy. Therefore, animal experiments were conducted to prove whether *n*-hexane could cause polyneuropathy. It was very difficult to cause peripheral nerve impairment in the experimental animals by injection of *n*-hexane into hypoderm, muscle, or peritoneum. But the continuous inhalation of *n*-hexane vapor for about 1 year in mice could cause peripheral nerve impairment which was dose-dependent from 2000 to 100 ppm of *n*-hexane exposure (Miyagaki, 1967). The animal experiments confirmed that *n*-hexane exposure could produce peripheral nerve impairment in mice comparable to the polyneuropathy in the workers exposed to *n*-hexane.

FIELD SURVEYS

In 1968, a patient with severe polyneuropathy was referred to our department, with suspected solvent poisoning. The patient had severe muscle atrophy and could not walk by herself. She was engaged in manufacturing vinyl sandals using adhesives which contained a large amount of *n*-hexane. Therefore, the patient was suspected to have been poisoned by *n*-hexane.

A total of 1662 vinyl sandal manufacturers were checked by questionnaire and medical examination was performed on 296 persons with suspect symptoms. By clinical examination, 95 patients with polyneuropathy were classified by mode of involvement into three groups. Group 1 was sensory polyneuropathy, group 2 was sensorimotor polyneuropathy, and group 3 was sensorimotor polyneuropathy with amyotrophy (Yamamura, 1969). Their working conditions were investigated. The ambient concentrations of organic solvents in their workplaces were measured (Inoue *et al.*, 1970). The time-weighted average concentrations (TWA) of *n*-hexane in the workplaces of the patients were calculated. Table 1 shows *n*-hexane concentrations (TWAs) by each mode of involvement (Takeuchi *et al.*, 1980).

TABLE 1
EXPOSURE CONCENTRATIONS (TWAs) OF *n*-HEXANE IN EACH MODE OF INVOLVEMENT

Mode of involvement	Number of patient	Concn. (TWA) of *n*-hexane (ppm)
Sensorimotor polyneuropathy with amyotrophy (3)	8	578
Sensorimotor polyneuropathy (2)	32	495
Sensory polyneuropathy (1)	53	118

TABLE 2
CLINICAL COURSE OF THE PATIENTS

Year of clinical examination	Mode of involvement				Missing	Death
	3	2	1	0		
1968	8	32	53			
1970	0	5	34	51	3	
1972	0	0	7	82	3	1

The clinical courses of the patients are shown in Table 2 (Iida *et al.*, 1973). Clinical findings revealed that neurological impairment was gradually improved. Prognosis of *n*-hexane polyneuropathy was not bad, although it often required more than a year for cure.

THRESHOLD LIMIT VALUE (TLV)

From our case studies and animal experiments, the Japan Association of Industrial Health (JAIH) revised the TLV of *n*-hexane in 1967 from 500 to 100 ppm to prevent polyneuropathy due to *n*-hexane (JAIH, 1966). After that, an electrophysiological study of 14 workers exposed to 40 to 88 ppm (mean, 58 ppm) time-weighted average concentrations of *n*-hexane revealed that the workers had subclinical impairment of peripheral nerve without overt polyneuropathy (Sanagi *et al.*, 1980). Due to these subclinical findings of low level exposure to *n*-hexane and many other pathological studies on its neurotoxicity, JAIH revised the TLV of *n*-hexane from 100 to 40 ppm in 1986 (JAIH, 1985).

BIOLOGICAL MONITORING

n-Hexane metabolites in the urine of the workers exposed to *n*-hexane can be measured by gas chromatography according to Peribellini's method (Peribellini *et al.*, 1981). The metabolites in the urine of the workers exposed to *n*-hexane and ambient concentrations of *n*-hexane produced a good correlation (Iwata *et al.*, 1983). Fetdke *et al.* revealed that most 2,5-hexanedione was transformed into 4,5-hydroxy-2-hexanone and excreted as its glucuronide in the urine, and the conjugated 4,5-hydroxy-2-hexanone was transformed into 2,5-hexanedione or 2,5-

TABLE 3
URINARY 2,5-HEXANEDIONE CORRESPONDING TO *n*-HEXANE EXPOSURE LEVEL BY AUTHOR

Author (reported year)	Number of surveyed persons	Correlation coefficient (r)	Urinary 2,5-hexandione level (mg/liter) in	
			40 ppm *n*-hexane	50 ppm *n*-hexane
Peribellini *et al.* (1981)	41	0.67	4.5	5.4
Iwata *et al.*[a] (1983)	22	0.90	2.2	2.7
Mutti *et al.* (1984)	10	0.97	2.4	3.2
DeRosa *et al.* (1988)	20	0.87	3.4	4.2
Ahonen *et al.* (1988)	12	0.96	1.3	1.6
Saito *et al.*[a] (1991)	50	0.97	2.2	2.7

[a] Reported from Japan.

TABLE 4
BRIEF HISTORY OF *n*-HEXANE POISONING AND ITS PREVENTIVE MEASURES IN JAPAN

Year	Brief history
1963	First patient with suspected *n*-hexane polyneuropathy referred to us
1964–1967	Case reports published
1965–1967	Animal experiments conducted
1967	Results of animal experiments published
1967	Threshold limit value (TLV) of *n*-hexane revised from 500 to 100 ppm
1968	Outbreak of polyneuropathy occurred in vinyl sandal manufacturers
1969–1970	Results of field surveys published
1980	Subclinical findings at low-level exposure reported
1982–1983	Urinary 2,5-hexanedione in workers analyzed
1986	TLV of *n*-hexane revised from 100 to 40 ppm
1989	Urinary 2,5-hexanedione adopted as an indicator for biological monitoring
1990	Analytical method of urinary 2,5-hexanedione further improved

dimethylhydrofuran by acid hydrolysis (Fetdke and Bolt, 1987). We found that acid hydrolysis of *n*-hexane metabolites in the urine formed mostly 2,5-hexanedione below pH 1 and 2,5-dimethylfuran above pH 3. It is thought that the main urinary *n*-hexane metabolites are converted into 2,5-hexanedione by acid hydrolysis below pH 1. The urinary 2,5-hexanedione concentration measured by our improved method and *n*-hexane exposure concentration produced good correlation in the workers exposed to *n*-hexane (Saito *et al.*, 1991). The regression is as follows: $Y = 0.053X + 0.078$ ($r = 0.0971$), where Y is 2,5-hexanedione in the urine (mg/liter) and X is the *n*-hexane exposure level (ppm (TWA)/8 hr). The results show that about 2.2 mg/liter of 2,5-hexanedione measured by the improved method corresponds to exposure to 40 ppm (TWA) *n*-hexane. The Ministry of Labor in Japan adopted urinary 2,5-hexanedione as biological monitoring indicator in 1989. However, the values for urinary 2,5-hexanedione that correspond to *n*-hexane exposure differ among authors, as shown in Table 3 and remain controversial.

CONCLUSIONS

I review a brief history of our investigations of *n*-hexane poisoning and its preventive measures in Japan and summarize them in Table 4. The following conclusions are reached.

(1) Over 100 ppm *n*-hexane exposure could cause overt polyneuropathy in workers.

(2) The present threshold limit value of 40 ppm in Japan is considered low enough to prevent subclinical impairment of peripheral nerve due to *n*-hexane.

(3) Urinary 2,5-hexanedione may be a good indicator for biological monitoring of *n*-hexane exposure. About 2.2 mg/liter of 2,5-hexanedione measured by our improved method corresponds to exposure of 40 ppm (TWA) of *n*-hexane.

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Neurotoxic Syndromes and Occupational Exposure to Solvents[1]

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Neurotoxic syndromes due to occupational solvent exposure present a worldwide health problem, the magnitude of which varies from country to country. Apart from the relatively clear-cut exposure–effect relationships in acute solvent intoxications, those caused by long-term, low-level occupational exposure to solvents are more difficult to detect. Controversial opinions and even debate are frequently encountered in literature on this matter. This is partly due to differences in neurobehavioral methods used, partly to difficulties in obtaining accurate information about exposure. These effects can be studied in humans using biochemical, clinical, and epidemiological methods. It is thus quite conceivable that direct comparison of the results obtained by different methods is not always possible. Moreover, exposure to a variable mixture of solvents is frequent in an occupational setting which is problematic from the toxicological point of view. The clinical pictures of "chronic" occupational solvent intoxications are, with few exceptions, quite nonspecific in nature and share several common features regardless of the underlying chemical exposure. The development of manifest disease is insidious and high interindividual variation of symptoms and signs exists. Some solvents cause primarily peripheral neuropathy. Deterioration in many psychological and neurophysiological functions can be seen. The most common subjective symptoms of solvent intoxication are headache, tiredness, memory disturbances, and dizziness. Clinical findings comprise signs of the central nervous system depression (psychoorganic syndrome, tiredness), dizziness, disturbances in coordination, and general neurasthenic signs. From the clinical point of view, it is important to define the criteria for a diagnosis. In different countries the diagnostic criteria for solvent intoxication may vary considerably, which provides additional difficulties in interpreting the results of studies in this field.

INTRODUCTION

Neurotoxicity in general has become a very important issue during the last few years. It is among the top priority areas in the programs of the "Decade of The Brain," as the 1990s has been designated (NIH, 1989). Neurotoxic syndromes associated with occupational exposure to organic solvents provide a good example of difficulties arising in etiologic diagnosis in clinical neurotoxicology. The extensive application of new organic solvents and their mixtures in industrial communities each year makes this issue complicated and persistent. Acute outbreaks of specific neurotoxic syndromes among particular groups of exposed workers are well known and a relationship between the disease and exposure in these cases is usually obvious. A diffuse neuropsychiatric syndrome associated with long-term low-level occupational exposure to solvents is a matter of consid-

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erable controversy worldwide. In this article, a short review of the concept of occupational solvent intoxication is given with special emphasis on the diagnostic criteria.

NEUROTOXIC SOLVENTS

Organic solvents constitute a large group of volatile chemicals, mainly hydrocarbons and their derivates. They are characterized by their nonpolarity and lipid solubility. Their usual route of entry is by inhalation and the nature of work tasks, the state of cardiovascular and respiratory systems and individual factors determine their metabolic rate (e.g., Fiserova-Bergerova, 1985, Sato and Nakajima, 1987). There are few solvents which have been definitely shown to cause peripheral neuropathy in occupational setting; they include carbon disulphide, *n*-hexane, and methyl-*n*-butyl ketone (e.g., Spencer and Schaumburg, 1985). A number of solvents or solvent mixtures have been associated with central nervous system disturbances. The most common exposure at workplaces is to a mixture of solvents, such as lacquer thinners, petroleum ether, white spirit, mineral terpentine, and greasing oils. This provides additional difficulties in toxicological research in occupational health since we know very little about the possible interactions of different components of solvent mixtures.

EXPOSURE AT WORKPLACES

Estimation of exposure to solvents at the workplace includes careful consideration of the chemistry of the compounds used, the nature of work tasks and methods, the work conditions, and protective equipment used, and the results of ambient air measurements and biological monitoring. All these data should be weighed against previous experiences with similar exposures (e.g., Järvisalo and Tossavainen, 1982). Again, great difficulties arise when dealing with solvent mixtures, which may contain various solvents in many combinations, mostly toluene, methylisobutyl ketone, isobutanol, acetone, ethylene glycol, isopropanol, butylacetate, etc. Currently, in addition to hygienic threshold limit values, we have standards for biological monitoring of many solvents (ACGIH, 1985).

For scientific purposes it is necessary to use exposure indices. Individual measures constituting the index should be analyzed separately against effects. Exposure indices are, of course, solvent-specific and should be used cautiously. An example is the index for styrene exposure used in a recent study (Juntunen *et al.*, 1989) seen in Table 1.

NEUROTOXICITY OF SOLVENTS: GENERAL CONSIDERATIONS

The response of the nervous system to organic solvent toxicity can be classified as structural toxicity (general responses of the neuron and the supporting cells), biochemical toxicity (hypoxic or histotoxic changes, the effects of metabolic products of the absorbed chemical), and the functional toxicity (sensory, motor, and integrative functions). The responses, or effects, may be different in developing, degenerating, or aging nervous tissue. An interesting, yet unsolved problem in neurotoxicology is the relationship between "effect" and disease. Particularly in human epidemiologic studies, the outcome parameters of toxicity may appear as

TABLE 1
EXPOSURE INDEX FOR STYRENE

1. Working method[a]	FA	CS	OS	ML	SL
Score	1	2	3	4	5
2. Years at work	<5	<10	<15	<20	>20
Score	1	2	3	4	5
A. Hours per day (lamination)	Occasionally	<4	<6	>6	Often extra job
Score	1	2	3	4	5
B. Concentration in air ppm	<20 (TLV_{8h})	<50	<100	<150	>150
Score	1	2	3	4	5
3. Dose (A × B)[b]	1	4	9	16	25
C. Mandelic acid mmol/liter	<3,2	<7,0	<10	<15	>15
Score	1	2	3	4	5
4. Response (A × C)[c]	1	4	9	16	25
Total Score 1 + 2 + 3 + 4	4	12	24	40	60
Score divided by the smallest number = index					
Score	1	3	6	10	15

Note. From J. Juntunen *et al.* (1990). Health Effects of Chemical Exposure in Reinforced Plastics Industry," Report 88243. The Finnish Work Environment Fund.

[a] Working method principally used: FA, fully automatic; CS, closed process; OS, open process; ML, manual laminating; SL, spray laminating.

[b] Dose (A × B) = hours per day × styrene concentration, ppm.

[c] Response (A × C) = hours per day × mandelic acid concentration, mmol/liter.

early disability, changes in social life, and behavioral changes. These are issues generally attributed to the clinical entity of psychoorganic syndrome (Lipowski, 1980). A survey of the vast literature shows that most studies dealing with neurotoxicity have considered only one or two of these aspects and has, for obvious reasons, led to many reports with apparently conflicting results (Savolainen, 1982; Grasso *et al.*, 1984; Spencer and Schaumburg, 1985; Örbäck *et al.*, 1985; Waldron, 1986; Iregren, 1986; Errebo-Knudsen and Olsen, 1986).

SOME EPIDEMIOLOGIC ASPECTS

Epidemiologic evidence suggests that the risk for developing a neurotoxic syndrome is increased among workers exposed to occupational solvents. A pioneering study by Axelson *et al.* (1976) suggested that workers occupationally exposed to solvents had a disabling neuropsychiatric disease more often. Since then, a large number of reports on solvent toxicity have been published, particularly from Scandinavian countries (Olsen and Sabroe, 1980; Lindström, 1981; Axelson, 1983;

Mikkelsen, 1980, 1988; Van Vliet, 1989; Van Vliet *et al.*, 1989, 1990; Parkinson *et al.*, 1990; Riise and Moen, 1990; Bolla *et al.*, 1990; Brackbill *et al.*, 1990; Gupta *et al.*, 1990). When the literature on the effects of solvents on the nervous system is reviewed one is confused by the highly controversial results of different studies that have employed a wide spectrum of neurobehavioral methods (Hernberg, 1980; Friedlander and Hearne, 1980; Juntunen, 1983; Grasso *et al.*, 1984; Hogstedt and Axelson, 1986). Questionnaires and a number of psychological and psychometric performance test batteries (Iregren, 1982; Lindström, 1982; Baker *et al.*, 1983; Cherry and Waldron, 1984; Gamberale, 1985; Maizlish *et al.*, 1985; Cherry *et al.*, 1985; Triebig *et al.*, 1988; Hartman, 1988; Morrow *et al.*, 1989; WHO, 1989; Milanovic *et al.*, 1990; Ng *et al.*, 1990; White *et al.*, 1990), and sophisticated neurophysiologic and other techniques (LeQuesne, 1982; Avanzini *et al.*, 1983; Bleecker, 1983; Risberg and Hagstadius, 1983; Juntunen *et al.*, 1985; Seppäläinen, 1982, 1988; Muijser *et al.*, 1988; Mergler *et al.*, 1988; Massioui *et al.*, 1990; Schwartz *et al.*, 1990; Mergler, 1990; Dyers, 1990) have been employed in these studies.

The assessment of whether the differences in the functions of the nervous system between the exposed group and the referents obtained with some of these methods are clinically meaningful is a very difficult task indeed. In particular, psychological studies are problematic. Exposed and nonexposed individuals may not be equally well motivated for psychological testing: some individuals tend to blame external factors for their poor health, partly due to compensational aspects involved (Hessel and Slvis-Cremer, 1987), selection of hypersusceptible individuals from among solvent-exposed groups (Omenn, 1982; Gyntelberg *et al.*, 1986), and many potential confounding and effect-modifying factors such as alcohol, drugs, and aging.

Large interindividual variation in behavioral tests, qualitative and quantitative differences in exposure, and the possibility of systematic bias, either positive or negative, further complicates the interpretation of psychological studies. A co-twin control study design has been applied recently to clarify these issues (Juntunen *et al.*, 1987; Hänninen *et al.*, 1991). The results of these studies suggest that exposure to solvents affects verbal learning and memory and the cognitive functions required by a visuoconstructive task and also increases the probability of other types of dysfunctions. Particularly lowered performance in associative learning, digit span, and block design seem to discriminate between the exposed and nonexposed individuals (Hänninen *et al.*, 1976, 1991, Fidler *et al.*, 1978, Valciukas *et al.*, 1985; Eskelinen *et al.*, 1986; Örbäck and Lindgren, 1988; Hänninen, 1990).

In this context, the important question of specificity and sensitivity of the examination technique used in clinical and epidemiologic studies on neurotoxicity should be considered. (Schoenberg, 1982; Juntunen, 1983). More sophisticated and sensitive methods are continuously introduced for studies of the nervous system function. Since the nonspecificity of a test increases along with increasing sensitivity, the validity of a new technique must be very carefully tested with clinically well-defined disease cases and healthy persons (Eskelinen *et al.*, 1986; Hänninen, 1990; White, 1990).

CLINICAL ASPECTS

In acute solvent intoxications, a relatively clear-cut exposure–effect relationship, ranging from a mild feeling of "drunkenness" to a narcotic stage is usually present. Disturbances in psychometric and neurophysiologic functions and balance disturbances are common in acute solvent intoxications (Savolainen and Linnavuo, 1979; Gamberale, 1985; Iregren, 1988). Transient attacks of mild acute intoxications may occur among workers exposed to solvents, while more severe intoxications are usually accidental. Diagnosis of "chronic" occupational solvent intoxication is more problematic. From the clinical point of view, neurological examination and differential diagnostics and assessment of the results of different ancillary tests are very important. Standardized neurological testing is required in scientific studies (Juntunen, 1983; Albers, 1990). In fact, neurological examination by an experienced neurologist yields the optimal specificity and sensitivity in detecting subtle neurological disturbances. Moreover, only clinical examination reveals reliably slight cerebellar dysfunction, which seems to be an early and very common sign of toxicity of the central nervous system (Juntunen, 1986).

The concept of chronic solvent intoxication in occupational health has caused much debate in the literature (Grasso *et al.*, 1984, Spencer and Schaumburg, 1985; Errebo-Knudsen and Olsen, 1986). The inherent problems in clinical, psychological, and neurophysiological study designs concerning exposure–effect relationships are obvious. Many researchers in this field still doubt whether a syndrome of chronic occupational solvent intoxication exists (e.g., Waldron, 1988). This, however, is a matter entirely depending on the diagnostic criteria used. In different countries the diagnostic criteria for solvent intoxication vary considerably. If we adapt the strict requirement of neuropathologic evidence in terms of structural changes of the nervous tissue to prove neurotoxicity (Spencer and Schaumburg, 1985), we probably lose most cases. If we employ subjective symptoms as a basis for a diagnosis, we may overdiagnose a number of cases (Clemmesen *et al.*, 1991). In the future, multicenter studies (e.g., Triebig *et al.*, 1990) will probably help in unifying the diagnostic criteria between different countries in future.

Peripheral Neuropathy Due to Solvent Exposure

Toxic polyneuropathy due to occupational exposure to methyl-*n*-butyl ketone (Allen *et al.*, 1975; Spencer *et al.*, 1975), carbon disulphide (Vigliani, 1954), a lacquer thinner (Means *et al.*, 1976), *n*-hexane (Cianchetti *et al.*, 1976), allyl chloride (He *et al.*, 1980), styrene (Lilis *et al.*, 1978), and trichloroethylene (Feldman, 1979) have been reported. Peripheral nerves provide a relatively easy target for neurotoxicity studies (Thomas, 1980). Therefore, a number of solvents have been studied regarding their toxicity to peripheral nerves. By using sensitive neurophysiological techniques many solvents and solvent mixtures have been suspected as having neurotoxic properties but convincing evidence is still lacking. At an individual level the diagnosis of polyneuropathy is essentially a clinical one and the diagnostic criteria should be defined (e.g., Thomas, 1980; Juntunen and Haltia, 1982). Peripheral autonomic involvement has been reported among workers exposed to a mixture of solvents (Matikainen and Juntunen, 1985; Matikainen *et al.*, 1987).

Solvents and the Central Nervous System

Most solvents exert their actions through the central nervous system. Psychoorganic syndrome associated with long-term low-level occupational exposure to solvents is very difficult from the diagnostic point of view (Juntunen, 1986). Clinical studies have revealed the frequent occurrence of a mild psychoorganic syndrome, motor and sensory disturbances, and cerebellar dysfunction (Lilis *et al.*, 1978; Arlien-Søborg *et al.*, 1979, 1981; Husman and Karli, 1980; Struwe *et al.*, 1980; Juntunen *et al.*, 1980b; 1982a, 1986; Flodin *et al.*, 1984; Triebig, 1990). Many neurological disease entities such as dementia and multiple sclerosis have been associated with solvent exposure (Arlien Soborg *et al.*, 1979; Axelson *et al.*, 1976; Mikkelsen, 1980; Amaducci *et al.*, 1982; O'Flynn *et al.*, 1987; Juntunen *et al.*, 1988). Brain atrophy is frequently found among solvent-exposed workers, but the problems discussed earlier make it very difficult to draw any causal conclusions (Elofsson *et al.*, 1980; Juntunen *et al.*, 1980a; Juntunen, 1985). Sophisticated analysis of the cerebrospinal fluid cells and proteins of patients exposed to solvents showed slight nonspecific changes suggesting immunoactivation of the central nervous system (Juntunen *et al.*, 1982b; Wikkelsø *et al.*, 1984; Barregård *et al.*, 1990; Moen *et al.*, 1990). Vestibular disturbances (Ödkvist *et al.*, 1980; Arlien-Sorborg *et al.*, 1981; Binaschi and Cantu, 1983, Möller *et al.*, 1990), disturbances in cerebral blood flow (Arlien-Soborg *et al.*, 1982; Risberg and Hagstadius, 1983), autonomic disturbances (Matikainen and Juntunen, 1985; Matikainen *et al.*, 1987), and posturographic changes have been described among solvent-exposed workers. It has to be emphasized again that association does not necessarily imply causal relationship.

The frequent clinical finding of cerebellar dysfunction and disturbances in gait and station among solvent-exposed workers is very interesting (Juntunen *et al.*, 1982a, 1987; Antti-Poika *et al.*, 1989). Review of the most common clinical findings among solvent-exposed workers shows that many of them can be attributed to cerebellar and brain stem dysfunction (Workshop on Neurobehavioral Effects of Solvents, Washington, 1985). It seems as if the multisynaptic structures of brain stem and cerebellum are susceptible to toxic actions of many chemicals, as has been reported among glue sniffers exposed to high concentrations of toluene (e.g., Fornazzari *et al.*, 1983) and alcoholics (Juntunen, 1984). Low-level occupational exposure to toluene does not affect the nervous system appreciably (Juntunen *et al.*, 1985; Antti-Poika *et al.*, 1985).

The prognosis of occupational solvent poisoning has been considered in some clinical studies. The results have been rather controversial: both improvement of the subjective symptoms and signs (Bruhn *et al.*, 1981) and deterioration of the signs after cessation of exposure (Antti-Poika, 1982; Antti-Poika *et al.*, 1982; Juntunen *et al.*, 1982a; Gregersen *et al.*, 1987; Örbäck and Lindgren, 1988) have been reported. No clear-cut picture of prognosis has emerged from these studies, and further longitudinal studies are needed to clarify this issue.

Considering the multitude of chemical exposures taking place simultaneously among workers, the possibility of unexpected toxic interactions is interesting (Iregren, 1986). For example, benzene and toluene seem to suppress each other

(Inoue *et al.*, 1988). Alcohol (Hills and Venable, 1982; Juntunen, 1982, 1984; Antti-Poika *et al.*, 1985) and anaesthetics (Juntunen *et al.*, 1984) can interact with solvents. In some cases serious neurological diseases may emerge when two apparently less toxic solvents are acting together (Altenkirch *et al.*, 1977; Juntunen *et al.*, 1984).

Diagnostic Criteria for Solvent Intoxication

Figure 1 shows the flow-chart of the diagnostic procedure employed in individual diagnostics of solvent intoxications in Finland (Juntunen, 1982). Suspected

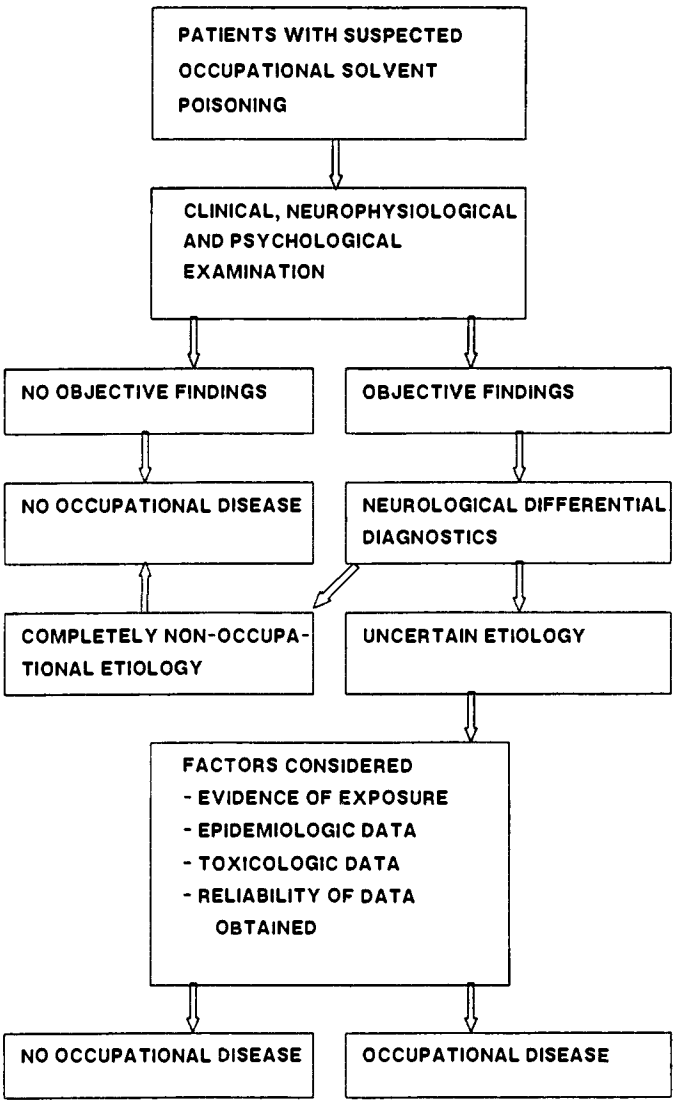

FIGURE 1

cases are admitted to the Institute of Occupational Health, Helsinki, for further examinations. Whenever some, even subtle, objective signs of nervous system dysfunctions are observed, a careful differential diagnostics is performed by an experienced occupational neurologist. If the cause of the nervous system disturbance still remains obscure, a comprehensive assessment of the case is performed, with particular emphasis on the available data on that particular exposure. Workers nowadays are well aware of the possible health effects of solvents and may seek help deliberately. Consequently, a selection of neurasthenic or hypersusceptible persons may occur. The final diagnosis of occupational solvent intoxication is always a probability diagnosis. In many cases a few months' follow-up is necessary to confirm the diagnosis. In Finland, a country with a relatively well-established occupational health care system, the following criteria for a diagnosis of occupational solvent intoxication have been used (Juntunen, 1978):

(1) Verified relevant exposure to solvents;
(2) Clinical picture of the nervous system involvement, which include
—typical nonspecific symptoms,
—signs of the nervous system disturbances;
(3) Other neurologic and/or psychiatric etiology reasonably well excluded.

These general criteria are applicaple for all cases. From the clinical point of view it is difficult to set any limits for the minimum time required for the chronic solvent intoxication to develop. It usually takes years, but depending on individual susceptibility and exposure there is high variation. The crucial issue is, however, which symptoms and signs of nervous system dysfunction are considered relevant for a diagnosis. Based on personal clinical experience of some 2000 cases of suspected occupational solvent intoxication, and on of data available from different studies, this author feels justified in stating that a clinical syndrome of chronic solvent intoxication exists. The following symptoms are frequently present: headache, fatigue, dizziness, and neurasthenic signs. The most common signs are mild psychoorganic syndrome, cerebellar disturbances, disturbances in gait and station, and neurasthenic signs. These can be considered as very nonspecific and are common for many solvents and solvent mixtures. The argument that we cannot make a diagnosis without knowledge about the solvent-specific nervous system effects is plausible. The above symptoms and signs are like fever in bacterial or viral infection: We can diagnose an infection without specific etiological diagnosis! We should, of course, have sound knowledge about those few solvent-specific neurological pictures.

CONCLUDING REMARKS

There is substantial evidence suggesting that long-term occupational exposure to solvents may cause diffuse neurotoxic syndromes, although perhaps less frequently than is generally believed in some countries. Taking this fact into account, we must accept that there is a spectrum of subclinical and clinical manifestations of occupational solvent intoxications ranging from nonobservable effects to even fatal forms. The clinical picture strongly depends on individual susceptibility and extent and nature of exposure. The stage at which these cases are diagnosed depends on the activity of occupational health professionals, the examination

techniques used, and, ultimately, the diagnostic criteria used. There is a strong need for unifying the criteria used in diagnostics and in field studies on effects. In this respect, psychological tests seem to be most problematic. Factors underlying individual susceptibility of the central nervous system to toxic effects of solvents are largely unknown. These are the most important challenges for researchers in the field of occupational neurotoxicology in the Decade of the Brain.

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Neurobehavioural Effects of Solvents: The Role of Alcohol[1]

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Results from three recent studies suggest that solvent-exposed workers are particularly at risk of disabling psychiatric illness associated with alcoholism. A case-referent study of psychiatric admissions in Quebec found an odds ratio of 5.5 for solvent exposure in patients with a diagnosis of alcoholic dementia, while cohort studies of Swiss and Swedish painters showed greater than expected numbers with alcohol-related psychiatric diagnoses. Simple mislabeling of disease related to solvent exposure does not appear to be the explanation; in the Quebec study the excess risk was essentially confined to those reporting heavy alcohol use. This would suggest that exposure to one substance might potentiate the effect of the other. Concurrent exposure to alcohol and solvents slows clearance of the solvent and might be expected to prolong internal exposure to the neurotoxin. In regular drinkers, elimination of solvent metabolites appears to be faster than in nondrinkers, presumably through enzyme induction. With heavy exposure to alcohol, hepatic function may become impaired, with decreased capacity to detoxify organic solvents. Alcoholic dementia was not included as a diagnosis in the initial studies of the long-term effects of solvents; however, it appears that the interrelation between the two exposures may be more important than previously suspected.

INTRODUCTION

Several studies in recent years (for example Olsen and Sabroe, 1980; Mikkelsen, 1980; Brackbill and Maizlish, 1990) have supported the initial Swedish observation (Axelson *et al.*, 1976) that workers in trades associated with solvent exposure, particularly painting, are more likely than those in unexposed trades to be retired early on psychiatric grounds. Two case-referent studies of deaths from senile dementia (O'Flynn *et al.*, 1987) and of Alzheimer's disease (Shalat *et al.*, 1988) did not show an excess of solvent exposure among the cases. Neither study had great power and that of Shalat *et al.*, systematically excluded cases with a high alcohol intake; however, together they might suggest that solvent exposure played no part in severe organic disease. In contrast a large case-referent study from Quebec (Cherry *et al.*, 1988, 1992) found an increased risk for solvent exposure in 254 patients with organic dementia or cerebral degeneration; the higher risk was, however, confined to those whose primary or secondary diagnosis was of alcoholic psychosis (ICD-9, 291) or alcohol dependency syndrome (ICD-9, 303). In this group the odds ratio for exposure was 5.5 (90% CI 1.6–19.5) with other psychiatric patients as the referent group and 5.0 (90% CI 1.4–17.9) with referents from general hospital admissions. The odds ratios for patients with organic psychosis or cerebral degeneration but without an alcohol-related diagnosis were 0.9 and 1.1, respectively.

Two subsequent studies also suggested an increase in alcohol-related problems

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in men exposed to solvents. A cohort study from Geneva (Guberan *et al.*, 1989) found that 20 (of 1916) painters but only 10 (of 1948) electricians had received a disability pension for neuropsychiatric disease, giving an age-standardized relative risk of 1.8. Among these pensions, however, 12 of those for painters were for alcohol-related disease, compared to only 1 of those for electricians. This excess of alcohol-related diagnoses accounted completely for any apparent increase in risk in the cohort of painters.

Results from the third study, a cohort study of painters and carpenters in Stockholm (Lundberg *et al.*, 1992), showed that painters were more likely to appear with an alcohol-related diagnosis in registers of psychiatric patients, early retirement, or mortality. They were also more likely to have repeated registrations in a register of alcohol-related crime. The authors suggest that alcohol damage, but not necessarily alcohol consumption, was increased among the painters, a conclusion that can be applied equally to the results of the Quebec and Swiss studies.

It remains to be considered why such an excess of alcohol diagnoses might occur among people in solvent-exposed trades. The first possibility is that the exposed men do indeed drink more, either because men attracted to these trades are those with a predisposition to heavy drinking or because something about the job, for example, frequent change of work site or informal supervision, might permit drinking habits less easily followed in other trades. Conceivably, too, working with solvents might lead to an increased need for, or tolerance of, alcohol. Guberan *et al.* (1989) found a significant excess of deaths from cirrhosis in their cohort of painters, but most studies find no difference in reported drinking habits of exposed and nonexposed groups. In the Quebec study, for example, heavy drinking was reported by 17% of both exposed and nonexposed workers.

A second possible explanation for an excess of alcohol-related diagnoses is that this label is mistakenly applied to disease resulting from organic solvent exposure. This hypothesis is less easily tested in existing studies but again the Quebec study provides some relevant data (Cherry *et al.*, 1992). An increased risk was found only in solvent-exposed workers where the patient (or his family) reported that he had drunk at least 42 units of alcohol (for example 42 bottles of beer) a week or whose alcohol intake could not be quantified but was described in a way that suggested excess ("drank like a fish"). For this group, exposed to both solvents and large amounts of alcohol, the odds ratio was 4.2 (90% CI 1.3–14.0). Solvent-exposed workers who reported more moderate alcohol consumption were at no increased risk of organic dementia or cerebral degeneration (odds ratio 1.1; 90% CI 0.6–1.9). Had the excess risk of alcohol-related diagnoses been simply due to mislabeling, an increased risk would be expected also in those with moderate alcohol consumption. Among the heavy drinkers, however, the damage labeled as alcohol related may well be due, in part, to solvent exposure rather than to alcohol alone.

A third explanation for an excess of alcohol-related diagnoses among solvent workers is that effect of one substance potentiates the effect of the other. At the simplest level, those with solvent-induced damage may be less able to tolerate heavy loads of alcohol and so be more likely to show its effects. Another possibility is that solvents and alcohol interact during the years of exposure, through changes in metabolism of the potentially neurotoxic substances.

It has been recognized for some time that a bolus of alcohol given during

experimental exposure to certain solvents, for example styrene (Wilson *et al.*, 1983) or toluene (Waldron *et al.*, 1983) slows the metabolism and clearance of the solvent. Internal exposure to a neurotoxic solvent (or early metabolite) would thus be increased. The effect in regular drinkers, however, is less clear; field studies suggest that those with moderate alcohol consumption eliminate these substances more quickly (Waldron *et al.*, 1983), presumably through enzyme induction, and that this may possibly protect from some neurotoxic effect (Cherry and Gautrin, 1990).

The effect of prolonged heavy drinking on solvent metabolism is unknown but those with hepatocellular damage, from alcohol or other causes, may well be less able to detoxify solvents. In such circumstances, the combination of solvents with a long period of excess drinking will be more potent than exposure to solvents or alcohol in a subject whose hepatic system is intact. If that is so and if, further, the effect of such poorly detoxified solvent were to cause a syndrome suggestive of organic dementia or cerebral degeneration, this would be sufficient to explain the findings of the various studies discussed here. It also raises another interesting question. Several of the earlier investigations (Axelson *et al.*, 1976; Lindstrom *et al.*, 1984; van Vleit *et al.*, 1990) excluded dementia associated with alcohol (ICD-9, 291) from the case series. If the effect of solvent exposure in heavy drinkers is to shift the risk of disease from alcohol dependency syndrome (ICD-9, 303) to alcoholic psychosis (ICD-9, 291) studies which excluded diagnosis 291 but included 303 will have systematically underestimated the effect of solvents on the risk of disabling psychiatric disease.

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A Prospective Cohort Study of the Chronic Effects of Solvent Exposure[1]

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The issue of the health effects of long-term exposure to solvents still attracts considerable debate, particularly among regulators and policymakers. This is especially true for studies of neurobehavioral effects. A major reason for this debate is that cross-sectional research designs are almost universally used in this area. Cross-sectional studies suffer from problems of possible confounding by a range of unknown factors and also usually from poor estimates of exposure. This study is an attempt to solve this problem by using an inception cohort design in which the subjects are measured at the beginning of their exposure and then at intervals while their exposure continues. Two hundred first-year apprentice vehicle spray painters were entered into the study within 6 months of beginning their apprenticeship together with a comparison group of 76 first-year apprentice electricians and 49 first-year apprentice metal fabricators. Measures of neurobehavioral function using a test battery based on an information-processing model were made at study entry and will be made annually. In addition apprentice's assessments of their own exposure are being made annually and compared with workplace-exposure assessments. So far measures have been made at the beginning of the second year for the entire cohort and at the beginning of the third year for one-third of the cohort. This paper is a description of the results for this one-third. The results suggest that there were no significant changes in neurobehavioral function in the first 2 years of exposure to solvents. However, the results of workplace monitoring suggest that exposure is considerably below current exposure standards for solvents during this time.

INTRODUCTION

There is little dispute that short-term exposure to solvents can produce adverse, transient effects on behavior (Dick, 1988). The consequences for behavior of long-term exposure to solvents, however, remains contentious (Triebig, 1989). Some studies have demonstrated effects of solvent mixtures (Hane *et al.*, 1977; Orbaek *et al.*, 1985; Gregersen *et al.*, 1987) and specific solvents, such as styrene (Lindstrom, *et al.*, 1976; Harkonen *et al.*, 1978) and toluene (Iregren, 1988; Hanninen *et al.*, 1987), but others have failed to demonstrate any effects (Cherry *et al.*, 1984; Maizlish *et al.*, 1985; Triebig *et al.*, 1988). Furthermore, there have been inconsistencies in the pattern of effects on neurobehavioral function among those studies that have demonstrated effects (Triebig, 1989).

Despite the large amount of research that has aimed to resolve the problem, methodological problems have marred the conclusions of many studies. Most studies have used cross-sectional or case–control methods to compare separate groups of exposed and nonexposed workers on a range of measures that reflect

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nervous system function. This necessarily leads to problems in interpreting impairments in neurobehavioral function or status as due to the effects of exposure or to some other factor, either known or unknown, on which individuals in the two groups differ but which can affect or influence neurobehavioral function in its own right. In addition, differences between individuals in the degree and type of exposure can also compromise interpretation of results.

Regardless of the specific problems of each study, some authors argue for a "weight-of-evidence" approach to evaluating the effects of chronic solvent exposure. They maintain that, on balance, the accumulated evidence supports the contention that chronic solvent exposure produces neurobehavioral effects or chronic toxic encephalopathy (Gamberale, 1985; Baker *et al.*, 1985). Other authors take a more cautious approach and argue that the evidence on the long-term effects of solvents on the nervous system are not yet clear (Grasso, 1984).

There is, however, widespread agreement that longitudinal studies involving repeated measurements of performance of the same individual over time are needed to overcome some of the more significant problems of interindividual differences that beset previous studies (Gamberale, 1985; Firnhaber White and Feldman, 1987). Unfortunately, due to difficulty in conducting longitudinal studies, there have been very few in the whole area of toxic effects on neurobehavioral function, most particularly for longitudinal studies from the onset of exposure. A recent study by Hanninen *et al.*, (1991) was an attempt to reduce some of the interindividual variation in behavioral test measurement by comparing monozygotic twins where one was solvent-exposed and the other nonexposed. Despite a relatively small sample size and low levels of solvent exposure, exposed twins showed significantly poorer performance on a number of tests compared to their sibling and some evidence of increasing dysfunction with greater exposure. While these findings provide fairly strong support for the weight of evidence conclusion regarding the effects of low to moderate exposure to solvents over time, the results were still affected by confounding by such interindividual differences as education and occupation.

The study described in this paper was an attempt to overcome problems due to interindividual differences by studying a group of workers from the beginning of their exposure to solvents and then annually until the end of their apprenticeship. The results reported in this paper are preliminary findings from a large-scale study of the health effects of occupational exposure to solvents in a group of apprentice vehicle spray painters. A reference group of nonexposed apprentices is also being followed from the beginning of their apprenticeship. The aim of the study is to determine whether solvent exposure produces neurobehavioral effects in occupations like vehicle spray painting and, if so, under what conditions.

MATERIALS AND METHODS

Subjects

Fifty first-year apprentice vehicle spray painters and 50 first-year apprentice electricians were recruited into the study in early 1989, the first year of their apprenticeship training. In New South Wales, training for these two trades involves spending 1 day per week during the teaching year at a technical college and the rest of their working time in a workplace. The apprenticeship is over 3 years, with a fourth year spent working full time in the trade. Apprentices were recruited

for the study from four different technical colleges, each covering different sections of the Sydney metropolitan area.

As virtually all apprentices in these two trades are male, all participants in the study were male. A summary of the general characteristics of the two groups is displayed in Table 1. The two groups were very similar in age, ethnic origin, and background of residence in Australia; percentage who smoked and the amount smoked per week; percentage consuming alcohol and the amount consumed per week; and the length of previous exposure in their respective jobs. The groups differed on their level of education. Electricians had received approximately 1 more year of education than the spray-painting apprentices.

Testing Procedure

All testing was performed during the apprentice's day at the technical college, usually in the morning. Testing occurred between late February and the end of April of each year, 1989, 1990, and 1991. Neurobehavioral performance testing was one part of a number of facets of each session with each apprentice. The session also included a comprehensive demographic questionnaire, a medical examination, and blood and urine tests.

The neurobehavioral tests were from a battery that included the following tests (for details, see Williamson *et al.*, 1982; Williamson, 1990):

1. *Critical Flicker Fusion (CFF).* A visual perception test in which a light cycling on and off, usually between 70 and 20 Hz, was used to measure an individual's ability to discriminate the onset of flicker as the on–off rate reduces by 2 Hz/sec. The fusion threshold in Hz is recorded for each eye.

2. *Hand Steadiness.* A visual–motor coordination test was conducted in which the individual was required to hold a thin metal stylus for 1 min in a 5-mm-diameter hole without touching the edges. The number and duration of touches to the upper and lower edges of the hole are recorded as off-target corrections and fatigue, respectively.

3. *Simple Reaction Time test.* A perceptual speed test in which the individual

TABLE 1
CHARACTERISTICS OF SPRAY-PAINTING AND ELECTRICAL TRADES APPRENTICE GROUPS AT BEGINNING OF THE STUDY (YEAR 1)

	Spray painting apprentices (n = 50)	Electrical trades apprentices (n = 50)
Age (years)	17.8	18.2
	(1.1)	(1.2)
Education level	10.2	11.1
	(0.62)	(0.98)
% <5 years residence in Australia	4.0	4.0
% Smoke for longer than 12 months	54.0	48.0
Amount smoked (No. of cigarettes per week)	78.2	95.8
	(72.5)	(63.7)
% Consuming alcohol	74.0	86.0
Amount alcohol consumed	10.3	13.6
	(10.8)	(15.9)
Length of time in job	0.76	0.60
	(0.56)	(0.44)

responded to the onset of light as quickly as possible by pressing a button was conducted. The speed of response is measured in milliseconds.

4. *Visual Pursuit test.* This is a psychomotor test requiring the individual to track a moving light within a circular pathway at each of two separate speeds. Time on-target is recorded.

5. *Sternberg Memory test.* This is a short-term memory test in which individuals are asked to remember a varying number of single digits (2, 3, 4, or 5 digits) and then respond positively (Yes button) if the digit was present in their to-be remembered set or negatively (No button) if the digit was not present. Speed of response and the number of errors are recorded.

6. *Paired Associates, short-term memory test.* This involves the recall of five pairs of three-letter consonant–vowel–consonant trigrams in which all pairs are shown once, followed immediately by the first member of each pair. The individual's task is to complete each pair. A parallel form of the test was used in each study year in order to minimize effects of learning across test sessions. The number of words correct on the first trial is recorded as a measure of short-term memory. The sequence is repeated until the subject correctly recalls all pairs and the number of trials to recall all words correctly is recorded as a measure of learning.

7. *Paired Associates, delayed recall.* This requires the individual to recall the five words learned earlier (approximately 30 min before) when presented once only with the first member of each pair. The number of words recalled correctly is recorded.

In addition, each apprentice was asked to complete the neuropsychiatric Questionnaire 16 (Hogstedt *et al.*, 1982) and a short questionnaire about details of their daily routine such as waking time and sleep time; use of alcohol, tobacco, caffeine, and other drugs; and their history of neurological illness and injury.

Exposure Assessment

Exposure was measured in two ways, by workplace assessment (including personal monitoring) and by calculation of an "exposure index" from a questionnaire.

Personal monitoring. Exposure was measured during a workplace inspection which involved a workplace assessment using a checklist questionnaire of potential problems and personal monitoring of the apprentice's breathing zone. Personal monitoring was performed using a charcoal tube sampler which was placed in the apprentice's breathing zone and allowed to remain for at least 6 hr of the work day (SA, 1987). The sample was later analyzed by gas chromatography (NIOSH, 1984).

Exposure index. Each spray-painting apprentice was asked to complete a solvent-exposure index questionnaire similar to one developed by Fidler *et al.* (1987), which asked for details of his history of using solvent-based paints before his apprenticeship and during it and exposure to paints outside work. The questionnaire asked for specific detail of exposure at work, the frequency and duration of time spent spraying, the amount of paint used per week, the proportion of time spent spraying in a spray booth, and the proportion of spraying time that engineering controls and personal protective equipment such as spray booths, ventilation fans, dust masks, respirators, and airline masks or hoods are used.

These factors can interact to modify exposure in a number of ways, such as

additively or multiplicatively. The use of control measures such as spray booths or personal protection may also reduce exposure. An estimation of exposure was calculated using the following general interactions:

Additive factors	Prior exposure
	Exposure outside work
Multiplicative factors	Duration of exposure
	Frequency of spraying
	Duration of spraying
	Location of spraying
Reducing factors	Use of engineering controls
	Use of personal protection

This calculation is based on a modification of the method of Fidler *et al.* (1987).

RESULTS

Subjects

Between Year 1 and 2 of the study the overall dropout rate was 31%, which was equally distributed across the spray-painting and electrician groups (32% and 30% respectively). Between Year 2 and 3, a further 32% dropped out. This loss was mainly from the electrical trades, which had a 40% dropout whereas the spray painter group only showed a 24% loss.

The main reasons that subjects dropped out of the study had to do with their attendance at the participating technical colleges and not to do with apprentices leaving the trade. This included apprentices who changed to colleges which were not participating in the study, those who failed the course, and those who changed the format of their attendance (for example, changing to night classes or to attendance in blocks of weeks). This accounted for 55% of dropouts.

More spray painters than electricians dropped out because they left the trade (45.8% and 13.8%, respectively). Approximately 21% of spray-painting apprentices left the trade because of money or working conditions compared to 7% of electrical apprentices. Several reasons for leaving the trade were unique to the spray-painting group. Approximately 17% of spray-painting apprentices left the trade for health reasons and 8.3% left because they were sent to prison. No reason could be determined for 15.1% of the dropout sample.

Examination of the main characteristics of the apprentices who dropped out compared to those who participated in the full 3 years of the study showed that the two groups were very similar in age, education level, years of residence on Australia, the frequency of those consuming alcohol and on the amount of alcohol consumed per week, and on the length of time in the job. The only differences between the groups were (i) that more dropouts from both spray-painting and electrician groups tended to smoke and (ii) those smokers smoked more per week than full study participants.

Analysis of Performance Tests

Table 2 shows the 1-year test–retest correlations (Year 1 compared to Year 2) for each test for the reference group. Statistically significant positive correlations were found for the CFF tests for both eyes, for both tests of Visual Pursuit, for both number of touches and time off-target for the Hand Steadiness test, for the

TABLE 2
TEST–RETEST CORRELATIONS FOR PERFORMANCE ON YEAR 1 AND YEAR 2 FOR EACH OF THE NEUROBEHAVIORAL TESTS FOR THE REFERENCE GROUP

Test	Correlation between Year 1 and Year 2 (r)
Critical Flicker Fusion (n = 34)	
Left eye	0.68*
Right eye	0.67*
Reaction Time (n = 34)	0.34
Visual Pursuit (n = 34)	
Slow test	0.51*
Fast test	0.72*
Hand Steadiness (n = 34)	
Off-target touches	0.64*
Time off-target	0.69*
Paired Associates (n = 35)	
Mean No. correct	0.34
Mean trials to criterion	0.52*
Delayed recall	−0.03
Sternberg memory test (n = 32)	
Positive set	0.56*
Negative set	0.48*
Questionnaire 16 (n = 50)	0.54*

* $P < 0.05$.

learning measure of the Paired Associates test, for both positive and negative memory set performance on the Sternberg memory test, and for Questionnaire 16. There was no significant test–retest relationship for the simple reaction time test, for the Paired Associates short-term memory test, nor for the delayed recall measure of the Paired Associates test.

The test results were analyzed using multivariate analysis of variance with repeated measures. The effect of main interest in all analyses was the interaction between study group (i.e., type of apprentice) and year of apprenticeship. For all analyses only subjects with complete data for all 3 years were included.

Analysis of the results for the CFF test (See Table 3) showed no significant interaction between group and year ($F_{(2,72)} = 0.69$, ns), nor a main effect of group ($F_{(1,36)} = 1.2$, ns), of year of apprenticeship ($F_{(2,72)} = 0.5$, ns), or of eye tested ($F_{(1,36)} = 1.5$, ns). Neither were there any significant two-way interactions or three-way interactions between group, year of apprenticeship, and eye tested. It appears that spray-painting apprentices were no different from electrical apprentices over the study time on this test.

For the Simple reaction time test (see Table 3) there was no interaction between group and year of apprenticeship ($F_{(2,68)} = 1.09$, ns) and no main effect of group ($F_{(1,34)} = 1.5$, ns). There was, however, a significant main effect of year of apprenticeship ($F_{(2,68)} = 30.2$, $P < 0.0001$) but no significant interaction effects. This indicates that there was a significant increase in reaction time for Year 2 apprentices relative to the other years, but that it was the same for both spray-painting and electrical apprentices.

The results for the Visual Pursuit test (see Table 3) also showed no significant interaction between group and year of apprenticeship ($F_{(2,76)} = 0.92$, ns) indicating that there were no differences between the spray-painting and electrical ap-

TABLE 3
RESULTS OF THE CRITICAL FLICKER FUSION, REACTION TIME AND VISUAL PURSUIT TESTS FOR SPRAY-PAINTING AND ELECTRICAL TRADES APPRENTICES FOR EACH YEAR OF THE STUDY SHOWING MEANS AND (STANDARD DEVIATIONS)

	Spray painters	Electricians
Critical Flicker Fusion		
Year 1		
Left eye	47.0 (5.7) (n = 50)	48.3 (5.6) (n = 50)
Right eye	47.9 (5.8) (n = 50)	49.9 (6.2) (n = 50)
Year 2		
Left eye	48.2 (10.4) (n = 34)	49.1 (4.8) (n = 35)
Right eye	48.0 (10.4) (n = 34)	50.1 (4.4) (n = 35)
Year 3		
Left eye	48.6 (2.9) (n = 26)	48.1 (10.6) (n = 21)
Right eye	48.9 (3.1) (n = 26)	49.8 (6.6) (n = 21)
Reaction Time		
Year 1	351.8 (56.1) (n = 50)	326.2 (37.1) (n = 50)
Year 2	416.8 (58.0) (n = 34)	401.4 (53.1) (n = 35)
Year 3	328.7 (163.2) (n = 25)	349.0 (131.1) (n = 20)
Visual Pursuit		
Year 1		
Slow	51.9 (4.3) (n = 50)	52.3 (5.0) (n = 50)
Fast	20.6 (5.8) (n = 49)	21.8 (5.7) (n = 50)
Year 2		
Slow	52.5 (4.7) (n = 33)	53.5 (4.4) (n = 34)
Fast	25.2 (5.7) (n = 33)	27.2 (5.2) (n = 34)
Year 3		
Slow	43.1 (9.8) (n = 23)	46.9 (7.8) (n = 21)
Fast	16.6 (7.0) (n = 23)	20.5 (8.1) (n = 21)

prentices over the duration of the study. The differences found for this test were all within-group differences. There was a main effect of group ($F_{(1,38)} = 8.85$, ns), with spray painters showing significantly faster performance than electrical apprentices at each year of the study. Both year of apprenticeship and speed of the test also showed significant main effects ($F_{(2,76)} = 20.25$, $P < 0.0001$; $F_{(1,38)} = 1388.66$, $P < 0.0001$, respectively) and a significant interaction between these two factors ($F_{(2,76)} = 12.88$, $P < 0.0001$). This shows that there was an overall significant improvement in performance from Year 1 to Year 3, but that the performance of all apprentices in Year 2 was significantly poorer than at the other test

sessions. This analysis also demonstrates, not surprisingly, that overall, apprentices performed better at the slow-speed test.

The results for the Hand Steadiness test are shown in Table 4. Analysis of the number of off-target touches over the test period showed no interaction between group and year of apprenticeship ($F_{(2,70)} = 2.49, P < 0.09$). There were, however, main effects for year of apprenticeship ($F_{(2,70)} = 43.48, P < 0.0001$) and for time period in the test ($F_{(2,70)} = 4.67, P < 0.01$) and a significant interaction between group, year of apprenticeship, and time period in the test ($F_{(4,140)} = 0.15$, ns). These results indicate that while there was a significant increase in the number of off-target touches over the time of the study, and an overall decrease over the time period of the test, spray painters showed significantly fewer off-target touches compared to electricians for Year 3 of the study. The remaining main effect, position of off-target touches, was not significant, nor were any other interactions statistically significant.

Analysis of the results for the Hand Steadiness test measure, time off-target, again showed no interaction between group and year of apprenticeship ($F_{(1,35)} = 0.88$, ns), but a significant main effect of year of apprenticeship ($F_{(2,70)} = 18.55, P < 0.0001$) and an interaction between year of apprenticeship and time period in the test ($F_{(4,140)} = 2.56, P < 0.04$). Unlike the results for the off-target touches measure, the main effect of time period in the test was not statistically significant ($F_{(2,70)} = 2.06$, ns), nor was the three-way interaction of group by year of apprenticeship by time period in the test ($F_{(4,140)} = 2.03$, ns). This shows that while there were significant differences in performance between years of the study, particularly between Year 2 and the other 2 years, this was so for all apprentices; spray painters were not different from electrical trades apprentices on this measure.

The results of the Paired Associates set of measures are shown in Table 5.

TABLE 4

HAND STEADINESS TEST RESULTS FOR SPRAY-PAINTING (SP) AND ELECTRICAL TRADES (ELEC.) APPRENTICES FOR EACH YEAR OF THE STUDY SHOWING THE NUMBER OF OFF-TARGET TOUCHES AND THE TIME OFF-TARGET FOR EACH 20-SEC BLOCK OF THE TEST SHOWING MEANS AND (STANDARD DEVIATIONS)

	Year 1		Year 2		Year 3	
	SP $n = 43$	Elec. $n = 50$	SP $n = 27$	Elec. $n = 34$	SP $n = 24$	Elec. $n = 21$
Off-target touches						
First block	22.5	70.8	53.4	52.6	46.3	70.8
	(20.0)	(334.0)	(36.6)	(40.6)	(36.9)	(42.0)
Second block	20.3	20.7	43.1	51.3	51.2	60.4
	(19.0)	(21.1)	(32.4)	(35.4)	(32.3)	(41.0)
Third block	16.4	16.0	41.9	46.8	46.0	58.5
	(18.2)	(18.3)	(28.4)	(28.5)	(33.0)	(33.0)
Time off-target						
First block	868.6	1051.1	1828.3	2042.9	834.6	1111.5
	(892.4)	(1019.0)	(1571.5)	(1797.5)	(901.7)	(763.2)
Second block	810.4	858.0	1545.9	1949.9	1067.9	1114.4
	(897.0)	(941.7)	(1417.9)	(1539.3)	(879.7)	(865.5)
Third block	634.8	726.7	1721.8	1846.0	877.7	1158.1
	(705.7)	(1038.0)	(1711.6)	(1661.5)	(837.6)	(779.1)

TABLE 5
RESULTS FOR THE PAIRED ASSOCIATES MEASURES OF NUMBER OF TRIGRAMS CORRECT, TRIALS TO CRITERION, AND LONG-TERM RETENTION FOR SPRAY PAINTERS AND ELECTRICAL APPRENTICES

	Year 1		Year 2		Year 3	
	SP $n = 50$	Elec. $n = 50$	SP $n = 33$	Elec. $n = 35$	SP $n = 24$	Elec. $n = 21$
Mean No. correct	1.8	2.7	1.9	2.7	2.1	3.6
(5 max.)	(1.4)	(1.3)	(1.2)	(1.8)	(1.5)	(1.4)
Mean trials to criterion	3.9	3.0	3.7	2.4	3.5	2.1
(all 5 correct)	(2.1)	(1.9)	(2.5)	(1.2)	(1.8)	(1.2)
Delayed recall	3.9	4.3	4.4	4.1	3.9	4.5
(mean No. correct)	(1.2)	(0.9)	(0.9)	(1.2)	(0.9)	(0.8)

Note. Values are means and standard deviations.

Analysis showed that for the short-term memory measure, number of trigrams correct, there was a significant main effect for the group factor ($F_{(1,40)} = 25.37$, $P < 0.0001$) but the year of apprenticeship factor and the interaction between these two factors was not statistically significant ($F_{(2,80)} = 2.62$, ns; $F_{(2,80)} = 0.66$, ns, respectively). This indicates that spray painters were significantly poorer than electricians across the time of the study and that both groups improved significantly between Year 1 and Year 3.

The same results were found for the learning measure, trials to criterion. There were significant main effects for the group factor ($F_{(1,40)} = 14.45$, $P < 0.0001$) and the year of apprenticeship factor ($F_{(2,80)} = 8.67$, $P < 0.007$), but not for their interaction ($F_{(2,80)} = 0.48$, ns). Spray-painting apprentices took longer to learn this task than electrical apprentices, but again both groups improved significantly over the study period.

The results for the long-term retention measure of the Paired Associates task showed similar significant main effects for group ($F_{(1,39)} = 4.52$, $P < 0.04$) but not for the year of apprenticeship factors ($F_{(2,78)} = 0.16$, ns). In addition there was a significant interaction between these two factors ($F_{(2,78)} = 4.59$, $P < 0.013$) showing that spray painters were poorer on this task in Year 3 of the study compared with electrical apprentices.

Analysis for the Sternberg task (see Fig. 1) showed no significant interaction between group and year of apprenticeship ($F_{(2,62)} = 2.3$, ns), but significant main effects for group ($F_{(1,31)} = 6.3$, $P < 0.02$), for the year of apprenticeship ($F_{(2,62)} = 24.1$, $P < 0.0001$), for memory set size ($F_{(3,93)} = 77.8$, $P < 0.0001$), and for type of response ($F_{(1,31)} = 281.58$, $P < 0.0001$). There were no significant interactions. These findings show that, as expected, reaction time increases with increasing memory set size and with the requirement to make a negative response. The effect of the year of apprenticeship on Sternberg task performance is shown in the overall increase in reaction time for apprentices in Year 2 and the effect of the groups is seen in the overall slower reaction times of the spray-painting apprentices on this test.

The results of the Questionnaire 16 are shown in Table 6. There was very little change in reports of neuropsychological symptoms across the time of the study in either group. For spray painters, the percentage reporting 2 or more symptoms decreased by about one-third between Year 1 and Year 3.

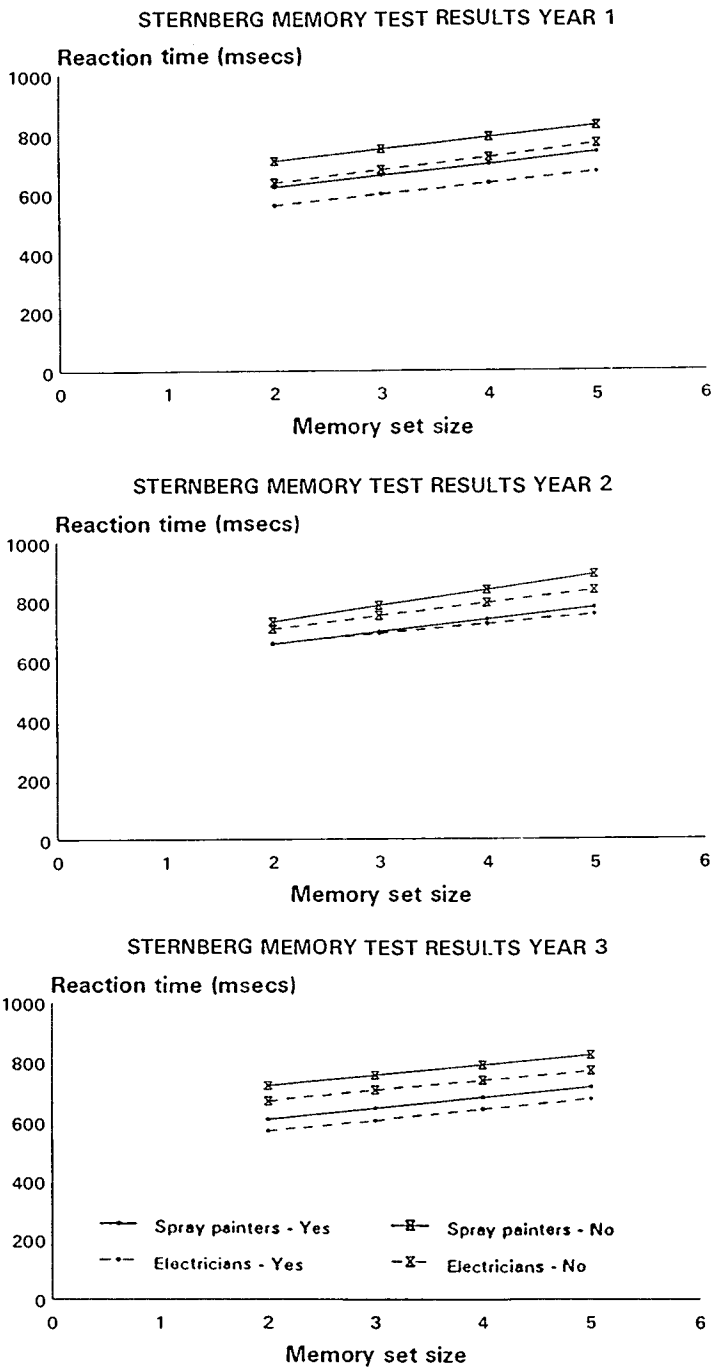

FIG. 1. Results of the Sternberg memory test for spray-painting and electrical trades apprentices at the beginning of Years 1, 2, and 3.

Analysis of Exposure Results

Personal monitoring. To date, workplace assessments have been performed for 20 apprentice spray painters. Table 7 shows details of the "significant contaminants" that were found in the breathing zone of the apprentices. A significant

TABLE 6
QUESTIONNAIRE 16 RESULTS FOR SPRAY-PAINTING AND ELECTRICAL TRADES APPRENTICES FOR EACH YEAR OF THE STUDY

	Spray Painters	Electrical Trades
Year 1	2.6 (2.5)	2.3 (2.6)
	61.5%	47.6%
	(n = 43)	(n = 44)
Year 2	2.5 (2.6)	2.3 (2.8)
	50.0%	52.4%
	(n = 27)	(n = 31)
Year 3	1.9 (2.1)	2.2 (2.1)
	38.5%	57.1%
	(n = 25)	(n = 20)

Note. Means and standard deviations and the percentage of apprentices reporting at least 2 items on the questionnaire are shown.

contaminant was defined as one which had a concentration of more than 1% of its exposure standard in at least one sample. Analysis of these results from the workplace monitoring showed that the common aromatic, aliphatic, ketone, and ester solvent ingredients in chemical products in the automobile-refinishing industry were the major components of all apprentices' exposures. The most common contaminant found was toluene, though significant quantities of acetone, xylene, butanol, and *n*-butyl acetate were observed. In all cases measured so far, composite exposure levels to groups of paints were well below a "recommended composite exposure standard" of 100%, ranging from 1 to 58%, with most below about 25% (Winder and Yeung, 1991).

Exposure Index. The responses on the subjective exposure questionnaire are summarized in Table 8. They show that a considerable percentage of the Year 2 group had exposure to painting prior to beginning their apprenticeship. For the Year 3 group few spray painters had worked in the industry before beginning their apprenticeship; however, those who had prior exposure had worked for longer periods but fewer had used solvent-based paints during this time. This suggests that many of the subjects had been exposed previously on the job, but that they had shorter prior exposure than the apprentices who dropped out and the exposure was less likely to have involved solvent-based paints.

After the beginning of the apprenticeship the reported patterns of use of solvent-based paint changed. More than half of the apprentices reported using solvent-based paints at least 50% of the time and the majority reported spraying every day. Approximately one-third reported spraying for a least 8 hr/week and using at least 4 liters paint/week. Nearly half reported always spraying indoors but less than half reported using a spray booth at least 50% of the time. The reported use of protective equipment was high for airline masks, but considerably less for the less-efficient methods of respirators, dust masks, and ventilation fans.

The mean exposure for apprentices in the second year of the study was 103.3 months (SD = 44.3) and for third-year apprentices, 144.6 months (SD = 39.9).

Figure 2 indicates that there is a significant correlation between the exposure index and personal monitoring, (r = 0.572, $F_{(1,19)}$ = 8.25, P = 0.011).

DISCUSSION

These results suggest that there were no significant changes in neurobehavioral performance that could be attributed to solvent exposure in this group of vehicle

TABLE 7

INDIVIDUAL APPRENTICE SPRAY PAINTER EXPOSURE TO INDIVIDUAL SOLVENTS (mg/m^3)

Apprentice:	1	1[a]	2	3	4	5	6	6[a]	7	8	9	9[b]	10	11	12	13	14	4[b]	15	16	16[a]	18	19	10[c]	20	21	22	23	24
Aromatic solvents																													
Benzene											1	1																	
Toluene	29	8	7		47	53	63	30	10	10	94	30	12	21	30	21	43	21	7	32	15	61	28	61	162	76	7	44	11
Xylene	6				6	6	6				6	5			5	5	11	7		5	5	11	9	7	24	17	4	13	
Trimethyl benzene	1		2												7	2	15	1		1		4	3	9	19	4	2	4	
Aliphatic solvents																													
C_5–C_7 Aliphatics																	19				36	71	45	28	19	46	45	17	
Ketone solvents																													
Acetone							12				68	12											16			24			
Methyl ethyl ketone							5	2			14	4					4												
Cyclohexanone											2																		
Alcohols																													
n-Butanol																									5	3			
iso-Butanol							4																						
Esters																													
n-Butyl acetate																		11											

[a] Repeat measure on the same day.
[b] Repeat measure on repeat visit.
[c] Repeat measure on the same apprentice at another workplace.

TABLE 8
SPRAY PAINTERS' REPORTS OF THEIR HISTORY OF EXPOSURE TO SOLVENTS FOR YEAR 2 AND YEAR 3 OF THE STUDY

	Year 2	Year 3
Solvent exposure before apprenticeship		
% With prior exposure	40.5	28.0
Amount of exposure (months)	14.9	22.9
	(12.2)	(17.1)
% Using solvent-based paints	37.8	24.0
Solvent exposure since beginning apprenticeship		
Amount exposure (months)	24.4	33.7
	(6.3)	(6.1)
% Using solvent-based paints more than 50% of time	45.9	48.0
% Spraying every day	81.1	96.0
% Spraying more than 8 hr/week	32.4	40.0
% Using more than 4 liters paint/week	76.7	28.0
% Always spraying inside	43.2	48.0
% Using spray booth more than 50% of the time	28.1	43.4

spray painters. Most of the observed changes were mirrored by similar changes in electrical trades apprentices. For example, both groups showed a better performance in CFF thresholds, in time on target in the Visual Pursuit task, and in the Paired Associates group of tests, in the number of items correct in the short-term memory test and in the number of trials to criterion or learning test between Years 1 and 3. These results could be due to increased familiarity with the tests; however, this is unlikely since the procedures for each of these tests are very simple such that they can be picked up easily in one session, and the interval between

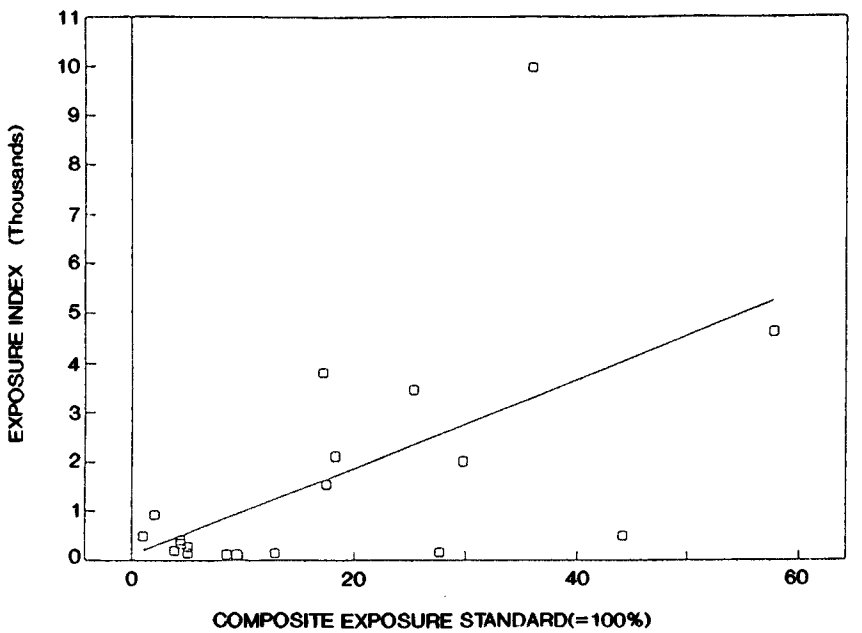

FIG. 2. Relationship between the subjective exposure index and results from personal monitoring in the workplace.

tests was relatively long. These improvements could also be due to increasing maturity of the apprentices over the study period.

For some tests both groups showed poorer performance over the study period. Between Years 1 and 3 all apprentices showed poorer performance on the Hand Steadiness test as evidenced by the increasing number of off-target touches in the later years of the study. For Year 2 alone, both groups showed poorer performance in the Simple Reaction Time test, Visual Pursuit test, the time off-target measure of the Hand Steadiness test, and the Sternberg memory test. It may be significant that in Year 2 the amount of alcohol consumed by both groups of apprentices increased markedly. From Year 1 to Year 3 spray painters consumed on average 10.3, 25.0, and 12.1 "standard" alcoholic drinks, respectively (one standard drink = about 10 g alcohol), and electrical trades apprentices over the same period consumed 13.6, 19.33, and 16.1 standard alcoholic drinks. This large increase in drinking in Year 2 could be a factor in the poor performance on the hand–eye coordination tests seen in both groups in Year 2.

For the Paired Associates tests spray painters performed differently from electricians at the beginning of the study on the short-term memory and learning measures of the test. This was most likely due to the slightly higher education level of the electricians. Previous research has demonstrated that education level is a significant covariate of performance on this test (Williamson, 1990). The delayed memory Paired Associate test did not show initial differences between the groups; however, by Year 3 spray painters were significantly poorer than electricians on this measure. It must be noted that this significant effect was due to improvements in performance of the electrical trades group as the spray painters remained at the same level on this test.

The results of the Paired Associates test suggest that electrical trades apprentices are not the best reference group for spray painters due to educational differences. For the larger study, an additional reference group of metal fabrication apprentices have been included to overcome the effects of differences in education level, as they appear to have a background similar to spray painters.

Some tests might be expected to produce inconsistent performance from one testing to another. The test–retest correlations suggested that the short-term and delayed recall measures from the Paired Associates test and the slow test for Visual Pursuit in particular are prone to more state-dependent influences than the tests with good test–retest correlations. If this is so, it could be argued that these tests may not prove to be useful indicators of any effects on these functions due to solvent exposure. This point should be resolved with further follow-up of the apprentices and with a larger sample size.

An important influence on the results of any longitudinal study is the effect of dropouts. With just over 50% of cases dropping out in this study over 3 years, the power of study is reduced. For this reason, the main study was started with 200 spray painters and 100 controls. The dropout rate for the section of the study described in this paper is higher than it should be as an additional 14 apprentices have yet to be contacted for Year 3 measurements as they had changed to night classes. It is hoped that the overall dropout rate over 3 years for the main study will be about 35%.

A consistent report from previous research has been of increasing neuropsychiatric symptomatology in spray painters (Juntunen, 1986; Ng *et al.*, 1990) and of high rates of criminal behavior (Lidberg *et al.*, 1987). The results of Questionnaire

16 showed, if anything, reduced symptom reporting by spray painters over the time of the study. This suggests that the limited exposure of spray painters in this study both in time and degree is not enough to produce changes in psychiatric symptoms. It is important to note, however, that two of the spray painter dropouts were in prison, both for aggressive criminal acts. Examination of their Questionnaire 16 results for the year before they dropped out showed that both reported psychiatric symptoms.

From the 20 workplaces assessed so far, it seems that in spray-painting apprentices, the level of exposure to solvents is within recommended exposure standards. However, it must be recognized that these results may not be characteristic of all spray-painting apprentices because not all workplaces have been assessed. Results of the workplace assessment also indicate that the use of engineering controls such as spray booths in some workplaces may reduce exposure to solvents even further. The close correlation of a subjective "exposure index" with the results of personal monitoring is promising and suggests that estimation of exposure through a questionnaire may be a valid measure of exposure in studies of this nature therefore supporting the findings of Fidler *et al.* (1987).

The results of the study suggest so far that with only 2 to 3 years of what may be fairly low exposure to solvents, spray-painting apprentices show very little neurobehavioral function effect. With the exception of effects on delayed recall, which showed deterioration in spray painters relative to electricians, but not relative to their own Year 1 performance, spray painters were as good as or better than electrical apprentices on all measures.

These results should be viewed with some caution. While they suggest that solvent exposure does not significantly affect neurobehavioral function, at least at the levels seen in this study, the small final sample size and the relatively short exposures have reduced the power of the study. As Hanninen (1988) points out, there is very large variation between individuals in their sensitivity to solvents. To establish whether chronic occupational exposure to solvents produces significant effects on neurobehavioral function, larger numbers of spray painters are needed and they need to be followed for longer than 2 to 3 years. It remains to be seen if the continuation of this longitudinal study will confirm these findings over a term longer than at least 3 full years. It is hoped that the main study will provide more definitive findings.

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Occupational Diseases Developed as a Result of Severely Injured Nervous System: Acute and Chronic Neurotic Effects[1]

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Some results of multiple epidemiological, clinical, electroneurophysiological, and psychological studies that reveal different types of neurotoxicoses are presented in this work. Under conditions of present-day industry, when a worker is exposed to concentrations close to but below the maximum allowable concentrations, or MAC values, of toxic substances, acute neurotoxicoses rarely develop, occurring perhaps only in catastrophies. Acute intoxications may be accompanied by mass hysteria, which impedes etiological diagnosis and prognosis. Vegetative polyneuropathy is the most common type of chronic intoxication where central vegetative disorders sometimes precede the peripheral ones. Clear forms of intoxication caused by neurotrophic poisons show stable torpid development during subsequent phases of a disease despite the absence of contact with the toxicant.

INTRODUCTION

Industrial neurotoxicoses are occupational poisonings in which the clinical picture is characterized by a predominant disturbance of the central and peripheral nervous systems. They may develop when a body is exposed to neurotrophic or polytrophic poisons or toxic allergens. Neurotoxicoses can be acute or chronic; their long-term effects are also the subject of different studies (Drogochina, 1968; Rizhkova and Dumkin, 1980; Izmerov *et al.*, 1983; Monaenkova *et al.*, 1988).

MATERIALS AND METHODS

Prospective and retrospective analyses of 1500 in-patients, including those poisoned by neurotrophic intoxicants such as carbon bisulfide, mercury, manganese, lead, aromatic hydrocarbons, pesticides, were conducted. In addition to regular clinical examinations, bioelectric analysis of cerebral activity (EEG) and electromyography (EMG) were performed. Psychological tests were used to assess nervopsychic state, mental capability, attention, memory, and intellectual level.

Specificity of Acute Neurotoxications

Development of acute intoxications as a result of simultaneous exposure to high concentrations of toxic substances is not characteristic of the contemporary industry and may occur only in extreme situations.

More than 150 accidents have been recorded in the USSR during the past two

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decades. Each could have been disastrous. It is noteworthy that a number of relatively new questions emerged from this. This most important concerns validation of toxic substances, especially during multiple complex exposure. This is significant both to etiology and to proper treatment.

We experienced difficulties in etiological diagnosis assessing mass intoxications from insecticides during the potatoe and onion harvests of 1989 in the Sverdlovsk region. Acute poisonings of miners and mountain rescuers by a complex of toxic substances (chlorobenzene plus acetone, etc.) occurred in 1990 in the Donetsk region. These toxicants, from the waste of the chemical plant, penetrated into the mine, which was immediately under the plant.

It is difficult to determine etiological factors when chemicals of foreign manufacture are involved and their toxicity is not well known. In addition, clinical and experimental evidence indicates that under certain conditions different insecticides in the soil may form new, highly active combinations with unpredictable biological effects.

It is important to realize that a syndrome of "mass tragedy" may develop during a so-called "ecological disaster" and acute poisonings may cause hysteria on the part of both industrial workers and the population of nearby residential areas, such as occurred in Erevan (Armenia) and Tbilisi (Georgia) in 1988 and 1989, respectively. Thus, there is a need to develop appropriate legal sanctions and special program to prevent accidents as well as to deal with their consequences in the country.

Multi-year retrospective studies of chronic neurotoxicoses conducted for more than 20 years in both clinical and industrial settings have shown the behavior of nervous system to be quite uniform during chronic exposure to different neurotrophic poisons at low concentrations close to MAC values.

Two phases may be distinguished during chronic intoxications. The primary phase is characterized by functional nervous disorders; their essence lies in weakened regulating activity of the highest gray matter centers which influence vegetative and neurohumoral processes. The main syndromes are vegetative vascular dysfunction, asthenic effects, asthenovegetative syndrome, and vegetative sensory polyneuropathy.

Organic impairment of the nervous system, or toxic encephalopathy developed under chronic exposure to industrial substances, has not been noted as an independent primary phase of intoxication. It is instead a progressive transformation of compensated neurodynamic disorders into dystrophic processes.

Transformations of one phase into the other are caused by changes in general body reactivity and systems responsible for adaptative body reactions. Characteristic of this phase is aggravation of asthenia together with work ability deficiency, decrease in intellectual level, insomnia, and apathy. Moreover, compensation can be broken not only by exposure to a toxicant but also by some adverse factors that influence body reactivity. For instance, infection and psychic trauma may aggravate the state of a poisoned patient. Of special importance are endocrine changes (pregnancy, delivery, climax, etc.).

The clinical syndrome of toxic encephalopathia is extremely polymorphic, which testifies to the diffuse impairment of the nervous system.

Certain clinical manifestations, which can be explained by selective action of some poisons on the predominantly targeted CNS organ, are observed at the stage of organic impairment.

Clear forms of chronic intoxications are characterized by torpidity, low reversibility of processes, and significant deficiency in social adaptation. Thus, early diagnosis is an important means of prevention. However, early diagnosis is complicated by the prevalence of nonspecific syndromes in the early phases of occupational intoxications which require additional research methods, such as electroneurophysiological techniques.

Electroencephalography allows assessment of bioelectric cerebral changes and physiological evaluation of the functional state of gray matter centers for which the majority of industrial poisons possess special attraction. The EEG signs most typical of hypothalamic insufficiency are synchronized symmetric splashes of rhythmic (4–8/sec; 1–4/sec) waves as well as pointed waves and their complexes. In accordance with the degree of neuron dysfunction of upper trunk and diencephalic level, rhythmically slowed or paroxysmal activity may emerge at rest or during functional tests. This EEG paroxysmal type may occur on average in 40% of the persons, according to our observations, with signs of occupational neurointoxications, in 20% of manganese intoxications, and in 70% of mercury intoxications (Fig. 1). Changes in bioelectric cerebral activity during exposure to neurotrophic poisons are often characterized by a decrease in α-range waves or a 10- to 20-microwave reduction in α waves. Parameters of β activity, which is a more frequent phenomenon, may be slightly changed: enforced or reduced. Thus, EEG evidence of neurointoxication may be either paroxysmal or "plane." According to our observations, plane EEGs occur more often (in 40% of the cases) during chronic manganese intoxications (Fig. 2); "irritative" EEGs prevail in persons with mercury intoxications. More profound changes are evident in polymorphic slowed activity and occur predominantly during chronic carbon bisulfide intoxications (Fig. 3). However, the changes described do not indicate a definite nosologic specificity. Therefore, EEG evidence must be combined with clinical hygienic examinations followed by more specific diagnostic tests, such as those measuring the toxicity of substances and their metabolites in biological media.

Clinical data on the nature of polyneuropathy accumulated in recent years have led to it revision (Antoniuzhenko and Krashennikova, 1991). For instance, classical division of toxic polyneuropathy into motor and sensor types is not quite true because in accordance with the electrophysiological and clinical examinations simultaneous impairment of both motor and sensor fibers is present, although of a different severity. The most informative criteria of EMG vegetosensory polyneuropathy are increased reflex musculus tone, reduction in amplitude, EMG break in the form of II–III peaks, changes in coordination, and slowed conductivity of impulses in distal units of sensory peripheral nerves which rarely occurs with motor fibers.

Psychic tests have been widely used in medicine. They have become even more sensitive than the costly diagnosis using different medical equipment. In accordance with the data from our studies, patients with chronic neurointoxications showed impaired psychic activity, impaired ability to work, and decreased func-

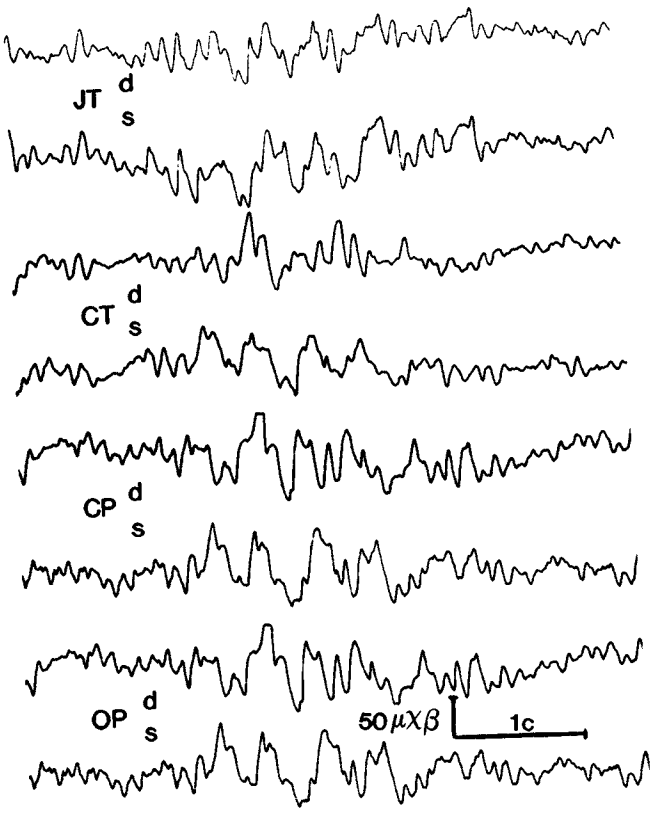

FIG. 1. EEG for mercury intoxication: high amplitude, 2–3 waves/sec, α waves at a background of general cerebral changes. "Paroxysmal" EEG type.

tions of attention, generalization, abstraction, and deduction. Lability of psychic activity, emotional changes, and nonspecific modal memory disturbance were also present. Individual tests in groups with different intoxications have revealed certain differences that can be explained by predominant localization of a process and selectivity of action. For instance, emotional excitement combined with absentmindedness, indecisiveness, and exhaustion is typical of encephalopathy due to mercury intoxication.

Encephalopathy caused by carbon bisulfide is characterized by decreased activity (slowed associative processes), emotional lability, deficiency, and sluggishness. Specific for manganese intoxication is the development of apathy. Psychic disorders were of a diffusive character which spoke of a toxic nature. Recommendations for the treatment of above-stated intoxications have been made.

Long-Term Effects of Neurotoxicoses

One of the more urgent remaining problems is a study of long-term effects of neurointoxications. It is a top-priority problem of occupational medicine. In accordance with the opinion of experts from the WHO Regional Office for Europe, its importance is evident in the prevalence of long-term effects of neurointoxications.

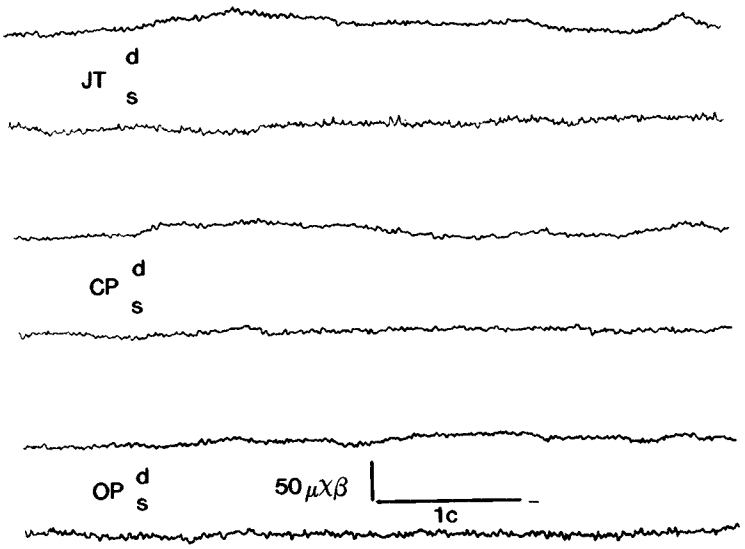

FIG. 2. EEG for manganese intoxication. General cerebral changes are decreased amplitude of bioelectric activity and weakened rhythms. "Encephalopathic" EEG type.

Health disorders that persist after the end of exposure to acute or chronic industrial intoxication are called long-term occupational effects. Studies of these effects are complicated by the specific clinical picture, pathogenesis, ineffective diagnostic methods, and difficult rehabilitation periods.

Vegetative vascular disorders combined with the aging, endocrine, and meta-

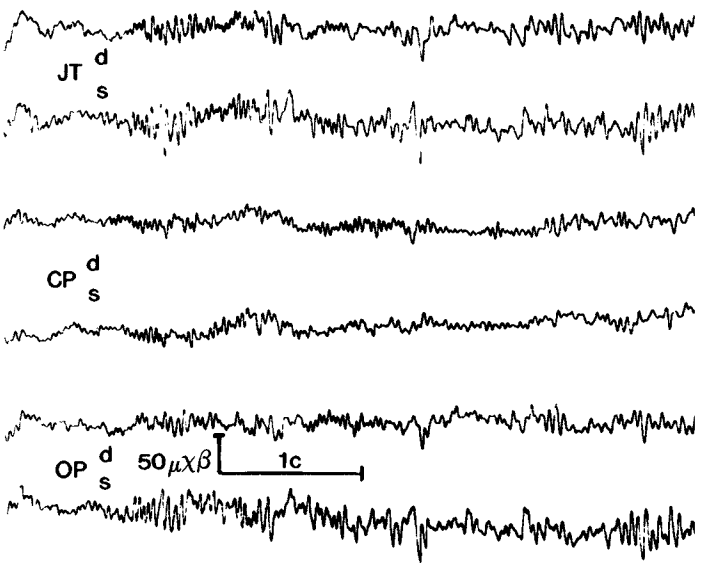

FIG. 3. EEG for carbon bisulfide intoxication. Frequent sinusoidal β waves, 18–20 waves per sec. Pointed waves. "Irritative" EEG type.

bolic changes caused by long-term effects are difficult to diagnose due to nonspecific neurosis-like states.

DISCUSSION

Long-term prospective analysis of patients who had suffered from neurotrophic intoxications made it possible to define certain phenomena of pathological processes after exposure. The main stages of a disease are as follows: complete or partial health rehabilitation and stable or prospective development of a process. The process correlates with dose–time relationship parameters and physical and chemical properties of a substance (target organs are CNS and cerebral disorders). The most important stages here are the subsequent stages of treatment, work challenge, combination of intoxication with concurrent general disease, etc.

Specific to the expressed neurotrophic intoxication is the impossibility of complete health rehabilitation even though the contact with the toxic substance has been eliminated. We have been observing expressed neurosis-like vegetative–vascular and endocrine disorders in such patients for many years.

Studies of deep trunk–hypothalamus cerebral centers conducted at in-patient occupational centers are of the utmost importance because cerebral centers are responsible for central regulatory mechanisms that provide the homeostasis and compensatory–adaptative functions of the body. When the hypothalamic syndrome was studied at the encephalopathy clinic, patients showed changes in the contents of corticosteroids, catecholamines, thyroid hormones and impairments in different mediators (cholinesterase, acetylcholine activity, etc). It is of note that impairment of nonspecific cerebral structures leads to emotional and psychic disorders.

CONCLUSIONS

(1) Measures to prevent ecologic catastrophies and accidents that lead to mass acute poisonings should be developed.

(2) Improvement in early diagnosis prevents severe forms of chronic neurotoxicoses.

(3) Top-priority studies of long-term effects should include:

- clinical studies of occupational acute and chronic diseases and poisonings,
- incidence studies of general morbidity in persons with long-term effects from an occupational disease,
- mortality in persons with occupational pathology.

Long-term dynamic studies are needed to solve all these problems.

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Occupation and the Prevalence of Major Depression, Alcohol, and Drug Abuse in the United States[1]

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Alcohol abuse, drug abuse, and mental illness (ADM) are major public health problems in the United States. Regier and associates (1988) have reported that of the population 18 years of age and older, 15.4% have fulfilled criteria for at least one alcohol or drug abuse or other mental disorder in the previous month and 32.2% have had at least one such problem during their lifetime. The impact of this illness burden is enormous, in terms of medical care, loss of productivity, loss of property, criminal activity, and human suffering. The economic cost of ADM disorders has been estimated to have exceeded $270 billion in 1988 in the United States. ADM morbidity costs, the value of reduced or lost productivity, are estimated to be in excess of $80 billion annually (Rice *et al.*, 1990).

Not surprisingly, programs to assist workers with ADM problems or to prevent such problems have become widespread among businesses and corporations (Warner *et al.*, 1988; Walsh and Hingson, 1985; Bromet and Parkinson, 1989). Yet, despite the magnitude of the overall ADM problem, and the increasing attempts to reduce their impact in work settings, basic data on the prevalence of ADM problems among workers in diverse occupational pursuits are still lacking (Eaton *et al.*, 1990; Bromet *et al.*, 1990).

That is the purpose of this study, to examine the prevalence of depression, alcohol abuse, and drug abuse among different occupational groups in the United States, and to assess differences in the burden of these problems among occupational categories. We focus on these ADM categories for several reasons. First, they constitute as a group the largest burden of serious ADM disorders in community (noninstitutionalized) populations (Regier *et al.*, 1988; Robins and Regier, 1991). Second, they are disorders which have received the most attention in work settings (Bromet *et al.*, 1990; Bromet and Parkinson, 1989). Third, there is considerable comorbidity along depressive, alcohol, and drug disorders (Bromet *et al.*, 1990; Helzer and Pryzbeck, 1988; Robins and Regier, 1991).

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METHODS

The ECA Program

The data presented are taken from the Wave I survey of the Epidemiologic Catchment Area (ECA) Program of the National Institute of Mental Health (Eaton and Kessler, 1985). The ECA was a five-site collaborative study of the prevalence, incidence, and associated risk factors for most categories of clinical psychopathology. The five sites were New Haven, Connecticut; Baltimore, Maryland; St. Louis, Missouri; Durham, North Carolina; and Los Angeles, California. The completed sample size was 18,572 subjects 18 years of age or older. The sample was 59% female. The modal educational level, both for the total sample and across all five sites, was 12 years of schooling. In terms of ethnic composition, the sample was 65% white, 24% black, and 8% Hispanic. Data on ADM were collected using the Diagnostic Interview Schedule (DIS), a highly structured interview schedule designed for use by trained lay interviewers to inquire about symptoms and episodes of mental disorders using diagnostic standards of the American Psychiatric Association (DSM-III, 1980). Most DSM-III disorders were covered, with the exception of personality (Axis II) disorders. The DIS was administered to a probability sample of persons residing in households at each of the five sites. One person was selected from each sampled household. For the analyses reported here, the five-site sample was restricted to those respondents 18 to 64 years of age who were last employed within 6 months prior to being interviewed (n = 8592). For further information on the design of the ECA program, see Eaton and Kessler (1985) and Eaton *et al.* (1981).

Measurement of ADM Disorders

As noted above, the focus here is on major depression, alcohol abuse, and drug abuse. As pointed out by Eaton *et al.* (1990), estimates of prevalence in psychiatric epidemiology depend on both the diagnostic criteria used and the instrument used to collect data on the criteria. Studies using the DIS methodology usually refer to estimates of psychological dysfunction as "DIS/DSM-III disorder." In this research, prevalence of DIS/DSM-III major depression, alcohol abuse, and drug abuse was estimated for four time periods: previous month, previous 6 months, previous year, and lifetime.

To qualify as a case of major depression a person had to report a spell of 2 or more weeks of sadness, accompanied by symptoms in four or more of the following eight groups: appetite, sleep, fatigue, slowing of bodily movements or of thought, feeling worthless or sinful, loss of pleasure in something usually enjoyed, difficulty concentrating, and suicidal thoughts, desires, or attempts. In keeping with DSM-III diagnostic criteria, symptoms attributable to use of alcohol, drugs, physical injury, or illness did not contribute to the diagnosis of DIS/DSM-III major depressive disorder (Weissman *et al.*, 1991).

A DIS/DSM-III diagnosis of alcohol dependence requires three out of nine symptoms persisting for at least 1 month, or recurring repeatedly over a longer period of time. The diagnosis of alcohol abuse requires persistent drinking despite recurrent social, psychological, or physical problems or drinking in physically hazardous contexts for at least 1 month or recurrently. In the ECA surveys, the symptoms that occurred most often among those receiving a DIS/DSM-III diagnosis of alcohol problems included drinking the equivalent of a fifth (750 ml) of

alcohol per day, having blackouts, suffering family complaints about drinking, and being in fights attributed to drinking. A very rare symptom for those with a diagnosis was losing a job because of drinking (Helzer, *et al.*, 1991). In these analyses, dependence and abuse were combined for analyses.

A DIS/DSM-III diagnosis of abuse of a psychoactive drug requires a pattern of pathological use and impaired functioning due to drug use. A diagnosis for dependence on a psychoactive drug requires signs of either tolerance or withdrawal. DSM-III also requires persistence of drug-related disturbance for at least 1 month (Anthony and Helzer, 1991). Drugs covered by the DIS interview include the following: (1) Cannabis (marijuana, hashish, pot, grass); (2) stimulants (amphetamines, uppers, speed); (3) sedatives (barbiturates, downers, sleeping pills, Seconal, Quaaludes, tranquilizers, Valium, Librium); (4) cocaine; (5) opioids (heroin, codeine, Demerol, morphine, Methadone, Darvon, opium); and (6) hallucinogens (LSD, mescaline, peyote, psilocybin, DMT, and PCB). As in the case of alcohol, dependence and abuse are combined for analyses.

Measurement of Occupation

Data on occupation were elicited using four standard open-ended questions concerning current (or, if not currently employed, most recent) full-time job: For whom do/did you work? What kind of business or industry is/was this? What kind of work are/were you doing? What are/were your most important activities? Answers to these questions were written out verbatim by the interviewers during the interview. Specially trained coders used these verbatim responses to categorize the job into 1 of the 502 detailed occupation categories of the 1980 census (Bureau of the Census, 1980).

Following Census Bureau procedures, these 502 detailed occupations were grouped into 13 broad occupational categories for analytic purposes. These categories and their 1980 Standard Occupational Classification codes are: (1) Executive, administrative, and managerial (codes 003–037); (2) professional specialty (codes 043–199); (3) technicians and related support (codes 203–235); (4) sales (codes 243–285); (5) administrative support, including clerical (codes 303–389); (6) household services (codes 403–407); (7) protective services (codes 413–427); (8) other services (codes 433–469); (9) farming, forestry, and fishing (codes 473–499); (10) precision production, craft, and repair (codes 503–699); (11) machine operators, assemblers, and inspectors (codes 703–799); (12) transportation and material moving (codes 803–859); and (13) handlers, equipment cleaners, helpers, and laborers (codes 863–889).

The categories classify a respondent's occupation (kind of work) as provided in the 1980 Census of Population, the Current Population Survey, and other demographic surveys conducted during the 1980s. The classification system groups similar occupations into relatively homogeneous categories (Bureau of the Census, pp. III–IV). Since, to our knowledge, this is the first study to report rates of clinical ADM disorders across the full range of occupational categories, we chose to use the 13 categories to facilitate comparisons with other types of health data tabulated by occupation. The number of cases of DIS/DSM-III disorders in the ECA study are not sufficient to sustain analyses using the specific occupations across the broad array of occupations reported.

Data Analytic Procedures

Our analytic strategy is basically descriptive. That is, we present estimated

prevalence rates (per 100) by occupational category, separately for major depression, alcohol abuse/dependence, and drug abuse/dependence. We present first crude prevalence rates, and then odds ratios, crude and adjusted, to compare prevalence ratios between occupational groups.

Sample weights are used in all our analyses to take into account the complex sample design used in the ECA survey. Sample weights were developed to reflect multistage cluster sampling procedures at four sites and systematic cluster sampling at one site, and to permit adjustment for nonresponse. Errors due to failure to complete an interview (nonresponse) were adjusted by a weight multiplied by the selection bias weight (the inverse of the selection probability) to make the interviewed sample equivalent to census figures for each site with respect to age, gender, and ethnic status. These two weights made the ECA samples representative of the population of the catchment areas from which the samples were selected. Since the selection probabilities are not equal for each sample person and the nonresponse rates vary by demographic characteristics, the sample weight varies. Therefore, all statistics presented in this paper are weighted using procedures described by Leaf *et al.*, (1991).

The odds ratios for occupational group are calculated with reference to the first group (executives). The adjusted odds ratios are derived by taking the antilog of the coefficient estimated from the logistic regression analysis of binary coded depression (alcohol or drug abuse) on occupational categories, age, gender, and educational level. The 99% confidence intervals are calculated for odds ratios. The odds ratio is significantly different from the reference group if the confidence interval does not contain 1. The reason for choosing the 99% instead of 95% confidence interval is the recognition of the design effects which tends to increase sampling variance. The design effect is not estimated directly in this study.

In our logistic regression analyses, adjustment is made for differences among occupation groups in age, gender, and education. For these analyses, the variables are coded as follows: gender (male = 0 or base; female = 1); education (0–11 years = 1; 12 = 2; 13–15 = 3; 16–17 = 0 or base); age (18–24 = 0 or base; 25–34 = 1; 35–44 = 2; 45–54 = 3; 55–64 = 4).

RESULTS

Crude prevalence rates for major depression are presented in Table 1. As can be seen, the rates are higher among women and those under 45 years of age, consistent with the results of other research on these factors, for all four measures of period prevalence. The pattern for education is unclear; only in the case of lifetime prevalence is there a linear trend, i.e., higher education with higher depression. Overall, the lifetime prevalence of major depression was 6.2%, the 1-month prevalence was 2.2%. The occupational group with consistently the highest prevalence was private household service workers (Job 6). Other occupational groups with high rates across most prevalence measures were professional specialties (Job 2), sales (Job 4), administrative support including clerical (Job 5), machine operators/assemblers/inspectors (Job 11), and other service workers (Job 8). Three occupational groups had lifetime rates clearly above the remainder: professional specialty workers, administrative support and clerical workers, and household service workers.

Crude prevalence rates for alcohol abuse/dependence are presented in Table 2. Two things stand out in this table: the high lifetime rates in a number of occupa-

TABLE 1
PREVALENCE (PER 100) OF MAJOR DEPRESSION

Variable	N	Prevalence			
		1 month	6 months	12 months	Lifetime
All	8592	2.2	2.9	3.5	6.2
Male	4470	1.6	2.0	2.2	3.8
Female	4122	3.0	4.3	5.2	9.5
Age					
18–24	1444	2.8	3.7	4.2	5.6
25–34	3044	2.6	3.6	4.3	7.9
35–44	1820	2.3	3.0	3.7	8.3
45–54	1113	1.7	1.9	2.1	3.9
55–64	1171	1.2	1.5	2.0	3.0
Education					
<12 years	2212	2.1	2.7	3.1	4.6
12	2614	2.3	3.0	3.5	5.6
13–15	1891	2.8	3.5	4.2	7.6
16+	1948	1.6	2.6	3.2	7.3
Job					
1 (Executive)	935	1.8	2.4	2.8	6.6
2 (Professional)	1212	1.9	3.0	4.1	8.6
3 (Technicians)	352	1.2	1.4	2.2	4.8
4 (Sales)	641	3.2	3.8	4.1	6.0
5 (Administrative Support)	1592	2.7	3.6	4.5	8.6
6 (Household Services)	99	5.3	7.0	7.0	8.3
7 (Protective Services)	144	.5	.5	.5	.7
8 (Other Services)	893	2.8	3.8	4.3	6.5
9 (Farming)	132	2.5	2.5	4.3	5.6
10 (Production)	977	1.2	1.7	1.7	3.1
11 (Operators)	941	3.0	3.6	4.0	6.0
12 (Transportation)	296	2.2	2.4	2.8	3.1
13 (Laborers)	377	1.9	2.6	2.6	4.0

tional groups and the consistently high rates across the four prevalence periods for four occupational groups. Illustrating the former pattern, 9 of the 13 groups have lifetime rates in excess of 10 per 100. Five of these groups have a one-in-five chance or better of having a diagnosable problem with alcohol use sometime during their lifetime. Four occupational groups—farming/forestry/fishing, production/craft/repair, transportation/material moving, and handlers/cleaners/helpers/laborer—have the highest rates across all four prevalence measures. In regard to gender and age, the usual patterns are observed, i.e., higher rates for males and for those under 45 years of age. There is an inverse relationship with education, i.e., the higher the educational level, the lower the prevalence of alcohol abuse/dependence.

Crude prevalence rates for drug abuse/dependence are presented in Table 3. In terms of age and gender, the usual patterns are observed. That is, the rates are much higher for males and for those under 35 years of age, and particularly high among those under 25. In the case of education, there is a consistent curvilinear relationship. The rates are low for those with less than a high school education, higher for those who graduated from high school and for those with some college, and then lower for those who have completed 4 years or more of college. Three occupational groups had higher current rates of drug abuse: production, opera-

TABLE 2
PREVALENCE (PER 100) OF ALCOHOL ABUSE/DEPENDENCE

Variable	N	Prevalence			
		1 month	6 months	12 months	Lifetime
All	8571	3.5	5.8	7.2	16.0
Male	4458	5.3	8.8	11.0	23.9
Female	4113	1.0	1.5	2.0	5.1
Age					
18–24	1443	4.2	7.4	10.2	15.9
25–34	3039	3.8	6.4	8.0	17.6
35–44	1816	4.0	6.1	7.3	18.5
45–54	1113	2.9	4.6	5.1	14.7
55–64	1160	1.6	2.7	3.4	10.3
Education					
<12 years	2202	5.2	8.7	10.7	21.3
12	2609	3.8	5.8	7.4	15.8
13–15	1890	2.9	5.3	6.7	15.4
16+	1943	1.7	3.1	3.9	10.8
Job					
1 (Executive)	933	3.3	4.6	5.7	13.6
2 (Professional)	1206	1.1	2.2	2.8	8.9
3 (Technicians)	352	2.3	3.6	4.2	9.7
4 (Sales)	639	4.1	7.3	8.0	16.2
5 (Administrative Support)	1590	1.6	2.8	4.2	9.8
6 (Household Services)	98	1.3	2.0	2.0	7.5
7 (Protective Services)	144	1.1	3.2	5.2	19.0
8 (Other Services)	893	2.9	4.8	6.6	14.1
9 (Farming)	132	4.9	8.1	10.1	24.1
10 (Production)	975	6.1	10.6	12.7	26.3
11 (Operators)	937	4.4	6.0	8.1	17.8
12 (Transportation)	295	8.6	15.0	17.6	32.6
13 (Laborers)	376	6.4	10.5	13.6	26.0

tors, and laborers. Four groups had much higher lifetime rates: production, laborers, transportation, and other services.

The next stage of our analyses involved estimating the risk of the three ADM disorders in terms of 6-month and lifetime prevalence rates using the odds ratio. We first estimated the unadjusted odds ratio, and then estimated it adjusting for the effects of age, gender, and level of education. In each contrast, the risk of disorder was estimated relative to Job 1 (the occupational category composed of executive/administrative/managerial workers).

For major depression, those occupations with the highest relative risk were Job 6 (household service), Job 8 (other service), Job 4 (sales), Job 5 (administrative support), and Job 11 (operators), all with risks 50% or more above Job 1 (data not shown). Those occupations with lowest relative risks were Job 7 (protective services), Job 3 (technicians), and Job 10 (production). Highest risks for episodes of alcohol abuse/dependence in the previous 6 months were among Job 12 (transportation), Job 10 (production), and Job 13 (farming). Lowest risk occupations were Job 6 (household service) and Job 2 (professional). Highest risks for drug abuse/dependence were for Jobs 9–13, all with odds ratios in excess of 2. By contrast lowest risks were Jobs 3, 6, 7, and 2.

In terms of crude lifetime risk of major depression, three occupational groups

TABLE 3
PREVALENCE (PER 100) OF DRUG ABUSE/DEPENDENCE

Variable	N	Prevalence			
		1 month	6 months	12 months	Lifetime
All	8558	1.6	2.6	3.1	7.6
Male	4450	2.2	3.3	3.9	8.4
Female	4108	.8	1.6	2.0	6.3
Age					
18–24	1435	3.8	6.8	8.0	13.4
25–34	3028	2.0	3.0	3.7	11.8
35–44	1814	.9	1.2	1.4	5.1
45–54	1113	.2	.2	.4	1.0
55–64	1168	0.0	0.0	0.0	.3
Education					
<12 years	2200	1.6	2.5	2.9	5.2
12	2609	2.3	2.9	3.4	7.1
13–15	1884	1.7	3.5	4.2	10.9
16+	1937	.9	1.3	1.8	7.1
Job					
1 (Executive)	929	1.4	1.6	2.0	6.7
2 (Professional)	1209	.7	1.3	1.7	6.8
3 (Technicians)	351	1.1	1.1	1.1	6.2
4 (Sales)	638	1.4	2.6	3.4	7.0
5 (Administrative Support)	1582	1.4	2.3	2.7	7.5
6 (Household Services)	99	1.3	1.3	1.3	4.2
7 (Protective Services)	143	.8	1.3	2.1	3.1
8 (Other Services)	893	1.5	2.5	2.7	8.3
9 (Farming)	132	1.5	6.0	6.0	7.6
10 (Production)	974	2.5	4.7	5.9	10.2
11 (Operators)	940	2.6	3.3	4.1	6.3
12 (Transportation)	294	1.7	3.3	3.8	9.3
13 (Laborers)	373	2.6	3.3	3.3	9.3

were higher than Job 1 (executives): Jobs 2, 5, and 6, each with about a 30% greater risk (data not shown). Lowest risks for lifetime episodes of major depression were for Jobs 7, 12, and 13, with less than half the risk of Job 1. For lifetime risk of alcohol abuse/dependence, the highest odds ratios were for Jobs 12, 10, 13, and 9, in that order, all with O.R. greater than 2.0. For the lowest risks, the occupations were Jobs 6, 2, 3, and 4, with risks only 50–60% that of Job 1. For drug abuse/dependence, the highest odds ratios were for Jobs 10, 12, and 13, with risks 40% or more about Job 1. The lowest risk, on a lifetime basis, were for Jobs 6 and 7, with odds ratios 40–60% below that of Job 1.

The adjusted odds ratios are presented in Table 4 for 6-month prevalence. As can be seen, there are significant differences among occupational groups. For major depression, there were three occupational groups with risks from 38 to 140% higher than the baseline group, Job 1: Job 6 (2.40), Job 11 (1.41), and Job 4 (1.38). However, all of the differences among occupational groups were statistically significant ($P < 0.01$), whether lower or higher than the baseline group. For alcohol abuse/dependence, the adjusted odds ratios for 6-month prevalence were quite elevated for Job 12 (1.96), Job 4 (1.67), Job 13 (1.44), and Job 10 (1.41), all at least 40% higher than Job 1. After adjustment, three of the 6-month prevalences

TABLE 4

ODDS RATIOS FOR 6-MONTH PREVALENCE OF MAJOR DEPRESSION, ALCOHOL, AND DRUG ABUSE/DEPENDENCE, ADJUSTED FOR AGE, GENDER, AND EDUCATION, BY OCCUPATIONAL GROUP

Occupational group	Coefficient	Odds ratio	CI
	Major depression		
1 (Executive)	—	—	—
2 (Professional)	−0.08	1.09	(1.02, 1.14)
3 (Technicians)	0.80	0.44	(0.41, 0.50)
4 (Sales)	−0.32	1.38	(1.32, 1.45)
5 (Adm. support)	−0.08	1.08	(1.04, 1.13)
6 (Household serv.)	−0.88	2.40	(2.18, 2.64)
7 (Protective serv.)	1.40	0.25	(0.20, 0.31)
8 (Other services)	−0.25	1.28	(1.22, 1.34)
9 (Farming)	−0.19	1.21	(1.09, 1.35)
10 (Production)	0.21	0.81	(0.77, 0.86)
11 (Operators)	−0.34	1.41	(1.35, 1.48)
12 (Transportation)	−0.18	1.20	(1.10, 1.30)
13 (Laborers)	−0.10	1.11	(1.04, 1.20)
	Alcohol abuse/dependence		
1 (Executive)	—	—	—
2 (Professional)	0.42	0.66	(0.62, 0.69)
3 (Technicians)	0.18	0.83	(0.78, 0.89)
4 (Sales)	−0.52	1.67	(1.61, 1.74)
5 (Adm. support)	0.14	0.87	(0.84, 0.91)
6 (Household serv.)	0.37	0.69	(0.59, 0.82)
7 (Protective serv.)	0.77	0.46	(0.42, 0.51)
8 (Other services)	−0.05	1.05	(1.01, 1.09)
9 (Farming)	0.08	0.93	(0.87, 0.99)
10 (Production)	−0.34	1.41	(1.37, 1.45)
11 (Operators)	0.01	0.99[a]	(0.96, 1.03)
12 (Transportation)	−0.67	1.96	(1.88, 2.03)
13 (Laborers)	−0.36	1.44	(1.38, 1.49)
	Drug abuse/dependence		
1 (Executive)	—	—	—
2 (Professional)	0.10	0.91	(0.85, 0.97)
3 (Technicians)	0.90	0.40	(0.36, 0.45)
4 (Sales)	−0.06	1.06	(1.00, 1.12)
5 (Adm. support)	−0.07	1.07	(1.02, 1.12)
6 (Household serv.)	0.17	0.84[a]	(0.69, 1.04)
7 (Protective serv.)	0.58	0.56	(0.48, 0.65)
8 (Other services)	0.00	1.00[a]	(0.94, 1.05)
9 (Farming)	−0.53	1.70	(1.57, 1.83)
10 (Production)	−0.60	1.83	(1.75, 1.91)
11 (Operators)	−0.42	1.52	(1.45, 1.59)
12 (Transportation)	−0.17	1.19	(1.10, 1.27)
13 (Laborers)	0.05	0.95[a]	(0.89, 1.01)

[a] Not significantly different from 1 (confidence interval includes 1).

for drug abuse remained 50–80% higher than baseline: Job 10 (1.83); Job 9 (1.70), and Job 11 (1.52).

Adjusted odds ratios for lifetime prevalence are presented in Table 5. After adjustment, the lifetime prevalence for major depression remained 35 to 67% higher than baseline (Job 1) for Job 9 (1.67), Job 6 (1.50), Job 2 (1.40), and Job 5 (1.35). For lifetime prevalence of alcohol abuse/dependence, the adjusted odds

TABLE 5

ODDS RATIOS FOR LIFETIME PREVALENCE OF MAJOR DEPRESSION, ALCOHOL, AND DRUG ABUSE/DEPENDENCE, ADJUSTED FOR AGE, GENDER, AND EDUCATION, BY OCCUPATIONAL GROUP

Occupational group	Coefficient	Odds ratio	CI
Major depression			
1 (Executive)	—	—	—
2 (Professional)	−0.34	1.40	(1.35, 1.45)
3 (Technicians)	0.27	0.77	(0.72, 0.81)
4 (Sales)	−0.13	1.14	(1.09, 1.18)
5 (Adm. support)	−0.30	1.35	(1.31, 1.39)
6 (Household serv.)	−0.41	1.50	(1.38, 1.63)
7 (Protective serv.)	1.71	0.18	(0.15, 0.22)
8 (Other services)	−0.16	1.17	(1.13, 1.21)
9 (Farming)	−0.51	1.67	(1.55, 1.80)
10 (Production)	0.19	0.83	(0.79, 0.86)
11 (Operators)	−0.23	1.26	(1.22, 1.31)
12 (Transportation)	0.15	0.86	(0.81, 0.93)
13 (Laborers)	0.03	0.97[a]	(0.92, 1.02)
	Alcohol abuse/dependence		
1 (Executive)	—	—	—
2 (Professional)	0.37	0.69	(0.68, 0.71)
3 (Technicians)	0.43	0.65	(0.63, 0.68)
4 (Sales)	−0.15	1.16	(1.14, 1.19)
5 (Adm. support)	0.06	0.94	(0.92, 0.96)
6 (Household serv.)	0.38	0.87	(0.80, 0.95)
7 (Protective serv.)	0.11	0.89	(0.85, 0.93)
8 (Other services)	−0.10	1.10	(1.08, 1.13)
9 (Farming)	−0.07	1.07	(1.03, 1.12)
10 (Production)	−0.20	1.22	(1.20, 1.24)
11 (Operators)	−0.03	1.04	(1.01, 1.06)
12 (Transportation)	−0.44	1.55	(1.51, 1.60)
13 (Laborers)	−0.29	1.33	(1.30, 1.37)
	Drug abuse/dependence		
1 (Executive)	—	—	—
2 (Professional)	0.04	0.96	(0.93, 0.99)
3 (Technicians)	0.40	0.67	(0.64, 0.70)
4 (Sales)	0.05	0.95	(0.92, 0.99)
5 (Adm. support)	−0.07	1.07	(1.04, 1.10)
6 (Household serv.)	0.02	0.98[a]	(0.87, 1.10)
7 (Protective serv.)	0.87	0.42	(0.38, 0.46)
8 (Other services)	−0.27	1.31	(1.27, 1.35)
9 (Farming)	0.03	0.97[a]	(0.91, 1.03)
10 (Production)	−0.43	1.54	(1.50, 1.58)
11 (Operators)	−0.05	1.05	(1.02, 1.09)
12 (Transportation)	−0.33	1.40	(1.33, 1.46)
13 (Laborers)	−0.20	1.22	(1.17, 1.27)

[a] Not significantly different from 1 (confidence interval includes 1).

ratios were significantly different from baseline for every occupational group, lower or higher. For two occupational groups, risks were 33%, (Job 13, 1.33) and 55% (Job 12, 1.55) higher than baseline. For drug abuse/dependence, there were three occupational groups with adjusted odds ratios more than 30% above baseline: Job 10 (1.54), Job 12 (1.40), and Job 8 (1.31).

SUMMARY AND CONCLUSIONS

These data from the ECA research program clearly indicate considerable variation in prevalence among different occupational groups in the United States for major depression, alcohol abuse/dependence, and drug abuse/dependence. For example, the crude lifetime prevalences for depression ranged from 0.7 to 8.6 per 100; for alcohol it was 7.5 to 32.6, and for drugs it was 3.1 to 10.5. The results also indicate that some occupations are associated with much higher rates of ADM problems. By way of illustration, the 6-month crude prevalence of major depression for the total sample of employed persons 18–64 years of age was 2.9. Six of the occupational groups had prevalences which exceeded this rate. The overall 6-month prevalence of alcohol abuse/dependence was 5.8, and six occupational groups had prevalences in excess of this. Similarly, the overall 6-month prevalence was 2.6 for drug abuse/dependence, and five occupational groups had prevalences which exceeded this.

Translated into terms of relative risk, it is clear that among the occupational groups some are at markedly increased risk of ADM disorders. For these comparisons, we used as the baseline group in logistic regression analyses, Job 1, composed of executive, administrative, and managerial occupations. This group is the first in the Census classification, had crude rates near the overall prevalence rates for depression, alcohol, and drugs, and is also one of the occupational categories with the highest prestige. Logistic regression analyses were done with and without controls for differences among occupational groups in gender, age, and educational level. As might be expected, adjustment in general narrowed differences in prevalence among occupational groups, and even on occasion, changed the rank order of groups slightly. However, in general, those groups with higher odds ratios based on crude rates also had higher adjusted odds ratios. Table 6 summarizes our findings, based on the adjusted odds ratios. In this table, we list occupational groups which had a relative risk of 30% or greater above baseline (Job 1), for both 6 month and lifetime prevalences, for each of the three ADM disorders. As can be seen, there is virtually no overlap between risk of depression, on the one hand, and risk of alcohol or drug abuse. The exceptions are Job 4 (sales) which ranked third for major depression and second for alcohol abuse in terms of 6-month prevalence, and Job 9 (farming, fishing, forestry), which had the

TABLE 6
OCCUPATIONS WITH ADJUSTED ODDS RATIOS OF 1.30 OR GREATER, 6-MONTH AND LIFETIME PREVALENCE

	6-month	Lifetime
Major depression	Job 6 (2.40)	Job 9 (1.67)
	Job 11 (1.41)	Job 6 (1.50)
	Job 4 (1.38)	Job 2 (1.40)
		Job 5 (1.35)
Alcohol abuse	Job 12 (1.96)	Job 12 (1.55)
	Job 4 (1.67)	Job 13 (1.33)
	Job 13 (1.44)	
	Job 10 (1.41)	
Drug abuse	Job 10 (1.83)	Job 10 (1.54)
	Job 9 (1.70)	Job 12 (1.40)
	Job 11 (1.52)	Job 8 (1.31)

highest lifetime risk for major depression and the second-highest 6-month risk for drug abuse/dependence. The distribution of risk across occupational categories also is attested to by the fact that 10 of the 13 groups had an elevated risk for at least one of the three ADM disorders on either 6-month or lifetime prevalence.

There are other patterns clear from Table 6 as well. Job 6 (household service workers) is at higher risk in terms of more proximal (6 months) as well as more distal (lifetime) risk for episodes of major depression. Likewise, workers in Job 12 (transportation and material moving) are at highest risk for alcohol abuse on both 6-month and lifetime prevalence. Job 10 (production, craft, and repair) has the highest odds ratios for drug abuse on both 6-month and lifetime prevalence.

By the same token, the results also indicate three occupational groups are at very low risk of the three ADM disorders. Job 7 (protective service) has the lowest odds ratios, 6-month (0.25) and lifetime (0.18), for major depression, followed by Job 3 (technicians and related), 0.44 6-month and 0.77 lifetime. Job 3 also has the lowest odds ratios, 6-month (0.40) and lifetime (0.67), for drug abuse, and Job 7 is second for 6-month prevalence of drugs (0.56). Job 7 has the lowest 6-month odds ratio for alcohol (0.46) and Job 3 the lowest lifetime odds ratio (0.65). Job 2 (professional specialty) has the next to lowest odds ratios for both 6-month (0.66) and lifetime (0.69) prevalences for alcohol abuse.

To our knowledge, this is the first study which has used the ECA data to examine the prevalence of major depression, alcohol abuse/dependence, and drug abuse/dependence among the major occupational categories used by the Bureau of the Census. A recent paper by Eaton *et al.* (1990) focused only on depression, and this in relation to specific types of jobs, using the ECA data. Other analyses of the ECA data (see Robins and Regier, 1991) have generally focused on employment or socioeconomic status as correlates of psychiatric disorder. The exception is one table in Helzer *et al.*, (1991), which examined 1-year prevalence of alcoholism by six broad occupational categories. They report that the lowest prevalence of alcohol abuse for males was among managers/professionals (6.0) and the highest prevalence was among skilled (14.8) and unskilled (13.9) laborers. Among women, the lowest prevalence was among farm/rural (0.0) and the highest was among service occupations (3.4).

In the only other study, to our knowledge, that has attempted to estimate the prevalence of ADM clinical psychiatric disorders in the work force, Bromet *et al.* (1990) report 1-year prevalences of major depression of 8.6 (per 100) for male and 16.6 for female managerial and professional workers; lifetime prevalences were 22.9 and 36.0, respectively. These rates are much higher than our rates using the ECA data for Job 1 (executive, administrative, and managerial) and Job 2 (professional), the categories which correspond most closely to the Bromet sample. In contrast, the 1-year prevalences for alcohol abuse were 4.1 for males and 3.5 for females, in contrast to 5.7 and 2.8 for Job 1 and Job 2 in the ECA data. It is difficult to reconcile the discrepancy between the rates of depression in the Bromet and ECA data sets, although there are a number of possibilities. From a measurement perspective, the ECA used the DIS and DSM-III criteria. Bromet and her colleagues used the SCID and DSM-III-R criteria (Spitzer *et al.*, First, 1987). The Bromet sample was selected from one corporation; the ECA sample was selected from five diverse communities. The effects of these differences are unknown.

From a planning and intervention perspective, the data provide strong support

for the development of work site programs designed to assist employees with psychiatric and substance abuse problems (Warner *et al.*, 1988; Walsh and Hingson, 1985; Bromet and Parkinson, 1989). Based on analyses of the ECA data, we can expect about 3.5% of workers to have experienced an episode of major depression during the previous 12 months (the modal rate was 4–5%). For alcohol problems, the figure is 7.2% (a number of occupational groups had rates in excess of 10%). For drug problems, about 3.1% of workers had an episode in the past 12 months, and in some job groups the rate is 5 or 6%. If these rates are projected across the United States working population, the sheer numbers of workers involved is staggering. As noted earlier, this illness burden (for ADM disorders) is costing Americans over $80 billion annually in lost productivity alone.

More resources clearly need to be directed at reducing this illness burden, particularly resources to increase early detection and intervention as well as to prevent the occurrence of ADM problems. In addition, we need more epidemiologic research to identify risk factors in particular jobs or occupational groups which increase vulnerability to ADM problems, and to suggest intervention strategies.

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Work Stress in Japanese Computer Engineers: Effects of Computer Work or Bioeducational Factors?[1]

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To examine whether computer work and bioeducational factors (age and school career) have significant effects on work stress in computer engineers in Japan, we administered a stress questionnaire to 764 male computer engineers and 211 male office workers in a computer-manufacturing factory in Tokyo. Four scales of perceived psychological stress at work examined were work overload, poor human relationships at work, unsuitable job, and competition–dismissal anxiety. The results of the three-way analysis of variance, in which age (20–29, 30–39, and 40–49 years), school career (high school and university graduates), and computer work (computer engineers and office workers) were three variation factors, indicated that: (1) there were no significant differences in all scores of work stress between computer engineers and office workers ($P > 0.05$); (2) scores for unsuitable job and poor human relationships at work were significantly higher in high school graduates than in university graduates ($P < 0.05$); and (3) there were significant age differences in scores for three scales of work stress (unsuitable job, competition–dismissal anxiety, and work overload: $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively). These findings suggest that computer work has no significant effect on perceived work stress in computer engineers; on the other hand, age and school career do have effects.

INTRODUCTION

Much attention has been paid to mental health of computer engineers since Brod proposed the concept of technostress in 1984 (Brod, 1984). Technostress is defined as the stress and concomitant psychosomatic disorder induced by the introduction of high computer technology. It has been argued that the mental state of workers may be changed by computer work. Subjective symptoms such as depression and anxiety have been attributed to computer operation in computer engineers and office workers using visual display terminals (Watanabe, 1986; Mino *et al.*, 1989; Hayashi and Kosugo, 1987; Shoji *et al.*, 1990a; Noda, 1987; Uchiyama and Noda, 1990). In one study, Shoji *et al.* classified occupational stressors of software engineers ascertained in the interview into "quantity of job," "quality of job," "role in organization," "relations with others," "work conditions," "career development," "reward," "organizational structure and climate," "low social support," and "lack of decision making" and reported that quality of job (35%), quantity of job (30%), and role in organization (33%) were the most common stressors for the subjects and that among the occupational stress-

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ors, only quantity of job was significantly related to psychiatric disorders (Shoji *et al.*, 1990b). The results of this study are difficult to interpret, since there was not a control group. Few epidemiologically well-documented studies have been reported on the effects of computer work on work stress computer engineers. On the other hand, despite the importance of bioeducational factors (e.g., age and school career) as determinants in reaction to work stress, their relations to work stress in computer engineers have not been sufficiently understood.

In the present study, to examine whether computer work and bioeducational factors (age and school career) have significant effects on work stress in computer engineers in Japan, we administered a stress questionnaire to computer engineers and office workers.

SUBJECTS AND METHODS

Subjects

The 975 study subjects consisted of a group of 764 male computer engineers and a group of 211 male office workers (control subjects) employed at a computer-manufacturing factory with 2083 full-time employees in a western suburb of Tokyo. In 1989, 2078 employees from this factory (99.8%) were surveyed using a self-administered questionnaire; further analyses focused on the 895 male computer engineers and 239 male office workers, aged 20 to 49 years, who graduated from high schools or universities, including postgraduate courses. The questionnaires were completed for all items in the 764 male computer engineers (85.4%) and in the 211 male office workers (88.3%); further studies were conducted for these workers (Table 1). The mean (±SD) ages of the subjects were as follows: computer engineers graduated from high schools, 33.2 ± 7.2 years; computer engineers graduated from universities, 30.8 ± 6.5 years; office workers graduated from high schools, 37.4 ± 7.4 years; and office workers graduated from universities, 36.6 ± 8.6 years. Computer engineers were composed of 56 computer architects, 407 software engineers, and 318 hardware engineers. The computer architects developed and administered the operating system of computers; the software engineers engaged in the planning of computer systems, design of programs, coding, debugging, etc.; and the hardware engineers designed systems and structures of computers, produced equipments made on an experimental basis, and standardized computers. These three kinds of job overlapped one another. Office workers included managerial and clerical workers. They engaged in control of corporate accounts, personal management, and administration of the factory.

TABLE 1
STUDY POPULATIONS

Age groups (years)	Computer engineers (764)		Office workers (211)	
	High school graduates (278)	University graduates (486)	High school graduates (157)	University graduates (54)
20–29	76	275	25	18
30–39	161	137	71	11
40–49	41	74	61	25

Note. Numbers in parentheses indicate total.

Work Stress

Cooper and Davidson classified stressor variables in the work arena into (1) factors intrinsic to the job (work overload, physical danger, etc.), (2) role in the organization (role ambiguity, responsibility for people, etc.), (3) career development, (4) relationships/social support, and (5) organizational structure and climate (Cooper and Davidson, 1987). Since there was no complete standardized scale of the work stress measure in Japan, we developed an original questionnaire of work stress with reference to Cooper and Davidsons' concept.

Fifteen items of psychosocial stressors at work were assessed by means of a questionnaire. They were scored using a Likert-type scoring of 1–2–3–4 for the response categories; the scores for each item were transformed so that higher scores indicated higher perceived stress. In order to obtain empirically derived measures for this sample, these items were subjected to principal components factor analysis with varimax rotation. The factor loadings of the 15 variables after rotation of common factors extracted are shown in Table 2. Four common factors were extracted for these variables in computer engineers and office workers. On the basis of these four factors extracted, the 15 variables were classified into four groups, i.e., work overload (6 items: first 6 listed in Table 2), poor human relationships at work (4 items: 7–10th listed in Table 2), unsuitable job (3 items: 11–13th listed), and competition–dismissal anxiety (2 items: last 2 listed). A total score of the items classified was used as a measure of corresponding work stress. The internal consistency of the four work stress factors was not good; the α for the four scales were as follows: 0.80 in work overload, 0.73 in poor human relationships at work, 0.44 in unsuitable job, and 0.38 in competition–dismissal anxiety.

TABLE 2

FACTOR LOADINGS OF 15 VARIABLES OF WORK STRESS FOR FOUR COMMON FACTORS (FC1, FC2, FC3, AND FC4)[a] EXTRACTED BY FACTOR ANALYSIS IN 764 MALE COMPUTER ENGINEERS AND 211 MALE OFFICE WORKERS

Work stress	FC1	FC2	FC3	FC4
A large quantity of work	0.810	0.010	0.098	−0.057
Heavy responsibility for work	0.815	0.031	−0.097	−0.013
Severe checks on time and amount of work	0.503	−0.105	0.372	0.273
Severity in the time limit of work	0.745	−0.052	0.137	0.090
Wide sphere of work	0.649	0.003	−0.228	0.021
Complex job content and procedure	0.633	0.053	−0.083	0.047
Poor relationships with supervisors	0.008	0.680	0.320	−0.017
Poor relationships with colleagues	−0.032	0.795	−0.054	0.064
Poor relationships with subordinates	0.006	0.770	−0.035	0.060
Uncomfortable atmosphere in the workplace	0.053	0.665	0.229	0.111
Limited worker control of job demands	0.214	−0.013	0.727	0.006
Role ambiguity on the work	−0.333	0.188	0.568	−0.035
Feelings of being unfit for one's work	−0.127	0.292	0.518	−0.039
Keen competition with colleagues at work	0.193	0.078	−0.188	0.743
Fear of dismissal	−0.087	0.114	0.130	0.782

[a] FC1, FC2, FC3, and FC4 represent the first, second, third, and fourth factors, respectively. Eigenvalues were 3.2, 2.6, 1.4, and 1.1 for FC1, FC2, FC3 and FC4, respectively. Cumulative proportions were 0.22, 0.39, 0.48, and 0.55 up to the first, second, third, and fourth factors, respectively.

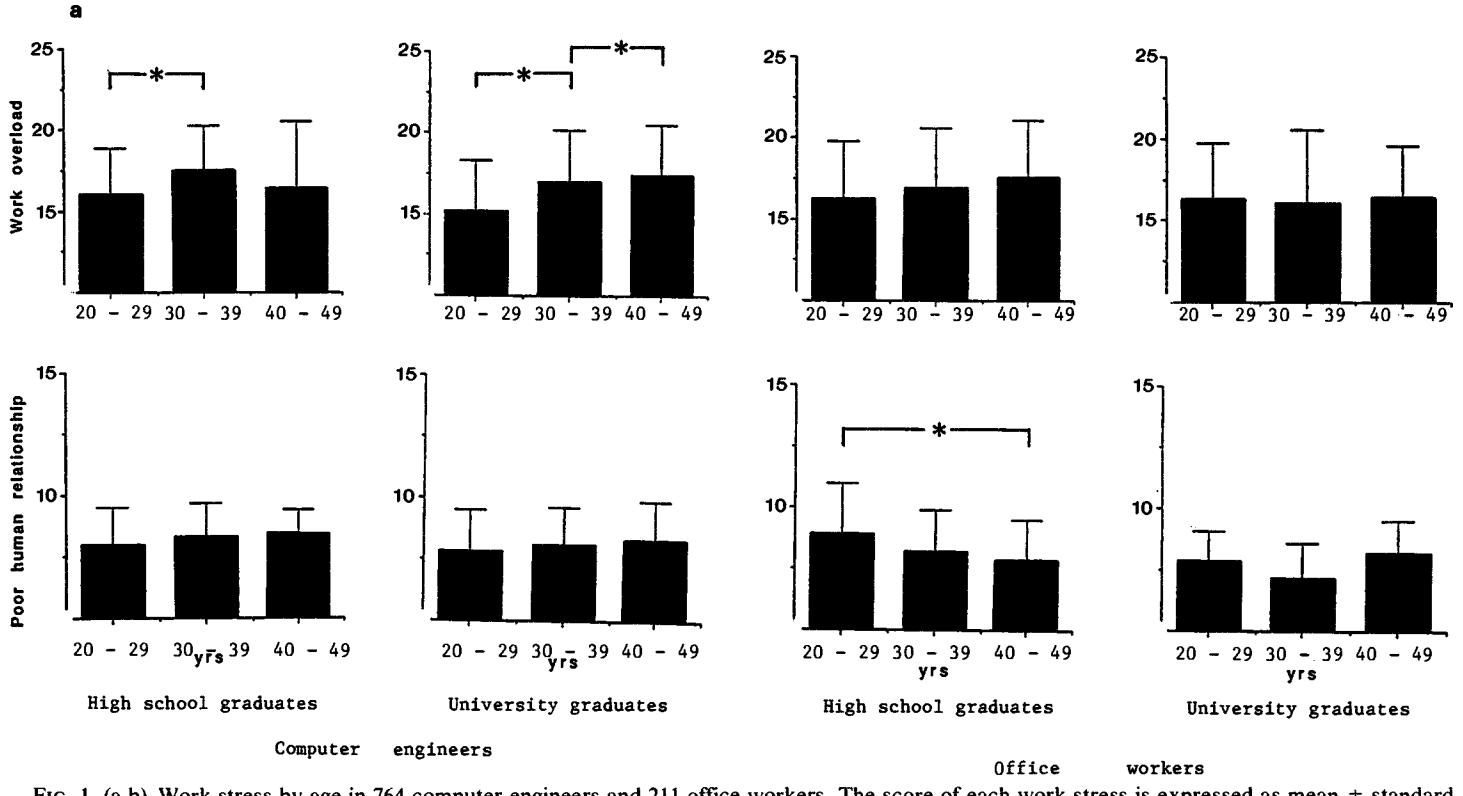

FIG. 1. (a,b) Work stress by age in 764 computer engineers and 211 office workers. The score of each work stress is expressed as mean ± standard deviation. $^*P < 0.05$ (Scheffe's multiple comparison test).

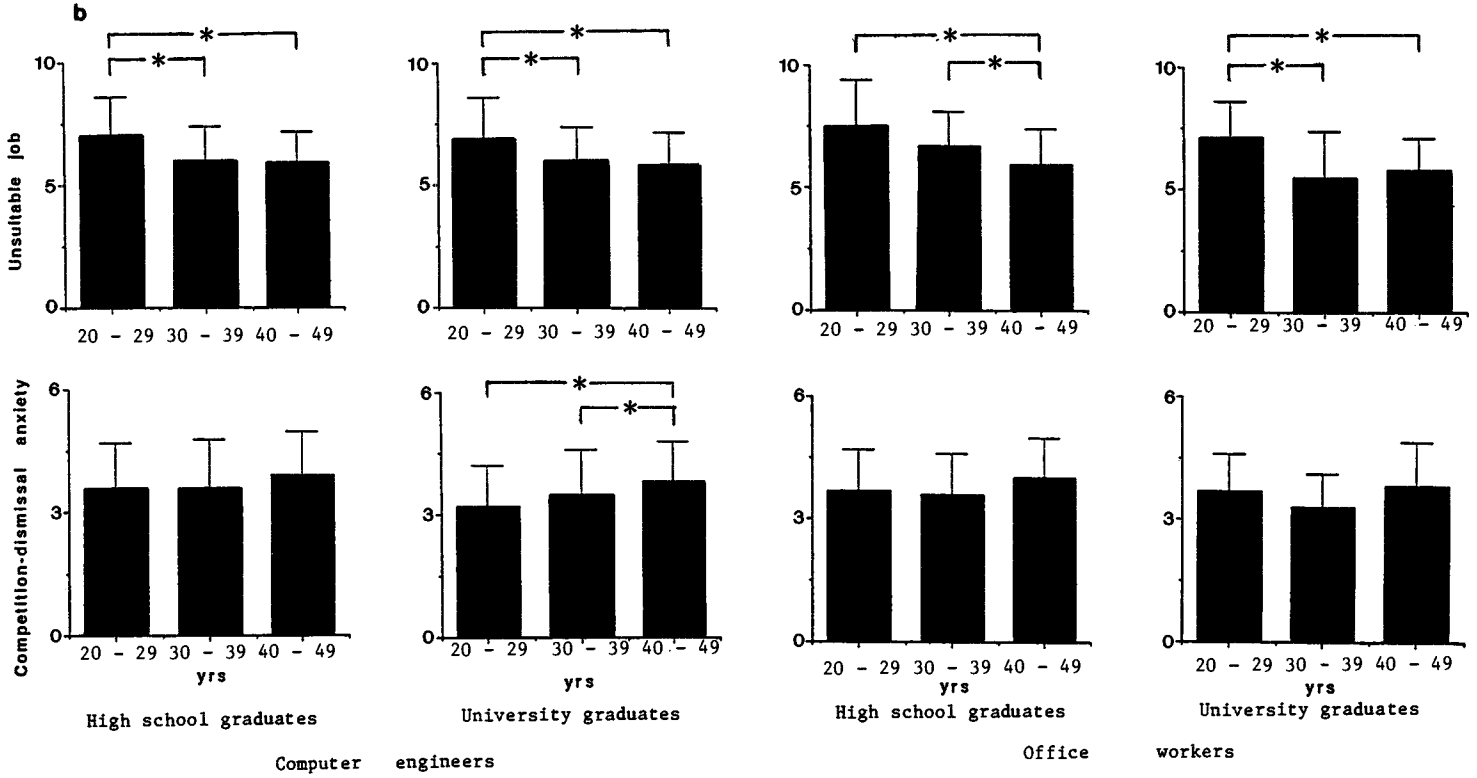

FIG. 1—*Continued*

Data Analysis

To examine relative strength of the effects of age (20–29, 30–39, and 40–49 years), school career (high school and university graduates), and computer work (computer engineers and office workers) on perceived psychological stress at work (work stress), the three-way analysis of variance (3-ANOVA) was carried out for each value of the work stress and the adjusted means, controlling for the effects of computer work, age, or school career, were also estimated. Furthermore, differences in the work stress among three age groups (20–29, 30–39, and 40–49 years) were analyzed by Scheffe's multiple comparison test in high school and university graduates of computer engineers and office workers, respectively.

These analyses were conducted using the SPSS-X computer program (SPSS Inc., 1988) at the Computer Center of the University of Tokyo (Faculty of Medicine).

RESULTS

Scores for four scales of work stress in computer engineers and office workers are shown in Fig. 1. The score for unsuitable job decreased significantly with increasing age in both high school and university graduates of computer engineers and office workers, respectively. On the other hand, the score for work overload significantly increased with age in high school- and university-graduated computer engineers; the score for competition–dismissal anxiety also increased with age in university-graduated computer engineers.

The results of the 3-ANOVA indicated that there were significant age differences in scores for work overload, unsuitable job, and competition–dismissal anxiety (Table 3). Similarly, no significant differences were found in all scores of work stress between computer engineers and office workers ($P > 0.05$); there were significant differences in the scores for poor human relationships at work and unsuitable job between high school and university graduates (Table 4).

DISCUSSION

In the present study, the score for unsuitable job significantly decreased with increasing age in both high school- and university graduated computer engineers

TABLE 3

DIFFERENCES IN SCORES OF WORK STRESS AMONG THREE AGE GROUPS ACCORDING TO THREE-WAY ANALYSIS OF VARIANCE[a] FOR 781 COMPUTER ENGINEERS AND 214 OFFICE WORKERS (CRUDE AND ADJUSTED MEAN SCORES)

	20–29 years		30–39 years		40–49 years		
Work stress	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted	*F* Value
Work overload	15.5	16.0	17.2	16.9	17.1	16.9	4.38*
Poor human relationship at work	7.9	8.2	8.2	8.0	8.2	8.2	0.90
Unsuitable job	7.0	7.1	6.1	6.0	5.8	5.8	30.56***
Competition–dismissal anxiety	3.3	3.5	3.6	3.5	3.9	3.9	6.37**

[a] Three variation factors are age (20–29, 30–39, and 40–49 years), school career (high school and university graduates), and computer work (computer engineers and office workers).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

and office workers. The higher score for unsuitable job in younger workers may reflect their psychological stress due to less experience at work and unestablished vocational identity. The older worker must have more adaptive potential, such as his greater professional experience and knowledge, sense of responsibility, and ability to resolve problems. That is why an age-related change was found in the stress due to unsuitable job.

However, another possibility should be noted, as this finding is based on cross-sectional data. The trend in unsuitable job with age could be due to selective survival of more contented workers in the jobs or a cohort phenomenon among all workers. A prospective cohort study is needed to ascertain whether this is the case.

On the other hand, the increasing scores for work overload and competition–dismissal anxiety with age in computer engineers may suggest the decrease of their competence for designing tasks and physical strength due to increasing age. Numerous investigations of work stress among older workers in the Federal Republic of Germany have also shown that, with advancing age, industrial workers complain of strain at work to an increasing extent (Hadžiolova, 1987). The computer industry, in particular, is growing so fast every year that it is more difficult for older workers to catch up with new computer knowledge; thus, they may feel more work overload and competition–dismissal anxiety.

Contrary to our expectation, there were no significant differences between computer engineers and office workers in all the scores of perceived psychological stress at work. Also in a previous study by us, a significant difference was not found between computer engineers and office workers in either psychiatric symptoms or maladaptive personality traits defined by the DSM-III-R (Ezoe *et al.*, 1992). These findings suggest that computer work itself has no significant effects on mental status in computer engineers.

In the United States, psychiatric symptoms and specific personality traits in computer engineers have been regarded as a stress reaction attributed to computer work: the syndrome seen among these persons is named techno-centered (Brod, 1984). On the other hand, only a few techno-centered computer engineers have been reported in Japan (Mitsubishi-sogo-kenkyujo, 1990) and there have been few epidemiologically well-documented studies which indicated that com-

TABLE 4
DIFFERENCES IN SCORES OF WORK STRESS BETWEEN HIGH SCHOOL AND UNIVERSITY GRADUATES ACCORDING TO THREE-WAY ANALYSIS OF VARIANCE[a] IN 764 COMPUTER ENGINEERS AND 211 OFFICE WORKERS (CRUDE AND ADJUSTED MEAN SCORES)

	High school graduates		University graduates		
Work stress	Crude	Adjusted	Crude	Adjusted	*F* Value
Work overload	17.0	16.8	16.1	16.4	1.80
Poor human relationship at work	8.2	8.3	8.0	7.9	6.24*
Unsuitable job	6.3	6.5	6.5	6.2	4.84*
Competition–dismissal anxiety	3.7	3.7	3.4	3.6	3.63

[a] Three variation factors are the same as those in Table 3.
* $P < 0.05$.

puter work itself had a significant effect on work stress in computer engineers. Thus the mental health status of computer engineers in Japan may not be so greatly influenced by computer work as that in the United States.

Another explanation for our findings of no difference in work stress between computer engineers and office workers in the present study should be considered. First, the subjects in this study were employed at a factory of one of the biggest electrical companies in Japan. Their working conditions should be better than those of computer engineers employed at smaller companies, who might perceive more psychological stress attributed to computer work. Second, the apparent lack of effects associated with computer engineering may be attributed to several methodological limitations in this study. The limitations of the present study are as follows: (1) heterogenous jobs were included in the two comparison groups (computer engineers and office workers); (2) more contented computer engineers may have survived selectively; and (3) the work stress scales we used may have lacked reliability. Thus, to examine whether computer work itself has a significant effect on work stress in computer engineers, further research should be undertaken by means of standardized scales of work stress in homogeneous comparison groups in different sizes of computer companies.

Table 4 shows that the scores for poor human relationships at work and unsuitable job were significantly higher in high school graduates than in university graduates when the effects of age and job were controlled. These findings suggest that the grade of stress due to unsuitable job and human relations might be higher in workers with less schooling. This result might reflect their role ambiguity and poorer career development, since, in general, workers graduated from high schools suffer worse working conditions than those graduated from universities in Japan. Further study is needed to clarify differences in working conditions and responsibilities associated with educational background.

CONCLUSION

The data obtained in this study suggest that computer engineering has no significant effect on perceived work stress in computer engineers; on the other hand, age and school career affect work stress.

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Relations of Work Stress to Alcohol Use and Drinking Problems in Male and Female Employees of a Computer Factory in Japan[1]

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To examine the effects of work stressors on alcohol use and drinking problems as well as a model of stress-induced drinking in Japanese male and female workers, a total of 2581 employees of a computer factory were surveyed using mailed questionnaires. Five psychosocial work stressors, overtime, rotating shift, frequency of drinking, amount of alcohol consumed per drinking occasion, and drinking problems, and depressive symptoms were assessed. The hierarchical linear and logistic regression analyses were conducted in 1043 male and 255 female current drinkers aged 20 years or older. The results suggested that overtime and lack of intrinsic work rewards are main factors for heavy and problem drinking in Japanese male workers and that ambiguity about job future is a factor for heavy drinking in Japanese female workers. However, the model of stress-induced drinking was supported neither in males nor in females, suggesting that the effects of these work stressors on heavy and problem drinking are not mediated by depressive symptoms.

INTRODUCTION

Heavy alcohol drinking and alcohol abuse/dependence have been recognized as an important public health problem in industrial settings (Roman and Trice, 1976). A number of studies have suggested that work stress is a risk factor for heavy alcohol consumption (Ames and Janes, 1987; Aro, 1981; Cooper *et al.*, 1990; Fennell *et al.*, 1981; Ferguson, 1974; Hammer and Vaglum, 1989; Harris and Fennell, 1988; Hingson *et al.*, 1981a,b; House, 1980; House *et al.*, 1986; Margolis, 1974; Mensch and Kandel, 1988; Parker and Farmer, 1988; Savada *et al.*, 1978; Seeman *et al.*, 1988; Seeman and Anderson, 1983; Syrotuik and D'Arcy, 1982; Violanti *et al.*, 1983). Three major work stressors have been consistently suggested by these studies as risk factors for high alcohol consumption: (1) job demand, such as job overload and time pressure, (2) job future ambiguity or poor prospects for promotion, and (3) lack of intrinsic work rewards. Besides, high control over job has been reported to be associated with heavy drinking by several studies (House, 1980; Mensch and Kandel, 1988).

Far fewer studies have been conducted on the effects of work stress on drinking problems (Bromet *et al.*, 1988; Cooper *et al.*, 1990; Markowitz, 1984, 1987; Parker and Farmer, 1988; Seaman, 1981; Seeman *et al.*, 1988; Seeman and Anderson, 1983). Here, lack of intrinsic work rewards (Bromet *et al.*, 1988; Markowitz, 1987;

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Seaman, 1981) and job demands, such as long working hours (Seaman, 1981), time pressure (Parker and Farmer, 1988) and heavy job responsibility (Markowitz, 1984), have been reported as risk factors for drinking problems.

Only one study has incorporated a model of stress-induced drinking in its design (Cooper *et al.*, 1990), where psychological distress acts as a mediator between work stressors and alcohol use and drinking problems. Since the study failed to support the model of stress-induced drinking, it has been not clear how work stressors influence alcohol use and drinking problems. Also, only a few studies have examined the effects of work stressors on other outcome measures, such as psychological distress, together with those on drinking behaviors (Bromet *et al.*, 1988; House, 1980). To date, the specificity of the effects of work stressors, vis-á-vis, alcohol use and drinking problems, has not been adequately investigated.

Furthermore, most of the studies were made used only male subjects or combined samples of both sexes. Six studies examined the effects of work stress on alcohol consumption (Aro, 1981; Hammer and Vaglum, 1989; Hingson *et al.*, 1981a; House *et al.*, 1986; Mensh and Kandall, 1988; Parker and Farmer, 1988) and only one (Parker and Farmer, 1988) examined the effects on drinking problems in females. All of the studies were made in the Western countries, most in the United States. In Japan, per capita alcohol consumption and number of alcoholic inpatients have been increasing during the past 2 decades (Japan Health and Welfare Statistics Association, 1988). Drinking problems are believed to have become a significant public health problem in Japanese industry.

We have conducted a study on the effects of work stressors on alcohol use and drinking problems in male and female working populations of an electrical factory in Japan. The objectives of this research were (1) to assess the effects of work stressors on alcohol use and drinking problems, comparing their effects on psychological distress, and (2) to examine whether psychological distress mediates their effects according to a theoretical model of stress-induced drinking.

SUBJECTS AND METHODS

Subjects

In January 1985, a questionnaire survey was conducted of 2581 employees in a computer factory in a suburb of Tokyo. A total of 2109 (81%) returned the questionnaires. Two hundred twenty-eight employees under 20 years old were excluded because by law they are not permitted to drink in Japan. Two hundred and thirty-one were excluded due to missing data on measures of demographic variables, work stressors, frequency of drinking, drinking problems, and psychological distress. The older subjects tend to have missing values on the questions, while no clear difference was observed between males and females. In 1273 male and 377 female respondents, 1043 (82%) males and 255 (68%) females were current drinkers; 36 (3%) and 16 (4%) were past drinkers, respectively. Only the current drinkers were subjected to the present analysis.

Methods

The questionnaires were mailed to the subjects a week prior to the annual health checkup and collected by nurses at the occupational health service center where the checkup was made. The questionnaires included four groups of measures, i.e.,

(1) work stressors, (2) alcohol use and drinking problems, (3) psychological distress, and (4) other covariates.

Work stressors. The questionnaire included 15 three-point items on work stressors. Each item was scored from 0 to 2, so that higher score indicated higher stress. Five scales of psychosocial work stressors were developed on the basis of their face validity and the results of a principal component factor analysis of these items with Varimax rotation. (1) Job overload scale measured quantitative overload and consisted of 3 items inquiring about amount of work load, pressure of deadlines, and frequency of thinking about job at home. (2) Lack of intrinsic work rewards was assessed using a 3-item scale. The items were "job is suitable to my interest," "job is suitable to my skills and ability," and "I can learn new knowledge." These items were similar with those used in previous measures of intrinsic work rewards (House, 1980) and job latitude (Karasek, 1979). (3) Job future ambiguity scale consisted of 2 items, i.e., poor promotion in position/pay and anxiety about future of working life. (4) Lack of social support at work was measured by a 4-item scale, assessing lack of a good relationship with supervisor, no conversation at work, feeling unfamiliar with co-workers, and feeling lonely at workplace. The internal consistencies (Cronbach's alphas) of these four scales were 0.60, 0.59, 0.59, and 0.52, respectively. (5) Lack of control over workpace was measured using a single item. Rotating work shift and overtime hours in the past month were similarly assessed and used as objective work stressors. The five scales of psychosocial work stressors intercorrelated weakly or moderately (Pearson r, 0.07–0.38). The correlation between job overload scale and overtime was also weak or moderate (Pearson r, 0.39 in males and 0.07 in females).

Alcohol use. Current drinking status was assessed by a single question and categorized into non-, past, and current drinkers. In current drinkers, two kinds of drinking patterns were assessed, i.e., frequency of drinking and amount of alcohol consumed per drinking occasion. Frequency of drinking was assessed using a single question and responses were coded as follows: "several times per year" = 1, "1–2 times per month" = 2, "once per week" = 3, "2–3 times per week" = 4, "4–6 times per week" = 5, "everyday" = 6. Amount of alcohol consumed per occasion was measured by inquiring how much subjects usually drank per drinking occasion. The subjects were asked to choose one of their favoritie types of alcohol and indicate the amount they drank. Amount of pure ethanol consumption per occasion was calculated by multiplying the concentration of ethanol with the amount of the beverage. Twenty eight (3%) male and 73 (29%) female subjects did not complete this question and were omitted from the analyses on amount of alcohol consumed per occasion. Past drinkers were similarly asked about the frequency of drinking and amount of alcohol they consumed when they used to drink.

Drinking problems. Drinking problems were measured using the Kurihama Alcoholism Screening Test (KAST) (Saito and Ikegami, 1978), a 14-item self-rating scale developed in Japan which assesses the severity of drinking problems during the past 6 months. The item analysis indicated that the internal consistency (Cronbach's alpha) of the KAST was improved from 0.64 to 0.70 by using unweighted item scores (e.g., yes = 1 and no = 0), instead of the original weighted item scores. The item No. 10 inquiring about job pressure to drink had little contribution to the scale reliability. Thus, in the present study, the number of drinking problems reported of 13 items, except for item No. 10, was used as a measure of

drinking problems. The internal consistency (Cronbach's alpha) of this 13-item scale was 0.71. The total score and the original KAST score highly intercorrelated (Pearson's *r*, 0.97). Because of the skewed distribution, the subjects were dichotomized into problem drinkers and nonproblem drinkers on the basis of the score in males (3+ and 0–2, respectively). However, since only 17 (6%) female subjects had the scores of 3+, problem drinkers were defined as 2+ in the score in female subjects. Past drinkers were also similarly asked about their drinking problems when they used to drink.

Psychological distress. Psychological distress was measured as levels of depressive symptoms by means of the Japanese version of Zung Self-Rating Depression Scale (SDS) (Zung, 1965, 1969). The internal consistency reliability (Cronbach's alpha) of the SDS was 0.78.

Other covariates. Six demographic variables were assessed, i.e., gender, age, marital status, education, family income, and occupation. Mean ages are shown in Table 1. Marital status was classified into married and not married (41 and 59% of the subjects, respectively). Education was scored as 9 years or less = 1, 10 to 12 years = 2, and 13 years or more = 3 (18, 46, and 36%, respectively). Family income had five categories, i.e., less than 3,000,000, 3,000,000 to 4,999,999, 5,000,000 to 6,999,999, 7,000,000 or more yen, and unknown (31, 27, 20, 10, and 12%, respectively). Subjects' occupation was categorized into two groups, i.e., white- and blue-collar workers (52 and 48%, respectively).

TABLE 1
DISTRIBUTIONS OF AGE, WORK STRESSORS, AND VARIABLES ON DRINKING BEHAVIORS IN MALE AND FEMALE DRINKERS

	Males (1043)			Females (255)		
	Mean	SD	%	Mean	SD	%
Age (years)	32	8		23**	5	
Objective work stressors						
Overtime (hr/month)	42	25		11**	8	
Rotating shift (%)			21			0**
Psychosocial work stressors						
Job overload	3	2		2**	2	
Lack of intrinsic rewards	3	2		4**	1	
Lack of control over pace	1	1		1**	1	
Job future ambiguity	2	1		2*	1	
Lack of social support at work	2	2		2	1	
Drinking behaviors						
Frequency of drinking	4	2		2**	1	
Amount of alcohol consumed per occasion (ml)[a]	50	33		48	37	
Drinking problems						
0–1			70			86**
2			11			7
3+			19			6
Depressive symptoms	42	7		46**	6	

Note. Parentheses indicate number of subjects.

[a] Only 1015 males and 182 females answered this question.

*,** Significance for gender difference, $P < 0.05$ and $P < 0.01$, respectively (χ^2 test for rotating shift and drinking problems; *t* test for all others).

Two other covariates were also assessed using the questionnaire. Perceived health status was measured using a four-point scale (i.e., from very good to poor) and the scale score (1–4) was used in the analyses. Flushing response at drinking was assessed using a two-item scale inquiring about the experience of flushing on their face when they drank in the past and at present. The flushing response is considered an indicator of lack of activity of the acetaldehyde dehydrogenase type I, which has been reported as a low-risk factor for heavy drinking and alcoholism and was observed in 30–40% of Japanese (Ohmori *et al.*, 1986). The internal consistency (Cronbach's alpha) of the scale was 0.91. The subjects were dichotomized into flushing or nonflushing type on the basis of the score (35 and 65%, respectively).

Analysis. A theoretical model of stress-induced drinking was built on the basis of previous models (House *et al.*, 1980; Cooper *et al.*, 1990). According to this model, objective work stressors cause psychosocial work stressors, which, in turn, increase psychological distress. Then, at the last step, the psychological distress promotes alcohol use and drinking problems.

Hierarchical multiple linear regression analyses were employed to examine the relationship between the work stressors and the frequency of drinking and amount of alcohol consumed per occasion, based on this model. Dummy variables were designed for rotating shift (i.e., yes = 1 and no = 0), marital status, family income, occupation, and flushing pattern (Cohen and Cohen, 1983). At the first step, the eight covariates except for gender were simultaneously entered into the equation. At the second step, overtime and rotating shift data were entered and their contribution to the equation was examined. At the third step, five scales of psychosocial work stressors were entered. At the fourth and final step, psychological distress (i.e., depressive symptoms) data were entered. Hierarchical multiple logistic regression analysis of drinking problems was conducted, following the same steps 1–4.

Hierarchical multiple linear regression analysis was conducted to predict depressive symptoms from work stressors in male and female subjects, using steps 1–3. All these analyses were made separately for males and females.

To examine the magnitude of possible selection bias due to exclusion of past drinkers from the subjects, the same series of analyses were also conducted in a sample of "ever-drinkers," i.e., current and past drinkers.

RESULTS

Mean values or frequencies of the seven work stressors are shown in Table 1. Male subjects worked significantly longer overtime and had higher scores of job overload and job future ambiguity than female subjects did. On the other hand, females had significantly higher scores on lack of intrinsic work rewards than males. Frequency of drinking was significantly higher in males than in females. The number of drinking problems were significantly greater in males than in females. No significant gender difference was observed in the amount of alcohol consumed per occasion.

Overtime related significantly and positively with frequency of drinking in males, while rotating shift related significantly and negatively with frequency of drinking (Table 2). None of the work stressors related significantly with frequency of drinking in females. Overtime and lack of intrinsic work rewards were significantly and positively related with amount of alcohol consumed per occasion in

TABLE 2
HIERARCHICAL MULTIPLE LINEAR REGRESSION ANALYSIS PREDICTING FREQUENCY OF ALCOHOL DRINKING FROM WORK STRESSORS IN MALE AND FEMALE DRINKERS (STANDARDIZED REGRESSION COEFFICIENT)[a]

	Males (1043)			Females (255)		
Variables	Step 1	Step 2	Step 3	Step 1	Step 2	Step 3
Objective work stressors						
Overtime		0.134**	0.125**		−0.018	−0.019
Rotating shift		−0.135**	−0.134**		—	—
Psychosocial work stressors						
Job overload			0.027			0.041
Lack of intrinsic rewards			0.009			0.060
Lack of control over pace			0.016			0.063
Job future ambiguity			0.047			0.087
Lack of social support			−0.023			−0.133
R^2	0.186	0.213	0.216	0.129	0.129	0.154

Note. Parentheses indicate total number of subjects. R^2, squared multiple regression coefficient.
[a] Step 1, Demographic variables (i.e., age, marital status, education, occupation, income); perceived health status and flushing reaction were entered (the coefficients are not shown). Step 2, Objective work stress variables were entered. Step 3, Psychosocial work stress variables were entered.
* $P < 0.05$.
** $P < 0.01$.

males (Table 3). Job future ambiguity related significantly and positively with amount of alcohol consumed per occasion in females. Overtime and lack of intrinsic work rewards were significantly and positively associated with problem drinking in males (Table 4). None of the work stressors were significantly associated with problem drinking in females. The standardized coefficients for overtime and rotating shift in the equations predicting the three variables of drinking

TABLE 3
HIERARCHICAL MULTIPLE LINEAR REGRESSION ANALYSIS PREDICTING AMOUNT OF ALCOHOL CONSUMED PER OCCASION FROM WORK STRESSORS IN MALE AND FEMALE DRINKERS (STANDARDIZED REGRESSION COEFFICIENT)[a]

	Males (1015)			Females (182)		
Variables	Step 1	Step 2	Step 3	Step 1	Step 2	Step 3
Objective work stressors						
Overtime		0.056	0.082*		0.011	0.023
Rotating shift		−0.024	−0.025		—	—
Psychosocial work stressors						
Job overload			−0.042			−0.018
Lack of intrinsic rewards			0.106**			0.079
Lack of control over pace			−0.055			−0.150
Job future ambiguity			0.036			0.204*
Lack of social support			−0.019			0.031
R^2	0.108	0.111	0.124	0.092	0.081	0.161

Note. Parentheses indicate total number of subjects. R^2, squared multiple regression coefficient.
[a] Step 1–3, see footnote *a* for Table 2.
* $P < 0.05$.
** $P < 0.01$.

TABLE 4
HIERARCHICAL MULTIPLE LOGISTIC REGRESSION ANALYSIS PREDICTING PROBLEM DRINKERS FROM WORK STRESSORS IN MALE AND FEMALE DRINKERS (STANDARDIZED REGRESSION COEFFICIENT)[a,b,c]

Variables	Males (1043)			Females (255)		
	Step 1	Step 2	Step 3	Step 1	Step 2	Step 3
Objective work stressors						
Overtime		0.240*	0.235*		0.272	0.258
Rotating shift		0.005	0.052		—	—
Psychosocial work stressors						
Job overload			0.147			−0.053
Lack of intrinsic rewards			0.376**			0.031
Lack of control over pace			−0.172			0.309
Job future ambiguity			0.012			−0.054
Lack of social support			−0.073			0.275
χ^2	71.0	77.4	102.3	13.7	15.7	22.1
Degree of freedom	10	12	17	10	11	16

Note. Parentheses indicate total number of subjects. χ^2, model goodness of fit χ^2.

[a] Step 1–3, see footnote *a* for Table 2.

[b] Problem drinker was defined as 3+ in the problem drinking score for males (19%) and 2+ for females (14%).

[c] Standardized regression coefficient was calculated by multiplying the unstandardized coefficient with standard deviation of the independent variable.

* $P < 0.05$.

** $P < 0.01$.

behaviors remained almost same between step 2 and 3, i.e., before and after the psychosocial work stressors were entered, suggesting the effects of overtime and rotating shift are independent of the psychosocial work stressors.

When depressive symptoms were added at step 4 into each equation (data not shown), depressive symptoms did not correlate significantly with frequency of drinking or amount of alcohol consumed per occasion in either males or females ($P > 0.05$), resulting in little change in the coefficients of other independent variables previously entered. In the equation predicting drinking problems, depressive symptoms were significantly associated with drinking problems in both males and females (standardized regression coefficient, 0.274, $P < 0.01$ for males; 0.607, $P < 0.05$ for females). The standardized coefficient for lack of intrinsic work rewards slightly decreased from 0.376 to 0.318 in males, although it was still significant ($P < 0.01$). The results indicated that few of the effects of lack of intrinsic work rewards on drinking problems were mediated by depressive symptoms in males.

The five psychosocial stressors significantly and positively related with depressive symptoms in males (Table 5). Lack of intrinsic work rewards, job future ambiguity, and lack of social support at work related significantly and positively with depressive symptoms in females.

Past drinkers reported significantly lower frequency of drinking when they used to drink than the current drinkers in males ($P < 0.05$, t test). No significant difference in amount of alcohol consumption per occasion or drinking problems was found between past and current drinkers in males ($P > 0.05$, t test or χ^2 test). No significant difference in any of these three drinking variables was found be-

TABLE 5
HIERARCHICAL MULTIPLE LINEAR REGRESSION ANALYSIS PREDICTING DEPRESSIVE SYMPTOMS FROM WORK STRESSORS IN MALE AND FEMALE DRINKERS (STANDARDIZED REGRESSION COEFFICIENT)[a]

Variables	Males (1043)			Females (255)		
	Step 1	Step 2	Step 3	Step 1	Step 2	Step 3
Objective work stressors						
Overtime		−0.004	−0.011		−0.023	−0.019
Rotating shift		−0.018	0.030		—	—
Psychosocial work stressors						
Job overload			0.081**			0.050
Lack of intrinsic rewards			0.236**			0.157**
Lack of control over pace			0.121**			0.061
Job future ambiguity			0.139**			0.206**
Lack of social support			0.293**			0.264**
R^2	0.195	0.195	0.478	0.210	0.210	0.427

Note. Parentheses indicate total number of subjects. R^2, squared multiple regression coefficient.
[a] Step 1–3, see footnote *a* for Table 2.
* $P < 0.05$.
** $P < 0.01$.

tween past and current drinkers in females ($P > 0.05$, t test or χ^2 test). The same series of regression analyses in ever (i.e., current and past) drinkers showed similar results. The work stress variables significantly related to three drinking variables were unchanged in males and females between the previous analyses in current drinkers and these additional analyses.

DISCUSSION

Overtime was found to be associated with frequency of drinking, amount of alcohol consumed per day, and drinking problems in male drinkers. Lack of intrinsic work rewards was also associated with amount of alcohol consumed per occasion and drinking problems in male drinkers. It is suggested that overtime and lack of intrinsic work rewards are risk factors for heavy drinking and problem drinking in male Japanese workers. The results are consistent with previous studies reporting the association of job overload (Fennell *et al.*, 1981; Harris and Fennell, 1988; Hingson *et al.*, 1981a; Margolis *et al.*, 1974; Ferguson, 1974; Mensch and Kandel, 1988; Parker and Farmer, 1988; Seaman, 1981) and lack of intrinsic work rewards (Bromet *et al.*, 1988; Hingson *et al.*, 1981a,b; House, 1980; House *et al.*, 1986; Markowitz, 1987) to alcohol use and drinking problems.

Job future ambiguity was significantly associated with amount of alcohol consumed per occasion in female drinkers, while the association was weak and not significant in male drinkers. House *et al.* (1986) suggested that extrinsic work rewards, i.e., amount of pay and good promotion, were associated with alcohol consumption in females, but not in males. It is suggested that job future ambiguity is an important risk factor for heavy drinking in females in Japan, as well as the United States. Female workers may be more susceptible to job future ambiguity than male workers.

Higher job centrality in males than in females in Japanese workers (Lascocco and Kalleberg, 1988) may explain the significant association between lack of

intrinsic work rewards and drinking behaviors observed only in males. On the other hand, female workers are more likely to experience job turnover and have little chance for promotion in Japan (Lascocco and Kalleberg, 1988). This may help to explain the effects of job future ambiguity found only in female subjects. Gender difference in the effects of overtime may be attributable to small variations in overtime. In addition, low frequency of drinking and drinking problems in female subjects, as well as the small number of female subjects, may be responsible for gender differences.

Frequency of drinking in male subjects under rotating shift was significantly less than that in dayshift workers. Previous studies have reported greater amount of alcohol consumed per day and greater drinking problems in shift workers (Gordon *et al.*, 1986; Smart, 1979). These previous findings are not necessarily inconsistent with the present study, because rotating shift related positively with amount of alcohol consumed per occasion and number of drinking problems in males before controlling other variables (point biserial r, 0.14 and 0.11, respectively, both $P < 0.01$). The negative association between rotating shift and drinking frequency might be explained by the limited number of places to drink outside the home after night shifts. Further research is needed to replicate the results and examine the possible explanation.

Like a previous study (Cooper *et al.*, 1990), the present study failed to support the model of stress-induced drinking, suggesting that depressive symptoms are not likely to be a mediating variable between work stressors and alcohol use/problems. Furthermore, it is suggested that the effects of overtime on heavy/problem drinking are not likely to be mediated by psychosocial work stressors. Overtime may be a relatively specific risk factor for heavy drinking and drinking problems, not for depressive symptoms. Its effects are speculated to be mediated by physical, rather than psychological, strain. On the other hand, lack of intrinsic work rewards may be a common risk factor for heavy/problem drinking and for depressive symptoms in males. This is consistent with the findings by House (1980). Job future ambiguity may be so for heavy drinking and depressive symptoms in females. However, it is suggested that heavy/problem drinking and depressive symptoms are two independent outcomes of these psychosocial work stressors, not sequential components of the stress process.

The interpretation of the present results is greatly limited by the cross-sectional study design: the causality is not clear. An experimental study (Conway *et al.*, 1981) reported that acute increase in job overload was associated with decrease in amount of alcohol consumed per day, suggesting a difference in the effect of acute and chronic job stressors on alcohol use/problems. A longitudinal study design should be employed in future research to clarify the causal relationships between both acute and chronic work stressors and alcohol use/problems. Furthermore, possible selection bias introduced by nonrespondents and subjects excluded due to missing values should be also considered.

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Depressive States in Workers Using Computers[1]

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There have been few reports investigating the depressive states in workers using computers. We describe the depressive states observed in workers using computers and discuss the sources of their occupational stresses. The first subject is a 34-year-old male manager of a manufacturing company who had customarily worked until 9 PM. In 1985, it became necessary for him to work until midnight; symptoms of depression began to appear during this period, exacerbated after trouble with a computer. In 1986, he visited a psychiatrist and his condition was diagnosed as Major Depression according to DSM-III. The second subject is a 26-year-old male VDT (visual display terminal) operator in a general hospital. Before the onset, he had had to work until 8 PM and, at the end of each month, until midnight. Two months later, he became depressed and his condition was diagnosed as Major Depression according to DSM-III. The third subject is a 32-year-old male chief in the computer programming section of a bank. He had had to work until 8 PM, became depressed, and visited a psychiatrist who diagnosed his condition as Major Depression according to DSM-III. The authors discuss these cases from the standpoint of occupational stresses, as they are associated with work overload, and the important role these stresses played in the onset of the workers' depressive states.

INTRODUCTION

Recently, many computers have been introduced into the workplace and many workers are using them in Japan (VDT Sagyo ni kansuru Kento Iinkai, 1985). Health hazards associated with visual display terminal (VDT) work, representative of the hazards work with computers involves, have been pointed out (Kogi, 1982). It has been suggested that the increase of occupational stresses associated with computer operation gives rise to psychological ill health (Kogi, 1982; VDT Sagyo ni kansuru Kento Iinkai, 1985) and some cases have been reported in Japan (Hayashi and Matsumoto, 1985; Natsume and Fujii, 1986; Watanabe, 1986).

It has been suggested that those suffering from depression or depressive states have increased recently in Japan (Nishizono, 1983) and this has become one of the foremost problems in the field of mental health in industry, as it applies to occupational stress (Levi, 1984). However, there have been few reports demonstrating depressive states among workers using computers. The aim of this paper is to report on three computer workers suffering from depressive states and to discuss sources of occupational stress which might be associated with the onset of the disease.

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CASE REPORTS

Case 1

The first case involves a 34-year-old manager of a confectionery company. His premorbid personality could be characterized as being serious and punctilious.

When he was 25 years old, he was selected to become the manager, as his sincere attitude relative to his work was evaluated highly by the owner of the company. At this time, a computer system was introduced into his office. He mastered the use of the computer immediately, although the rest of the staff were unable to. As a result, responsibility for operating the computer fell upon him and his work load increased to include both input and output, as well as educating others on how to operate the computer, in addition to his ordinary work. To do all this, it became necessary for him to work until 10–12 PM. Although, during this same period, two of his subordinates quit, the company did not replace them, because it was assumed that the computer would be able to fulfill their functions.

When he was 33 years old, during the Christmas season when many Christmas cake orders were being processed, a new computer system was introduced. Therefore, he had to work until 10–12 PM and continued to endure this schedule, because he felt a strong sense of responsibility. For the entire 2 days before Christmas day, he sat up all night to complete work on the order processing. However, because of a failure in the computer program, half of the cake orders were not made up, causing a great deal of economic damage to the company.

The next day, he was not able to go to the office, due to severe feelings of fatigue and depression. After an absence of 2 weeks, the symptoms diminished and he returned to the office. However, he had to continue to work until 10 PM, as work remained which had not been completed during his absence. He repeatedly took 1- to 2-week absences several times during the next 6 months and, finally, sought psychiatric help. During the first interview he complained of depressed mood, psychomotor retardation, irritability, and sleep disturbances including early waking and delayed sleep. His condition was diagnosed as depression, which appeared to be a reaction to the exhaustion induced by overwork and the events of the preceding Christmas. His condition met the diagnostic criteria for Major Depression according to DSM-III (American Psychiatric Association, 1980). He was admitted to a hospital and treated with antidepressant and anxiolytic drugs as well as supportive psychotherapy. He felt that his depressive state was strongly associated with his overwork.

Although he returned to work after in-patient treatment, his situation at work did not change and he relapsed into depression. Eventually, he was assigned to another department, where he did not have to work overtime, and he recovered from the depression.

Case 2

The second subject is a 26-year-old VDT operator in a general hospital. His premorbid personality could be characterized as being serious, punctilious, and nervous.

When he was 23 years old, he graduated from university and obtained a job in the hospital. The following spring, he was assigned to a computer input unit and engaged in VDT operation. His work consisted mainly of processing the methods of treatment for the 100 in-patients into the computer. His work schedule began at

8:30 AM ending at 8 PM on weekdays, and from 8:30 AM to 5 PM on Saturdays. For a few days at the end of each month, he had to work until 12 midnight.

Two months later, in June, he began to feel depressed, lacked enthusiasm, and suffered from sleep disturbances including delayed sleep and early waking. He visited a psychiatrist and his condition was diagnosed as depressive state; the psychiatrist prescribed antidepressants. He managed to continue his work, although the symptoms did not diminish. In December, the symptoms including psychomotor retardation and his depressed mood became more severe which finally resulted in absence from work. Afterward, he explained his feelings at the time, "I had no vitality and no desire to do anything. I couldn't move any more."

The following January, he visited the Mental Health Center. His condition was diagnosed as depression as it met the diagnostic criteria for Major Depression according to DSM-III (American Psychiatric Association, 1980). Although he continued to receive pharmacotherapy with antidepressants, the symptoms did not diminish. In autumn, he was assigned to the general secretary section and he found that his new work was easier than his previous job and that there was no need for any overtime work. As a result, he recovered immediately from his depression. He said that he believed his depression had been caused by overwork.

Case 3

The last subject is a 34-year-old bank employee. After he graduated from university, he got a job in a city bank.

When he was 31 years old, he was assigned to the computer programming unit, as he had been evaluated highly due to his proposals and reports using a personal computer. His employer's expectations of his performance were very high and this assignment was quite an advancement. His work mainly dealt with computer programming and systems development and he worked from 9 AM to 8 PM. Although the work was strongly associated with a computer, he worked at a desk. He used a computer privately at home for at least 2 hr a day.

In a short time, feelings of fatigue, events of early waking, insufficient subjective thinking patterns, and anxiety appeared, disturbing his work routine. The following year, he visited the psychiatric unit of a general hospital and his condition was diagnosed as depression. He was treated as an out-patient for 6 months. Despite pharmacotherapy and supportive psychotherapy, his symptoms did not diminish and he attempted suicide. He was then admitted to the psychiatric unit and remained there for 3½ months, his condition having been diagnosed as Major Depression according to DSM-III (American Psychiatric Association, 1980). However, he continued to suffer, from some neurotic and physical symptoms for 2 years, recovered, and was prescribed an increased dosage of antidepressants.

DISCUSSION

There have been several researchers who have reported mental illnesses observed in workers associated with computers in Japan. Hayashi and Matsumoto (1985) reported two cases of psychogenic fugue and discussed their work overload. Watanabe (1986) described four cases of ill mental health and he focused on the subjects' relationships with fellow employees in the workplace. However, diagnoses of the subjects' ailments were not clarified in the paper. Natsume and Fujii (1986) studied occupational maladjustment syndrome, which is classified

under Adjustment Disorder according to DSM-III (American Psychiatric Association, 1980), and identified 13 cases in which the illnesses were associated with technostresses. They found illnesses related to work overload in 6 of the 13 cases. However, there have been relatively few reports on the depressive states observed in computer workers in Japan, which have been clinically diagnosed, providing impetus for the authors' report.

Depression is one of the most prevalent of all mental illnesses and the point prevalence of depressive disorders is estimated at 3% for men and at 5 to 9% for women (Gelder *et al.*, 1983). In Japan, there have been some suggestions of a recent increase of the prevalence (Nishizono, 1983) and, as a result of this, depression has been focused on by mental health workers in industrial fields.

The etiology of depression has been studied vigorously from the standpoints of psychopharmacology, genetics, psychology, epidemiology, and sociology. The study of social causation in depression has now reached the stage where both its methods and its findings are widely accepted; many researchers have suggested that the psychosocial environment has a major influence on its manifestation (Bebbington and McGuffin, 1989). Among these psychosocial factors (Brown and Harris, 1978) such as life events, loss of parents in early life, and similar experiences, factors associated with workplace and job description might be important. In DSM-III and DSM-III-R (American Psychiatric Association, 1980, 1988), psychosocial stress sources include stress on the job, such as changes in work hours, job dissatisfaction, loss of job, retirement, and trouble with boss. Relative to working with computers, a positive correlation between work load and the intensity of computer use has been clarified (World Health Organization Meeting, 1989). In Japan, Ohara and his colleagues (1970) studied 56 patients suffering from depression and found that job changes, unemployment, and work overload were the precipitating factors in the illnesses among middle-aged males.

With regard to our cases, characteristic occupational stress sources were found to be work overload and excessive overtime; both clearly related to one another. In our first subject's case, since he was able to operate the computer, he did so by himself. The company chose to ignore the necessity of employing two employees to make up for the two who had quit, asserting that the computer was capable of handling the work. As a consequence, his work load increased, causing more overtime. In our second case, manpower assignments were based on the efficiency of the computer system, under the assumption that a reduced staff would be just as efficient. However, this reasoning resulted in work overload and excessive overtime for the employees, as the computer system did not come up to expectation. In our third case, the subject was selected for an important position and worked until 8 PM to prove his work. In all our cases, the level of expectation that the employers placed in the subjects was very high and the subjects did their best to meet these expectations. These occupational stress sources, such as work overload and overtime, might play an important role in the occurrence of depression in similar cases.

In Japan, Kawakami and his colleagues (1990) found job stress due to job unsuitability to be significantly associated with the occurrence of major depression among workers in factories; however, work overload was not found to be associated with it. Although our second case complained of job unsuitability, the others did not. Difference in size of the companies might account for the disagreements. In the study of Kawakami and his colleagues workers in a large company

were chosen as subjects and, in contrast, the companies for which our cases worked were relatively small.

Work overload may demand careful attention in workers using computers, especially in the Japanese, who are famous for their hard work and excessive overtime. However, we should be prudent in asserting this interpretation, considering the following problems in this study: (1) This study is not epidemiological and gives no information concerning the amount of work load and overtime among "non-patient" computer-related workers (2). As to the diagnosis for the subjects, the condition was diagnosed clinically according to DSM-III, without any structured interview. This might cause misclassification in diagnosis. Further epidemiological studies are needed in order to clarify the relationships between job stresses and depression among computer workers.

CONCLUSIONS

The authors presented the cases of three computer workers who developed depressive states which met the diagnostic criteria for Major Depression by DSM-III (American Psychiatric Association, 1980). Work overload and overtime were identified as their occupational stress sources and may be assumed to play an important role in the occurrence of depression. Considering the problems in this study, further epidemiological studies are needed in order to confirm the finding.

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Relationships between Health Status and Working Conditions and Personalities among VDT Workers[1]

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A total of 486 visual display terminal (VDT) workers were surveyed on their health status, working conditions, type A state, and depression state through questionnaires. They were also divided into three groups by self-assessment: technocentered (TC), technoanxious (TA), and neither (N). The weekly working hours and daily VDT operating hours of the type A group were longer than those of non-type A group. Type A subjects had more symptoms than non-type A subjects. The mean weekly working hours of depressive group was 61.3 hr, much longer than that of the others. The TC subjects worked daily with the VDT for longer hours than the other subjects. The TA subjects felt most dissatisfied with their computer training (TC, 47.4%; N, 64.5%; TA, 91.7%). Awkward VDT operators were more often in the TA group (36.1%) than in the others (TC, 3.8%; N, 10.5%). The TA subjects had a higher previous history of duodenal ulcer than the others (TA, 13.9%; N, 4.6%; TC, 3.8%).

INTRODUCTION

Visual display terminals (VDTs) have become widespread in Japan since the early 1980s. Since the International Ergonomics Association Congress in 1982, many studies have found that VDT workers complain of increased workload, intensive work, and high levels of stress, and that VDT workers are apt to suffer from visual disturbances and musculoskeletal problems (Nishiyama, 1990). Therefore, the Investigative Committee on VDT Work and Occupational Health organized by the Japanese Association of Industrial Health issued recommendations regarding VDT work and occupational health in 1985. Soon after that, the Japanese Ministry of Labor also issued an official notice about guidelines for occupational health on VDT work. However, they do not have legal force. Japanese VDT workers are still working under poor conditions (Japanese Ministry of Labor, 1988). VDT workers have been also reported to suffer from psychological distress such as computer anxiety and behavioral distress (Smith, 1987; Billette and Piché, 1987; Landau, 1987; WHO, 1989). However, very few studies have investigated the relationships between health status of VDT workers and their attitudes toward computers (Igbaria and Chakrabarti, 1990).

The purpose of this article is to examine the relationships between the health status and working conditions and the attitudes toward computers among VDT workers. Furthermore, we have presented some comments on promoting occupational health for VDT workers.

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MATERIALS AND METHODS

Of 865 VDT workers, 486 (56.6%) participated in the health survey held at a local government office in Ehime, Japan, in August 1990. They were all local government workers who were engaged in VDT work more than 1 hr a day.

We interviewed each person to collect information by a questionnaire consisting of 128 questions. The subject's biological information (10 items), life history (10 items), working conditions (30 items), subjective symptoms, and signs including awkwardness with VDT (50 items), brief type A scores (12 items; Maeda, 1985), and self-rating score for depression (SRQ-D) (12 items; Abe *et al.*, 1972) were collected through the questionnaires. The subjects were also asked to assess their current attitudes toward computers, with response options ranging from (1) considerably technocentered to (5) considerably technoanxious.

All ratio or interval data were analyzed using the Kruskal–Wallis test with the Scheffe's multiple comparison, and all categorical data were analyzed by the χ^2 test.

RESULTS

Attributes of the Subjects

Four hundred eighty-six (male, 418; female, 68) local government VDT workers, ages 18–58 (mean 33) were examined. They engaged in VDT work for 1 to 10 hr daily (mean 2.4 hr). Type of VDT work among subjects is shown in Table 1. Most subjects did word processing, followed by data entry and acquisition. Most of the subjects who did programming were male.

One hundred forty-eight (30.3%) VDT workers were found to have some health problems through the health examination. Most of the problems, such as mild hypertension and lowered visual acuity, were seen among middle-aged and elderly people and were not severe.

Type A Behavioral Characteristics

Sixty-five male (15.4%) and nine female (13.2%) subjects were classified as type A based on their answers to a questionnaire. Demographic characteristics, lifestyle, working conditions, and health status of type A and non-type A are presented in Table 2. With regard to working conditions, mean weekly working hours, mean daily VDT hours, and mean continuously working hours of the type A group were significantly longer than those of non-type A. The percentage of

TABLE 1
TYPE OF VDT WORK[a]

	% Male (422)	% Female (68)
Word processing	86.0	76.5
Data entry	42.7	50.0
Data acquisition	31.0	33.8
Programming	21.3	4.4
Interactive processing	2.4	4.4
CAD/CAM	2.8	—
Surveillance	2.6	—
Others	2.8	—

[a] Multiple answer.

TABLE 2
DEMOGRAPHIC CHARACTERISTICS, WORKING CONDITIONS, AND HEALTH INDICES IN TYPE A GROUP AND NON-TYPE A GROUP (MEANS ± SD)

	Type A (male, n = 65; female, n = 9)	Non-type A (male, n = 357; female, n = 58)
Age (years)	33.7 ± 8.6	32.7 ± 8.0
Work experience with VDT (years)	4.0 ± 4.0	3.3 ± 2.5
Weekly working hours	51.0 ± 9.6	47.7 ± 7.7*
Daily VDT hours	2.8 ± 1.8	2.2 ± 1.5*
Continuously VDT hours	1.6 ± 1.1	1.3 ± 0.9*
Sleeping hours	7.0 ± 1.1	7.1 ± 1.2
Programming (%)	21.6	18.5
Awkward VDT operators (%)	13.5	11.3
Dissatisfied computer training (%)	31.1	18.5†
Good sociability (%)	71.6	88.5†
Microcomputer owner (%)	24.3	20.2
Home overtime worker (%)	25.7	17.5
Nonathlete (%) (exercise less than once a week)	67.5	75.7
Eye symptom score[a]	3.0 ± 2.8	1.7 ± 1.9*
Musculoskeletal symptom score[b]	2.4 ± 2.4	1.3 ± 1.6*
Gastrointestinal symptom score[c]	1.6 ± 2.0	0.9 ± 1.5
Respiratory symptom score[d]	1.0 ± 1.3	0.6 ± 1.1*
Cardiovascular symptom score[e]	0.4 ± 0.9	0.3 ± 0.8*
SRQ-D score	7.4 ± 3.7	4.4 ± 3.3*
Previous duodenal ulcer (%)	9.5	4.3
Underwent a personality change (%)	24.3	9.9*

[a] Total score of eyestrain, eye pain, redness, epiphora, dim, and chromopsia (no = 1, sometimes = 2, often = 3).

[b] Total score of low back pain, arthralgia, forearm numbness, forearm dullness, leg dullness, shoulder or neck pain (no = 1, sometimes = 2, often = 3).

[c] Total score of constipation, diarrhea, appetite loss, postcibally pain, hunger pain, stuffed stomach, get thin, sour eructation, uncomfortable throat (no = 1, sometimes = 2, often = 3).

[d] Total score of cough, sputum, sore throat, shortness of breath (no = 1, sometimes = 2, often = 3).

[e] Total score of palpitation, precordial oppression, shortness of breath, dizziness (no = 1, sometimes = 2, often = 3).

* $P < 0.05$ (t test).

† $P < 0.05$ (χ^2 test).

type A subjects who felt dissatisfied with their computer training was higher than that of non-type A. The mean scores of type A subjects in eye, musculoskeletal, respiratory, and cardiovascular symptoms were significantly higher than those of non-type A. The mean SRQ-D score of type A subjects was also higher than that of non-type A. The percentage of subjects who stated that they had undergone subjective personal change was higher in the type A group than in the non-type A group. Distribution of VDT work type was unrelated to the type A behavior.

Depressive State

According to SRQ-D score, 389 male and 62 female subjects, 28 male and 5 female subjects, and 5 male and 1 female subjects were classified into normal (from 0 to 10 points), borderline (from 11 to 15 points), and depressive group (from 16 to 36 points), respectively. Demographic characteristics, life-style, working

conditions, and health status of each categorical group are presented in Table 3. Mean weekly working hours of the depression group was 61.3 hr, much longer than that of the other groups. The percentage of subjects who stated that they believed their current work required much more training than their ability increased with depressive state: normal (8.9%), borderline (42.4%), and depression group (100%). The percentage of subjects who did data entry was high (83.3%) in the depression group. The percentage of subjects who kept a good relationship with other workers was smaller in depression group than that in the other two groups. Each mean symptom score of depression and borderline group was significantly higher than that of normal group. Mean type A behavioral scores of depression and borderline group were also greater than those of normal group.

Attitude toward Computers

The subjects were further grouped into following five categories according to

TABLE 3
DEMOGRAPHIC CHARACTERISTICS, WORKING CONDITIONS, AND HEALTH INDICES BY DEPRESSION SCORES[a]

	Depression grade (SRQ-D scores)		
	Normal (0–10) (male, n = 389; female, n = 62)	Borderline (11–15) (male, n = 28; female, n = 5)	Depression (16–36) (male, n = 5; female, n = 1)
Age (years)	32.9 ± 8.1	31.9 ± 7.3	35.2 ± 10.0
Work experience with VDT (years)	3.4 ± 2.9	3.6 ± 2.4	4.1 ± 0.9
Weekly working hours	48.1 ± 7.9	47.6 ± 5.7	61.3 ± 19.3*
Daily VDT hours	2.3 ± 1.6	2.4 ± 1.3	2.7 ± 2.0
Continuously VDT hours	1.3 ± 0.7	1.3 ± 0.8	1.8 ± 1.3
Sleeping hours	7.1 ± 1.2	6.7 ± 1.4	7.1 ± 0.9
Programming (%)	18.6	24.2	16.7
Awkward VDT operators (%)	10.6	27.3	0†
Dissatisfied computer training (%)	62.1	81.8	83.4†
Good sociability (%)	89.1	51.5	33.3†
Microcomputer owner (%)	20.4	30.3	0
Home overtime worker (%)	18.6	24.2	0
Nonathlete (%) (exercise less than once a week)	64.3	57.6	50.0
Eye symptom score	1.7 ± 1.9	3.9 ± 3.3	4.3 ± 1.9*
Musculoskeletal symptom score	1.3 ± 1.6	4.1 ± 2.1	4.3 ± 2.3*
Gastrointestinal symptom score	0.8 ± 1.3	3.5 ± 2.9	3.5 ± 2.2*
Respiratory symptom score	0.6 ± 1.0	2.1 ± 1.6	2.8 ± 1.5*
Cardiovascular symptom score	0.2 ± 0.6	1.3 ± 1.2	1.0 ± 1.7*
Previous duodenal ulcer (%)	4.9	9.1	0
Underwent a personality change (%)	10.0	39.4	16.7†

[a] Means ± SD.
* $P < 0.05$ (Kruskal–Wallis test). $P < 0.05$ (Scheffe's test).
† $P < 0.05$ (χ^2 test).

self-assessment of attitude toward computers: (1) considerably technocentered (male, 8; female, 1), (2) somewhat technocentered (male, 62; female, 7), (3) neither (male, 314; female, 58), (4) somewhat technoanxious (male, 28; female, 2), and (5) considerably technoanxious (male, 6). The two technocentered and two technoanxious categories were then merged into a respective single category, resulting in three groups: technocentered (TC), technoanxious (TA), and neither (N).

Demographic characteristics, life-style, working conditions, and health status of each group are presented in Table 4. The TC subjects worked for longer daily VDT hours than other subjects did. The percentage of programmers in the group was greater than that in the other two groups. The percentage of personal computer owners was also greater among TC subjects. The TA subjects felt most dissatisfied with their computer training. The percentage of awkward VDT operators in the TA group (36.1) was much more than that in the other groups (TC, 3.8; N, 10.5).

TABLE 4
DEMOGRAPHIC CHARACTERISTICS, WORKING CONDITIONS, AND HEALTH INDICES IN EACH TECHNOSTRESS GROUP[a]

	Technocentered (male, n = 70; female, n = 8)	Neither (male, n = 317; female, n = 55)	Technoanxious (male, n = 34; female, n = 2)
Age (years)	32.5 ± 7.1	33.9 ± 8.3	30.9 ± 5.7
Work experience with VDT (years)	4.5 ± 2.8	3.3 ± 2.8	3.0 ± 2.7*
Weekly working hours	48.7 ± 7.7	48.1 ± 8.3	48.2 ± 7.3
Daily VDT hours	2.7 ± 1.4	2.3 ± 2.3	2.0 ± 2.0*
Continuously VDT hours	1.4 ± 0.9	1.3 ± 0.9	1.2 ± 0.9
Sleeping hours	7.1 ± 1.0	7.1 ± 1.1	7.0 ± 1.5
Programming (%)	37.2	16.4	8.3†
Awkward VDT operators (%)	3.8	10.5	36.1†
Dissatisfied computer training (%)	47.4	64.5	91.7†
Good sociability (%)	75.6	88.7	77.8†
Microcomputer owner (%)	33.3	19.1	13.9†
Home overtime worker (%)	23.1	18.5	11.1
Nonathlete (%) (exercise less than once a week)	39.7	35.4	36.1
Eye symptom score	2.6 ± 2.4	1.8 ± 2.1	1.6 ± 1.7*
Musculoskeletal symptom score	1.7 ± 1.8	1.5 ± 1.8	1.2 ± 1.7
Gastrointestinal symptom score	1.2 ± 1.5	1.0 ± 1.6	0.8 ± 1.4
Respiratory symptom score	1.1 ± 1.5	0.6 ± 1.1	0.6 ± 1.0*
Cardiovascular symptom score	0.5 ± 1.0	0.3 ± 0.7	0.4 ± 0.8*
SRQ-D score	5.4 ± 3.4	4.7 ± 3.5	5.1 ± 3.9
Previous duodenal ulcer (%)	3.8	4.6	13.9†
Underwent a personality change (%)	16.7	9.7	27.8†

[a] Means ± SD.
* $P < 0.05$ (Kruskal–Wallis test). $P < 0.05$ (Scheffe's test).
† $P < 0.05$ (χ^2 test).

With respect to health, 13.9% of the TA subjects had a history of diagnosed duodenal ulcer. This rate was higher than that in the other groups. The percentage of subjects who answered that they underwent a subjective personality change was greater among both the TC and the TA groups than among the N group.

Age, weekly working hours, sleeping hours, habitual exercise frequency, type A scores, and self-rating scores for depression (SRQ-D) were unrelated to the attitudes toward computers.

DISCUSSION

We surveyed 486 VDT workers by questionnaires to investigate the relationships between health status and working style and personality characteristics in terms of type A behavior, depressive state, and computer anxiety.

Although the Japanese Ministry of Labor had issued an official notice about a special health check for VDT workers in 1985, it was the first time the local government had conducted a survey. Consequently, the notices about the health check were not as adequate and the participation rate of 56.6% was relatively low.

In light of the high percentage of awkward VDT operators and the high incidence of past duodenal ulcer in technoanxious group, technoanxiety was assumed to be a potent stress factor. For an underskilled and underqualified worker, a new computer system can cause considerable anxiety and embarrassment. Recent research shows that a computer training program considerably decreases computer anxiety and that management support has a good effect on computer anxiety.

Long-term occupational stress may induce peptic ulcer. Some studies have shown that peptic ulcer, in particular duodenal ulcer, is a kind of psychological disorder (Dotevall, 1985). In humans, peptic ulcer may be linked to a dependence–independence conflict (Alexander, 1950). With regard to the high incidence of previous duodenal ulcer in the technoanxious group, we assume that the technoanxious condition is similar to the dependence–independence conflict or avoidance–avoidance conflict state. Therefore, a peptic ulcer is apt to be formed. There is, however, a possibility that this general anxiety trait is a risk factor for both technoanxiety and duodenal ulcer or dissatisfaction with training in computer use.

The results of this study suggest that the main cause of the technoanxious condition was the lack of computer training. Thus, adequate computer education and training should be given to VDT workers.

The mean scores of eye, respiratory, and cardiovascular symptoms were significantly higher in technocentered groups than in the other groups. In view of longer daily VDT hours and high personal computer owner rate in the technocentered group, we believe that the high workload is associated with such many symptoms. The number of personal computer owners has increased steeply during the past 10 years in Japan, and many VDT workers spend time at home with their personal computers for extra work (Watanabe *et al.*, 1989). According to the Labor Security and Health Law in Japan, a company must appoint a health supervisor and an occupational doctor. Health supervisor, occupational doctor, and other health-related staff must pay more attention to VDT workers' attitudes toward computers, overwork, and also overtime work at home.

The percentage of depressive subjects evaluated by the SRQ-D in this study group was not significantly higher than that in other occupational groups reported in the previous studies. It is obvious that VDT work itself does not lead to

depression directly. Since subjects worked weekly for longer hours, it is possible that both long working hours and long daily VDT hours are major factors associated with depressive state and subjective symptoms. A vicious cycle may occur because masked depressions also induce various somatic symptoms. Moreover, our study shows that too many quotas, poor relationships with colleagues, and monotonous work are also related to a depressive state.

The most widely discussed personality characteristic with respect to occupational stress is the type A and type B differentiation described first by two American cardiologists, Friedman and Rosenman. In our previous survey, a high rate of type A was seen among workers using computerized techniques (Watanabe *et al.*, 1989). However, this study showed a normal type A distribution. This discrepancy was ascribed to management differences between private enterprise and government offices. In our investigations, type A subjects had many more symptoms than non-type A. Furthermore, the percentage of type A subjects who stated that they had experienced subjective personal change was higher than that of non-type A. Further research is needed to evaluate effects of VDT work on type-A behavior pattern.

CONCLUSIONS

The percentage of type A subjects estimated by the brief questionnaire was 15.2%. The subjects classified as type A worked longer hours than non-type A subjects. Type A subjects had more eye, musculoskeletal, respiratory, and cardiovascular symptoms.

The health status and the working conditions of VDT workers are significantly affected by the attitudes toward computers. We found that the technoanxious subjects had a much higher previous history of duodenal ulcer (13.9%) than the others. In addition, the technoanxious group showed the high prevalences of awkward VDT operators and dissatisfaction with computer training. From these results, it is suggested that technoanxiety is a considerable stress factor, which is provoked by the lack of computer training.

The prevalence of depression estimated by SRQ-D is 1.2%. The mean weekly working hours of depressive group was 61.3 hr, much higher than that of the other groups. It seems that long working hours, high quotas, poor relationships with colleagues, and monotonous work are also linked to a depressive state.

To promote VDT workers' health, health supervisors, occupational doctors, and other health-related staffs must pay more attention to VDT workers' attitudes toward computers, overwork, and relationships with other workers.

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Occupational Influences Relative to the Burnout Phenomenon among Japanese Nursery School Teachers

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To identify and evaluate recent working conditions and job content of nursery school teachers in Japan, as well as the prevalence of the burnout phenomenon and the occupational influences responsible for it, a questionnaire survey was carried out. The subjects consisted of 719 nursery school teachers and 204 municipal clerical workers as the control group. Working conditions and workload burdens were more severe among nursery school teachers than those of the clerical workers. The burnout phenomenon among the nursery school teachers was characterized by emotional exhaustion. Moreover, the rate ratio and multivariate analyses indicated that a great variety of occupational factors, not only interpersonal relationships, but also the general working conditions and specific physical or mental workloads, influenced the burnout phenomenon as well. Therefore, in examining measures dealing with the burnout phenomenon among nursery school teachers, it is important to evaluate the occupational factors systematically and comprehensively.

INTRODUCTION

Recent accelerated social changes in Japan such as the aging population, the transformation into an information-oriented society, an obsession with educational credentials, a decrease in family size, and an increase in the number of working women, have had a profound effect on the part played by nursery school teachers in the process of teaching and caring for children. For example, there has been a reduction in the amount of time spent with children by their parents; a reduction in the involvement in the administration of education on the part of families, relatives, and the social network; and an increasing need to redefine values and objectives as they pertain to education for nursery school children.

The most obvious result of all this has been the wide-ranging and complex role entrusted to nursery school teachers by society as well as the anxiety felt by them relative to the meaning and aims of their job. However, school administrations have not come up with any strategies for coping with this new situation. This increase in teachers' responsibilities has not been accompanied by necessary changes in their training; changes which would enable them to cope with these new demands.

In addition, the fertility rate in Japan has rapidly decreased since the late 1970s. This situation has created an imbalance between the number of children and the number of nursery schools and has led nursery schools to become highly competitive in trying to get nursery school children. Nursery schools have prolonged the care time of children for working mothers and set up more attractive and sophisticated curricula. However, these measures were supported neither by an

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increase in staff nor by an effort to further their education and training. Further, to improve financial conditions, schools have reduced the number of nursery school teachers as well as replaced them with part-time teachers. Consequently, working conditions and workloads among nursery school teachers have become much more difficult than before.

Recently, teacher stress and the burnout syndrome among educational workers have received considerable attention. A large number of studies indicated that teaching in schools is one of the most stressful professions (Kyriacou and Sutcliff, 1977; Cummings, 1983; Kyriacou, 1987). Although nursery school teachers fall in the same category of workers, the prevalence and characteristics of burnout, and its occupational influences, among them has not as yet been examined.

In Japan, research on health problems among nursery school teachers has mainly been focused on musculoskeletal disorders (Nagira *et al.*, 1981). The main purpose of this study is to identify and evaluate the recent working conditions and workloads among nursery school teachers. Moreover, the prevalence and characteristics of burnout phenomenon and its occupational risk factors among these teachers were examined.

SUBJECTS AND METHODS

A questionnaire survey was conducted. The subjects consisted of all 896 nursery school teachers of all nursery schools in K prefecture and all 220 municipal clerical female workers as a control group. Although subjects are different between the subject and the control groups, the clerical workers also deal with people such as residents. To avoid sociocultural confounding factors, the control group was selected from the workers at the nearest municipal office branch to each nursery school

The questionnaire dealt with general demographic characteristics, working conditions and environments, job content, and burnout symptoms. The Maslach Burnout Inventory (MBI) (Maslach and Jackson, 1981) was used to evaluate the burnout phenomenon. The valid responses (and response rates) were 719 (80.2%) and 204 (92.7%), respectively. The mean age and duration of job experience among the nursery school teachers was 36.3 (SD = 8.9) and 15.0 (SD = 8.6), respectively. That among the clerical workers was 37.9 (9.4) and 15.8 (9.2).

In analyzing the data, the working conditions and workloads were first compared between the nursery school teachers and the clerical workers. The statistical significance was evaluated by the χ^2 test.

Second, to evaluate the prevalence and the magnitude of the burnout phenomenon, the average scores of MBI, classified by its four subscales (Emotional Exhaustion, Personal Accomplishment, Depersonalization, and Personal Involvement), were compared between the nursery school teachers and the clerical workers. They were also compared by job category among the nursery school teachers. The Emotional Exhaustion subscale assesses feelings of being emotionally overextended and exhausted by one's work. The Personal Accomplishment subscale measures feelings of competence and successful achievement in one's work with people. The Depersonalization subscale assesses lack of feeling and impersonal response toward recipients of one's service. The Personal Involvement assesses feelings of involvement with others. High degrees of burnout are reflected in high average scores on the Emotional Exhaustion, Depersonalization, and Personal

Involvement scales and low average scores on the Personal Accomplishment scale. The distribution of scores of Emotional Exhaustion, Personal Accomplishment, and Personal Involvement were a dome type (kurtosis <3, skewness $= 0$). That of Depersonalization was an unsymmetric type (kurtosis >3, skewness >0). Although the scores of MBI were not normally distributed, normal distribution was assumed according to the central limit theorem. The statistical significance was evaluated by the Student t test.

Taking the differences in workloads and burnout phenomenon according to job category into consideration, ordinary nursery school teachers who took care of the children directly, and who were in the largest job category, were selected as subjects for analysis of the risk factors in the burnout phenomenon. High-scoring prevalence rates in the subscale of MBI, Emotional Exhaustion, were calculated according to demographical and occupational items among the 478 ordinal nursery school teachers. The criterion of high score is more than 35 points in total in the nine items of the subscales of MBI. This criterion, which is 4 points on average per item, means that nursery school teachers feel emotional exhaustion several times per month. Moreover, it exceeds the range of the high burnout category (more than 30) (Maslach and Jackson, 1981; McGrath *et al.*, 1989). On the other hand, although the gold standard of burnout has not established, its external validation has been obtained by use of observers such as physicians, psychologists, and spouses (Perlman and Hartoman 1982; Maslach and Jackson, 1981; Rafferty, 1986).

Rate ratios for this prevalence rate, similar to the concept of relative risk, and their confidence intervals (95%) among these items were computed to evaluate occupational influences on burnout phenomenon (Miettinen, 1985; Karvonen and Mikheev, 1986; Checkoway *et al.*, 1989; Hisashige *et al.*, 1989). The standard prevalence rates used as denominators for the rate ratios were those for the ordinal or interval category which was considered to produce a workload at the lowest level within each item. For example, in the ordinal category such as workload, the category, where workload was not intense, was used as the standard, taking the distribution of each category into consideration.

Finally, to simultaneously examine the effect of multiple risk factors on a burnout symptom, Emotional Exhaustion, and confirm the result of univariate rate ratio analysis, multiple stepwise regression analysis was performed (SPSS:PC+ V3.0, Norusis, 1986). The variable selection procedures were straightforward. The selection criterion is the probability with a default of 0.05. The frequency score of Emotional Exhaustion (MBI) was used as a dependent variable while 30 occupational factors, where rate ratios were relatively high, were used as independent variables. Since many ordinary variables were included among the explanatory variables, dummy variables were used in this analysis (Kleinbaum *et al.*, 1990).

RESULTS

The Working Conditions and Workloads of Nursery School Teachers

As shown in Table 1, the proportion of working hours exceeding 7 hr, staggered working hours, overtime work exceeding 10 hr per month, and after-hours work were higher among the nursery school teachers than among the clerical workers ($P < 0.001$). Moreover, the availability of break periods and vacation time was

limited among the nursery school teachers when compared to the clerical workers ($P < 0.001$). This inability to take a break frequently hampered nursery school teachers from being able to use the lavatory.

Table 2 shows a comparison of workloads between the nursery school teachers and the clerical workers. The frequency of unnatural postures required during work such as bending, deep bending, twisting, and squatting were higher among the nursery school teachers than the clerical workers ($P < 0.001$). Also, incidents of standing while being constrained from movement were more frequent among nursery school teachers. Handling heavy materials, including nursery school children, was performed more frequently by nursery school teachers. The weight of materials was higher among the nursery school teachers when compared to clerical workers.

In addition to these physical stressors, nursery school teachers were exposed to many psychological stressors. They felt more irritation, felt more compelled to act, and felt that more intense attention was required to make decisions by themselves when compared to the clerical workers ($P < 0.01$–$P < 0.001$). Regarding mental conflicts with others, a difference was observed in the conflict with those groups regarded as the objects of their attention (teachers to children, clerical workers to clients) ($P < 0.001$).

Characteristics of Workloads among Nursery School Teachers

Table 3 shows that the range of workload intensity among nursery school teachers varied widely according to their categories. The proportion of intense or very intense, relative to the psychological workload, was highest in the category of informing and explaining to children's parents. The second highest workload consisted of recording, connecting, and arranging. On the other hand, the proportion of intense or very intense in the physical workload category was the highest in that of teaching exercises.

Prevalence of Burnout Phenomenon and Its Occupational Influences

Figure 1 shows a comparison in the average scores of burnout symptoms arranged by subscales of MBI between the nursery school teachers and the clerical workers. Nursery school teachers (mean ± SD, 2.1 ± 1.1) scores significantly higher than clerical workers (1.3 ± 0.9) on the subscale reflecting Emotional

TABLE 1
WORKING CONDITIONS AMONG NURSERY SCHOOL TEACHERS

Items	Nursery school teachers (N = 719)	Clerical workers (N = 204)
Working hours (>7 hr)	645 (90.4)	105 (51.4)***
Staggered working hours	667 (92.8)	7 (3.5)***
Overtime work (>10 hr/month)	210 (29.2)	13 (6.4)***
After hours work	557 (76.3)	29 (14.3)***
Difficulties in taking paid vacation	121 (16.8)	19 (9.3)***
Lack of rest time during work	560 (77.9)	64 (31.4)***
Shortage of lunch time	317 (44.1)	6 (2.9)***
Difficulties in going to the lavatory	357 (49.7)	17 (8.3)***

*** $P < 0.001$ (by χ^2 test).

TABLE 2
WORKLOADS OF NURSERY SCHOOL TEACHERS

Items	% Nursery school teachers (N = 719)	% Clerical workers (N = 204)
Working postures (frequent)		
Bending	522 (72.6)	12 (5.9)***
Deep bending	229 (31.8)	4 (2.0)***
Twisting	257 (35.7)	5 (2.5)***
Standing	563 (78.3)	27 (13.2)***
Squatting	475 (66.1)	5 (2.5)***
Handling heavy materials		
Frequent	472 (65.6)	7 (3.4)***
Weight (>10 kg)	615 (85.6)	23 (11.3)***
Emotion felt during work (frequent)		
Irritation	194 (27.0)	31 (15.2)***
Intense attention	418 (58.1)	71 (34.8)***
Being compelled to act	285 (39.6)	59 (28.9)**
Being required to make decisions frequently	372 (51.7)	53 (26.0)***
Boredom	29 (4.0)	16 (7.8)*
Interpersonal conflicts (sometimes)		
Co-workers	372 (51.8)	79 (38.7)
Administrative personnel	289 (40.2)	72 (35.3)
Other staff	242 (33.6)	58 (28.4)
Clients (or children's parents)	375 (52.1)	70 (34.4)***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (by χ^2 test).

Exhaustion ($P < 0.001$). Also, they (1.5 ± 1.3) scored significantly higher than clerical workers (0.6 ± 0.5) in Personal Involvement ($P < 0.001$). Whereas clerical workers (0.44 ± 0.37) scored significantly higher than nursery school teachers (0.26 ± 0.22) in Depersonalization ($P < 0.001$). On the other hand, regarding Personal Accomplishment, the average score among nursery school teachers (2.7 ± 1.3) was higher than that among clerical workers (1.4 ± 1.2) ($P < 0.001$).

Figure 2 shows the average scores for each subscale of MBI classified by job category. Ordinary teachers (mean ± SD, 2.4 ± 1.0) scored significantly higher than substitute teachers (1.9 ± 1.0) and administrative personnel (1.7 ± 1.0) on the subscales of Emotional Exhaustion ($P < 0.001$). The average score of Depersonalization among ordinary teachers (0.28 ± 0.26) was significantly higher than among substitute teachers (0.14 ± 0.23) and administrative personnel (0.14 ± 0.15). Relative to Personal Involvement, ordinary teachers (1.7 ± 1.3) scored higher than the chief teachers (1.1 ± 1.3) and the administrative personnel (0.7 ± 1.0) ($P < 0.05$ or $P < 0.001$). The average score for Personal Accomplishment among the ordinary teachers (2.8 ± 1.3) was higher than among the administrative personnel (2.1 ± 1.5) ($P < 0.01$).

High-ranking rate ratios of Emotional Exhaustion among ordinary nursery school teachers are shown in Table 4. They consisted of diverse occupational factors, which included not only mental and physical workloads, but also working conditions, such as workloads involving recording, connecting, and arranging (psychological) (RR = 4.45); teaching basic living skills (3.21); difficulties in going to the lavatory (3.18); teaching exercises (psychological) (3.09); and the feeling of being compelled to act (3.07).

TABLE 3
INTENSITY OF WORKLOADS AMONG NURSERY SCHOOL TEACHERS

Items	Proportion (N = 719)	
	a (%)	*b* (%)
Directing play activities		
Psychological	20.7	58.0
Physical	11.7	52.0
Teaching basic living skills		
Psychological	22.9	66.8
Physical	20.9	60.0
Teaching exercises		
Psychological	28.8	65.8
Physical	21.6	61.4
Informing and explaining to children's parents		
Psychological	38.9	79.0
Physical	16.0	52.9
Recording, connecting, and arranging		
Psychological	29.9	83.2
Physical	20.0	61.2

[a] Very intense.
[b] Intense or very intense.

Rate ratios for confounding factors, such as age (1.28), marital status (1.12), number of children (1.01), and household matters (0.83), were not significantly high ($P > 0.05$). On the other hand, those of length of employment (1.43) and working hours (1.38) were also not significantly high ($P > 0.05$).

As shown in Table 5, eight occupational factors, such as the workloads involving recording, connecting, and arranging (standardized multiple coefficient = 0.180), mental conflict with co-workers (0.138), and feeling of being compelled to act (0.170), were selected as the main independent variables by utilizing the multiple stepwise regression analysis. This model's multiple correlation coefficient was 0.596 ($P < 0.001$). These factors show the same diverse characteristics in

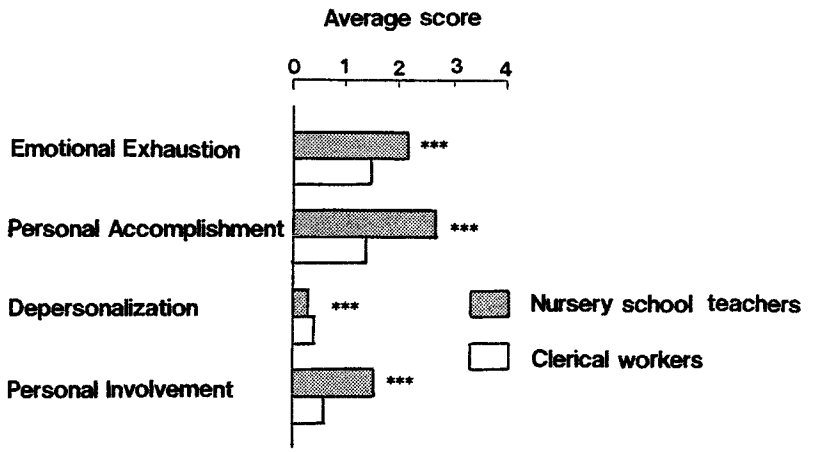

FIG. 1. Comparison of burnout phenomenon between nursery school teachers and clerical workers. ***$P < 0.001$ by t test.

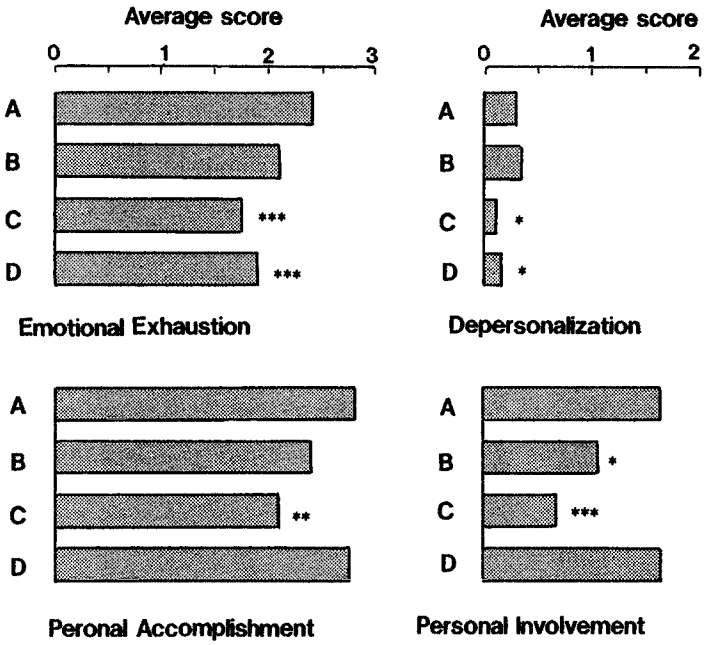

FIG. 2. Burnout phenomenon among nursery school teachers classified by job category. (A) Ordinary nursery school teachers (N = 478). (B) Chief nursery school teachers (N = 50). (C) Administrative personnel (N = 71). (D) Substitute teachers (N = 120). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with ordinary nursery school teachers by t test.

terms of workloads and working conditions mentioned before among the nursery school teachers. The results of the multivariate analysis confirmed those of the univariate analysis.

DISCUSSION

Over the past 10 years interest has continued to increase relative to occupational stress among school teachers in Western countries (Kyriacou and Sutcliffe, 1977; Kyriacou, 1987). A large number of studies have indicated that school teaching is one of the most stressful of professions, and the nature of the demand placed on teachers in their work will likely cause it to remain a stressful profession for many years (Kyriacou and Sutcliffe, 1977; Kyriacou, 1987; Cunningham, 1983). In Japan, however, teacher's stress has yet to be examined thoroughly.

The findings of this study indicate that nursery school teachers in Japan are experiencing considerable burnout on the job in comparison with municipal clerical workers relative to emotional exhaustion and personal involvement (Fig. 1). Maslach (1982) pointed out that emotional exhaustion is at the heart of the burnout syndrome and that it may develop into the second aspect of burnout, a dehumanized response to other people, and the third aspect of burnout, a diminished sense of self-worth. Nursery school teachers did not show the depersonalization tendency nor reduced feeling of personal accomplishment. It seems that the burnout syndrome among Japanese nursery school teachers does not progress to its final stage. The main reason for this is that subjects receiving the attention of the nursery school teachers were of a very specific age group and, naturally, much

TABLE 4
RATE RATIOS FOR EMOTIONAL EXHAUSTION BY OCCUPATIONAL ITEMS (ORDINARY NURSERY SCHOOL TEACHERS)

Items	Rate ratios
Working conditions	
Staggered working hours (none vs frequent)	2.22
Overtime work (none vs more than 1 hr/mo)	1.69
After hours work (none vs frequent)	1.70
Difficulties in taking paid vacation (seldom vs frequent)	1.54
Lack of rest time during work (seldom vs frequent)	1.53
Shortage of lunch time (moderate vs short)	1.93
Difficulties in going to the lavatory (seldom vs frequent)	3.18
Number of nursery school children (9 vs more than 9)	1.79
Age of nursery school children (under 3 years vs 3 years and more)	1.86
Workloads	
Intensity of workloads	
Directing play activities	
Psychological (moderate vs intense)	2.94
Physical (moderate vs intense)	2.57
Teaching basic living skills	
Psychological (moderate vs intense)	3.21
Physical (moderate vs intense)	2.74
Teaching exercises	
Psychological (moderate vs intense)	3.09
Physical (moderate vs intense)	2.85
Informing and explaining to children's parents	
Psychological (moderate vs intense)	2.81
Physical (moderate vs intense)	2.41
Recording, connecting and arranging	
Psychological (moderate vs intense)	4.45
Physical (moderate vs intense)	2.89
Working postures	
Bending (seldom vs frequent)	1.72
Standing (seldom vs frequent)	2.62
Squatting (seldom vs frequent)	2.60
Handling heavy materials (seldom vs frequent)	1.95
Emotion felt during work	
Being compelled to act (seldom vs frequent)	3.07
Being required to make decisions frequently (seldom vs frequent)	1.51
Interpersonal conflicts	
Nursery school teachers (seldom vs frequent)	2.78
Administrative personnel (seldom vs frequent)	1.77
Other staff (seldom vs frequent)	2.75
Children's parents (seldom vs frequent)	2.24
Working environment	
Noise (quiet vs noisy)	1.71

Note. High-ranking 30 items ($P < 0.05$).

different from those who receive attention from social welfare and health care workers.

The average score (2.70) for Personal Accomplishment among Japanese nursery school teachers in this study is extremely low in comparison with the results among teachers in other developed countries whose average scores exceeded 4.00 (Meadow, 1981; Belcastro *et al.*, 1983; Capel, 1987). Therefore, the burnout syn-

TABLE 5
THE RESULT OF MULTIPLE STEPWISE REGRESSION ANALYSIS FOR EMOTIONAL EXHAUSTION (ORDINARY NURSERY SCHOOL TEACHERS)

Items	β	*T*	*P* value
Intensity of workload: recording, connecting and arranging (psychological)	0.180	4.43	0.000
Interpersonal conflict: with nursery school teachers	0.138	3.39	0.001
Emotion felt during work: being compelled to act	0.170	4.87	0.000
Intensity of workload: directing play activities (psychological)	0.123	2.90	0.004
Working environment: noise	0.101	3.10	0.002
Interpersonal conflict: with other staff	0.118	2.90	0.004
Intensity of workload: teaching basic living skills (physical)	0.096	2.30	0.022
Working conditions: difficulties in going to the lavatory	0.072	2.15	0.032
Multiple regression coefficient		0.569	
Contribution ratio		0.324	
F value (*df* = 8)		38.973	

drome may be considered more serious among nursery school teachers in Japan than in developed Western countries. This phenomenon was observed also in the study of Japanese nurses (Hisashige *et al.*, 1989). How the nature of the work is evaluated may be dependent on the sociocultural background of each country. Differences of burnout among these countries must be examined from this cultural–anthropological aspect. In this sense, the differences in the burnout phenomenon between nursery school teachers and clerical workers, in this study, should be reanalyzed relative to job characteristics as they relate to social need and importance in Japanese society, as well as the situation in which burnout is likely to occur.

Working conditions such as overtime work, long working hours, and lack of adequate rest periods were poor among nursery school teachers (Table 1). Moreover, workload burdens which included not only physical loads (e.g., unnatural working postures, handling heavy materials) but also mental loads (e.g., intense psychological work load, emotions felt during work, mental conflict with others) were heavier among them (Tables 2 and 3). These occupational factors were pointed out as major risk factors in the burnout phenomenon (Hisashige *et al.*, 1989).

Occupational factors relative to the burnout syndrome among nursery school teachers have not been analyzed systematically in past research. Results of this study indicate a great variety of occupational factors; not only stress incurred from interpersonal conflicts, but also general working conditions and specific physical and mental workloads influenced the burnout phenomenon among nursery school teachers (Tables 1–3). Pines (1982) identified the dimensions of the organizational environment which have been found to play an important part in preventing or accelerating burnout. These include the psychological, physical, social, and work environment. The results of this study are consistent with those dimensions, and, it is hoped, will help to create a more encompassing understanding of occupational influences on burnout phenomenon among nursery school

teachers. In examining preventive measures in health care for problems such as the burnout phenomenon, it is important to evaluate the occupational influences systematically and comprehensively.

However, the results must be evaluated cautiously, as the design of this study was cross-sectional. The results may be vulnerable to information bias as well as selection bias. Relative to selection bias, the results of this study would underestimate relative risks as they pertain to occupational factors. The largest workload category does not necessarily show the highest prevalence rate among several items in examining a dose–response relationship even when this relationship exists. Regarding information bias, there is not sufficient data to enable a detailed examination. It is also very difficult to rule out the possibility that nursery school teachers, with burnout symptoms, estimated their own workload more severely than the healthy teachers did. Therefore, the risk factors suggested in this study must be evaluated next to a more concentrated study design focusing on the examination of causal relationship.

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Multidimensional Assessment of Mental State in Occupational Health Care—Combined Application of Three Questionnaires: Tokyo University Egogram (TEG), Time Structuring Scale (TSS), and Profile of Mood States (POMS)[1]

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We attempted to develop a brief test battery to assess mental state for use in occupational health care settings. As a first step, we focused on the following three psychosocial aspects: ego state, behavioral pattern, and mood state which were considered to be closely related to mental state. To evaluate these parameters, we selected three established self-rating questionnaires: the Tokyo University Egogram for ego state, the Time Structuring Scale for behavioral pattern, and the Profile of Mood States for mood state. The combination of these three questionnaires was applied on 300 healthy company employees and school teachers (170 males and 130 females). Five ego-state factors (Critical Parents, Nurturing Parents, Adult, Free Child, and Adapted Child), five behavioral pattern factors (Withdrawal, Rituals and Pastimes, Activities, Intimacy, and Games), and six mood state factors (Tension–Anxiety, Depression–Dejection, Anger–Hostility, Vigor–Activity, Fatigue–Inertia, and Confusion–Bewilderment) were scored. Of 85 correlations between test factors, 49 in males and 61 in females were not significant, indicating that each test in this battery assessed aspects of mental state rather independent of those assessed by the others. However, significant correlations ($P < 0.05$) were observed for remaining pairs of test factors, indicating that these three parameters were also interrelated with each other, indicating that mental state could be elucidated more comprehensively by assessing all three psychosocial parameters than by assessing only one. The possibility that this battery could be used in the future in worksite health promotion programs is discussed.

INTRODUCTION

According to the World Health Organization, "health" is defined as the presence of physical, mental, and social well-being, and not simply as the absence of disease or infirmity (World Health Organization, 1986). A human being lives in a complex biopsychosocial environment as individual organism with an integrated body and mind. In industrialized countries, however, occupational environments have recently become more complicated and stressful. Many employees confronted with such stressful circumstances complain of psychological problems, which have been recognized as a leading occupational health problem (Kalima, 1987; Sauter *et al.*, 1990). Conceptual aspects of psychologically mediated diseases have been discussed (Levi, 1987; Elkin and Rosch, 1990), and mental health and physical health are now thought to be inextricably linked (Scofield, 1990).

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With the recognition that mental health is becoming an important issue in occupational health care, much research has been performed. Methodologically, research on psychiatric epidemiology has been based mostly on self-rating questionnaires and/or semi-structured interview protocols. Several screening methods to detect psychological illness or to evaluate the outcome of intervention have been developed (Clark and Friedman, 1983; Hase and Luger, 1988; Liang *et al.*, 1989; Weinstein *et al.*, 1989). Among them, the General Health Questionnaire (Goldberg, 1972) is the most widely used for screening, and efforts are being made to increase its sensitivity and specificity (Berwick *et al.*, 1991). However, these screening methods were designed to detect only psychological illness. For preventive and therapeutic intervention to be effective, mental state must be described more clearly and comprehensively. At present, no useful brief test or test battery to assess individual mental state is available. Thus, we have attempted to develop a brief, self-rating test battery for assessing mental state for use in occupational health care settings.

Mental state is thought to be strongly influenced by personality, and mental state directly affects mood state and behavioral pattern. As a first step to our goal, we selected three psychological parameters: ego state, behavioral pattern, and mood state. The three parameters, respectively, can be evaluated separately with the following previously developed questionnaires: Tokyo University Egogram (TEG, Ishikawa *et al.*, 1984; Suematsu *et al.*, 1989), Time Structuring Scale (TSS, Nomura *et al.*, 1989), and Profile of Mood States (POMS, McNair *et al.*, 1971, 1981; Yokoyama *et al.*, 1990; Akabayashi *et al.*, 1991). According to the theory of Transactional Analysis, "ego state" is defined as the way in which one manifests a certain part of one's personality at a given time (Berne, 1961; see Appendix).

This is the first study in which the combination of these three questionnaires has been applied. Methods of evaluating the validity of this test battery and the possibility of its use in occupational mental health settings are discussed.

SUBJECTS AND METHODS

Subjects

Subjects were 170 males aged 33.9 ± 10.5 (range, 20–60) years and 130 females aged 39.2 ± 9.4 (range, 21–60) years who were free from any psychiatric disorders and were employed as white collar employees or school teachers at three Japanese industrial companies and three high schools. We considered them to be representative of white collar workers.

Tests

The TEG (see Table 1 and Appendix) is a questionnaire (60 items) to assess ego state developed by our group which assesses the following five ego-state factors: Critical Parents, Nurturing Parents, Adult, Free Child, and Adapted Child. The TSS (see Table 1 and Appendix) is a questionnaire (78 items) to assess behavioral pattern, which was also developed by our group, which assesses the following five behavioral factors: Withdrawal, Rituals and Pastimes, Activities, Intimacy, and Games. The Japanese edition of POMS (see Table 1 and Appendix; 65 items, Yokoyama *et al.*, 1990), which is a translation of the original (McNair *et al.*, 1971), assesses the following six mood-state factors: Tension–Anxiety, Depression–

TABLE 1
LIST OF TEST FACTORS

Test factors	Abbreviations
TEG factors	
Critical parents	CP
Nurturing parents	NP
Adult	A
Free child	FC
Adapted child	AC
TSS factors	
Withdrawal	W
Rituals and pastimes	RP
Activities	A
Intimacy	I
Games	G
POMS factors	
Tension–Anxiety	T
Depression–Dejection	D
Anger–Hostility	A
Vigor–Activity	V
Fatigue–Inertia	F
Confusion–Bewilderment	C

Dejection, Anger–Hostility, Vigor–Activity, Fatigue–Inertia, and Confusion–Bewilderment.

Administration of the Test Battery

The test battery consisting of TEG, TSS, and POMS was handed to each subject by a member of the health care staff at the subject's workplace. The subjects were randomly selected by the staff of each workplace. The subject was requested to complete all questionnaires within the next few days. Completed questionnaires returned to the health care staff were then mailed back to us; the recovery rate was 90%. We then scored the 16 factors and determined the 85 coefficients of correlation for interest factors. Significance of coefficients was determined by the method of Simultaneous Inference for Coefficients of Correlation (Bonferroni method, Morrison, 1976).

Feedback to Subjects

All subjects received questionnaire results, and the health care staff at each company received institution-wide data. The feedback report to each subject consisted of the subject's scores and normal range values for each factor, general evaluation of the results for each factor, and brief suggestions for daily life. The health care staff at each company received mean scores for the subjects at that company and general comments on them.

RESULTS

Mean Raw Scores on the Three Questionnaires

Mean raw scores on the 16 factors are shown in Table 2. Statistically significant sex differences were observed for the factors TEG-NP, TSS-RP, TSS-A, TSS-I, POMS-A, and POMS-F.

TABLE 2
SCORES ON TEG, TSS, AND POMS IN 300 SUBJECTS (MEAN ± SD)

	Males (N = 170)	Females (N = 130)	Sex difference[a]
TEG factors			
CP	8.0 ± 4.0	8.0 ± 4.3	N.S.[b]
NP	13.9 ± 3.5	15.4 ± 3.3	$P < 0.01$
A	12.5 ± 3.6	12.4 ± 3.8	N.S.
FC	10.4 ± 3.6	10.6 ± 3.9	N.S.
AC	8.2 ± 4.4	9.0 ± 4.5	N.S.
TSS factors			
W	8.8 ± 3.8	9.1 ± 3.5	N.S.
RP	20.0 ± 4.5	21.6 ± 4.1	$P < 0.01$
A	14.0 ± 5.2	12.0 ± 5.2	$P < 0.01$
I	20.9 ± 5.9	24.6 ± 6.1	$P < 0.01$
G	10.7 ± 6.0	11.4 ± 5.3	N.S.
POMS factors			
T	12.2 ± 6.0	12.0 ± 7.1	N.S.
D	11.4 ± 9.3	10.2 ± 9.1	N.S.
A	11.6 ± 8.9	9.6 ± 8.1	$P < 0.05$
V	14.4 ± 6.1	14.2 ± 6.0	N.S.
F	10.0 ± 6.4	8.1 ± 5.8	$P < 0.01$
C	8.6 ± 4.4	8.1 ± 4.6	N.S.

[a] Significance of difference between males and females was examined (t test).
[b] N.S., not significant ($P > 0.05$).

Relationships among Factors of Three Questionnaires

Table 3 presents the coefficients of correlation between TEG factors and POMS factors. Of 30 correlations, 14 in males and 10 in females were significant. Coefficients greater than 0.35 were observed for several pairs of factors, i.e., in males,

TABLE 3
CORRELATION COEFFICIENTS OF FACTORS ON TEG AND POMS

	TEG factors				
	CP	NP	A	FC	AC
Males (N = 170)					
POMS-T	0.33*	−0.02	−0.14	0.28*	0.44*
POMS-D	0.28*	−0.08	−0.19	0.32*	0.47*
POMS-A	0.40*	−0.15	−0.06	0.34*	0.27*
POMS-V	−0.01	0.25*	0.23	0.20	−0.27*
POMS-F	0.18	−0.11	−0.15	0.19	0.34*
POMS-C	0.17	−0.13	−0.28*	0.17	0.42*
Females (N = 130)					
POMS-T	0.26	−0.02	−0.12	0.21	0.29*
POMS-D	0.28*	−0.12	−0.20	0.13	0.40*
POMS-A	0.32*	−0.12	−0.11	0.23	0.30*
POMS-V	−0.08	0.31*	0.28*	0.25	−0.15
POMS-F	0.27	−0.04	−0.14	0.25	0.33*
POMS-C	0.29*	−0.12	−0.19	0.24	0.34*

* $P < 0.05$.

TEG-CP and POMS-A, TEG-AC and POMS-T, and TEG-AC and POMS-C, and, in both males and females, TEG-AC and POMS-D.

The correlation coefficients for TEG and TSS factors are shown in Table 4. Of 25 correlations, 10 in males and 7 in females were significant. Coefficients greater than 0.35 were observed for several pairs of factors, i.e., TEG-NP and TSS-RP in males, TEG-NP and TSS-I in both males and females, TEG-A and TSS-A in females, and TEG-AC and TSS-G in both males and females.

Table 5 shows the correlation coefficients for TSS and POMS factors. Of 30 correlations, only 12 in males and 7 in females were significant. Coefficients greater than 0.35 were observed for several pairs of factors, i.e., in males, TSS-W and POMS-D, TSS-W and POMS-C, and TSS-I and POMS-V, and, in both males and females, TSS-G and POMS-T, TSS-G and POMS-D, TSS-G and POMS-A, TSS-G and POMS-F, and TSS-G and POMS-C.

DISCUSSION

The present study was designed to develop a new tool for assessing mental state for use in occupational health care settings. These three psychological tests were chosen because of their brevity (the battery takes about 20 min to complete), ease of administration and scoring, established validity and reliability, comprehensive coverage of important aspects of mental states (ego state, behavioral pattern, and mood state), and the fact that they are all self-reporting tests the results of which can be explained to the subjects.

The mean raw scores obtained in this study were quite consistent with those obtained in previous studies (see Appendix). Data on sex differences were slightly different from the previous data, probably reflecting the demographic characteristics of the subjects in this study. Statistical analysis revealed that 49 of 85 factor pairs in males (58%) and 61 in females (72%) were not significantly correlated ($P > 0.05$). This finding indicates that this battery can be used to assess independent aspects of mental state.

However, significant correlations ($P < 0.05$) were observed for the remaining factor pairs. This result could be explained that the two factors in one pair were

TABLE 4
CORRELATION COEFFICIENTS OF FACTORS ON TEG AND TSS

	TEG factors				
	CP	NP	A	FC	AC
Males (N = 170)					
TSS-W	0.10	0.01	0.06	0.05	0.33*
TSS-RP	−0.12	0.42*	0.07	0.23	0.08
TSS-A	0.34*	0.19	0.28*	0.18	−0.02
TSS-I	−0.03	0.43*	0.26*	0.20	−0.24*
TSS-G	0.33*	−0.10	−0.26*	0.15	0.71*
Females (N = 130)					
TSS-W	0.13	−0.03	0.11	0.18	0.20
TSS-RP	0.08	0.31*	0.21	0.25	0.10
TSS-A	0.03	0.34*	0.36*	0.10	0.01
TSS-I	0.10	0.36*	0.25	0.27	−0.11
TSS-G	0.30*	−0.21	−0.28*	0.05	0.63*

* $P < 0.05$.

TABLE 5
CORRELATION COEFFICIENTS OF FACTORS ON TSS AND POMS

	TSS factors				
	W	RP	A	I	G
Males (N = 170)					
POMS-T	0.27*	0.11	0.21	−0.01	0.62*
POMS-D	0.41*	0.05	0.10	−0.12	0.70*
POMS-A	0.26*	0.02	0.13	−0.03	0.52*
POMS-V	0.02	0.22	0.32*	0.37*	−0.23
POMS-F	0.29*	0.04	0.07	−0.12	0.55*
POMS-C	0.36*	−0.02	0.05	−0.16	0.59*
Females (N = 130)					
POMS-T	0.20	0.04	0.07	−0.09	0.50*
POMS-D	0.22	−0.01	0.02	−0.17	0.56*
POMS-A	0.22	0.09	0.01	−0.08	0.43*
POMS-V	0.12	0.24	0.33*	0.33*	−0.24
POMS-F	0.27	0.12	0.02	−0.08	0.43*
POMS-C	0.13	−0.01	0.06	−0.03	0.50*

* $P < 0.05$.

detecting a certain mental state in different ways. We discuss those pairs with r greater than 0.35 from this perspective. TEG-CP was correlated with POMS-A, indicating that a critical or controlling ego state is accompanied by aggressive mood. TEG-NP was correlated with TSS-RP and TSS-I. TSS-RP reflects pleasant ways of filling time and getting to know people, and TSS-I reflects a loving relationship with another person. It is reasonable that these pleasant and comfortable ways of using one's time would be related to a nurturing and sympathetic ego state. The correlation between TEG-A and TSS-A can easily be explained by the assertion that, when one is engaging in work activities with others, one must have an adult ego-state. TEG-AC was correlated with POMS-T, POMS-D, POMS-C, and TSS-G. When one is highly tense, depressed, or confused, one does not know how to behave properly, which might result in dependence or avoidance. This could lead to the AC ego state, in which no responsibility is felt and behavior is designed to conform to what others expect. In AC ego state, one is also mentally suppressed. This frustration could lead to G behavior in an effort to feel superior to others.

TSS-W was correlated with POMS-D and POMS-C. It is easily understood that one who is depressed or confused is also withdrawn from the people around him. On the other hand, when one has intimate relationships with others, one is filled with vigor, which explains the correlation between TSS-I and POMS-V. When engaging in G behaviors, one would experience a variety of undesired, negative feelings, such as tension, anger, depression, and confusion. Thus, it is reasonable that TSS-G was relatively highly correlated with all POMS factors except V.

As discussed above, the parameters of ego state, behavioral pattern, and mood state are interrelated with each other. Thus, scores on this battery would more clearly and comprehensively elucidate the subject's complex and transient mental state than would separate use of each questionnaire. Such detailed information on employee's mental states is certainly useful not only in therapeutic intervention (e.g., employee assistance programs) but also in other preventive worksite health promotion projects.

Elkin and Rosch (1990) classified stress-related symptoms as physical, psychological, or behavioral. They also described a model of workplace stress, in which they stated that stress could best be understood as a dynamic process initiated by a stressor and mediated or interpreted by the individual which had physical, emotional, behavioral, and organizational consequences. The present battery is thought to assess the emotional (by POMS) and behavioral consequences (by TSS), as well as individual mediation by self-evaluation of transient personality (by TEG). Other stress-related consequences, such as physical problems, could be screened by routine physical health check-up programs. Organizational consequences could be evaluated by monitoring such indicators as absenteeism, accidents, and job turnover.

Another characteristic of this battery is that the results of three tests can be easily reported to the subjects. The subjects who get the results will be aware of their mental states. If they are not satisfied with the results, then they would be motivated to participate in worksite stress management programs or to take better care of themselves. If aware of their mental states, employees might improve their self-management abilities. This battery would also be a useful complementary strategy at worksite mental health promotion programs or employee assistance programs. In fact, TEG and TSS were designed to be scored by the subjects by themselves. (We are attempting to design a self-scoring version of the Japanese edition of POMS as well). If a stress management class is accompanied by a mental-state awareness session using this test battery, the class could be expected to be a more structured and effective one.

CONCLUSION

A test battery consisting of TEG, TSS, and POMS was applied to assess mental state in occupational health care settings. Of 85 correlations between test factors, 49 in males and 61 in females were not significant. This result indicated that this battery could be used to assess independent aspects of mental state. However, significant correlations were observed for several pairs of factors, indicating that ego state, behavioral pattern, and mood state are interrelated with each other. This finding indicates that mental state can be described more clearly and comprehensively by assessing all three psychosocial parameters than by assessing only one of them. Finally, there is a possibility that this battery is useful in worksite preventive mental health promotion programs.

Further investigation to validate this battery should include testing on mentally ill groups so that the results could be compared with those obtained in the present study. In addition, factor analysis on the 16 factors may reveal closely related factors which could be selected so that a briefer battery could be developed. Differences on several demographic factors, such as sex, age, occupational class, and education, should also be studied.

APPENDIX

Description of the Tokyo University Egogram (TEG)

Transactional Analysis is a theory of human personality, relationships, and communication developed by Berne (1961). According to his theory, ego state is defined as a consistent pattern of feeling and experience directly related to a corresponding consistent pattern of behavior (Stewart and Joines, 1987). In other

words, ego state is a way in which people manifest a part of their personality at a given time. This ego-state model recognizes three distinct ego states: Adult, Parent, and Child. Each state has its own ways of feeling and behaving.

If one is behaving, thinking, and feeling in response to what is going on around him here and now, using all the resources available to him as a grown-up person, he is said to be in his Adult ego state (A). If one behaves, thinks, and feels in ways which are a copy of one of his parents, he is said to be in his Parent ego state. Parent ego state consists of two states: Critical Parent (CP, sometimes called Controlling Parent) and Nurturing Parent (NP). When one behaves in ways which copy his parents when they were telling him what to do, controlling him or criticizing him, he is said to be in CP ego state. When one replays the behaviors that his parents showed when they were looking after him, he is said to be in NP ego state. Sometimes one may return to ways of behaving, thinking, and feeling which he used when he was a child. At that time, he is said to be in his Child ego state. Child ego state also consists of two states: Adapted Child (AC) and Free Child (FC). One may replay the ways of behaving that he decided on as a child to fit in with what his parents expected. Then he is said to be in his AC ego state. This ego state also includes rebellion against rules and expectations set by one's parents. In adults, AC behaviors may work in positive and in negative ways. By replaying these rule-following patterns, one can get what one wants comfortably in his social life (a positive way). However, when one is behaving only to fit others' expectations, he may be suppressed and then become dependent (a negative way). On the other hand, one might behave in ways which were independent of parental pressures. At those times, one was neither adapting to parental expectations nor rebelling against them. When an adult is in Child ego state, he may sometimes behave in these uncensored childhood ways. At these times one is said to be in the FC ego state.

The egogram is a bar-chart analysis of a person's functional ego states (Dusay, 1972). The TEG is a questionnaire (60 items) developed to assess the five ego-state factors listed above and to construct the egogram (Ishikawa *et al.*, 1984; Suematsu *et al.*, 1989). The TEG is self-administering for most patients and normals. Most subjects complete the TEG in about 5–10 min. They must choose one of three answers (yes, intermediate, and no) for each question. All items are keyed in the same direction. The TEG scores are calculated on the scale of 2 points for a "yes" answer, 1 point for an "intermediate," and 0 points for a "no."

The TEG scales were standardized with scores obtained from 4012 healthy subjects. By factor analysis, items have been categorized into the following five ego-state factors: factor CP (10 items: No. 8, 13, 22, 23, 27, 38, 43, 52, 53, and 57), factor NP (10 items: No. 10, 15, 20, 24, 25, 40, 45, 50, 54, and 55), factor A (10 items: No. 6, 7, 14, 17, 26, 36, 37, 44, 47, and 56), factor FC (10 items: No. 2, 4, 5, 16, 28, 32, 34, 35, 46, and 58), and factor AC (10 items: No. 1, 11, 12, 18, 21, 31, 41, 42, 48, and 51). The remaining items are used for the Deviation Scale, which assesses eccentricity (10 items: No. 3, 9, 19, 29, 30, 33, 39, 49, 59, and 60). The mean scores for 2605 males and 1407 females are 8.2 ± 3.6 and 7.1 ± 3.5 for TEG-CP (means ± SD; $P < 0.01$, by t test); 12.7 ± 3.7 and 13.1 ± 3.7 for TEG-NP ($P < 0.05$), 11.8 ± 3.8 and 10.3 ± 3.5 for TEG-A ($P < 0.01$), 9.8 ± 3.7 and 10.1 ± 3.9 for TEG-FC ($P < 0.05$), and 9.0 ± 4.3 and 10.0 ± 4.3 for TEG-AC ($P < 0.01$). The reliability and validity of these scales were reported elsewhere (Ishikawa *et al.*, 1984; Suematsu *et al.*, 1989). Briefly, reliability coefficients

(Cronbach's alpha) were 0.435–0.696 for six egogram scales. Several validity studies were also conducted. For example, a combined study with Cornell Medical Index (CMI, Japanese edition; Kaneshita and Fukamachi, 1972) and Yatabe–Guilford test (Y-G, Japanese edition; Tsujioka, 1962) revealed that there were reasonable correlations among the factors of TEG and other established personality trait tests. The neurotic subgroup assessed by CMI had high CP and AC scores and had low NP scores compared to the normal subgroup. TEG-A and TEG-FC had positive correlations with Y-G G (General Activity) and Y-G R (Rathymia) scales. TEG-AC had positive correlations with Y-G D (Depression), C (Cyclic Tendency), I (Inferiority Feelings), N (Nervousness), and O (Lack of Objectivity), and negative correlations with Y-G A (Ascendance) scales. Studies on patients with neurosis and psychosomatic diseases showed that the former had significantly higher CP, NP, and AC scores and the latter had significantly higher CP, A, and AC scores, when compared to the normal subjects. These results were considered to indicate that the TEG had good criterion-related validity.

TEG Test Instructions and Items

Please circle one of three answers (yes, intermediate, no) for each question, according to your first impression.

1. I can't say what I want to say.
2. I get rowdy and party with others.
3. I think that no one is born bad.
4. My mood fluctuates extremely.
5. I have a short temper and easily get angry.
6. I make plans for the future.
7. I observe people's behavior objectively.
8. I often assume a critical attitude toward others.
9. I think that teaching manners is important.
10. I sympathize easily with others.
11. I often have to force myself to do things.
12. I change my opinion in the face of somebody else's objection.
13. I rush people.
14. I make plans, then I act on my plans.
15. When I see someone in sorrow, I try to comfort them.
16. I say what I want to say without reserve.
17. I act after considering the possible losses and gains.
18. I force myself to endure unpleasant situations.
19. I think that success is the result of one's efforts.
20. I often take care of others.
21. I watch other people's faces closely.
22. I'm stubborn and inflexible.
23. I see people's faults rather than their strengths.
24. I'm gentle and indulgent toward people.
25. I often take care of children.
26. I judge everything based on the facts.
27. I use expressions like, "You should. . . ."
28. I can tell lies skillfully.
29. I enjoy singing and sports.

30. I think that life has its ups and downs.
31. I'm tactless and timid.
32. I'm open and free.
33. I'm honestly glad when other people are happy.
34. I easily join in with casual situations.
35. I'm good at making jokes and teasing.
36. I clear up any doubts.
37. I can express things simply.
38. I meddle in other people's affairs.
39. I take responsibility for what I've done.
40. I take pleasure in public service activities.
41. After experiencing an upset, I'm prone to frustration.
42. I'm sometimes puzzled about what to do.
43. I'm strict with my children and my subordinates.
44. I'm efficient at doing my work.
45. I value customs, courtesy, and manners.
46. I have a strong curiosity.
47. I take care of things efficiently.
48. I get caught up in regret.
49. I have felt envious of other people before.
50. When I see someone in trouble, I only think about wanting to help them.
51. I tend to be reserved and passive.
52. I'm hard on people when they are unjust or make mistakes.
53. I'm not satisfied unless I clarify whether things are right or wrong.
54. I have regard for human feelings.
55. I notice people's good points and admire them.
56. When I talk, I use numbers and data.
57. People feel an atmosphere of tension around me.
58. I'm selfish.
59. I set much value on human relations.
60. I consider the opinions of others.

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Description of the Time Structuring Scale (TSS)

According to the theory of transactional analysis, people spend their time in the process of taking and/or giving "strokes." A stroke is defined as any act of recognition when two person transact each other (positive stroke and negative stroke, Stewart and Joines, 1987). Whenever people get together in pairs or groups, there are six possible ways in which they can spend their time. These six modes of Time Structuring are Withdrawal (W), Ritual (R), Pastime (P), Activity (A), Game (G), and Intimacy (I). The intensity of stroking increases from W to I. The meanings of these six modes have been explained in detail elsewhere (Stewart and Joines, 1987). Briefly, when one stays with the group physically but does not transact with other group members and is mentally absent from the people around him, he is said to be in W. R is a familiar social interaction that proceeds as if it were preprogrammed. R is a fixed way of behaving toward others, a transaction that almost everyone uses. P, like R, proceeds in a way that is familiar. However, the content of P is not programmed as strictly as that of R. P is a pleasant way of

exchanging strokes, filling time, and getting to know people. In A, the communication between the group members is directed at achieving a goal or getting something done, not just talking about it. In A, people are directing their energy toward some material outcome. G is a way to obtain negative strokes when one does not get positive strokes. In G, one is not being straight and his message to the other is ulterior, for some hidden purpose, to have a feeling of defeating the other. When G is over, one feels superior, and the other feels put down. In G, people always end up feeling bad, although their social-level messages sound like an exchange of information. In I, people express their authentic feelings and wants to each other without censoring. There are no secret messages. The communications at the social and psychological levels are congruent. I is a close, loving relationship with other people.

The TSS was developed to evaluate one's behavioral patterns in everyday life (Nomura *et al.*, 1989). It is a self-administered questionnaire (78 items) useful for most patients and normals. Most subjects complete the TSS in about 5–10 min. They must choose one of three answers (often, sometimes, and rarely) for each question. The score is calculated by assigning 2 points for "often," 1 point for "sometimes," and 0 points for "rarely." All items except one (No. 20) are keyed in the same direction. By previous factor analysis, 78 items have been classified into the following five factors: factor W (15 items: No. 1, 9, 10, 11, 18, 22, 27, 30, 34, 46, 52, 53, 54, 62, and 78), factor RP (R and P, 15 items: No. 4, 8, 13, 25, 39, 40, 41, 43, 44, 49, 50, 55, 63, 70, and 74), factor A (15 items: No. 14, 15, 16, 17, 19, 21, 28, 38, 47, 48, 61, 64, 67, 76, and 77), factor I (18 items: No. 2, 6, 12, 26, 29, 31, 35, 36, 37, 45, 51, 56, 59, 60, 65, 68, 71, and 75), and factor G (15 items: No. 3, 5, 7, 20, 23, 24, 32, 33, 42, 57, 58, 66, 69, 72, and 73). The mean scores for 130 males and 199 females are 9.4 ± 4.5 and 8.8 ± 4.3 for TSS-W (mean ± SD; not significant (N.S.) by t test), 21.4 ± 4.1 and 21.2 ± 4.2 for TSS-RP (N.S.), 16.4 ± 4.8 and 13.3 ± 5.2 for TSS-A ($P < 0.01$), 22.7 ± 6.2 and 24.4 ± 5.8 for TSS-I ($P < 0.01$), and 9.3 ± 5.6 and 10.8 ± 5.6 for TSS-G ($P < 0.05$). The validity and reliability of these scales were assessed elsewhere (Nomura *et al.*, 1989). Those studies indicated that the TSS had good reliability and criterion-related validity. We have attempted to revise this test and detailed assessment of reliability and validity will be reported soon (Nomura *et al.*, submitted).

TSS Test Instructions and Items

1. This questionnaire, which is based on the theory of Transactional Analysis, is only for evaluating behavior patterns.
2. While reflecting candidly on your daily life, please circle the most appropriate of the three responses (often, sometimes, rarely).

1. I listen to a compact stereo while I walk.
2. I have many kinds of friends.
3. I get involved in trouble.
4. I gossip about people who are close to me.
5. I see things in a bad light.
6. I consult with someone that I can rely on about important matters.
7. I feel unpleasant or exhausted after interacting with others.
8. I talk about trendy things.
9. I watch TV by myself.

10. I go fishing by myself.
11. I prefer staying home to going out.
12. I chat with friends.
13. I talk about popular things.
14. I have so many things to do that I have no time to spare.
15. I don't have any free time.
16. I read books related to my business even on the train.
17. I read the newspaper on the train.
18. I keep a diary.
19. I am busy with my work.
20. I can clearly tell a person what I think.
21. I work even on holidays.
22. I watch movies alone.
23. I often think, "If only this person didn't exist. . . ."
24. I make the same mistake over and over again.
25. I have respect for my seniors.
26. I take trips with my spouse and/or my family.
27. I read books.
28. I am not satisfied unless I am doing something.
29. I spend lots of time in talking about important things with my spouse or my family.
30. I often doze off.
31. I have dinner at home.
32. I humble myself.
33. I can't do things as I want.
34. I am lost in reverie.
35. I place a high value on a happy home.
36. I dine with my spouse and/or my family.
37. I meet friends through my hobbies.
38. I go out drinking on the way back from work.
39. I regard my seniors with respect.
40. I try to get new information from ordinary conversations with people.
41. I greet people when I happen to meet them.
42. I get lost in thinking, "At that time, I should have done. . . ."
43. I respect the opinions of others.
44. I chat.
45. I have meals with friends.
46. I have meals alone.
47. I am driven by work.
48. I participate in social activities.
49. I chat over tea.
50. I can't refuse when I am asked to do something.
51. I talk for a long time on the phone.
52. I often fantasize.
53. I take trips by myself.
54. I drink alcohol alone.
55. I attend ceremonial occasions as much as possible.
56. I often feel better after a conversation with my spouse or family.
57. I feel nervous when I see people.

58. I remember frightening experiences or have bad memories.
59. I have a companion with whom I feel comfortable.
60. My family associates with other families.
61. I talk about current events.
62. I lose myself in the computer.
63. I place value on the rule of seniority.
64. I talk fast.
65. I see a close friend regularly.
66. Even if it is a small thing, I am upset about unpleasant aspects of situations.
67. I work even at home.
68. I have a feeling of satisfaction with my life.
69. I yield to self-hatred.
70. I use polite expressions.
71. I can have friendly talks with people.
72. I am worried about something.
73. I feel uneasy about taking on responsibilities.
74. I talk about sports.
75. I see a friend with whom I can talk about everything.
76. I have a sense of fulfillment with my work.
77. I lose myself in my work.
78. I read religious books or philosophic books.

Description of the Profile of Mood States (POMS)

POMS is a factor analytically derived inventory which measures six identifiable mood or affective states: Tension–Anxiety (T), Depression–Dejection (D), Anger–Hostility (A), Vigor–Activity (V), Fatigue–Inertia (F), and Confusion–Bewilderment (C). This questionnaire was developed to meet the need for a rapid, economical method of identifying and assessing transient, fluctuating affective states (McNair *et al.*, 1971, 1981).

The POMS consists of 65 5-point adjective rating items. It is virtually self-administering for most psychiatric outpatients and normals. It can be administered to individuals or to groups, and most subjects complete the POMS in about 3–5 min. To obtain a score for each mood factor, the sum of the responses (from 0 to 4) for the adjectives defining that factor is obtained. Factor T is defined by adjective scales descriptive of heightened musculoskeletal tension. The defining scales also include reports of somatic tension which may not be overtly observable (Tense, On edge), as well as of observable psychomotor manifestations (Shaky, Restless). Factor D represents a mood of depression accompanied by a sense of personal inadequacy. Factor A appears to represent a mood of anger and antipathy toward others. Factor V is defined by adjectives suggesting a mood of vigorousness, ebullience, and high energy. This factor V is negatively related to the other POMS factors. Factor F represents a mood of weariness, inertia, and low energy level. Factor C appears to be characterized by bewilderment and muddleheadedness.

The Japanese edition of POMS was produced by translating the original version (McNair *et al.*, 1971). Its reliability and validity have been assessed (Yokoyama *et al.*, 1990), and clinical applications have also been performed (Akabayashi *et al.*, 1991). The mean scores for 354 males and 152 females on the Japanese edition are 11.9 ± 5.6 and 10.7 ± 4.8 for POMS-T (mean ± SD; $P < 0.05$, by t test), 11.7 ±

8.4 and 10.3 ± 7.6 for POMS-D (N.S.), 11.8 ± 8.1 and 11.1 ± 7.1 for POMS-A (N.S.), 12.8 ± 5.6 and 11.7 ± 4.2 for POMS-V ($P < 0.05$), 9.2 ± 5.7 and 8.4 ± 5.4 for POMS-F (N.S.), and 8.4 ± 4.2 and 7.9 ± 3.8 for POMS-C (N.S.).

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Strategies for the Prevention of Environmental Neurotoxic Illness[1]

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Toxic chemicals in the environment can cause a wide range of neurological disease. High-dose exposures to environmental neurotoxicants have produced encephalopathy in children ingesting chips of lead-based paint, blindness in persons who ingested methanol, blindness and ataxia in persons who consumed organic mercury, spinal cord degeneration and peripheral neuropathy in persons exposed to triorthocresyl phosphate (TOCP), and Parkinsonism in persons exposed to MPTP or to manganese. Environmental neurotoxicants have also been shown to produce a wide range of subclinical neurotoxic effects, including reduction in intelligence, impairment in reasoning ability, shortening of attention span, and alternation of behavior. The first step in the prevention of environmental neurotoxicity is to test chemicals for their toxic potential. More than 70,000 chemicals are currently in commerce. However, except for pharmaceuticals, fewer than 10% of these chemicals have been tested for neurotoxicity. A logical approach to neurotoxicologic assessment of chemical substances will build on and extend currently available test systems. It will have a tiered structure. The first or screening tier will consist of tests to measure obvious structural and functional changes, often a functional observational battery. Subsequent levels of testing will be guided by the results of initial screening. Toxicologic testing must be supplemented by epidemiologic surveillance of populations exposed to known and suspect neurotoxicants. Screening programs in these populations designed to detect excessive absorption of a neurotoxic agent or subclinical neurological dysfunction can be useful in identifying affected individuals before severe disability occurs.

INTRODUCTION

The recognition that exposure to certain chemicals can cause neurologic injury first arose from the study of acute illnesses in people exposed to environmental toxicants at high doses. The illnesses included encephalopathy in children who ate chips of lead-based paint, blindness in persons who consumed wood alcohol (methanol), and coma, convulsions, and respiratory paralysis after exposure to organophosphorus pesticides. Epidemics of neurotoxic diseases related to environmental exposures have occurred: blindness and ataxia caused by organic mercury in fish from Minamata Bay, Japan, and in fungicide-treated grain in Iraq; spinal-cord degeneration and peripheral neuropathy caused by tri-*o*-cresyl phosphate (TOCP) in cooking oil in Morocco and in patent medicine (Ginger Jake) in the United States; tremors, anxiety attacks, and incoordination caused by the pesticide Kepone (chlordecone) in Hopewell, Virginia; and parkinsonism caused by MPTP, a contaminant of synthetic heroin, in California and Hawaii. In all, these epidemics affected tens of thousands of people and established clearly that toxic chemicals in the environment can cause neurologic and psychiatric illness.

Injury to the nervous system caused by toxic chemicals in the environment is an important public-health problem, but it is insufficiently studied and poorly defined. "Environment" is defined broadly in this construct to encompass a wide range of extragenetic factors that can cause injury to body systems, including diet, alcohol, tobacco, drugs, and occupational exposures, as well as exposures to components of the ambient environment—air, water, and soil. A major unanswered question is whether the causal associations observed in such epidemics

reflect isolated events or a widespread and pervasive association between toxic environmental chemicals and neuropsychologic impairment. That question is one of the central issues confronting neurotoxicology today.

SUBCLINICAL NEUROTOXICITY

The demonstration in recent years of subclinical neurotoxicity adds another dimension to the question. "Subclinical toxicity" refers to the concept that chemicals in the environment, many neurotoxicants among them, can cause dose-related adverse effects through exposures too small to produce signs and symptoms that are evident in a standard clinical examination. Effects on the nervous system can include lower intelligence, impaired reasoning ability, shorter attention span, alteration of a wide spectrum of behaviors, and fatigue. Environmental chemicals known to cause subclinical neurotoxicity include lead, organophosphorus pesticides, some chlorinated hydrocarbons, some solvent mixtures, and mercury. These are chemicals to which several million persons are regularly exposed at work and to which tens of millions more are exposed in smaller doses in the general environment. Although subtle in appearance, the changes in neurologic function produced by subclinical neurotoxicity can be devastating in effect. Moreover, because the central nervous system has little capacity for repair, the alterations caused by subclinical neurotoxicity can be permanent and irreversible.

The recognition of subclinical neurotoxicity raises the possibility that some undefined fraction of chronic neurologic and psychiatric illness in the human population—including such diseases as parkinsonism, motor neuron disease, demyelinating illness, and some forms of dementia—can be exacerbated or even caused by chronic, low-level exposure to environmental neurotoxicants.

BIOLOGIC MARKERS IN NEUROTOXICOLOGY

As recently defined by the National Research Council's Committee on Biologic Markers, biologic markers in environmental health are measures of changes or variations in biologic systems or samples. It is useful to classify biologic markers into three types: markers of exposure, of effect, and of susceptibility. A biologic marker of exposure is an exogenous substance, its metabolite, or the product of its interaction with some target molecule or cell that is measures in an organism. A biologic marker of effect is a measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can indicate established disease or potential health impairment. A biologic marker of susceptibility is an indicator of an inherent or acquired variation in an organism's ability to respond to the challenge of exposure to a specific substance. Biologic markers are valuable in augmenting the sensitivity and specificity of epidemiologic and clinical studies and in identifying early and subclinical neurotoxic injury.

The development of new biologic markers of neurotoxicity would assist recognition of neurotoxic injury or illness at earlier stages, when dysfunction might still be halted and when the occurrence of additional cases in other members of an exposed population could be prevented. In epidemiologic and clinical studies, markers can be used for the systematic monitoring of populations at high risk. Biochemical markers of neurologic dysfunction include measurements of specific

lipids and proteins or of neurotransmitters or their metabolites, as well as measurement of changes in the number or affinity of specific neurotransmitter receptors. Structural markers include changes observed in paraffin- or plastic-embedded tissues obtained at biopsy. Functional markers include results of tests of central neurologic function and of noninvasive tests of function in motor and sensory nerves. Objective and rapidly administered tests are finding wide application and appear to provide reliable and sensitive information on early injury to the nervous system.

Biologic markers of exposure to neurotoxic substances, such as body lead burden or extracellular or intracellular cholinesterase activity, need to be used increasingly in human studies along with biologic markers of individual variation in susceptibility to neurotoxicants. The use of accurate markers of exposure and susceptibility, in combination with biologic markers of neurologic dysfunction, will permit precise delineation of individual exposures and detailed assessment of dose–response relationships. The recent reports by the National Research Council Committee on Biologic Markers provide an important basis for the development of neurotoxicologic markers.

NEUROTOXICITY TESTING

About 70,000 chemicals are used in commerce, of which several hundred are known to be neurotoxicants. However, except for pharmaceuticals, less than 10% of the chemicals in commerce have been tested for neurotoxicity, and only a handful have been evaluated thoroughly. Therefore, it is not known how many untested toxic chemicals in the environment might have neurotoxic effects. It is possible that large numbers of people are exposed to these unrecognized neurotoxicants and are suffering injury as a result.

This gap in toxicity testing needs to be closed. Gathering information on the toxicity of untested chemicals is the essential first step in the process. However, the gap is large, and resources are not readily available to undertake across-the-board testing of all chemical substances already in commerce.

New strategies for neurotoxicologic assessment of environmental chemicals must therefore be developed. They will include the establishment of testing priorities among chemicals for hazard identification (with emphases on new chemicals, chemicals considered likely to be hazardous, and chemicals to which large numbers of people are exposed), refinement of existing neurotoxicity test systems, development and validation of efficient, sensitive new testing systems, and development of standard approaches to the interpretation of the results of neurotoxicity testing.

A new strategy for neurotoxicologic assessment will build on and extend currently available test systems. It will have a "tiered" structure—decisions to test chemicals at the higher tiers, as well as decisions concerning types of testing, will be guided by data from the initial, or screening, tier. The screening tier will consist of a set of tests to measure chemical, structural, and functional changes in an integrated fashion, including a functional observational battery. Such tests must be carefully validated at every stage. To address the board functional diversity of the nervous system, they must examine multiple end points; a highly specific effect on one function of the nervous system will not necessarily entail an effect

on another function. Because the testing strategy will be labor and resource intensive, quicker and more economical approaches must be developed, particularly for screening for potential neurotoxicity.

In vitro systems are available and appear suitable for detailed studies of some neurotoxic mechanisms. They have not yet been used for screening. A difficulty in using these systems is the need to establish a relationship between effects observed *in vitro* and the expression of effects at a structural or behavioral level in whole animals, particularly humans. Studies of the correlation between the results of *in vitro* systems and the results of functional *in vivo* tests are therefore essential. *In vitro* assays should be conducted in conjunction with whole-animal tests to determine the correspondence between the two types of assays, to validate the use of *in vitro* assays as quicker, more efficient methods for screening chemicals for neurotoxicity, and to develop a better understanding of the mechanisms of neurotoxic damage. More mechanistic studies of neurotoxic reactions at the molecular and cellular levels might also be used to generate the detailed mechanistic information needed for accurate risk assessment and for development of predictive structure–activity relationships.

The general objective of neurotoxicity testing both *in vivo* and *in vitro* is to identify neurotoxic potential before the occurrence of human exposure. The goal is the prevention of human disease.

EPIDEMIOLOGIC STUDIES AND NEUROTOXICOLOGY

Epidemiologic and clinical studies of populations exposed to potentially neurotoxic chemicals are needed to provide additional information on the human neurotoxic effects of environmental chemicals and to complement screening studies *in vitro* and in animals. High-risk populations must be identified and monitored. People diagnosed as having neurologic illnesses must be studied to identify possible environmental etiologies and to complement and extend the knowledge gained through *in vivo* and *in vitro* laboratory investigations. Public-health surveillance systems for the detection of people who are potentially exposed to environmental neurotoxicants are not well developed, and there is little information on the background incidence and prevalence of the major neurologic diseases in the American population.

Recognition of the neurotoxic effects of exposure to environmental chemicals through epidemiologic and clinical studies is made difficult by the enormous variety and subtlety of the possible reactions of the nervous system to toxic insult. The reactions are as varied as peripheral neuropathy, alteration in the sense of smell, and impaired mathematical ability. The changes are often subtle and subclinical. Moreover, months or years can elapse between exposure to a neurotoxicant and the appearance of dysfunction or disease. Thus, populations known to be exposed to potential neurotoxicants should be followed for long periods in prospective studies, and retrospective studies of people with neurologic illness must consider the possibility that exposures occurred many years previously. Epidemiologic studies will increasingly need to use biologic markers of exposure, of toxic effects, and of susceptibility.

RISK ASSESSMENT AND NEUROTOXICOLOGY

Risk-assessment techniques provide a means for estimating the risks to humans

associated with exposure to toxic chemicals in the environment. The estimation of the risks most often involves extrapolation from high experimental doses used in animal tests to lower environmental doses. Numerous assumptions must be made to bridge gaps in the available scientific data. Most risk-assessment procedures have focused on cancer as an end point, and techniques for assessing other types of risk are relatively undeveloped. The approach used most often now for non-cancer end points, which simply divides the dose below which effects were not seen by uncertainty factors to generate an exposure level presumed to be safe, must be considered inadequate. Virtually all neurotoxicologic risk assessment today is limited to qualitative hazard identification and to the early stages of hazard characterization; neither sufficient data nor adequate paradigms are yet available to permit quantitative evaluation of most neurotoxic risks.

Risk-assessment techniques are under development for the evaluation of neurotoxic illness that incorporate more quantitative information about dose–time–response relationships and mechanisms of toxicity. They will assist in appraising the benefits that would be gained for the human population with its diversity of susceptibilities by reducing exposure to specific neurotoxic agents. The construction of new models for neurotoxicologic risk assessment will be greatly facilitated by the acquisition of new knowledge of the fundamental mechanisms of action of chemical toxicants on the human nervous system. The molecular and subcellular mechanisms by which environmental chemicals cause neurotoxic injury need to be delineated. Such information will particularly improve prediction and quantification of risks that become evident only long after exposure.

CONCLUSION

Conclusion 1. Neurotoxic effects can be caused by exposure to chemical agents in the environment. Environmental chemicals have been shown to cause neurotoxic effects in individual cases and in epidemics. Neurotoxicity caused by environmental toxicants results in a range of neurologic and psychiatric disorders. The complexity of the disorders reflects the enormous diversity of the nervous system's functions and the presence in the nervous system of a large number of cellular and subcellular targets. Neurotoxic outcomes range from devastating illnesses, such as parkinsonism and dementia, to subtle changes, such as alterations in behavior and limitations on memory and cognition. In addition to immediate and progressively developing effects, there is increasing evidence that neurotoxic effects can occur after long latent periods. Intervals as long as many decades can elapse between exposure to a chemical and the appearance of neurologic illness. Concern over the potential neurotoxic effects of chemical substances is greatest for agents that cause irreversible or progressive changes. Chemicals can permanently alter brain development and cause subclinical dysfunction or they can reduce reserve capacity of the nervous system, which may become manifest as disease in the elderly. On the basis of the available evidence, the committee hypothesizes that a definite, but as yet unspecified, fraction of human neurologic and psychiatric disease is attributable to chemical agents in the environment.

Conclusion 2. A major obstacle to assessing the extent to which chemicals in the environment cause nervous system diseases and dysfunction is that little

qualitative or quantitative information is available on possible adverse effects of most environmental chemicals on the nervous system. Some chemicals in commerce are known to have neurotoxic potential, but most commercial chemicals have not been assessed for neurotoxicity. There is a particular lack of data on chronic and long-latency neurotoxic effects. Structure–activity relationships, now the most widely used approach to assessment of toxicity, provide a poor basis for predicting neurotoxic potential; however, greater fundamental understanding of mechanisms can be expected to lead to the discovery of more useful applications of structure–activity relationships.

Conclusion 3. Additional biologic markers for the assessment of subclinical neurotoxic effects are needed. Such markers can be biochemical, structural, or functional. They can be developed through *in vitro* analyses, through animal studies, or during observational studies in human populations exposed to environmental neurotoxicants. Although associations between biologic markers and disease are usually established initially in cross-sectional studies, a particular need exists to validate putative biologic markers in prospective studies. Only in longitudinal prospective studies can the ability of biologic markers to predict the occurrence of disease be accurately assessed.

Conclusion 4. Tests are available to construct a tiered approach to neurotoxicity testing. The first tier, or screen, is intended for hazard identification. The results of the screen and a chemical's exposure pattern would determine further characterization of dose–response (second tier) and mechanism (third tier).

There is no existing validated system that satisfies all the necessary requirements for a screening program to detect the neurotoxic potential of chemicals. The range of such a program should extend to the detection of neurodevelopmental effects and effects on cognitive function and of neuroendocrine effects. No comprehensive effort has yet been made to determine the predictive ability of individual screening tests by examining the relationship between test results and data from long-term studies in animals or epidemiologic and clinical studies in humans.

Conclusion 5. Attempts to quantify the exposure of populations to neurotoxic chemicals have been limited. Clinical evaluation of neurotoxic illness and epidemiologic surveillance of populations at high risk for neurotoxicity have been fragmentary and inadequate. Few attempts have been made to explore the possible relationships between chemical exposures and chronic or progressive neurologic and behavioral disorders. The disorders include developmental delays in the young and some forms of dementia and parkinsonism in the elderly.

Conclusion 6. Recognition of the possible environmental origin of neurologic and psychiatric disease is hampered by the inadequate training of most physicians and other health providers in occupational and environmental medicine. Greater uniformity in disease definition would improve identification of diseases of neurologic interest.

Conclusion 7. The commonly used paradigms for risk assessment do not accurately or adequately model the risks associated with exposure to neurotoxicants. Neurotoxicologic risk assessment has been largely limited to the application of no-observed-effect levels and uncertainty factors, which does not generate specific risks for given magnitudes of exposure.

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The Use of Behavioral and Psychophysiological Methods in the Monitoring of Health at the Worksite[1]

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The paper discusses the possibility of identifying and preventing certain occupational health hazards by assessing and evaluating a number of behavioral and psychophysiological responses induced by exposure to unfavorable environmental conditions at work. The main aspects of the research strategies and measurement methods used in this field of research are described to provide a background for the establishment of a policy of health promotion based on the monitoring of early behavioral and psychophysiological indices of functional CNS changes at the worksite. © 1993 Academic Press, Inc.

INTRODUCTION

It is a fact that the work itself and the work environment are factors of paramount importance for the health and well-being of the general population. Thus, epidemiological research has shown that physical and psychological factors in the working environment too often are associated with the incidence and prevalence of numerous disease and injuries. In these circumstances, it becomes necessary to orient our attention to the work and the work environment even if our goals are as broad as to include health promotion in the population as a whole.

To produce accurate definitions of concepts like *health* and *disease* is a very difficult enterprise. From the logical point of view, each of these concepts indicates a position on a continuum rather than an absolute condition. The position is determined by the results of the interaction between the individual and his environment.

In most occupational health programs, health is usually conceived as being the opposite of and the absence of disease. The advantages of using a negative concept of health are obvious since this allows health to be operationally defined and objectively measured. It is therefore not surprising that, until recently, the promotion of health in the workplace has relied almost exclusively on defensive strategies, the goal of which has been to provide satisfactory medical care and, at the same time, to find ways of avoiding occupational diseases.

Positive concepts of health, however, have been discussed and might be expected to have a greater influence on the strategies of occupational health promotion in the near future, at least in the developed countries. The direction to be taken has already been indicated by the World Health Organization on different occasions. Thus, according to a report of a WHO Study Group (1975), "Health

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does not mean only absence of disease but also optimum physical, mental, and social wellbeing. . . . [Health] not only means freedom from pain and disease, but also freedom to develop and maintain one's functional capacities." Statements of this kind imply a considerably more dynamic and positive concept of health than that suggested by traditional occupational medicine. They point out the need for a new approach to occupational health promotion and for alternative ideas and perspectives with regard to what is to be considered as a manifestation of health impairment and how the monitoring of health in the work environment should be performed.

The health aspects of the well-being of the workers have been discussed and formulated in Scandinavia by a working group sponsored by the WHO (WHO, 1980). According to this working group "adverse factors affecting the wellbeing of workers may be classified as psychosocial, physical, chemical, and biological; all are interrelated and have combined effects."

This is probably the first time in a formal document that psychosocial factors at the workplace have been considered as being a potential cause of occupational disease. Unfortunately, the report of the working group does not provide any guidance for distinguishing these psychosocial factors from the psychosocial consequences of an unhealthy physical work environment.

It would seem that the major problem in formulating and implementing a valid occupational health program is to find a balance between the challenge of applying a positive concept of health to promote workers' well-being and the necessity to avoid disease.

This paper deals primarily with the possibility of identifying and preventing potential occupational health hazards by assessing and evaluating certain behavioral and psychophysiological responses induced by exposure to unfavorable environmental conditions at work. It is beyond the scope of this paper to provide a comprehensive review of this field of research. For such a review the reader is referred to the proceedings of an international course on this subject (Gamberale and Kjellberg, 1990). In the presentation of this theme examples are primarily taken from our own research experience in this field which goes back to the beginning of the 1970s.

BEHAVIORAL AND PSYCHOPHYSIOLOGICAL INDICATORS OF HEALTH IMPAIRMENT

To make prevention of overt disease possible, criteria of health impairment should be based on early indicators of a reversible biological effect. A reduction of the nervous systems' functional capacity, of which the individual need not necessarily be aware, is an indicator of such a biological effect due to exposure to work environmental stressors. This emphasis on the nervous system is motivated by its vulnerability as a target organ.

Nowadays there is unequivocal evidence that even relatively small deviations from the optimum work environment can affect work efficiency and comfort. There is also evidence that adverse effects on the nervous system such as reduced functional capacity, alterations in the psychophysiological state, or other behavioral changes may be caused by exposure to different chemical or physical envi-

ronmental factors at the work site, as well as by factors linked to the physical workload, the organization of work, and the production processes. Today these effects are recognized to be warning signs of potentially serious job health hazards which can no longer be neglected. Adverse effects of this type may be present among the workers long before any clinical signs of occupational disease and pathology appear. By using an adequate monitoring program it is possible nowadays to detect these adverse effects at an early stage and to avoid serious consequences by intervening in the work environment with appropriate actions. The implementation and application of occupational health programs of this kind should become a task for modern occupational health practice. This task should be considered as important as the task of providing medical care in the case of manifest occupational disease.

THREE EFFECT DOMAINS

Early manifestations of adverse effects on the nervous system caused by exposure to unfavorable environmental conditions can be observed as changes in individual response which may occur as a result of impairments in the functional capacity of the nervous system. These impairments may be demonstrated by measurements in certain variables belonging to any or all of the three effect domains, illustrated in Fig. 1.

The perceptual domain includes all kinds of subjective reactions, e.g., sensation, feelings, symptoms. These reactions are usually assessed with the use of interviews, questionnaires, and rating scales or by psychophysical methods.

The performance domain includes all kinds of objective measures of the capacity of the nervous system with regard to sensory, perceptual, cognitive, and motor functions. These functions can be accurately assessed by psychometric tests. A remarkable improvement in the measurement of the performance functions has recently been brought about by the computerization of the tests. The use of

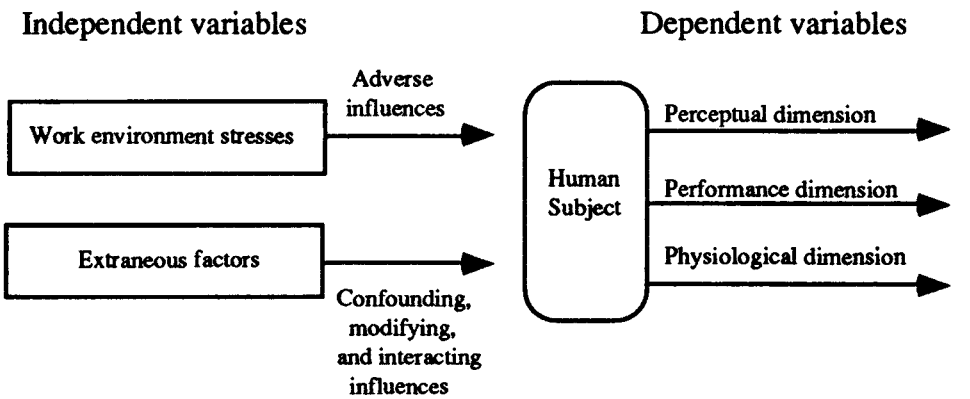

FIG. 1. Model of the three effect domains in the study of the effects of the work environment on the nervous system. The adverse influences of the work environment stresses and the confounding influences of extraneous factors (independent variables) are observed as changes in the perceptual, performance, and physiological variables (dependent variables).

computers has provided an opportunity for strict standardization of test procedures, possibilities to perform detailed measurement and analyses of single-response or response components, and an increased precision in the measurement procedure. One example of a computerized testing system specially developed for monitoring effects due to work environmental stresses is the system currently in use at our laboratory, the Swedish Performance Evaluation System (Gamberale *et al.*, 1989, 1990). This system has evolved over many years primarily for use in assessing the effects of exposure to neurotoxic substances in the work environment.

The physiological domain covers all measures, particularly electrophysiological ones, which might reveal functional changes in the central or peripheral nervous system. Of special interest in this context are the measures collected by electroencephalographic methods and the measures of nerve conduction in major sensory and motor pathways.

EARLY EXPERIENCE WITH THE BEHAVIORAL MEASURES

The use of behavioral measures in testing potential health hazards in the work environment began to emerge in the early 1970s. The starting point for our own interest in this topic was the widespread opinion among representatives of the Swedish Trade Union Confederation that exposure to industrial solvents was associated with complaints among the workers. A suspicion began to form that the complaint was not only due to discomfort, but could be a manifestation of the neurotoxicity of the solvents. The question then arose concerning the suitability of the current exposure standards for solvents. Under these circumstances a laboratory experiment was performed (Gamberale and Hultengren, 1972) in which human subjects were examined after periods of exposure to various concentrations of toluene, a commonly used solvent. The performance on tests of reaction time and of perceptual speed as well as the subjective complaints and heart rate variability were used as indicators of effects on central nervous functions. The results of the experiment confirmed our suspicions. Thus, the level of performance on each test was clearly affected by exposure to toluene concentrations corresponding to what many workers at that time were exposed to daily. The observed effects of toluene, of course, were reversible, but they demonstrated unequivocally that the inhaled solvent was biologically active in the nervous system.

The above experiment was followed by a series of experiments using single solvents such as methylchloroform, styrene, white spirit, methylenchloride, trichloroethylene, xylene, methylisobuthylketone, and combinations of solvents (Gamberale, 1976, 1985). Over the years, the experimental procedure became more sophisticated both with regard to the exposure procedure and the measurement of effects. For many of the solvents investigated unequivocal relations were revealed between reduced performance in psychomotor, perceptual, and cognitive tasks and the uptake of solvent in the organism.

The specific aim of the experiments also changed somewhat over the years. Whereas the first experiments were performed primarily to demonstrate the capacity of a work environment agent to impair nervous system functions in a way

which could not be done by traditional toxicological methods, the later studies were performed to evaluate current exposure standards (Iregren *et al.*, 1986; Iregren and Gamberale, 1990).

Nowadays experimentally induced acute effects on the nervous system of low-dose solvent exposure are considered as potential health hazards. Consequently, the results of such experiments are referred to in most criteria documents which are the basis for the establishment of hygienic limit values. From the beginning, however, the results of the first experiments were not given proper attention by the medical experts. They were questioned primarily on the grounds that the effects were reversible and could not be demonstrated to cause any known occupational disease. This skepticism was probably associated with the "softness" of the behavioral measures as compared to more traditional indicators of health impairment.

THE SEARCH FOR BEHAVIORAL EFFECTS AT THE WORK PLACE

One of the basic limitations of the experimental approach described above is that the environmental conditions at work, which can be simulated in a laboratory setting, are rarely if ever very representative of the conditions existing in the real work environment. In other words, laboratory experiments may lack ecological validity. Our first experiments had in fact been criticized on this ground and we felt challenged to demonstrate, if possible, the validity of our observations by examining workers at the worksite.

One way of studying the effects of unfavorable work environment factors and of overcoming most of the shortcomings of the experimental approach is to perform what might be termed a quasi-experimental field study, i.e., a study which simulates the experimental approach and is conducted directly in the field. A distinctive characteristic of the designs of this type of study is that the dependent measures are collected both at the beginning and at the end of a workday. This design is based on the underlying assumption that the adverse effect of the environment may manifest itself in an impairment of nervous system functions at the end of a workday, with a return to normalcy by the beginning of the following day, after approximately 16 hr free from exposure. If the action of exposure lasts long enough to bridge the exposure-free period between two consecutive workdays, the nervous system functions should be negatively affected also at the start of a workday.

From the beginning this type of study was performed to test specific hypotheses concerning the acute effects of low-dose exposure to neurotoxic substances. Recently, the same quasi-experimental design and research strategy has been used to investigate the potential adverse effects of physical aspects of the work environment. It should be possible to use a similar procedure to monitor for changes in nervous system functions among the workers for the purpose of supervising the conditions of work environments with known or suspected potential health hazards.

Our first quasi-experimental field study was performed among a group of styrene-exposed workers in four factories building fiberglass boats (Gamberale *et al.*, 1976). Age-matched workers in two light engineering companies served as the

reference group. The workers' reaction time was measured and their mood state assessed at the beginning and at the end of a working day. The workers exposed to styrene had a longer reaction time, a greater deterioration of reaction time over time, and greater irregularity in performance on the test than the nonexposed workers. The differences between the groups were highly significant and were still present 16 hr after the cessation of exposure. These results were regarded as remarkable and alarming, since the average exposure to the solvent did not exceed the hygienic standards considered safe at the time. The outcome of this study also confirmed the results obtained previously in the experimental laboratory. Thus, behavioral measures had proved to possess high sensitivity as indicators of an ongoing effect of the occupational environment on the nervous system.

The results of the field investigation described above were later confirmed in a study (Kjellberg *et al.*, 1979) in which a group of styrene-exposed workers was followed after the closing down of a fiberglass boat factory. The reaction time of the exposed workers was found to be prolonged compared with that of a reference group of unexposed workers. The deterioration was still observed 4 days after the cessation of exposure. No effect was noticeable 30 days later. Thus, the effect of low-dose exposure to styrene, and probably to several other industrial solvents, can be expected to last long enough to bridge the exposure-free period of the weekend. Additional support for this statement is found in a recent report of a Canadian study (Cherry and Gautrin, 1990). In this investigation reaction time was slower for the exposed workers with a larger body burden and for those who failed to clear the metabolites of the solvent during the weekend. In addition to this effect on the central nervous system, there was also an effect on the peripheral nervous system which manifested itself in the form of mild sensory nerve conduction deficits.

A further demonstration of the capacity of the behavioral measure to detect changes in the nervous system functions is found in a quasi-experimental field study conducted among workers in the paint industry who were exposed daily to a mixture of organic solvents (Anshelm Olson, 1982). On the average the exposed workers performed less well than the reference group on the behavioral tests used. The differences were evident for both morning and afternoon measurements. Particularly noticeable was a marked decrease in performance on a reaction time test over the course of the day for a group of young workers employed full time in the process of cleaning paint containers and who were exposed to the highest solvent levels (Fig. 2). Thus in this study a dose–effect-like relationship was found between the quality of the working conditions and the behavioral responses.

In another study (Anshelm Olson *et al.*, 1981) it was possible to demonstrate the validity of the behavioral measures to monitor for nervous system effects over a long period of time and of the potential use of these measures as a criterion to evaluate programs of improvement of the hygienic conditions of the workplace.

This study was performed in collaboration with the Department of Health Services of a Swedish steelworks, Domnarvets Jernverk, in Borlänge. Forty-two employees from the plastic coating line of the steelworks participated. At the start of the study, February 1976, these workers were exposed daily to varying con-

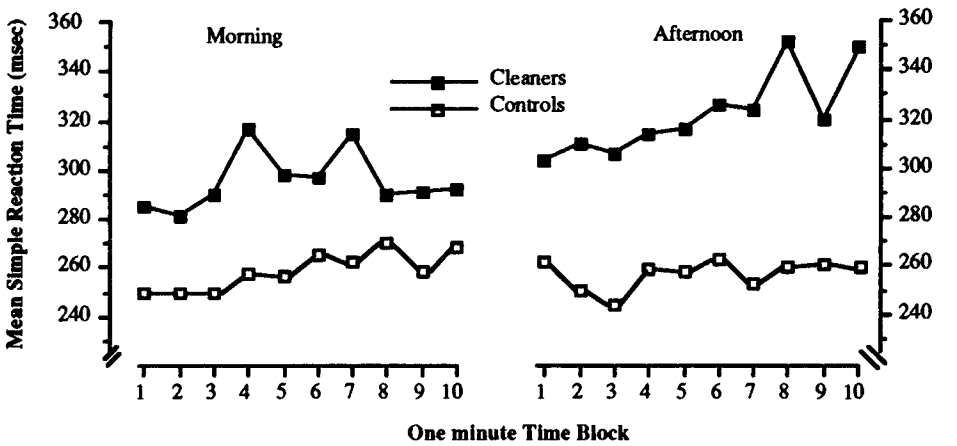

FIG. 2. Changes in simple reaction time over time (1-min time blocks) and test sessions for a group of workers employed in the cleaning process in a paint production factory and for an age-matched control group. (Adapted from Anshelm-Olson, 1982.) Subjects responded to a visual signal by pressing a button as quickly as possible. Each point in the figure is a mean of the 16 stimuli administered each minute during the 10-min reaction time test. Detailed information about the test are found in Gamberale *et al.* (1989).

centrations of solvent mixtures. The 10-min reaction time test used in the previous study (Fig. 2) was standardized so that it could be administered by the personnel of the Department of Health Services. The worker's reaction time was measured four times at different occasions during a period of 27 months. The solvent-exposed personnel of the coating department showed an improvement in reaction time over the four measurement occasions. It was evident that this improvement had taken place in conjunction with radical changes in the ventilation system of the coating department followed by changes in the conditions connected with the cleaning process. These changes had brought about a dramatic improvement in the hygienic quality of the work place.

Again behavioral measures had proved to be sensitive in detecting work conditions leading to an adverse effect on the central nervous system. It was clear that this effect could not as a matter of course be ascribed to a brief impairment of central nervous functions of the same type as that observed in experimentally exposed subjects. In the present case a condition of depression of the central nervous system had been affecting the workers for a long period of time. It is important to observe that neither the workers themselves nor the medical personnel of the steelworks had been completely unaware of the situation and they were not very surprised when confronted with the results of the investigation. Thus the results had given objective form to their suspicions.

MONITORING HEALTH AT THE WORK PLACE

So far, all examples of the use of behavioral and psychophysiological measures given here have dealt with possible effects of low-dose exposure to neurotoxic substances. In principle, however, the same research strategy and similar effects

variables may be applied to the investigation of early effects due to other work environment stresses. Thus, at present attention is being directed to physical work environment stressors such as those associated with exposure to, e.g., noise, vibration, heat, cold, and electromagnetic fields. Examples of field studies of the behavioral effects of stresses associated with the organization of work can be found in Åkerstedt (1990), Aronsson (1989), and Frankenhauser and Johansson (1986).

As a further illustration of the quasi-experimental field study design, a recent investigation of this type (Gamberale *et al.*, 1989) will be referred to in some more detail. The study was performed to investigate directly at the work place the hypothetical acute effects that might result from working in the proximity of high-voltage power lines generating low-frequency electric and magnetic fields. Results from previous studies on acute effects on the nervous system were not unequivocal and the occurrence of such effects could not be ruled out (for a brief review of these studies, see Gamberale, 1990). The abstract of the report of this study is given below.

In this quasi-experiment twenty-six experienced linesmen were studied during 2 working days while performing simulated routine inspection of insulators on steel poles of a 400-kV power line. During one of the working days the inspection was performed on a power line in operation and the other day the same work procedure was performed on an identical power line, which, however, was not in operation. The 2 days were found to be comparable with regard to the physical workload, which, on the basis of heart rate measurements was estimated to be very high. Exposure to the electric and magnetic fields was measured using a device designed for on-worker sampling on each linesman. The mean exposure for the working day was estimated to be 2.8 kV/min (SD = 0.35) and 23.3 μT (SD = 4.2). The possible effects of exposure were studied using a battery of four automated performance tests from the Swedish Performance Evaluation System (Gamberale *et al.*, 1989, 1990), EEG, a mood scale, and a questionnaire for the assessment of subjective symptoms. All workers were examined immediately before and after each working day. Furthermore, blood samples were collected for each subject on three different occasions during each working day. The battery of behavioral tests constituted a test of simple reaction time (SRT), a vigilance test (CWV), a test of short-term memory (digit span), and a perceptual test (symbol digit). The four EEG recordings for each worker were judged blindly and sorted with regard to amount and stability of alpha activity. The blood samples were used for an analysis of possible changes during the working day with regard to the following hormones: thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, prolactin, cortisol, testosterone, and neopterin. The schedule followed during the experiment is given in Table 1.

Detailed analyses of the results using both parametric and nonparametric tests did not reveal any statistically significant difference between the two conditions which could be attributed to exposure to electric and magnetic fields.

This investigation did not reveal any signs of acute effects on the nervous system caused by the working conditions. Although completely "negative," the results of this study still make a valuable contribution to our knowledge of po-

TABLE 1
TIME OF DAY FOR EACH ACTIVITY DURING THE TWO EXAMINATION DAYS

Time of day	Activity/meausrement
06:45–07:00	Blood samples
07:00–07:30	Breakfast
07:35–07:45	Safety regulation information
07:50–09:20	Individual psychometric testing (45 min) and EEG recording (45 min)
09:25–09:45	Coffee break
09:45–10:00	Transportation to work site by car
10:00–12:00	Simulated inspection of insulators
12:00–12:10	Blood samples
12:10–12:30	Lunch in a house-trailer placed under the line in order to expose the linesmen during the lunch break
12:30–14:30	Simulated inspection of insulators
14:30–14:45	Transportation from work site
14:45–15:00	Coffee break
15:00–16:40	Individual psychometric testing (45 min), EEG recording (45 min), subjective assessment of mood and symptoms
16:40–17:10	Blood samples
17:10–	Dinner

tential occupational health hazards. Of course, from a theoretical point of view, it is not possible to test a zero effect. However, due to the sensitivity previously shown by the quasi-experimental design adopted and due to the number and diversity of the highly reliable effect variables investigated, we consider the content validity of the study to be very high. Therefore, since exposure to the electric and magnetic fields during work with production and distribution of electricity seldom exceeds the level studied in this investigation, we feel confident in considering this type of work as safe, at least with regard to acute nervous system effects.

CONCLUDING REMARKS

One of the conclusions which can be drawn from the studies described above is that it is possible to use behavioral and psychophysiological measures to detect early effects on the nervous system of unfavorable work environment conditions. Of course, a better case could be made for the use of the behavioral and psychophysiological measures as a research tool if the available literature were fully utilized. The aim of this paper, however, was not to review the field but to suggest a more extensive use of these effect measures not only in research but also in long-term programs of health promotion at the workplace. Therefore, the choice of the studies described in this paper was also determined by the wish to illustrate the research strategy implicit in the design of the quasi-experimental field study and to indicate a method for the monitoring of health at the work place.

It would seem that the use of certain behavioral and psychophysiological measures together with the application of a suitable strategy to collect measurements directly at the work site have potentials which have not yet been fully exploited.

In fact, behavioral performance functions nowadays can be assessed in highly standardized ways and at low cost by the use of computerized techniques (Gamberale *et al.*, 1990). This makes it practically possible to perform reliable comparisons of nervous system functions over time among workers exposed to unfavorable working conditions. Our own experience clearly indicates that this strategy is practicable and that it can be used as a complement to traditional medical activities of health promotion as well as to modern programs of controlling psychosocial and other factors affecting well-being.

A health promotion program for monitoring unexpected changes in nervous system functions at the work place based on the present suggestions implies a concept of health which seems to be somewhere between the two extremes discussed early in the paper. Thus changes in behavioral and psychophysiological functions may be early signs of adverse biological effects, which may develop into a disease, as well as objective indications that the well-being of the workers is in need of improvement.

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Research Program for Neurotoxic Disorders and Other Adverse Health Outcomes at Hazardous Chemical Sites in the United States of America[1]

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INTRODUCTION

Environmental contamination and its potential threat to human health are issues of great concern in the United States, which has an estimated 32,000 hazardous waste sites, and an average of 6000 persons living near each site whose health may be affected (ATSDR, 1990). Neurotoxic agents are extremely prevalent at these sites (Johnson *et al.*, 1990). This report describes preliminary efforts by the Agency for Toxic Substances and Disease Registry (ATSDR) to characterize the adverse health effects of such agents, when present in hazardous waste sites, on persons and communities living or working nearby.

Background

An agency of the U.S. Public Health Service, the ATSDR was created by the U.S. Congress in 1980 and given expanded responsibilities in 1986. ATSDR's mission is to prevent or mitigate adverse effects to both human health and the quality of life resulting from exposure to hazardous substances in the environment.

By December 1988, the ATSDR had identified heavy metals at 59% and solvents at 54% of 951 high-priority sites (ATSDR, 1990). Where off-site migration of hazardous substances occurred, groundwater was the environmental medium most often contaminated. Children were deemed most likely to come in contact with soil-laden contamination. An estimated total of more than 2 million children, women of childbearing age, and elderly persons live near National Priorities List (NPL) sites.

Reorganization of the ATSDR in 1989 placed responsibility for studies of human health outcomes under the Division of Health Studies.

METHODS

Health Studies

The Division of Health Studies has developed a health research program to study the linkage between adverse health outcomes and environmental exposures to various hazardous substances (Stehr-Green and Lybarger, 1989). This program comprises the following activities:

Health investigations are designed to evaluate communities where residents

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may be exposed to hazardous substances in their environment, and typically measure biological indicators of exposure, symptom/disease prevalence, and putative clusters of morbidity or mortality.

Epidemiologic studies are designed to evaluate the nature of associations between exposure to hazardous substances and disease outcome by testing hypotheses. Case-control studies and comparative mortality studies are commonly employed.

Surveillance activities describe and monitor adverse health events through the periodic, systematic collection of health statistics from defined populations of persons who may be exposed to hazardous substances where they live or work.

Registries serve an important role in ensuring the uniformity and quality of collected data from well-defined populations. The National Exposure Registry lists persons who have been exposed to certain hazardous substances. The National Disease Registry is intended to serve as an official listing of diseases or serious illnesses found in people exposed to hazardous substances in the environment.

Medical Test Batteries

A consistent core of medical test batteries has been proposed for use in the health research program, as biologic markers (biomarkers) of subclinical effects in several major organ systems. The biomarkers are based on recommendations made by the National Academy of Sciences/National Research Council, the World Health Organization, and an interagency subcommittee formed by the Centers for Disease Control and the ATSDR. These recommendations established medical test batteries for several major organ systems, including effects on the central and peripheral nervous systems (Table 1).

Most of these tests are commonly used by health care providers for clinical diagnosis and patient evaluation. Although the tests have not been fully validated as environmental health study tools, they are clearly effective in detecting early dysfunction of their respective organ systems.

ATSDR Priority Health Conditions

A defined group of priority health conditions, including neurotoxic disorders, was established to focus ATSDR's health research efforts on the greatest potential

TABLE 1
BIOMARKERS RECOMMENDED FOR USE IN ENVIRONMENTAL HEALTH STUDIES 1989–1990

System	Sources of recommendation[a]
Immune system	ATSDR/CDC
Liver (hepatobiliary system)	ATSDR/CDC
Kidney (renal–urinary system)	ATSDR/CDC
Reproductive effects	NAS/NRC
Neurodevelopmental effects	NAS/NRC
Immune system effects	NAS/NRC
Ecologic effects (toxicity to plants and animals)	NAS/NRC

[a] Sources: ATSDR/CDC, Agency for Toxic Substances and Disease Registry and Centers for Disease Control Subcommittee on Biological Markers of Organ Damage and Dysfunction, Atlanta, Georgia; NAS/NRC, National Academy of Sciences, National Research Council, Board of Environmental Studies and Toxicology, Washington, DC.

benefit (Table 2). Experienced physicians and public health practitioners selected the health conditions to be studied based on a review of biomedical literature using four criteria: (1) the frequency of conditions resulting from some of the most hazardous substances (for example, heavy metals and solvents) found at waste sites in the United States, (2) the severity of the disorders, (3) the extent of public concern, and (4) the ability to treat or prevent illnesses through medical care or other intervention strategies.

IMPLEMENTATION

Multisite Health Studies

As of June 1991, more than 40 studies in 32 states had been implemented, proposed, or completed (Fig. 1). Multisite study protocols were developed for two major types of studies: biologic indicators of exposure studies and symptom/disease prevalence studies. The multisite protocols were designed to enhance statistical power using larger numbers of study subjects obtained by combining data from several study populations at comparable types of waste sites.

The first multisite study will be implemented during July, August, and September 1991, when data collection is scheduled for biologic indicators of exposure to heavy metals at mining and manufacturing sites in three states (Illinois, Kansas, and Missouri). Each study will collect four classes of data from the study populations for pooled analyses:

1. Biologic indicators of exposure to heavy metals
2. Environmental samples from individual residences
3. Major routes of exposure, based on questionnaire responses
4. Medical test batteries for four organ systems.

Field Trials of Medical Test Batteries

The medical test batteries for four organ systems (Table 3) were selected for initial field trials in the summer of 1991 based on feasibility and cost. These field trials will help define background levels of medical test values in populations with different levels of exposure to heavy metals (and eventually other hazardous chemicals) in the environment. Test batteries for additional organ systems will be considered for future studies as time and resources permit.

Training and Development

As a first step toward implementation of a neurobehavioral test battery for use in field studies of environmentally exposed communities, ATSDR investigators have received training in administration of the Neurobehavioral Core Test Battery

TABLE 2
ATSDR PRIORITY HEALTH CONDITIONS

1. Birth defects and reproductive disorders
2. Cancer (selected anatomic sites)
3. Immune function disorders
4. Kidney dysfunction
5. Liver dysfunction
6. Lung and respiratory diseases
7. Neurotoxic disorders

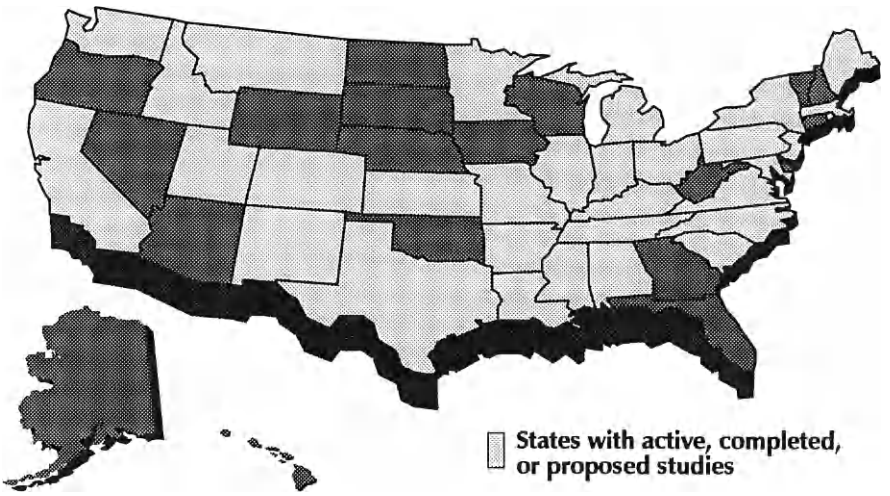

FIG. 1. Map shows states with ATSDR-supported environmental health studies as of June 1, 1991.

(NCTB) recommended by the World Health Organization. The training is intended to provide a practical knowledge of administration of the NCTB, the field requirements for its implementation, and its principal strengths and weaknesses as a measurement tool. It includes individual instruction on testing procedures and a presentation of research results from the NCTB Cross-Cultural Assessment.

FUTURE PRIORITIES AND INFORMATION NEEDS

To perform its public health mission, the ATSDR needs to characterize many detailed events in a continuum that begins with a pathway of environmental exposure and proceeds, if unimpeded, from human uptake to organ effect, disease, or death. Expanded use of neurobehavioral and other organ-specific test batteries will add precision to standards development, responsible decision making, and appropriate public health interventions. Accordingly, efforts are under way to support future priorities and information needs.

Hypothesis Definition

The ATSDR has initiated a university-based health studies initiative to define and test specific hypotheses regarding neurotoxic disorders and other adverse health outcomes associated with exposure to hazardous wastes, specific targeted chemicals, and a variety of routes of exposure. These studies will be used to establish the prevalence of discernable abnormalities in reference populations at background levels of exposure and at different levels of greater-than-background exposure to environmental toxicants.

These studies are structured to determine the prevalence of adverse health conditions among persons at hazardous waste sites and to determine whether persons living near hazardous waste sites have worse health than other persons. If so, those conditions may be defined and studied to determine their association with exposure to hazardous substances. To examine the prevalence of neurotoxic disorders, the ATSDR will need to establish and standardize fieldworthy neurobehavioral tests and conduct epidemiologic surveillance on exposed and reference

TABLE 3
BIOMEDICAL TEST BATTERIES, SUMMER 1991

System	Specimen
Liver (hepatobiliary system)	
Aspartate aminotransferase (AST, SGOT)	Serum
Alanine aminotransferase (ALT, SGPT)	Serum
Gammaglutamyl transferase (GGT)	Serum
Total protein	Serum
Albumin	Serum
Kidney (renal–urinary system)	
Creatinine	Serum
Blood urea nitrogen (BUN)	Serum
Electrolytes (Na, K, Cl, CO_2 content)	Serum
Total protein	Serum
Chemical urinalysis (dipstick)	Urine
Microscopic urinalysis	Urine
Osmolarity and specific gravity	Urine
β-*N*-Acetylglucosamine (NAGA)	Urine
γ-Glutamyltransferase (GGT)	Urine
Alanine aminopeptidase (AAP)	Urine
Immune system and hematopoietic system	
Total protein	Serum
Albumin	Serum
Globulin	Serum
Immunoglobulins (IgA, IgE, IgG, IgM)	Serum
Hemoglobin and hematocrit	Blood
White blood cell count and differential	Blood
Red blood cell count and morphology	Blood
Platelet estimate and morphology	Blood
Reticulocyte count	Blood
Leukocyte surface marker analysis	Blood
Total T cells (CD2 or CD3)	
Helper T cells (CD4)	
Mixed/natural killer cells (CD8)	
Suppressor/cytotoxic cells (CD8–CD3)	
B cells (CD19 or CD20)	

populations. Definitive studies may then be designed for those neurotoxic disorders that are identified.

Neurobehavioral Test Consultation

The ATSDR has scheduled a workshop in September 1991[2] to assist in developing standardized neurobehavioral test batteries for use in field studies of environmentally exposed communities. Additional tests of neurotoxicity are needed (especially for newborns, infants, and children) to more completely research this important class of disorders in communities located near hazardous waste sites. The workshop will address development of the following objectives:

1. A test battery or a strategy for using existing batteries for environmental

[2] *Note added in proof.* Test battery report available from the National Technical Information Service, Springfield, Virginia: (703) 487-4650. Specify order number PB93-145563.

neurobehavioral testing suitable for the ATSDR to use immediately in extensive field testing in environmental health field studies of adults.

2. A battery or batteries of tests for newborns, infants, and children applicable in environmental health field studies; or, if this is not currently possible, a strategy to develop such a battery or batteries as soon as possible.

3. Criteria and procedures to determine what tests are to be included in future modifications of standard neurobehavioral test batteries for environmental health field studies and regular review procedures to allow progressive modification.

The workshop also will help the ATSDR apply such methods, in the future, to special population groups, such as different cultures and non-English-speaking minorities. The detailed organ-specific information gained from validated neurobehavioral test batteries will improve considerably the quality of ATSDR's health research program and its ability to prevent or mitigate adverse health outcomes.

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Database Programs as a Literature Research Tool for the 1990s Scientist: Surveillance of Neurotoxicology Data[1]

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As the mass of data and literature in any scientific field grows, it becomes increasingly difficult to recall details of early experiments and to relate findings in different studies. Simple database programs are available for the individual scientist to record and manipulate information, to provide a reliable and flexibly updatable memory aid, and to reveal new relationships through (re)organizations of information. Such programs can increase the efficiency of the literature research process and increase writing productivity by orders of magnitude. The use of database programs for literature research is exemplified with a behavioral neurotoxicology database revealing previously unappreciated consistencies in the human worksite research literature.

INTRODUCTION

For most scientists, writing begins with reading literature (typically journals) and, as a memory aid, writing notes on small (often 3 × 5) cards, in log books, or in word-processing programs. Then, the scientist organizes and collates the information by assembling the notes from those articles judged scientifically sound, perhaps reorganizing the notes into related units of information. When the useful information is contained in a relatively small number of publications, this process is effective and efficient. When the useful information is distributed among dozens or hundreds of articles, this process will only work when the scientist's memory and relational abilities are of legendary proportions.

There is a type of computer software, the database program, which has the potential to stimulate a fundamental change in this literature research process for scientists. The database program is designed to manipulate masses of information and organize that information through alphabetization of key words or numerical ordering. The database program can easily be employed for recording information on a reliable medium (with copies in the event of catastrophic failure) that has immense advantages over note cards or other forms of paper media. The most noteworthy advantages are the ability to (a) Change the recording form *mid-search* (e.g., reorganize the visual presentation of the form into which information is typed, (b) add new categories of information (e.g., when the importance of a variable is newly recognized), and (c) interrelate information across masses of records. This is illustrated here to demonstrate the effectiveness of the database program as a tool that can fundamentally alter scientists' approach to literature research by making that process more efficient and thorough.

METHODS AND MATERIALS

A variety of database programs exist for every generic type of computer. Only

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the most basic features are needed in a database program to record and query a literature review. Software completely preprogrammed for a particular job would defeat the purpose, so an author should expect to learn the rudiments of working with the chosen database program. For microprocessors found in most educational institutions, simple databases in popular integrated programs are more than adequate for recording and manipulating relatively large collections of information (e.g., Claris Appleworks for the Apple II series, Microsoft Works for the Macintosh or IBM computers, and PFS Windows Works for IBM computers). (Integrated programs incorporate the advantage of electronically copying references and database elements into a manuscript and have the further advantage of utilizing the same basic commands across programs.) A database novice can be operational using simple database programs with less than a day of training using a tutorial.

It is obviously possible to use a sophisticated, multifeatured database on a mainframe (e.g., Oracle Corporation's Oracle) or a personal computer program (e.g., R: BASE 5000 or dBASE III) (Gaydosch, 1988). However, sophisticated and powerful database programs are procedurally complex, requiring a significant time investment to learn. Although a sophisticated program may be required for interrelating a very broad and diverse literature (e.g., Eckerman, 1991), virtually any set of records collected for one database can be transferred to a more sophisticated database program with no loss of information if the simple database program eventually proves inadequate for a given task. However, operational simplicity is the key to entry-level success for most people.

To begin using a database program for a literature search, the scientist constructs the basic format for recording information. Figure 1 exemplifies a simple record form (the basic collection unit of information—here recording a single publication). It is used for recording basic information in a vertical column down the screen. A slightly more sophisticated record form is listed in the top panel of Fig. 2; this program allows the scientist to recreate a note card format. What is powerful about both example database programs is the ability to add new categories at any time. Using the second example, the bottom panel of Fig. 2 depicts new categories melded with the old categories (in the top panel), and the "card" is reorganized. The reorganization may be used to put related pieces of information next to each other or to follow an order in which one wishes to enter information. This allows one to conveniently enter information as one reads an article.

In the process of recording the literature in the database, the data should periodically be organized alphabetically or numerically (as relevant) by its various categories. This allows rapid identification of spelling errors when adding infor-

Chemical—Mercury
Test Name—Simple Reaction Time
Function Tested—Speed, Coordination
Design—Cross-Sectional
Industry/Work Groups—57 Mercury distillation workers
Reference—Angotzi et al., 1983
Referent Analysis Basis—24 (approx) Workers; correlation with burden
Chemical Measure (exp)—32–109 μg/l urine
Chemical Measure (ref)—10–21 μg/l urine
Significant difference?—Yes

FIG. 1. Simple database record form.

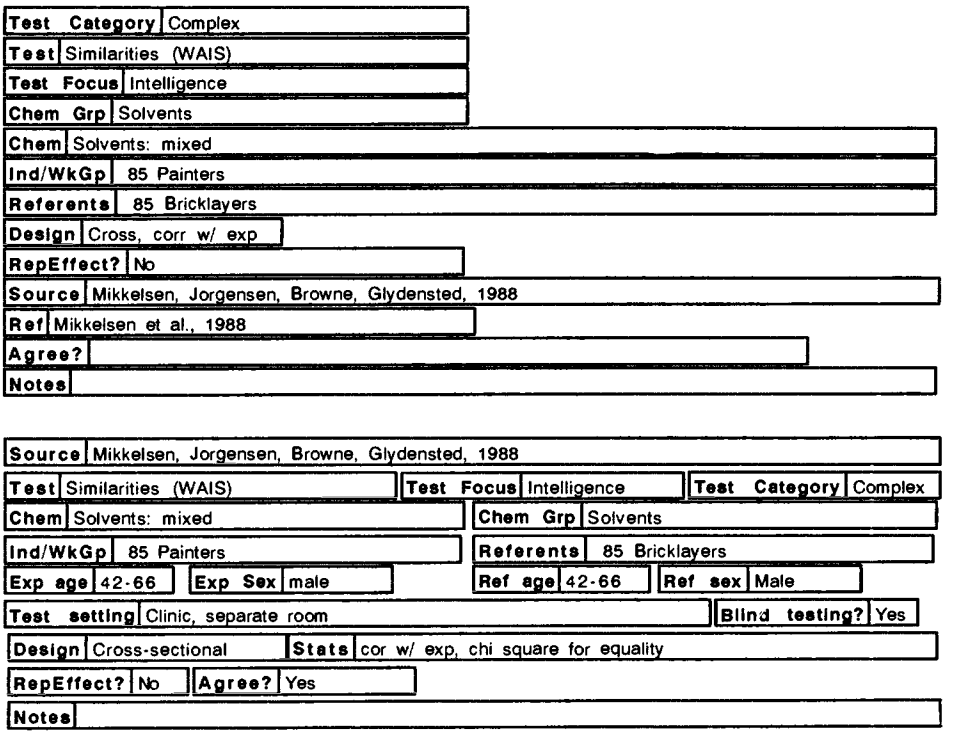

FIG. 2. More sophisticated database record form (top panel) and a revision of this record form (bottom panel).

mation, inconsistencies in categorization, and suggests needed categories that must be added (requiring rereading of the articles already added—better earlier than later in the process). Periodic ordering of the data also allows ongoing assessment of the basic questions that might be asked of the database, e.g., how many subjects are employed in most studies, which statistics are typically used, what levels of the independent variables (e.g., chemical dosage) are associated with significance.

RESULTS: AN EXAMPLE

The largest segment of the human behavioral neurotoxicology literature describes long-term (typically field) research in working populations. In the course of preparing a review article (Anger, 1990) on these findings, a search of the international literature identified some 185 studies published, primarily in English, from 1966 through 1989. The strategy for drawing conclusions for the review was focused on replication or consistency of results in several independent studies. Two basic questions were asked: (1) Which behavioral tests most consistently detect nervous system deficits in workers exposed to various chemicals and (2) what are the most frequently reported behavioral effects associated with the chemicals studied.

While reading each of the 185 articles, specific information was entered in a record form (similar to Fig. 1) on a user-constructed Appleworks database on an Apple II computer. Once the relevant information from all 185 studies was entered into the database, the two questions posed above could be answered very quickly.

Grooved Pegboard	Coord, speed	Lead	288 Battery wkrs	181 Truck frame mfrs; regress w/ burden	Parkinson *et al.*, 1986
Grooved Pegboard	Coord, speed	Lead	288 Battery wkrs	181 Auto, truck mfrs	Ryan *et al.*, 1987
Grooved Pegboard	Coord, speed	Organophosphates	100 w/ pesticide poisoning	100 Referents (various sources)	Savage *et al.*, 1988
Grooved Pegboard	Coord, speed	Solvents	17 Blue collar wkrs	17 Blue collar wkrs	Ryan *et al.*, 1988
Grooved Pegboard	Coord, speed	Solvents	42 Dockyard wkrs	42 Joiners (co-wkrs)	Cherry *et al.*, 1983, 1984
Grooved Pegboard	Coord, speed	Toluene	59 Rubber mat wkrs	59 Manual laborers (co-wkrs)	Cherry *et al.*, 1983, 1984
Grooved Pegboard	Coord, speed	Mercury	13 Dental wkrs (high exp)	13 Dental wkrs (low exp)	Uzzell & Oler, 1986
Grooved Pegboard	Coord, speed	Mercury	26 Dentists	17 Dentists	Shapiro *et al.*, 1982
Grooved Pegboard	Coord, speed	PBBs	21 Farmers (persistent complaints)	21 Hospital wkrs	Brown & Nixon, 1979
Grooved Pegboard	Coord, speed	Pentaborane	14 Emergency personnel exp acutely	Norms (source not described)	Hart *et al.*, 1984
Grooved Pegboard	Coord, speed	Solvents	19 Sewage treatment wkrs	Norms (clinical); corr w/ job yrs	Kraut *et al.*, 1988
Grooved Pegboard	Coord, speed	Solvents	29 Printers	29 Service wkrs	Braun *et al.*, 1989
Grooved Pegboard	Coord, speed	Solvents	44 Painters (dockyard)	44 Joiners (dockyard)	Cherry *et al.*, 1985
Grooved Pegboard	Coord, speed	Toluene	52 Ruberised asbestos mat mfrs	752 Asbestos products wkrs	Cherry *et al.*, 1985
Grooved Pegboard (Purdue)	Coord, speed	Formaldehyde +	305 Histologists	Multiple regress w/ exp hrs	Kilburn *et al.*, 1987
Grooved Pegboard (Purdue)	Coord, speed	Lead	24 Electrical component mfrs	29 Co-wkrs	Pasternak *et al.*, 1989
Hand-eye Coordination	Coord, speed	Tin +	10 Trimethyltin dichloride mfrs	8 Co-wkrs (minimal exp)	Ross *et al.*, 1981
Hand-eye Coordination (NES)	Coord, speed	Ethylene oxide	8 Hospital wkrs	8 Co-wkrs	Estrin *et al.*, 1987
Hand-eye Coordination (NES)	Coord, speed	Diazinon	46 Applicators (39 exp days)	56 Supervisors, inspectors	Maiztish *et al.*, 1987b
Hand-eye Coordination (NES)	Coord, speed	Lead	24 Electrical component mfrs	29 Co-wkrs	Pasternak *et al.*, 1989
Hand-eye Coordination (NES)	Coord, speed	Mercury	60 Chloralkali wkrs	60 Woodprocessing wkrs	Piikivi & Hänninen, 1989
Hand-eye Coordination (NES)	Coord, speed	Solvents	101 Painters	Regress w/ exp	Fidler *et al.*, 1987
Hand-eye Coordination (NES)	Coord, speed	Solvents	145 Painters & Allied Trades members	Regress w/ exp	Baker *et al.*, 1988
Index (Santa Ana & Pin Test)	Coord, speed	Solvents	32 w/ toxic enceph	32 Referents	Ørbæk *et al.*, 1987
Line Pursuing Test	Coord, speed	Lead	49 Polyvinyl chloride stabilizer mfrs	36 Wkrs	Jeyaratnam *et al.*, 1985, 1986
Michigan Eye-Hand Coordination	Coord, speed	Carbon monoxide	6 Toll booth collectors	Corr w/ COHb	Johnson *et al.*, 1974
Michigan Eye-Hand Coordination	Coord, speed	Lead	316 Battery mfrs	112 Light mfg wkrs	Repko *et al.*, 1975
Michigan Eye-Hand Coordination	Coord, speed	Mercury	77 Chloralkali, magnetic products mfrs	65 Co-wkrs	Miller *et al.*, 1975
Michigan Eye-Hand Coordination	Coord, speed	Mercury	79 Chloralkali wkrs	Corr w/ urine Hg	Langolf *et al.*, 1978
Michigan Eye-Hand Coordination	Coord, speed	Carbon disulfide	131 Viscose rayon wkrs	167 Co-wkrs (low exp)	Putz-Anderson *et al.*, 1983
Michigan Eye-Hand Coordination	Coord, speed	Lead	53 Battery mfrs	55 Light mfg, service wkrs	Repko *et al.*, 1978
Michigan Eye-Hand Coordination	Coord, speed	Lead	403 Smelter wkrs	305 Residents	Johnson *et al.*, 1980
Michigan Eye-Hand Coordination	Coord, speed	Methyl bromide +	74 Structural, soil fumigators (3 grps)	29 Co-wkrs, government wkrs	Anger *et al.*, 1986
Michigan Eye-Hand Coordination	Coord, speed	Methyl chloride	122 Foam products mfrs	49 Co-wkrs	Repko *et al.*, 1976
Pencil-flipping	Coord, speed	Mercury	77 Chloralkali, magnetic products mfrs	65 Co-wkrs	Miller *et al.*, 1975
Pins (Hogstedt)	Coord, speed	Lead	49 Lead smelter, battery wkrs	27 Wire, machine wkrs (low PbB)	Hogstedt *et al.*, 1983
Pins (Hogstedt)	Coord, speed	Solvents	45 Floor layers (2 grps)	50 Carpenters	Ekberg *et al.*, 1986

FIG. 3. Organization of data by function (Table 3 in W. K. Anger, 1990). Worksite behavioral research: Results, sensitive methods, test batteries and the transition from laboratory data to human health. *Neurotoxicology* 11, 629–720; reproduced by permission of the publisher).

Figure 3 illustrates one organization of the data. This organization was designed to group together tests of a given function (column 2). Several tests of coordination and speed are shown in Fig. 3. The test names (column 1) shown in **bold** print are findings in which statistically significant differences were reported between the groups assessed (columns 4 vs 5) with that test. The organization by test was accomplished in seconds for the data in the 185 studies by simply organizing (sequentially) the "test" and "test focus" columns in alphabetical order.

Figure 4 illustrates a second organization of the same database, by chemical (column 3). Here the findings for carbon disulfide are brought together, revealing the results of all tests administered to workers exposed to carbon disulfide. Figure 4 also illustrates the point that data can be excluded for an analysis; only the findings that are statistically significant (those in **bold** in Fig. 3) are shown in Fig. 4. As above, this can be revealed in seconds for the data in the 185 studies by simply organizing the "chemical exposure" column in alphabetical order.

Careful consideration of Figs. 3 and 4 reveals that there is extensive suborganization beyond the basic organizational principles described above. For example, in Fig. 3, each of the tests are in alphabetical order as are each of the chemicals within the individual test groupings. Such organization is accomplished simply by alphabetizing, successively, first the "chemical exposure" column, then the "test name" column, than the "test focus" column. In fact, each column in these figures was organized alphabetically in a predetermined succession, from the least important column to the most important column, the latter being the main focus of the analysis. A final benefit was that the tables were formatted and printed by the author for the journal. This provided creative control over table formatting which was critical to maximizing information. It also eliminated the need to proofread 50 pages of tables in the type size shown in Figs. 3 and 4.

Pauli Test	Calculations, speed	Carbon disulfide	17 Viscose rayon mfrs (high exp)	17 Viscose rayon mfrs (low exp)	Foà *et al.*, 1976
Pauli Test	Calculations, speed	Carbon disulfide	120 Viscose rayon wkrs	54 Co-wkrs	Cassitto *et al.*, 1978
Digit Symbol	Coding	Carbon disulfide	97 Viscose rayon wkrs	96 Paper mill wkrs	Tolonen, 1974
Digit Symbol	Coding	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Digit Symbol	Coding	Carbon disulfide	100 Viscose rayon wkrs	50 Co-wkrs	Hänninen, 1971, 1974
Digit Symbol	Coding	Carbon disulfide	120 Viscose rayon wkrs	54 Co-wkrs	Cassitto *et al.*, 1978
Picture Completion	Intelligence	Carbon disulfide	100 Viscose rayon wkrs	50 Co-wkrs	Hänninen, 1971, 1974
Picture Completion	Intelligence	Carbon disulfide	120 Viscose rayon wkrs	54 Co-wkrs	Cassitto *et al.*, 1978
Raven Progressive Matrices	Intelligence	Carbon disulfide	120 Viscose rayon wkrs	54 Co-wkrs	Cassitto *et al.*, 1978
Similarities	Intelligence	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Benton Visual Retention	Memory	Carbon disulfide	100 Viscose rayon wkrs	50 Co-wkrs	Hänninen, 1971, 1974
Digit Span	Memory	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Digit Span	Memory	Carbon disulfide	100 Viscose rayon wkrs	50 Co-wkrs	Hänninen, 1971, 1974
Memory (verbal, visual)	Memory	Carbon disulfide	100 Carbon disulfide wkrs (exp, pois)	50 Wkrs	Gharase, 1976
Rey PRM1	Memory	Carbon disulfide	17 Viscose rayon mfrs (high exp)	17 Viscose rayon mfrs (low exp)	Foà *et al.*, 1976
Rey Test	Memory	Carbon disulfide	120 Viscose rayon wkrs	54 Co-wkrs	Cassitto *et al.*, 1978
Block Design	Spatial Relations	Carbon disulfide	89 Viscose rayon wkrs	50 Co-wkrs, carpenters	Tuttle *et al.*, 1976
Block Design	Spatial Relations	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Block Design	Spatial Relations	Carbon disulfide	100 Viscose rayon mfrs	50 Co-wkrs	Hänninen, 1971, 1974
Block Design	Spatial Relations	Carbon disulfide	120 Viscose rayon wkrs	54 Co-wkrs	Cassitto *et al.*, 1978
Bourdon-Wiersma	Vigilance	Carbon disulfide	97 Viscose rayon mfrs	96 Paper mill wkrs	Tolonen, 1974
Bourdon-Wiersma	Vigilance	Carbon disulfide	100 Viscose rayon mfrs	50 Co-wkrs	Hänninen *et al.*, 1974
Bourdon-Wiersma	Vigilance	Carbon disulfide	206 Viscose rayon wkrs	152 Paper mill wkrs	Tolonen & Hänninen, 1978
Neisser Letter Search	Vigilance	Carbon disulfide	89 Viscose rayon wkrs	50 Co-wkrs, carpenters	Tuttle *et al.*, 1976
Vigilance +	Vigilance	Carbon disulfide	100 Carbon disulfide wkrs (exp, pois)	50 Wkrs	Gharase, 1976
Numeric square	Vigilance +	Carbon disulfide	265 Viscose rayon mfrs	Inferred	Herbig, 1973
Mira Test	Coord	Carbon disulfide	100 Viscose rayon mfrs	50 Co-wkrs	Hänninen, 1971, 1974
Mira Test	Coord	Carbon disulfide	206 Viscose rayon wkrs	152 Paper mill wkrs	Tolonen & Hänninen, 1978
Tapping (finger)	Coord	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Eye-hand coordination	Coord, speed	Carbon disulfide	100 Carbon disulfide wkrs (exp, pois)	50 Wkrs	Gharase, 1976
Santa Ana	Coord, speed	Carbon disulfide	89 Viscose rayon wkrs	50 Co-wkrs, carpenters	Tuttle *et al.*, 1976
Santa Ana	Coord, speed	Carbon disulfide	97 Viscose rayon wkrs	96 Paper mill wkrs	Tolonen, 1974
Santa Ana	Coord, speed	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Santa Ana	Coord, speed	Carbon disulfide	100 Viscose rayon wkrs	50 Co-wkrs	Hänninen, 1971, 1974
Santa Ana	Coord, speed	Carbon disulfide	206 Viscose rayon wkrs	152 Paper mill wkrs	Tolonen & Hänninen, 1978
Reaction Time (FIOH)	Speed, coord	Carbon disulfide	98 Viscose rayon wkrs	91 Machinery wkrs	Liang *et al.*, 1985
Simple Reaction Time	Speed, coord	Carbon disulfide	89 Viscose rayon wkrs	50 Co-wkrs, carpenters	Tuttle *et al.*, 1976
Visual Motor Speed Test	Speed, coord	Carbon disulfide	17 Viscose rayon mfrs (high exp)	17 Viscose rayon mfrs (low exp)	Foà *et al.*, 1976
Choice Reaction Time	Speed, coord, decis	Carbon disulfide	89 Viscose rayon wkrs	50 Co-wkrs, carpenters	Tuttle *et al.*, 1976
Choice Reaction Time	Speed, coord, decis	Carbon disulfide	100 Carbon disulfide wkrs (exp, pois)	50 Wkrs	Gharase, 1976
Farnsworth-Munsell 100-Hue Color Arrangement	Vision-color	Carbon disulfide	64 Viscose rayon wkrs	46 Co-wkrs	Raitta *et al.*, 1981
Cattell IPAT Anxiety Scale	Anxiety	Carbon disulfide	17 Viscose rayon mfrs (high exp)	17 Viscose rayon mfrs (low exp)	Foà *et al.*, 1976
Eysenck Maudsley Personality Inventory	Personality	Carbon disulfide	17 Viscose rayon mfrs (high exp)	17 Viscose rayon mfrs (low exp)	Foà *et al.*, 1976
Rorschach Inkblot	Personality	Carbon disulfide	100 Viscose rayon wkrs	50 Co-wkrs	Hänninen, 1971, 1974
Rorschach Inkblot	Personality	Carbon disulfide	206 Viscose rayon wkrs	152 Paper mill wkrs	Tolonen & Hänninen, 1978
Audiometer	Audition-absolute	Carbon disulfide +	60 Viscose rayon wkrs	205 Factory wkrs; corr w/ work yrs	Morata, 1989

FIG. 4. Organization of data by chemical (Table 4 in W. K. Anger, 1990). Worksite behavioral research: Results, sensitive methods, test batteries and the transition from laboratory data to human health. *Neurotoxicology* **11**, 629–720; reproduced by permission of the publisher).

DISCUSSION

Database programs can be easily and profitably employed to record a literature search on a permanent medium. For the scientist who is synthesizing a small assemblage of literature, the database program offers convenience and efficiency. For the scientist who needs to interrelate a large literature of articles (e.g., Anger, 1990; Eckerman, 1991), the database program offers far more advantages. The database program can be used to arrange information in a variety of ways to ask new questions about the studies reviewed. As sophistication increases or needs change, one can add new categories to a previously created database, returning to the original articles to enter the data previously omitted. In addition, new literature can be added to the database as the years go by, and the same questions can be asked to determine if the answers are changing with new literature. For the scientist who studies large masses of related literature, this process can increase immensely the efficiency of periodically writing new "update" reviews of a growing literature.

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Factors Influencing the Assessment and Control of Occupational Hazards in Developing Countries[1]

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The principles of occupational health may be the same in the developed and developing countries. However, there can be a wide diversity in practice. The exposure to chemicals at the workplace in developing countries is usually of a different nature, and the level of exposure is generally of a higher magnitude. The leading occupational diseases in developing countries are also very different to those reported in industrialized nations. For hazard evaluation in developing countries, more factors need to be considered. Problems are usually more complicated as most workplaces are subjected to many factors which typify small-scale industries. Low capital investment often culminates in cutbacks on necessary expenses, especially on occupational or environmental health activities. Thus the health, safety, and welfare of the workers are usually overlooked. This situation helps only to promote greater risks to the workers. Furthermore, many workers in the developing countries suffer from poor nutrition, endemic diseases, and other debilitating conditions. For these reasons, it is possible that currently recommended occupational exposure limits could allow injury to workers in the developing nations. When carrying out health assessment, careful attention must be paid to cultural practices, genetic components, working conditions, and other predisposing factors. This paper reviews some of the current techniques commonly used for the monitoring of toxic substances and an in-depth discussion on various problems facing the developing countries concerning the usage of these techniques.

INTRODUCTION

The concerns for health at the workplace are to a large degree universal, and the principles of occupational medicine are the same in industrialized countries as in developing nations. Nevertheless, there is often a wide diversity in the occupational health practices and problems between the developing countries and the industrialized nations.

Information on occupational activities in various developed nations can be readily found. There is, however, very little information concerning assessment of occupational hazards in developing countries.

Estimation of exposure is a crucial element in toxicological study and can be one of the most challenging aspects of health protection and disease prevention. This paper attempts to identify some of the problems faced by the developing countries concerning the assessment and control of occupational health hazards. For hazard evaluation in developing nations, more factors need to be considered.

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Problems are usually more complicated than in the developed nations as most workplaces are subjected to many factors which typify small-scale industries. Low capital investment often culminates in cutbacks on necessary expenditures, especially on welfare and occupational health activities. This situation only helps to promote greater risks to the workers. Furthermore, workers in the developing countries suffer from poor nutrition, endemic diseases, and other debilitating conditions. These factors are not usually taken into consideration when adopting and implementing occupational exposure standards.

DEVELOPING COUNTRIES

The term "developing world" is one of the many synonyms in current usage to describe a group of nations which is also known as "the newly industrializing countries," "the Third World," "the underdeveloped nations," and "the nations of the South."

The First-World countries consist of nations of the industrialized market economics of the Western Europe, North America, and Japan. The Second-World countries comprised the once centrally planned economies of Eastern Europe, now undergoing rapid political and economic changes. The Third-World countries include most of the countries in Asia, Africa, the Middle East and South America.

The geographical configuration of this grouping has led to a parallel distinction of north (First and Second World) versus south. To a certain extent, this is a distinction between nations of sufficiency and nations of deprivation. Notable exceptions are the Asian countries of Taiwan, South Korea, Hong Kong, and Singapore, which have developed beyond their previous Third-World status.

Many developing countries are financially disadvantaged and have largely agricultural and rural economies. On the other hand, many of these countries are rich in terms of culture, history, and civilization (Gillies *et al.*, 1983). Although the developing countries may have common economic characteristics, they are widely different in many ways with diverse aspirations and political systems and varying stages of industrial growth.

The world's population in 1987 was estimated to be 5 billion people. About 6 of every 10 people live in Asia. Another 2 in 10 live in Africa or Latin America. Thus, more than 80% of the world's population live in the developing countries. The health status of people in developing countries is often poor, with high infant mortality rates and low life expectancies (WHO, 1986).

Infectious Diseases and Malnutrition

People in the developing nations are often of poorer health status compared to those of the developed world and may therefore be more susceptible to effects of workplace hazards. Approximately one-third of all deaths in the developing world are due to infectious diseases (Hakulien, 1988). For example, in Mexico (population 70 million), for the year 1980 there were over 80,000 deaths attributed to infectious diseases and only 28,000 deaths due to malignant neoplasms (WHO, 1986).

Lucas and Gillies (1973) have expressed the view that the health problems of workers in the developing countries are more complex than those of industrialized

nations because of the high prevalence of endemic and epidemic diseases. Infectious and parasitic diseases may reduce the resistance of those infected. This has implications for the development of occupational standards for chemicals as they could cause detriment to the health in such individuals at lower levels of exposure.

Malaria and dengue haemorrhagic fever may account for far more deaths and ill health among people in developing countries than occupational cancers or poisoning. Tuberculosis and other infectious diseases are common causes of sickness absence. In the Philippines, tuberculosis was considered to be one of the more prominent occupational diseases (Reverente, 1985), and epidemics of cholera, typhoid, and yellow fever have been known to devastate lumber camps and plantations and even lead to their shut-down (Phoon, 1983).

Nonalcoholic liver disease is widespread among Africans and Asians. A damaged liver will be less able to detoxify poisons in the work environment. Many of those cases of liver disease are sequelae of hepatitis B viral infections, which predispose both to hepatic cirrhosis and to hepatoma (Blumberg, 1980). It is therefore sensible to screen for liver function in preemployment of those who will be exposed to liver toxic compounds which may adversely affect it (Tamburro and Liss, 1985).

Threshold limit values (TLVs) or their equivalents presuppose that the exposed workers are "normal" and "healthy." Unfortunately, many workers in the developing countries suffer from malnutrition. Malnutrition lowers the resistance of the body to both infections and toxic substances. It may not be easy to motivate anemic workers with a hemoglobin value of 8–9 gm/100 ml due to poor nutritional status or hookworm infestation to take up a physically demanding job.

Small-Scale and Traditional Industries

Small-scale industries (less than 100 employees) account for a large proportion of the manufacturing activities in many of the developing countries, in terms of both the number of factories and the number of workers employed (Kogi, 1985).

Over 40% of industrial settings in southeast Asia are made up of small enterprises with fewer than 50 on staff (Foo *et al.*, 1985) and many of these enterprises have difficulties providing workers with even basic welfare facilities. In Korea, 93% of factories are small-scale operations and the number of workers employed in this sector amount to 45% of the total work force. More than 45% of the work force in Singapore are involved with small-scale industries. Many developing countries also recognize that these small industrial operations are important in their economic development. However, workers in this industrial sector often work in unsatisfactory environmental conditions. Moreover, the methods and parameters of protection are usually less efficient or totally lacking (Su'mamur, 1985).

The World Health Organization has declared its objective of achieving health for all by the year 2000. This is a laudable aim that deserves universal support. It has been reported that in Asian and African developing countries, less than 25% of the working population has access to any form of occupational health service. In addition, health care in small industries is usually not regulated by national legislation. In most cases, there is a general lack of health, safety, and welfare

services. Thus the consequence is that occupational exposure is likely to be greater for these workers and their health problems often greater.

Let us now look at a few specific occupational health problems of small-scale industries. Effects of benzene on bone marrow depression and leukemia were first reported in 1897. In most of the western nations, new stringent occupational standards of 0.1 or 1 ppm has been recommended since 1987 to protect workers from the hazard of exposure to this chemical. A recent report revealed that occupational exposure to benzene in petroleum is still commonplace in many developing countries due to the shortage of electricity supply. Vendors of petroleum are constantly inhaling and swallowing petrol while siphoning. Motor mechanics in small workshops still use petrol as a solvent for cleaning their hands. Significantly high rates of anemia, microcytosis, and hypochromia were observed among these workers (Fleming, 1990).

Exposure to toxic substances in agricultural development has received somewhat less attention in the Third World. Exposure may occur from direct insult associated with insecticides, herbicides, or fungicides during formulation, application, harvesting, and ingestion of treated crops (Jeyaratnam, 1985).

Genetic and Ethnic Differences

Differences in susceptibility to potentially hazardous chemicals represent a significant parameter in the characterization of risk to human health. Genetic differences in the metabolization of chemicals should be analyzed in risk assessments. Genetic factors do not act in isolation from other biological variables such as immunological, biochemical, and nutritional status. They affect a worker's susceptibility to a broad spectrum of occupational hazards. Some genetic disorders are more prevalent in developing countries, and these disorders have been noted to be related to exposure to various chemicals.

There is little information regarding the influence on the genetic diversity on exposure to chemicals. This is unfortunate, particularly from the perspective of developing nations, because the ethnic diversity varies tremendously. An appropriate example is the anthropometric differences between Asian and Caucasian populations. The Asian workers are generally of smaller size and more slender build. This difference may have various health implications. In terms of physiology, there are differences in heat exchange due to a comparatively smaller skin surface for the same weight. Thus, reference values for heat stress published in Western texts may not be directly applicable to Asian populations.

Glucose-6-phosphate dehydrogenase (G-6PD) deficiency, is an inherited enzyme deficiency state common in many African and Asian countries, with a prevalence of about 15–26% in central Africa and of about 18% in South East Asia (WHO, 1990). It has been shown that persons with this enzyme deficiency are more susceptible to hemolytic episodes when exposed to ozone or other oxidizing agents (Stokinger and Mountain, 1967; Calabrese *et al.*, 1982).

Thalassemia, a common genetic disorder in the Mediterranean region, has a prevalence of about 4–17% among the Asian population (Wong, 1984; Sangani *et al.*, 1990). In India, it is estimated that about 7000 thalassemia infants are born annually. It presents clinically as an anemia and persons with this hematological

disorder have been advised not to be excessively exposed to hematotoxic agents such as arsine, benzene, and lead (Phoon, 1987). Whether the anemia is worsened by occupational exposures to these chemicals at low concentrations has yet to be fully investigated.

Religious, Social, and Cultural Factors

The religious and cultural diversity in many developing countries may affect the implementation of occupational safety and health regulations. Religious and cultural traditions also influence health and life-style practices. Muslim employees fast during the month-long "Ramadan" period. They are forbidden by religion to eat or drink from sunrise to sunset during this period. This increases the likelihood of dehydration in hot climates and may affect the metabolism of absorbed toxic chemicals.

Eating immediately after a work period is common in industries in the developing countries. The use of hands and fingers for eating, instead of utensils such as forks and spoons, is practised by several Asian races. This practice can lead to the ingestion of toxic material from the workplace, especially if contaminated hands have not been adequately cleansed prior to meals (Chia *et al.*, 1991).

Female and Child Workers

Over 100 countries have stipulated protective regulations and limitations regarding women's employment (ILO, 1987). These regulations prohibit employment of women in dangerous, arduous, or unhealthy work. In many developing nations, standards and regulations that are available for the well-being of women seldom adequately address the issues of likely harm to the fetus in pregnant women. Exposure to various chemicals and environmental contaminants can be of greater risk to the fetus than to adults.

In developing countries, as well as in the developed countries, the women's dual responsibilities at work and at home mean that there is an additional burden of domestic work to aggravate problems arising at the workplace. Recent epidemiological studies have shown that Asian women worked for significantly longer hours than non-Asian women and they also suffered a higher risk of prenatal mortality than non-Asian women (Peel and Clarke, 1990).

In the developed nations, there are adequately enforced laws against the use of child labor. Unfortunately, child labor is still prevalent in some developing countries (Asogwa, 1986). It is lamentable that young children are required to work in dangerous situations, often toiling without sufficient rest and nourishment and living in squalid conditions. The Dickensian days of children working in mines and waiting at Paddington station to work in grim factories is consigned to history in the United Kingdom, but is still a reality in some Third-World countries.

Long Working Hours

In industrialized countries, working time is normally 8 hr a day for a 5-day week. This working schedule may not be the same for workers in developing countries, as a high proportion of the work force may have working hours in excess of these values (Ong and Kogi, 1990). In many industrial sectors, many of

the employees carry out work on two consecutive shifts because of the attractive financial incentives. Exposure to airborne chemicals during long shifts can be expected to produce tissue overburden (Paustenbach, 1985). As little as 10 hr overtime in a week will extend the exposure level by 25% (Howard, 1980).

Climatic Conditions

Many of the developing countries are located in the hot and humid tropics. As far back as 1948, Yant had already pointed out the necessity of considering "the great increases in respiratory rates arising from high levels of physical activity and work in hot climate." This hot and humid climatic condition makes it difficult to persuade workers to use personal protection, such as respirators and aprons, which are uncomfortable under such conditions. For many of the small enterprises, owing to poor environmental control, workers are often provided with personal protective equipment as a first line of defense against occupational exposures, instead of as a last resort.

There are several reasons why the hazards associated with field application of pesticides are difficult to control in the tropics, of which climatic conditions could be a major factor. The wearing of protective devices can be extremely uncomfortable because breathing in hot and humid air requires a greater effort than breathing in a cool drier atmosphere (Pulket *et al.*, 1980). On the other hand, high temperature increases the speed of skin absorption of many chemicals. In addition, washing facilities at the work sites are not usually adequate.

Pharmacological studies have shown that during exposure to high temperatures, there is an increase in susceptibility and in the rate of reaction to most drugs. Increased respiratory rate resulted in a faster rate of absorption, distribution, and metabolism of the chemicals and an altered level of normal body functions due to the work at high temperatures. Experimental studies have also reported the increased susceptibility to chemicals such as cadmium, lead, benzene, and parathion. These are some of the important factors to be considered when evaluating exposure to toxic substances.

Methodological Issues

An efficient way of preventing health impairment from exposure to harmful agents in the working environment is to assess the hazards at the workplace and to monitor the health effects on exposed persons. Estimation of exposure is a crucial element in toxicological study and can be one of the most challenging aspects of health protection and disease prevention.

In practice, exposure assessment may range from the use of environmental monitoring, biological monitoring, health record investigations and clinical diagnosis. Unfortunately, the selection of a practical method to be used in the developing nations is always difficult. Not only does the accuracy of these assessments vary widely, but the selection of an appropriate method for assessment is difficult as well.

A traditional approach for exposure measurement is through evaluation of health records. Epidemiological data such as medical records from medical institutions or from industries can be used as an alternative source for exposure

measurement. However, the specificity and sensitivity of using this approach have to depend on the completeness of the records. The use of this method in developing countries is limited as the medical records are almost nonexistent or far from complete.

The two most reliable methods for health risk assessment are: (1) measuring the concentration of the chemical in the environment (environmental monitoring) and (2) measuring some biological parameters among exposed persons (biological monitoring). The use of these procedures also poses problems.

Environmental monitoring. Owing to the shortage of funds and trained personnel, highly sophisticated procedures and monitoring techniques designed and developed in the industrialized countries may not be applicable in the context of developing countries. In addition, there are many problems which may affect instruments and measurements. Many of the delicate and sophisticated hygiene instruments require appropriate storage and a conducive operating environment, such as is needed for mass spectrograph gas chromatography. The high humidity of the atmosphere in many developing tropical countries may affect the result of air sampling, particularly those for aerosols and organic solvents (Ong *et al.*, 1991).

Biological monitoring. In recent years, efforts have been made to study not only the use of biological fluids as an indicator of internal dose, but also to examine biological effects in exposed persons. A variety of assays are now available for the monitoring of concentrations of toxic substances and their metabolites in blood, urine, and exhaled air. These tests are useful because they take into account the health conditions of the exposed persons. Some of the important parameters to be considered for biological monitoring of human toxicity are listed in Table 1. The main advantage of biological monitoring is that it reflects individual burdens of hazardous substances. Nevertheless, the personal habits of a person need to be considered. For a proper study of biological monitoring, two important practical factors require special attention. The first is the information on the normal or background levels of the general population. Unfortunately, their reference or baseline values are not available among many of the developing countries. The second factor pertains to the question as to what level constitutes an undesirable health effect. For example, it is well known that lead affects the production of red blood cells. The generally poor nutritional status of workers in

TABLE 1
PARAMETERS TO BE CONSIDERED FOR BIOLOGICAL MONITORING

1. Knowledge of the normal values
2. Intra-individual variability of the measurement
3. Inter-individual differences
4. Individual characteristics
   a. Sex
   b. Age
   c. Genetic compositions
5. Laboratory know-how
6. Route of exposure

developing countries, however, suggests that attention must be paid to the hemoglobin level of lead workers before clinical symptoms appear or before the blood lead level exceeds the statutory limit of 60 μg/100 ml.

In occupational toxicology, quality control is another important consideration for laboratories in developing countries. Participation in quality control programs is essential for the laboratory results to be validated against reliable benchmarks. However, the exorbitant cost of participation in quality control programs has deterred many laboratories in the developing nations.

Types of Occupational Disease

The types of occupational diseases that dominate the Third World particularly the least developed countries are very different from those in the First World. In the industrialized countries occupational diseases have become a thing of the past and many physicians are diverted into areas with which they were not familiar in the past such as work-induced stress, musculoskeletal problems related to sedentary work, minute exposure at the workplace that might affect behavior or reproduction, and the much wider implications of work-related diseases. On the other hand, anyone who takes the trouble to visit the small-scale industries in developing countries will, however, realize that the disease patterns are very different (Table 2). Many of the most hazardous chemicals are still commonly used throughout the less developed world.

There is a social basis for this, but even more importantly, there are economic reasons. It has been estimated that in the less developed countries of South America approximately one-third of imported pesticides are products prohibited for use in the United States because of their extreme toxicity (Coye and Fenske, 1988). Several studies have also revealed that very few agricultural workers in Latin America have access to protective clothing. Virtually all of the farmhands lived within 100 m of the farms, many in temporary housing with no walls for protection from pesticides sprayed from airplanes. Michaels and Mendes (1988) observed that the levels of organochloride pesticide residue found in adipose

TABLE 2
THREE LEADING WORK-RELATED DISEASES IN SELECTED INDUSTRIALIZED AND DEVELOPING COUNTRIES

United States[a]	China[b]	Singapore[c]	Sri Lanka[c]	The Philippines[c]
Musculoskeletal injuries	Occupational lung diseases	Noise induced deafness	Pesticide poisoning	Tuberculosis
Occupational lung diseases	Chemical intoxication	Industrial dermatitis	Lead intoxication	Industrial dermatitis
Occupational cancer	Noise induced deafness	Chemical intoxication	Industrial dermatitis	Occupational asthma

[a] Bureau of Statistics (1989).
[b] ILO (1985).
[c] Occupational Health in Developing Countries in Asia. SEAMIC, Tokyo.

tissue of Mexicans, as well as in residents of other Latin American countries, are far greater than pesticide residues present in the same tissue of North Americans and Europeans. The conditions of pesticide poisoning among agricultural workers in less developed countries in Asia did not show such differences (Jeyaratnam, 1985). Furthermore, the main victims of those diseases are the vulnerable groups, namely growing children and pregnant women.

The current U.S. standard for lead exposure mandates immediate medical attention if blood lead levels of exposed workers exceed 50 μg/dl. In a recent study, 71% of workers in two battery factories in Columbia were found to have blood lead above 50 μg/dl with one subject having blood lead level of 180 μg/dl. In another plant 13 of the 14 employees exceeded the 50 μg/dl removal level. Serious symptoms of lead poisoning were reported by over 70% of the workers in this study (Gacharna *et al.*, 1976). A cohort study of 8500 underground miners involved in tin mining with less than 15 years of exposure revealed that over 40% suffered from either silicosis or tubercular silicosis (Pinell, 1976). Based on an analysis by Mendes (1979), it was estimated that in 1977 alone there were 20,000 workers with silicosis in southern Brazil.

The shift of hazardous industries, such as the benzidine-based dye and asbestos textile industries from the highly regulated countries to less developed countries has been of concern in recent years (Jeyaratnam, 1990). By shifting hazardous production to countries with no or little environmental regulation the manufacturers could avoid investing in the equipment necessary to control hazardous exposures. This will further widen the gap of technology and disease patterns between the industrialized and less developed countries.

Furthermore, due to inadequate coverage of occupational medicine in the medical undergraduate curriculum in developing countries (Phoon *et al.*, 1988), most physicians have only the sketchiest knowledge of the way in which their patients' occupation and their state of health are interrelated. This leads to the results that many of the occupational diseases reporting systems in the developing nations are either incomplete or inaccurate.

Setting of Occupational Health Standards

Exposure limits are the basic guidelines for delineating healthy and unhealthy working environments. The interest of developing countries in this subject, however, reflect reservations on the applicability of the standards set by the Western nations mainly for Caucasians. The extrapolation and use of these standards are faced with serious limitations. Furthermore, many of the developing countries have not yet adopted any environmental standards or legislation for the control of environmental exposure.

Policies and legislation set without considering people, health, technology, and economics are fraught with difficulties in implementation. The setting of such standards should be free from undue external influences and it must also include substantial participation by representatives of exposed persons, management representatives, learned bodies, and government agencies.

CONCLUSION

Many of the occupational health problems and health needs faced by developing

countries today are similar to those faced by the developed nations in previous decades. They are closely related to other indices of health in a country or region. The high incidence of occupational diseases is associated not only with occupational exposure but also with societal problems, environmental contamination, and poor health care (WHO, 1990). In the developing countries, surveillance of exposed populations is still grossly inadequate (Ong 1986). The linking of existing records of employment, morbidity, and mortality data would greatly increase the knowledge of many industrial hazards. On the other hand, there is a need to continue the training of industrial physicians and to develop more effective research techniques.

A practical approach to workplace hazard control is to ensure that toxic compounds are being effectively handled, workplaces are carefully monitored, and clinical surveillance of the working population is conducted. At the factory level, it is essential to keep standardized records of workers' occupational histories and exposure conditions when chemicals are being used.

Compilation of basic data on the working population and the working environment is urgently needed. Evaluation of the health status of workers by using simple laboratory equipment should be considered. The active involvement of health personnel and the participation of government and research institutions are essential for the successful application of preventive programs in developing countries. Similarly, for a successful program to be conducted in a factory, the participation of management, workers and the government is required. The benefits will be tangible in terms of better work environments, better health, and a better quality of life. The health of future generations cannot be secured by excluding men or women from hazardous jobs, but by improving working conditions. The ultimate goal has to be a safe and healthy workplace for all.

Developing countries hunger for knowledge, but this hunger is paralleled by a shortage of resources. Nevertheless, the rapid industrial growth of developing nations should be of interest to toxicologists and industrial physicians. Despite their economic and cultural diversity, developing countries have many similar needs. It is surely not to the credit of occupational health that many of the above-mentioned problems still exist. Occupational health is a discipline practiced in the developed and less developed nations. The knowledge of occupational health and its applications is for sharing worldwide.

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A Study on the Neurobehavioral Effects of Occupational Exposure to Organic Solvents in Korean Workers[1]

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In order to investigate the neurobehavioral effects in workers occupationally exposed to organic solvents in Korea using WHO neurobehavioral core test battery (NCTB), a cross-sectional study of 113 male car painters and printers and 81 controls was conducted. Among the seven tests of the NCTB, only four tests—simple reaction time, Santa Ana dexterity, digit symbol, and Benton visual retention tests—were administered to the subjects because of time limitations. Painters and printers were exposed mainly to toluene, xylenes, and methyl ethyl ketone and often to solvent mixtures. The range of the solvent exposure level was 0.10–2.29 of hygienic effect. Poorer performance of Benton visual retention in the exposed group when compared to the control group was found after controlling confounders. No exposure-dependent effect was found. Further investigation with a matched control group regarding confounding factors is required for conclusive results.

INTRODUCTION

It was reported that about 100,000 workers were occupationally exposed to organic solvents in 1989 in Korea (Korean Industrial Health Association, 1990a). About 12.4% of 18,615 air samplings for organic solvent(s) concentration measurement from work sites were found to exceed the current threshold limit values (TLVs) of the Industrial Safety and Health Law of Korea (Ministry of Labor, 1989) in a cross-sectional nationwide study in the same year (Korean Industrial Health Association, 1990b). An increase in subjective symptoms as a function of exposure intensity was reported among toluene-exposed female workers (Lee *et al.*, 1988).

It has been reported that occupational exposure to organic solvent(s) has neurotoxic effects, and there are many studies concerning associations between solvent exposure and behavioral changes. Psychological performances declined in solvent-poisoned workers (Seppäläinen *et al.*, 1980), in workers exposed to solvent mixtures (Morrow *et al.*, 1990), and in those exposed to styrene (Härkönen *et al.*, 1978). Lindström and Wickström (1983) concluded that impairment in visual short-term memory and prolonged simple reaction times were found among workers exposed to solvent at levels much lower than the hygienic standard.

Psychiatric and neurological symptoms among solvent-exposed workers were reported to be evident in some of the studies (Lee *et al.*, 1988; Kim *et al.*, 1989).

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Therefore, we conducted a cross-sectional neurobehavioral study among workers exposed to solvents in painting and printing occupations.

SUBJECTS AND METHODS

Subjects

A total of 113 male workers occupationally exposed to organic solvents participated in this study. For the control group, 81 male subjects who never have been exposed to organic solvents volunteered. This group was composed of manual workers, guards, clerks, and technicians. Solvent-exposed workers were car painters from three factories and printers from two factories. They were exposed to toluene, xylenes, methyl ethyl ketone, trichloroethylene, perchloroethylene, gasoline, *n*-hexane, and often to mixtures of solvents for longer than 5 hr a working day. Because of the small sampling size and the skewed age distribution the subjects were from several different factories in both exposed and control groups. Therefore, we analyzed the data by controlling some known confounding factors, such as the level of education, age, and alcohol consumption (Fidler *et al.*, 1987; Maizlish *et al.*, 1985).

The solvent-exposed subjects were categorized into two groups—low exposure and high exposure—according to the information on the hygienic measurements of their work place during 1990 when this study was conducted. Air samples from the working stations were subjected to gas chromatography for solvent(s) concentration analysis. High- and low-exposure levels were defined by whether the exposure level exceeded the TLVs of the Korean law (Ministry of Labor, 1989). The range of the so-called hygienic effect (Struwe and Wennberg, 1983) of the low-exposure group (LE group, $n = 87$) was 0.10–0.86, and that of the high-exposure group (HE group, $n = 26$) was 1.14–2.29.

Table 1 presents the demographic characteristics of the study groups. They were all apparently healthy male workers and lived in the Seoul metropolitan area. No statistical differences were found between the three groups regarding age, alcohol consumption, and smoking. Mean education years for LE, HE, and the

TABLE 1
CHARACTERISTICS OF THE STUDY GROUP

Variable	Solvent-exposed group		Control	Total
	Low	High		
Number	87	26	81	194
Age, years	32.7 ± 6.0	33.8 ± 7.2	34.7 ± 8.6	33.7 ± 6.9
Education, years	10.3 ± 2.2	9.7 ± 2.7	12.9 ± 2.5	11.3 ± 2.8
Working duration, years	7.2 ± 5.9	9.6 ± 10.4	7.9 ± 6.0	7.8 ± 6.8
Alcohol consumption[a]	7.6 ± 9.8	6.9 ± 4.9	10.4 ± 10.2	8.7 ± 9.6
Smoking[b]	9.8 ± 8.6	7.5 ± 8.8	11.6 ± 33.1	10.2 ± 22.4

Note. Data are means ± SD.
[a] Absolute alcohol (liters)/year.
[b] Cigarettes/day.

control groups were 10.3 ± 2.2 (SD), 9.7 ± 2.7, and 12.9 ± 2.5, respectively, and the education year was significantly different among the study groups ($P < 0.001$).

Methods

Four tests from the WHO neurobehavioral core test battery (NCTB) were performed at the work site using the operational guide of WHO (1986): simple reaction time (SRT) was used to estimate attention and response speed, Santa Ana dexterity (preferred hand (SAp), left hand (SAl)) to estimate manual dexterity, digit symbol (DS) for the perceptual–motor speed test, and Benton visual retention (BVR) for the visual perception and memory test. For SRT, the subjects were presented with 64 visual stimuli for 6 min from a standard reaction time tester (Software Science, USA).

Statistical Methods

The relationship between the results of neurobehavioral tests and the level of exposure was examined by the analysis of variance (ANOVA) and Scheffe's test. The relationships between the neurobehavioral performances and age, duration of exposure, the level of education, and alcohol consumption were measured by correlation coefficients (Pearson's r). In the former analysis (ANOVA), the effect of age and the level of education, alcohol consumption, and smoking were controlled by the factorial analysis of variance using the general linear models procedure of SAS.

RESULTS

Group Comparison of the NCTB Test Results

The means and standard deviations of raw scores of the NCTB test performances for the three groups are shown in Table 2. Three test results—BVR, DS, and SAl—of six tests revealed significant differences among the three groups. Low- and high-exposure groups performed poorer than the control group in the tests of visual perception and memory ability (BVR) and perceptual–motor speed (DS). However, there was no statistically significant difference between HE and

TABLE 2
COMPARISON OF MEAN PERFORMANCES OF 113 SOLVENT-EXPOSED (LOW- AND HIGH-EXPOSURE GROUPS) AND 81 CONTROL GROUPS, RAW SCORES

Performance	Low	High	Control	$F(df)$	P
Simple reaction time					
Mean (SRTm), msec	252.8 ± 30.8	247.8 ± 30.0	256.5 ± 32.9	0.80(2,191)	0.427
SD (SRTs), msec	49.9 ± 34.7	50.0 ± 30.1	46.5 ± 16.8	1.30(2,191)	0.276
Benton visual retention (BVR)	7.2 ± 1.5	7.0 ± 1.5	8.1 ± 1.5	8.62(2,190)	0.000
Digit symbol	49.8 ± 12.6	51.3 ± 9.8	59.0 ± 13.6	11.50(2,190)	0.000
Santa Ana dexterity					
Preferred hand (SAp)	46.5 ± 5.4	45.9 ± 4.1	45.6 ± 5.1	0.60(2,188)	0.549
Left hand (SAl)	44.3 ± 5.8	41.5 ± 5.5	42.7 ± 5.4	3.29(2,187)	0.039

LE group in either tests. The HE group showed poorer performance on SAl than the LE group.

As shown in Table 3, age was negatively correlated with BVR and DS, while the education level was positively correlated. Poor performance in the SAp test was related to older age. DS was also negatively correlated with duration of work ($r = -0.256$, $P = 0.0064$; data are not shown).

Significant differences of BVR and DS among the three groups were sharply diminished after factorial ANOVA on age and on the level of education (Table 4). Nevertheless, the effect of exposure on BVR was still significant and the effect on DS was marginally significant. Therefore, age and the level of education appeared to be confounders as shown in Tables 3 and 4. The difference of SAl between LE and HE was insignificant after the effects of the confounders were controlled using factorial ANOVA.

Intercorrelations between Neurobehavioral Performances

There were several differences in the correlation matrices between the exposed and the control groups. Most correlation coefficients between performances of the control group were higher than the coefficients of the exposed group with the exception of manual dexterity. Mean SRT (SRTm) and DS were significantly correlated with most of the other performances in the control group. However, in the exposed group only standard deviation of SRT was correlated with SRTm. BVR was correlated only with DS and showed no correlations with the other tasks in both the exposed and the control groups (Table 5).

DISCUSSION

Psychiatric and neurological symptoms, such as fatigue, nervousness, headache, dizziness, nausea, and drunken feeling, have been reported to be the effects of solvent exposure (Lee *et al.*, 1988; Struwe and Wennberg, 1983; Fidler *et al.*, 1987). A wide range of neurobehavioral deficits were documented after long-term low-level exposure to organic solvents (Hänninen *et al.*, 1976; Elofsson *et al.*, 1980). Short-term visual memory and reaction times declined among Swedish house painters (Hane *et al.*, 1977) and Finnish painters who were exposed to low levels of solvent mixtures (Lindström and Wickström, 1983). Significantly poor performance among solvent-exposed workers was found only in the digit span

TABLE 3
PERFORMANCES SHOWING A SIGNIFICANT CORRELATION WITH INDEPENDENT VARIABLES OTHER THAN SOLVENT EXPOSURE

Performance	Variable	$r^a(n)$	P
BVR	Age	−0.174(190)	0.016
	Education	0.340(183)	0.000
DS	Age	−0.420(190)	0.000
	Education	0.635(183)	0.000
SAp	Age	−0.155(188)	0.034

[a] Pearson's r.

TABLE 4
RESULTS FROM FACTORIAL ANOVA FOR PERFORMANCES SHOWING A SIGNIFICANT DIFFERENCE BETWEEN THREE GROUPS

	Source of variation				
	Main effect of solvent exposure		Other variable		
Performance	*F*	*P*	Variable	*F*	*P*
BVR	3.15	0.045	Education	3.48	0.064
			Age	2.03	0.156
DS	2.62	0.075	Education	38.01	0.000
			Age	13.53	0.000
SAl	1.36	0.260	Education	1.290	0.258

after confounding variables were controlled (Maizlish *et al.*, 1985). Symbol digit substitution and digit span showed associations with solvent exposure (Fidler *et al.*, 1987).

In this study, only four tests among WHO NCTB were administered to the subjects because of limited time. The tests were administered during work hours. As mentioned earlier, there was a difference in the level of education between the exposed and the control groups due to difficulties in sampling the control group which was comprised of volunteers. We analyzed the data by factorial ANOVA to control this difference as well as other possible confounders.

Visual perception and memory ability and perceptual–motor speed declined in the exposed group in this study. The results of the Santa Ana dexterity test for preferred hand in the HE group were lower than those in the LE group. Only visual memory ability declined in the solvent-exposed group; however, the association between DS and solvent exposure was marginally significant after the effects of confounders were controlled. The solvent-exposed group was not exposed to lead or mercury. Therefore, it is presumable that there is an association between solvent exposure and decreased visual perception/memory. This result is

TABLE 5
CORRELATION MATRIX BETWEEN PERFORMANCES WITHIN THE EXPOSED (RIGHT UPPER) AND THE CONTROL (LEFT LOWER) GROUPS

	SRTm	SRTs	BVR	DS	SAp	SAl
SRTm	—	0.541***	−0.037	0.099	−0.108	−0.047
SRTs	0.685***	—	−0.011	−0.001	−0.085	−0.130
BVR	−0.005	−0.073	—	0.286**	0.127	0.051
DS	−0.362**	−0.255*	0.555***	—	0.388***	0.408***
SAp	−0.241*	−0.160	0.054	0.234*	—	0.727***
SAl	−0.260*	−0.115	−0.094	0.179	0.695***	—

Note. Data determined using Pearson's *r*.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

similar to the study result of Lindström and Wickström (1983) who found short-term visual memory decline among painters exposed to low levels of solvent mixtures. A significant association of the solvent exposure with simple reaction times was not found in this study.

However, because we were not able to evaluate the effects of the job type of the subjects and preexposure intellectual level of the exposed group (Lindström and Wickström, 1983), it is difficult to be conclusive. Precise measurements of the personal solvent exposure level would also be required. Hence, we are planning a further investigation in a forthcoming study on this topic.

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Evaluation of Brain Function in Acute Carbon Monoxide Poisoning with Multimodality Evoked Potentials[1]

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The median nerve somatosensory evoked potentials (SEP), pattern reversal visual evoked potentials (VEP), and brain stem auditory evoked potentials (BAEP) were studied in 109 healthy adults and in 88 patients with acute carbon monoxide (CO) poisoning. The upper limits for normal values of peak and interpeak latencies of multimodalities of evoked potentials in the reference group were established by a stepwise multiple regression analysis. SEP changes selectively affecting N32 and N60 were found in 78.8% of patients. There was prolonged P100 latency of VEP in 58.2% of the cases examined. The prevalence of BAEP abnormalities in comatose patients (36%) was significantly higher than that (8.6%) in conscious patients. BAEP abnormalities were most frequently seen in comatose patients who had diminished brain stem reflexes (77.8%). It has been found that a consistent abnormality involving N20 and subsequent peaks in SEP, a remarkable prolongation of P100 latency in VEP, or a prolongation of III–V interpeak latency in BAEP as well as the reoccurrence of evoked potential abnormalities after initial recovery all indicate unfavorable outcomes in patients with acute CO poisoning. The multimodality evoked potentials have proved to be sensitive indicators in the evaluation of brain dysfunction and in the prediction of prognosis of acute CO poisoning and the development of delayed encephalopathy.

INTRODUCTION

The annual rates of morbidity and mortality from acute carbon monoxide (CO) poisoning are the highest among all acute occupational poisonings in China (Li, 1989). Acute carbon monoxide poisoning is also a common life-threatening disorder in inhabitants of north China because of indoor coal fire used for heating in winter. About 11% of the acute CO poisoning patients admitted to hospitals have developed delayed encephalopathy after a period of "pseudorecovery" lasting for 2–60 days which usually manifests as mental disorders, loss of intelligence, and extrapyramidal signs. Its occurrence and prognosis are not predictable and the pathogenesis remains to be solved (Choi, 1983; He, 1984; Thom and Keim, 1989).

Recent advances in brain evoked potentials (EPs) have led to their widespread use as an adjunct to neurological examinations in many brain diseases. It has been shown that EPs can provide accurate and objective data on sensory system function and are nontraumatic, reproducible, and more sensitive than clinical evalu-

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ation (Anderson *et al.*, 1984; Arrezo *et al.*, 1985; Chiappa, 1983; Owen and Davis, 1985). However, few data on the brain evoked potentials in acute CO poisoning have been reported.

In this study, the median nerve somatosensory evoked potentials (SEP), visual evoked potentials (VEP), and brain stem auditory evoked potentials (BAEP) were studied and followed up in 88 patients with acute CO poisoning including those with delayed encephalopathy. The results were compared with those of 109 healthy adults and used to detect possible alterations of brain function related to the somatosensory, visual, and auditory pathways and to predict the prognosis of acute CO poisoning and the occurrence of delayed encephalopathy.

SUBJECTS AND METHODS

Subjects

Reference group. A total of 109 healthy adults, 54 males and 45 females, with ages ranging between 20 and 77 years (mean, 43.6 years) were selected. Neurological, hepatic, and renal diseases, as well as diabetes, alcoholism, and malnutrition, had been excluded in all referents.

Patient group. A total of 88 patients with acute CO poisoning, 51 males and 37 females, were selected from a hyperbaric oxygen treatment center in Beijing. Their ages ranged between 18 and 72 years and the average age was 44.9 years. In this group, 53 cases were admitted at the acute stage of CO poisoning (subgroup A) and 35 patients showed delayed encephalopathy upon admission (subgroup B). They all received hyperbaric oxygen and supportive treatments during hospitalization.

Methods

Evoked potential recording. The multimodality EPs were studied with Dantec 2000c Neuromatic equipment for each subject in the reference group. Follow-up studies of the three modalities of EP were conducted in the patient group upon admission, at the partial recovery stage, and at the full recovery stage at an interval of 2 months on average (1–12 months). A total of 85 patients underwent SEP studies, 84 BAEPs, and 60 VEPs.

Median nerve SEPs were obtained after stimulation of the median nerve at the wrist with a square-wave pulse of 0.2 msec duration, strong enough to produce a moderate thumb twitch (8–12 mA), and delivered at a rate of 3/sec. The sweep time and bandwidth were set at 100 msec and 20–2000 Hz, respectively. Left and right median nerves in each subject were tested separately with skin temperature in the hands ranging between 30 and 34°C. The recording electrodes were placed over each Erb's point, the seventh cervical (C7) vertebra, and the scalp at bilateral C3′, C4′ (2 cm posterior to the C3 and C4 according to the conventional "10–20" system in EEG), with the reference electrode at the midforehead (Fpz). Two hundred to five hundred responses per trial were averaged and duplicate trials were conducted to assure reproducibility.

The BAEPs were recorded at the same time as the SEP testing and in response to repeated click stimuli of alternating rarefaction and condensation phases de-

livered monoaurally by an earphone of 20 L 01 type. The click intensity was adjusted to 65 dB above the click hearing threshold. The contralateral ear was masked with white noise at 65 dB. Recording electrodes were placed over the lobe of the stimulated ear and on the top of the head (Cz). The ground electrode was placed over the forehead. The sweep time and bandwidth were set at 10 msec and 100–2000 Hz, respectively. Two thousand clicks were averaged per trial and reproducibility was assured by duplicate trials.

The VEPs were elicited in response to stimuli of a black and white reversal pattern with a check size of 3.1 cm at a frequency of 1.0/sec from a DISA 25000 television screen with a viewing distance of 1.5 m. The stimulus luminance for white squares was 255.5 cd/m^2 and for black squares 42.7 cd/m^2. The subjects were asked to keep their sight fixed on the central target throughout the period of recording for each eye. Recording electrodes were placed over the midoccipital (O_z), left-occipital (O_1), and right-occipital (O_2) scalp. The sweep time and bandwidth were set at 300 msec and 2–1000 Hz, respectively. The reference electrode was attached to the midforehead (Fpz). The impedance was kept at less than 5 kOhm. Sixty to one hundred responses were averaged per trial and duplicate trials were carried out.

Statistics for evoked potentials studies. The peak and interpeak latencies of the three modalities of EP, the difference between the left and right, as well as the peak-to-peak amplitudes of SEP and VEP were measured. The results were statistically evaluated by SYSTAT statistical software on an IBM PC AT computer. The evoked potentials of the reference group were analyzed by utilizing a stepwise multiple regression analysis to obtain a regression equation with 2.5 residual standard deviation and to establish the upper limits of the normal values of variables of each modality in healthy adults. The evoked potentials of the patient group were interpreted by comparison with the reference group.

CT Scanning. Twenty-one cases in subgroup A and 16 cases in subgroup B had cranial CT scanning at the time of the evoked potential study. CT scans were obtained using a scanner in standardized 5-mm cuts above the orbitomeatal line.

RESULTS

Clinical Status of the Patient Group

The consciousness status of patients in subgroup A upon admission is shown in Table 1. The duration of coma in comatose patients was more than 4 hr in all but 6 cases, with a mean of 26.7 hr. In subgroup A, 8 cases (15%) developed delayed encephalopathy after a pseudorecovery period (2–35 days), and 5 comatose cases died. The remaining 40 cases (75.5%) recovered satisfactorily.

Subgroup B consisted of 35 patients with delayed encephalopathy who had regained consciousness from coma during acute stage of CO poisoning and then developed mental disorder, impairments of intelligence, and extrapyramidal signs after a pseudorecovery period from 2 to 35 days (mean, 21 days). Among the 35 cases in subgroup B, 25 cases (71.4%) also recovered, but 5 cases (14.3%) died and 5 cases remained unchanged during hospitalization.

TABLE 1
CONSCIOUSNESS STATUS OF 53 CASES WITH ACUTE CO POISONING (SUBGROUP A) ON ADMISSION

	Consciousness status					
	Normal	Impaired				
		Mild[a]	Moderate[b]	Severe		
				Slight coma[c]	Moderate coma[d]	Deep coma[e]
	(n = 12)	(n = 13)	(n = 1)	(n = 16)	(n = 9)	(n = 2)
%	22.6	24.5	1.9	30.1	17.0	3.7

[a] Including cloudiness, somnolent status, twilight state.
[b] Including confusion or delirium state.
[c] Brain stem reflexes and tendon reflexes exist. Scored 6–7 on Glasgow coma scale (Robin *et al.*, 1986).
[d] Brain stem and tendon reflexes are slugglish. Scored 4–5 on Glasgow coma scale.
[e] Brain stem and tendon reflexes disappear. Scored 3 or less on Glasgow coma scale.

Six of the 21 patients in subgroup A (28.5%) and 11 of the 16 cases in subgroup B (68.7%) showed abnormalities in CT scanning. Decreased density in bilateral subcortical white matter and globus pallidum was the prominent finding shown by CT and was seen mainly in patients who had coma persisting for 3 days in an acute stage of CO poisoning or after developing delayed encephalopathy for at least 2 weeks.

SEPs

Reference group. The negative waves N9 and N13 were recorded at the Erb's point and C7 vertebra, respectively. The "w" configuration of the SEP, consisting of N20, P25, N32, P40, and N60 in sequence, was recorded over the C3′ and C4′ and, except for the last wave (N60), which emerged in 94.5% (102/108) of the healthy adults, was recorded in all subjects (Fig. 1). All peak and interpeak latencies were significantly related to age, sex, and height. Therefore these variables were adjusted by a stepwise multiple regression analysis. The regression equation and 2.5 SD of the residual standard deviation were chosen to establish the upper limits of normal values of SEP parameters (Table 2).

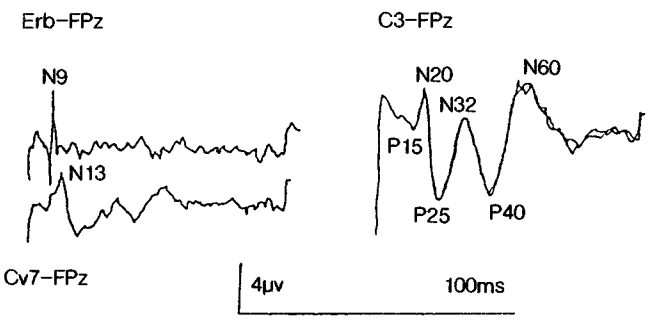

FIG. 1. Normal SEPs elicited by stimulating the median nerve and recorded at Erb's point (N9), C7 (N13), and over the contralateral scalp.

TABLE 2
UPPER LIMITS OF SEP LATENCY IN HEALTHY ADULTS (n = 108)

Peak latencies (msec)	
N9	$= 0.018X + 0.050Z - 0.202Y + 0.117 + 1.24$
N13	$= 0.029X + 0.064Z - 0.246Y + 0.698 + 1.65$
N20	$= 0.047X + 0.059Z - 0.504Y + 7.087 + 1.92$
N25	$= 0.069X + 0.055Z + 12.440 + 4.18$
N32	$= 0.044X + 0.065Z + 19.202 + 7.14$
P40	$= 0.065X + 0.067Z + 25.554 + 8.41$
N60	$= 0.140X + 0.097Z + 30.061 + 12.74$
Interpeak latencies (msec)	
N9–13	$= 0.011X + 0.016Z + 0.023 + 1.56$
N13–20	$= 0.018X + 0.026Z + 1.115 + 1.64$
N9–20	$= 0.031X + 0.033Z + 2.769 + 1.66$
N20–32	= 19.46*
N32–60	$= 0.093X + 16.26 + 10.86$
N20–60	$= 0.089X + 29.66 + 12.67$

Note. Equations are derived from the formula $L = b_1 X + b_2 Z - b_3 Y + a + c$, where L = latency (ms); b_1, b_2, and b_3 = regression coefficients; X = age (years); Y = sex (male = 0, female = 1); Z = height (cm); a = intercept; c = 2.5 SD of the residual standard deviation.

* Mean ± 2.5 SD.

Patient group. A comparison of the SEP parameters between the reference group and the patient group either at acute stage or at recovery stage was made by analysis of variance (ANOVA). There was no difference in the short-latency components of SEP including N9, N13, N20. However, the peak and interpeak latencies of long-latency SEPs (N32, N60, N32–60, N20–N60) in the patient group at the acute stage were significantly longer than those of the reference group, but these variables were delayed to a lesser extent in the patient group during the recovery stage (Table 3). There was no difference in amplitudes of SEP between the reference group and the patient group by Student's t test.

In follow-up studies, the peak latencies of N9 and N13 in all patients were consistently normal. The prevalences of short-latency SEP (N20, N9–20, and their right–left difference) abnormalities in subgroup A and subgroup B were 9.4% (5/53) and 3.1% (1/32), respectively. However, the prevalence of long-latency SEP abnormalities (prolongation of latency or abnormal configuration of N32, N60) was 79.2% (42/53) in subgroup A and 78.1% (25/32) in subgroup B and then decreased to 10% at recovery stage for both subgroups, indicating a correlation between the SEP changes and the clinical course. There were 2 patients in subgroup A who showed abnormal N32 and N60 again after their initial recovery just 3 days prior to the onset of delayed encephalopathy (Fig. 2). The results of follow-up SEP studies provided valuable information for predicting the occurrence of delayed encephalopathy.

The SEP abnormalities in the patient group were also found to be correlated with the clinical severity of CO poisoning. There were three types of SEP changes. Type I involved only N60 and was observed mainly in patients with acute carbon monoxide poisoning with mild or moderate disturbance of consciousness prior to coma. Type II showed abnormal N32 and N60 and was usually

TABLE 3
COMPARISON OF PEAK AND INTERPEAK LATENCIES (msec) OF SEP BETWEEN THE REFERENCE GROUP AND THE PATIENT GROUP

Median nerve SEPs	Reference group			Patient group					
				Acute stage			Recovery stage		
	n	Mean	±SD	*n*	Mean	±SD	*n*	Mean	±SD
Right									
N32	108	32.0	3.1	75	35.6*	5.2	60	32.7	2.3
N60	102	52.5	6.0	60	68.8*	12.3	60	56.8**	5.5
N32–N60	102	20.5	4.8	58	34.7*	11.1	59	24.0**	5.6
N20–N60	102	33.8	5.6	60	50.3*	12.3	59	38.0**	5.6
Left									
N32	108	31.7	2.8	74	35.0*	4.9	59	32.4	2.2
N60	104	52.6	6.3	58	66.0*	12.0	59	57.0**	7.1
N32–N60	104	20.9	2.6	56	32.3*	11.3	59	24.6**	6.8
N20–N60	104	34.2	5.9	56	47.3*	11.9	59	38.2**	7.1

* $P < 0.05$, compared with referents by ANOVA test.
** $P < 0.05$, compared with referents and the patients at recovery stage by ANOVA test.

seen in patients in subgroup A with slight and moderate coma and in patients in subgroup B with intelligence loss. Type III consisted of abnormal N20, N32, and N60 and was found only in patients with moderate or deep coma. The prognosis for patients with different types of abnormal SEPs also varied significantly, the worst being for type III and the best being for type I. Thirty-four out of 37 patients with type I SEP changes recovered fully. In 24 patients with type II SEP changes, 2 died, 6 had partial recovery, and 16 recovered. All 6 cases with type III SEP abnormalities had moderate coma: 4 of them were fatal, 1 retained severe residual mental disorders, and 1 developed hemiparesis.

BAEPs

Reference group. Five waves of BAEP were recorded over the vertex (Fig. 3C). The latencies of peak I, III, V and their interpeaks were closely related to age and sex. Hence the upper limits of normal values for peak and interpeak latencies of BAEP were established by a stepwise multiple regression analysis (Table 4) similar to that for SEP.

Patient group. BAEP changes in the patient group were significantly correlated with loss of consciousness. It was found that the III–V interpeak latency in the comatose patients was remarkably longer than that in the reference group and that in the conscious patients in either the acute stage or the recovery stage (Table 5). In the meantime, the prevalence of BAEP abnormality in the comatose patients was the highest (36%, 9/25), followed by that in the conscious patients in subgroup B (8.6%, 3/35), and that in the conscious patients in subgroup A (8.3%, 2/24) ($P < 0.05$ by χ^2 test). The BAEP abnormalities in the clinically recovered patients of both subgroups decreased to 2.5% (1/40).

Nine of 25 patients in moderate or deep coma had abnormal brain stem reflexes; i.e., their orbital, corneal, and pupillary reflexes were sluggish or absent. Of those

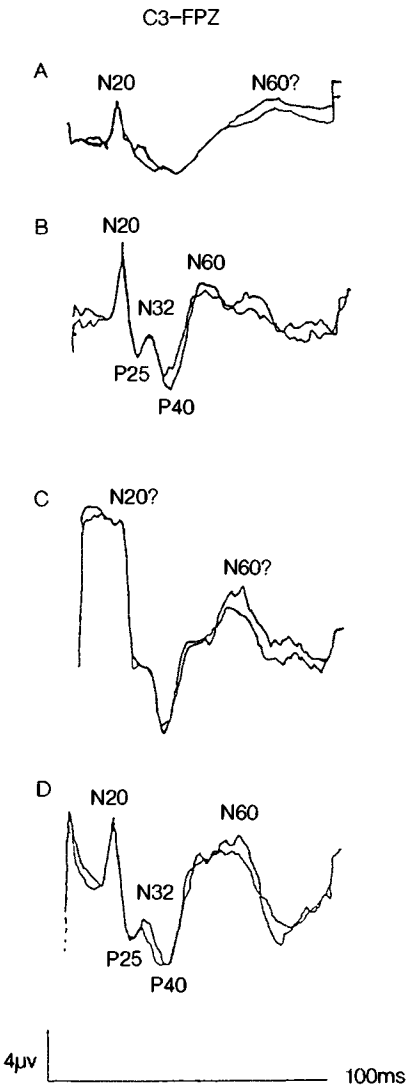

FIG. 2. A series of SEP recordings in a female patient aged 64 with acute CO poisoning. (A) December 24, 1987, acute CO poisoning with coma: Normal N20, N32 absent, delayed N60. (B) December 25, 1987, patient revived for 12 hr after 10 hr of coma: SEPs recovered to normal. (C) January 7, 1988, 3 days prior to the development of delayed encephalopathy after 16 days of "pseudorecovery": All components of SEP changed. (D) March 14, 1988, SEPs recovered again at 2 months of patient's recovery from delayed encephalopathy.

showing abnormal BAEPs, 7 of the 9 (77.8%) were in a persistently comatose state for 12–148 hr (mean, 34 hr) and 4 progressed to death. On the other hand, only 2 of 16 comatose patients (12.5%) with normal brain stem reflexes had abnormal BAEP. The difference was significant ($P < 0.05$ by χ^2 test). This strongly suggests that an abnormal BAEP in comatose patients usually indicates an unfavorable prognosis, particularly in those having abnormal brain stem reflexes.

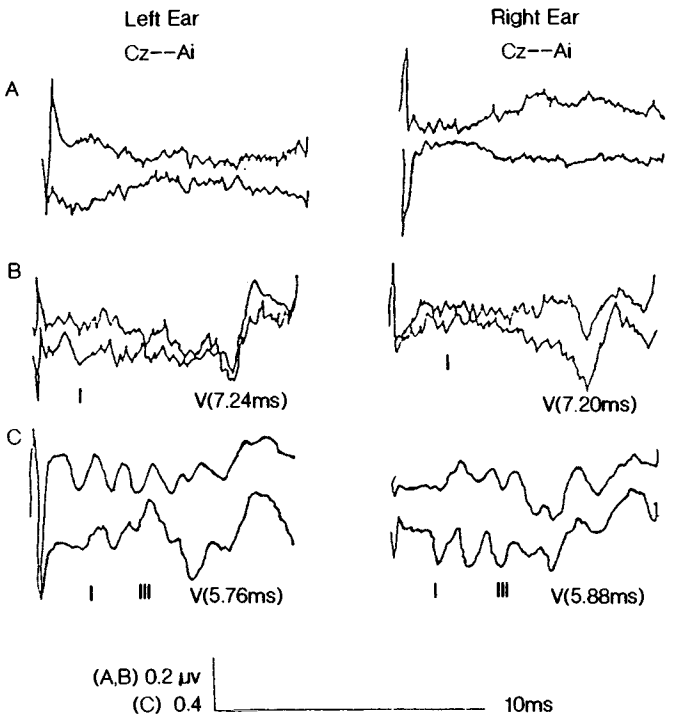

FIG. 3. A series of BAEP recordings in a patient with acute CO poisoning (male, aged 30). (A) November 9, 1987, BAEP disappeared on the first day of acute CO poisoning with moderate coma. (B) November 13, 1987, a delayed I–V interval appeared 3 days later while patient was no longer comatose but in confusion. (C) December 2, 1987, BAEP became normal upon clinical recovery 2 weeks later.

VEPs

Reference group. A positive wave with a latency of about 100 msec was recorded over the occipital scalp (O_1, O_2 and O_z) in 105 healthy adults (Fig. 4C). The latency of P100 was found to be significantly related to sex and age, and the P100 latency of the right eye was longer than that of the left eye. Therefore, the upper limits of normal values of P100 latency of both eyes were established by a step-

TABLE 4

UPPER LIMITS OF BAEP PEAK AND INTERPEAK LATENCY (msec) IN HEALTHY ADULTS (n = 105)

I[a]	= 2.05 (male) or 1.97 (female)
III[a]	= 4.46 (male) or 4.16 (female)
V[b]	= $0.004X - 0.163Y + 5.665 + 0.48$
I–III[b]	= $0.003X - 0.007Y + 2.076 + 0.43$
III–V[b]	= $0.002X + 1.835 + 0.41$
I–V[b]	= $0.005X - 0.106Y + 3.293 + 0.49$

[a] Mean + 2.5 SD.

[b] Equations are derived from the formula $L = b_1 X - b_2 Y + a + c$, where L = interpeak latency; b_1 and b_2 = regression coefficients; X = age in years; Y = sex (male = 0, female = 1); a = intercept; c = 2.5 SD of the residual standard deviation.

TABLE 5
COMPARISON OF INTERPEAK LATENCIES OF BAEP (msec) BETWEEN THE REFERENCE GROUP AND THE PATIENT GROUP

			Patients with CO poisoning					
	Referents (n = 109)		Conscious (n = 59)		Comatose[a] (n = 25)		Recovered (n = 40)	
BAEP	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Left ear								
I–III	2.17	0.18	2.12	0.19	2.15	0.24	2.15	0.18
III–V	1.92	0.17	1.99	0.19	2.05*	0.36	1.97	0.18
I–V	4.09	0.22	4.11	0.27	4.20	0.48	4.11	0.25
Right ear								
I–III	2.22	0.20	2.14	0.20	2.17	0.27	2.18	0.17
III–V	1.91	0.17	1.92	0.18	2.07*	0.32	1.92	0.16
I–V	4.12	0.21	4.06	0.22	4.24	0.35	4.10	0.19

[a] Excluding four cases of moderate or deep coma with absence of BAEP.
* $P < 0.05$ by ANOVA test.

wise multiple regression analysis and the following equations were derived from the formula $P100 = b_1 X - b_2 Y + a + c$, where X = age in years; Y = sex (male = 0, female = 1), b_1, b_2 = regression coefficients, a = intercept, c = 2.5 SD of the residual standard deviation:

$$\text{Right P100} = 0.151X - 3.4Y + 88.5 + 12.8$$
$$\text{Left P100} = 0.132X - 4.2Y + 88.9 + 13.2.$$

The difference in the latency of P100 between the two eyes was not related to age and sex. Its upper limit of normal value, 8 msec, was determined by mean + 3 SD.

Patient group. Thirty-two cases in subgroup A and 28 cases in subgroup B were selected for VEP testing because they were able to keep their sight fixed on the central target. The results showed that the mean latency of P100 was prolonged in subgroup A at acute stage (50%, 16/32) and in subgroup B at the onset of delayed encephalopathy (67.9%, 19/28), which was longer than that at the recovery stage and that of the referents (Table 6). The prolongation of P100 latency then decreased to 5% (1/20) and 22.2% (4/18), respectively in subgroups A and B at the recovery stage.

The follow-up studies also showed that the VEP changes were correlated with the patients' clinical situation and prognosis. The P100 latency, which is longer than 150 msec, or delayed over 10 msec in comparison with the previous recordings, or consistently abnormal, usually indicates a poor prognosis in patients with delayed encephalopathy. On the contrary, 14 patients with delayed encephalopathy whose P100 latency was at least 10 msec shorter than their previous recordings, irrespective of normal or abnormal, showed favorable prognosis which was well correlated with the clinical recovery of their dementia syndrome. There was a patient in subgroup A who revived from coma and was apparently normal, having

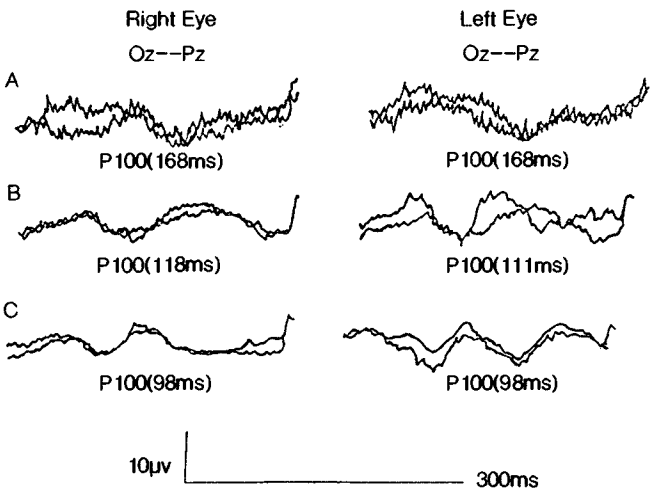

FIG. 4. A series of VEP recordings in a 72-year-old male patient having delayed encephalopathy that developed 25 days after acute CO poisoning. (A) January 4, 1988, a month after the development of delayed encephalopathy, very prolonged P100 latency. (B) January 29, 1988, a shorter P100 latency than that recorded in (A). (C) March 11, 1988, normal VEP appeared as patient recovered remarkably.

a reoccurrence of a remarkable prolongation of P100 just 2 days prior to the onset of delayed encephalopathy. Such types of VEP changes in the pseudorecovery period of acute CO poisoning are a strong predictor of the occurrence of delayed encephalopathy.

DISCUSSION

The three modalities of evoked potentials, SEP, VEP, and BAEP, allow the exploration of anatomical condition and functional status of somatosensory, visual, and auditory pathways, respectively. On the other hand, they share many properties and tend to be similarly affected in several disorders that affect the central nervous system diffusely, such as coma and toxic–metabolic conditions

TABLE 6
COMPARISON OF P100 LATENCY (msec) BETWEEN THE REFERENCE GROUP AND THE PATIENT GROUP

P100 latency	Reference group (n = 105)		Patient group: Acute stage (n = 60)		Patient group: Recovery stage (n = 30)	
	Mean	±SD	Mean	±SD	Mean	±SD
Right eye	92.5	4.5	108.3*	21.0	96.6**	5.6
Left eye	93.1	5.9	110.1*	25.5	97.2**	5.4
Difference between two eyes	0.64	2.2	2.25	5.3	0.55	3.6

* $P < 0.05$ compared with referents and the patients at recovery stage by ANOVA test.
** $P < 0.05$ compared with referents by ANOVA test.

(Anderson *et al.*, 1984; Facco *et al.*, 1985; Lai, 1985). A number of studies have evaluated the use of single or multimodality evoked potentials and the results indicate that the combined use of more than one modality shows a higher diagnostic and prognostic power than a single modality in the assessment of brain dysfunction (Owen and Davis, 1985).

Carbon monoxide has an affinity for hemoglobin 200–250 times that of oxygen, and the symptoms and signs that follow inhalation of carbon monoxide have been hypothesized to result from tissue hypoxia. Since the central nervous system has been found to be the most vulnerable organ to hypoxia, the severity of acute CO poisoning usually correlates well to the severity of cerebral hypoxia which can be clinically evaluated by the severity of impairments of consciousness (Choi, 1983; He, 1984; Thom and Keim, 1989).

In this study, the evoked potentials have been applied in the assessment of brain dysfunction and in the prediction of prognosis for patients with acute CO poisoning and delayed encephalopathy. A comparison of multimodality evoked potentials between the patient group and a reference group consisting of 109 healthy adults was made. Based on the results of this study, the following points under discussion merit attention.

Determination of Normal Limits of EPs

Many factors have significant effects on EP latencies and amplitudes (Chiappa, 1983; Owen and Davis, 1985). Therefore, the methodology for the determination of the normative data of EPs is of paramount importance. To avoid the effects of the testing technique, the same parameters should be used with the patients as have been used with the normal subjects.

However, EPs in healthy adults have been found to be related to age, sex, and height by previous investigations (Allison *et al.*, 1983) and by the present study. In order to adjust the combined effects from the three physiological indices (age, sex, and height) and avoid statistical bias, a stepwise regression analysis and multiple regression equations with 2.5 SD of the residual standard deviation were chosen to establish the upper limits of normal values of peak and interpeak latencies. Results of EP studies on patients with acute CO poisoning in terms of age, sex, and height were brought into the equations individually to determine whether a patient's test results fall within the range of normal. The results of this study have proved that these equations are highly reliable and sensitive. This allows the application of EPs not only for provision of a quantitative assessment of human central sensory function, but also for use in individual diagnosis without the confounding factors of age, sex, and height.

Assessment of the Severity of CO Poisoning

In this study, the long-latency components of SEP (N32 and N60) were selectively impaired in patients with acute CO poisoning and delayed encephalopathy. The three types of SEP changes, namely, type I (involving only N60), type II (affecting both N32 and N60), and type III (affecting N20 and the subsequent peaks) in sequence, were well correlated with the severity of impairment of consciousness from mild to severe. These findings are similar to the three types of

SEP changes in various cerebral lesions described by Yamada *et al.* (1985). We also found that the III–V interpeak of BAEP in comatose patients with acute CO poisoning was longer than that in conscious patients. In patients with moderate or deep coma whose brain stem reflexes were impaired, the prevalence of BAEP abnormalities (77.8%) was significantly higher than that (12.5%) in patients with slight coma and intact brain stem reflexes. The VEP was also shown to have a prolonged latency in 67.9% of the patients with delayed encephalopathy with dementia and then recovered along with the clinical improvement of mental syndrome.

All these data indicate that evoked potentials, particularly SEP and BAEP, are useful in monitoring patients of acute CO poisoning with consciousness disturbance. VEP and SEP are valuable in evaluating the severity of delayed encephalopathy.

Prediction of Prognosis

The results of this study have shown that the outcome of patients with SEP abnormalities of type III is the worst, that of type II is better, and that of type I is the best. The follow-up studies on BAEP showed that almost all the comatose patients with a normal BAEP revived within 6 hr and those having abnormal BAEP usually regained their consciousness later than 12 hr and tended to have higher mortality. The P100 latency of VEP in patients with delayed encephalopathy being 150 msec longer, delayed over 10 msec longer, than the previous recordings, or consistently abnormal indicates a poor prognosis exclusively. On the other hand, favorable outcome was seen in patients with delayed encephalopathy whose P100 latency was at least 10 msec shorter than their previous recordings, irrespective of normal or abnormal. The results indicate that remarkable changes in the three modalities of EP in follow-up studies are of importance in the prediction of prognosis of acute CO poisoning.

The occurrence of delayed encephalopathy has long been thought to be unpredictable. In our follow-up studies on patients with CO poisoning at the acute stage, the SEP or VEP in four patients showed recovery of initial abnormalities after regaining consciousness; however, they became abnormal again just 3 days prior to the onset of delayed encephalopathy. This strongly suggests that both SEP and VEP merit follow-up use in the acute stage of CO poisoning for the prediction of occurrence of delayed encephalopathy.

Pathological Implications

The selective damage to bilateral N32 and N60 of SEP without impairment of short-latency components (N9, N13, N20) in patients with acute CO poisoning with disturbance of consciousness conforms with a diffuse cerebral cortical dysfunction and suggests that the long-latency components of SEP originate from the cerebral cortices (Chiappa, 1983; Owen and Davis, 1985; Yamada *et al.*, 1985). Four out of 25 comatose patients whose brain stem reflexes were sluggish or absent showed abnormalities not only in long-latency SEPs, but also in short-

latency SEPs as well as in BAEPs, suggesting that not only the cerebral cortices but also the brain stem was involved when cerebral hypoxia aggravated in these cases.

Six patients who showed an abnormal N20 of SEP were found to have subcortical lesions confirmed by CT scanning. In patients with delayed encephalopathy following acute CO poisoning, our findings using CT scanning are identical to the neuropathological characteristics which were reported to be extensive demyelination in subcortical white matter and softening of bilateral globus pallodum (Lapresle and Fardeau, 1967). The facts that the prevalence of both SEP and VEP abnormalities was significantly high and that there was prolongation of P100 latency without changes of configuration and amplitudes of VEP support the existence of demyelination in the subcortical portion of somatosensory and visual pathways.

Combined Use of Multimodality EPs

It has been found that each modality of evoked potentials, SEP, VEP, or BAEP, has its diagnostic and prognostic accuracy in the assessment of severity of CO poisoning and the prediction of clinical outcome. In patients with milder disturbance of consciousness examined by SEP and BAEP simultaneously, the prevalence of abnormalities of SEP was found to be much higher than that of BAEP. The prevalence of abnormal BAEP increased only in patients with moderate and deep coma with sluggish brain stem reflexes. It can be postulated that the cerebral cortex is more vulnerable to CO poisoning than the brain stem; hence, the SEP is more sensitive than the BAEP. However, the BAEP changes are more persistent and reliable in severe patients. BAEP in combination with SEP appears to be more valuable in the assessment of severity of consciousness impairment in acute CO poisoning.

In 27 cases of delayed encephalopathy simultaneously examined by both SEP and VEP, the prevalence of abnormality of SEP and VEP was identically high, being 74.07% and 70.37%, respectively. The recovery of their SEP and VEP was correlated with improvement of dementing syndrome. VEP changes seemed to be more persistent than SEP changes in partially recovered patients. In addition, VEP is more reliable as its variation in normal referents is minimal. It is strongly suggested that both SEP and VEP should be employed to yield the best prognostic power in patients to determine delayed encephalopathy with intelligence impairments.

CONCLUSIONS

Brain evoked potentials have provided us with valuable diagnostic and prognostic information for monitoring patients with consciousness disturbance and for predicting the clinical outcome of acute CO poisoning and the development of delayed encephalopathy. Combined use of multimodality evoked potentials should be encouraged. Information from EPs must be integrated with that from clinical history, physical examinations, CT scanning, and other laboratory tests in order to increase the accuracy in the evaluation of brain function and in the diagnosis and prognosis of acute CO poisoning.

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Acute and Chronic Neurological Symptoms among Paint Workers Exposed to Mixtures of Organic Solvents[1]

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The purpose of this study was to determine the prevalence rates of acute and chronic neurologic symptoms among paint workers and the association of such symptoms with the severity of exposure to mixtures of solvents. Two paint manufacturing factories and 25 various kinds of spray painting factories were selected for study. Air concentrations of organic solvents were measured by personal samplers and were analyzed by gas chromatography. A total of 196 workers were given a screening neurological examination and a questionnaire on acute and chronic neurologic symptoms. A detailed personal medical history and a profile on alcohol consumption and medication were also included. The results showed that xylenes and toluene were the major solvents found in almost all the air samples with average contents of 50 and 24% on a weight basis of 73 air samples. We classified workers according to different exposure patterns and different air concentrations of breathing zones: high (8-hr hygienic effect, 0.25–9.86; median, 1.66), short-term high (hygienic effect, 0–3.38; median, 0.12), and low (hygienic effect, 0–0.38; median, 0.12). All workers showed no overt neurological signs such as ataxic gait, poor coordination, or muscle weakness. After excluding those workers who consumed more than 280 g of alcohol per week (n = 8), took antihypertensive medications (n = 4), or were treated with antipsychotic agents (n = 1), we found that the severity of exposure was associated with acute symptoms of headache and chest tightness and chronic symptoms of dizziness, easy fatigability, depressed mood, and palpitation. There was no association between peripheral neurological symptoms and the severity of exposure. Workers in the high exposure group were 2.7 times more likely to develop two or more acute symptoms and 3.3 times more likely to develop three or more chronic symptoms of the central nervous system than the low exposure group. After modeling by multiple logistic regression, we concluded that exposure to a medium level of mixtures of solvents (hygienic effect exceeding 1.66) may produce acute and chronic central neurological symptoms.

INTRODUCTION

Organic solvents have been widely used in various industrial processes since the middle of the last century, and their neurotoxic effects were recognized early (Frost *et al.*, 1885). Although neurological symptoms are usually regarded as the earliest health effect from solvent exposure (Axelson and Hogstedt, 1988), they are very nonspecific and can be confounded by medication, alcohol consumption, smoking, psychological stress, etc. (Triebig *et al.*, 1988; Johnson, 1987; Estrin and Parry, 1990). Differences in case definition, methods of exposure assessment,

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study design, and strategy of analysis further complicated the interpretation of a causal association between subjective symptoms and solvent exposure. Thus, it is not surprising that subjective symptoms alone have not been regarded as a general tool for the early recognition of solvent hazards. Although neurobehavioral tests may be a more objective tool for detecting the health effects of solvent exposure, they also suffer from the similar drawback of being nonspecific (Rosenberg, 1990).

As questionnaires of subjective symptoms are very inexpensive and convenient to administer at the workplace, it may be worthwhile to develop a standardized questionnaire which, in combination with good epidemiological design and analysis, may be useful for the early detection of solvent neurotoxicity.

Because exhaust ventilation is generally not appropriately designed or installed in Taiwan, we are concerned that workers exposed to mixtures of solvents might suffer from neurotoxicity in the workplace. The purpose of this study is to determine the prevalence rate of abnormal neurological symptoms among paint workers and the relationship between such symptoms and exposure to solvents. In addition, to falsify this causal and dose–response relationship, we try to control possible confounding by various factors. In order to achieve this goal, we have enrolled a reference group with the same socioeconomic status, education, and life-style.

MATERIALS AND METHODS

In Taipei city, there were 2 factories of paint manufacturing, 3 factories of video terminal painting, 2 factories of aircraft painting, 1 factory of trailer spray painting, 2 factories of model spray painting, and 1 painting department of a car assembly factory. All workers who had been working in these factories for over 1 year were included in our study. There were over 200 car-painting factories in Taipei city from which a random sample of 16 of them were selected for our study (through random numbers). Thus, a total of 196 workers were enrolled in our study.

We took a walk-through survey for each factory before we performed air sampling to determine exposure zones (Corn and Esmen, 1979). Air concentrations of organic solvents were measured by personal samplers and later analyzed by gas chromatography (GC) with a Perkin–Elmer Sigma 3B model and a flame ionization detector. The GC analytic conditions were as follows: the column, a Mega Bore OBWAX-30m with film thickness 1.0 μm, was programmed from 40 to 100°C with an incremental rise of 10°C/10 min. The injection temperature was 150°C, while the detector temperature was 200°C. The elevated nitrogen gas flow was set at 8 ml/min. Under this condition, we were unable to separate all isomers of xylenes, but were successful in distinguishing the major contaminants which were listed in Table 1. We took two to four air samplers at each factory according to the number of exposure zones. Because some factories were relatively small, only two exposure zones (e.g., paint spraying and filling and polishing) can be identified among them. Seventy-three air samples were collected, each representing at least 4 hr of continuous exposure during a complete workday. During the walk-through survey we found that car painters had the most serious exposure when working in a poorly ventilated painting booth; they must spend 0.5–1.5 hr in the

painting booth every workday. Because the fluctuation of solvent exposure should be taken into account, we took 9 additional air samples which were randomly chosen from the above 16 car painting factories. All of the 9 air samples were collected from workers' breathing zones when car painters were actually working in the painting booth.

All the workers were given a subjective symptoms questionnaire, a comprehensive physical examination, and a liver function test. The subjective symptoms included in our questionnaire were translated and modified from Hogstedt *et al.* (1984). They include acute central nervous system (CNS) symptoms experienced during the workday, chronic CNS symptoms experienced during the past month, and symptoms of the peripheral nervous system. All the above symptoms were recognized as "positive" only when they occurred at least once per week independent of any other known medical problems such as the common cold. In addition, the questionnaire contains a detailed personal medical history including alcohol consumption and smoking and an extensive occupational history about previous exposures and duration of employment. All the interviews of the questionnaire were conducted by two standardized interviewers in the field during the medical examination. The association between the solvent exposure and biochemical alterations of liver function has been discussed in another paper (Chen *et al.*, 1991).

The workers were exposed to a mixture of solvents. Eight different solvents were detected in the air. The average contents of xylenes and toluene in these air samples were 50 and 24%, respectively. The air concentrations were expressed as an 8-hr time-weighted average (TWA) (Table 1). The hygienic effect is used as a measure of total solvent exposure and is defined as the sum of the fractions of the respective threshold limit values (TLV) (ACGIH, 1990) that each solvent represents. We divided workers into three groups according to their different exposure patterns and different categories of air concentrations in our analysis. These three groups are defined as follows:

TABLE 1
DETECTED SOLVENTS AND THEIR CONCENTRATIONS IN THE AIR SAMPLES OF PAINT WORKERS

Solvents	Range (ppm)	Median (ppm)	Mean ± SD (ppm)	TLV–TWA[a] (ppm)	Number of samples exceeding the TLV
Xylenes	0–365	18	33 ± 77	100	7
Toluene	0–540	10	16 ± 67	100	2
Acetone	0–124	3	8 ± 25	750	0
Benzene	0–20	1	2 ± 4	10	3
Methyl isobutyl ketone	0–68	0	2 ± 10	50	1
Methyl ethyl ketone	0–70	1	2 ± 9	200	0
Ethyl acetate	0–29	1	1 ± 4	400	0
Butyl acetate	0–41	0	2 ± 7	150	0

Note. All figures are expressed as an 8-hr time-weighted average. (Number of samples, 73; sampling period, 4 hr).

[a] TLV, threshold limit value recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) in 1990.

Exposure index 0: This is the low exposure group, including 3 video terminal painting (39 workers), 2 aircraft painting (7 workers), 2 model painting factories (2 workers), and 1 painting department of a car assembly factory (10 workers). They were exposed to a relatively low level of organic solvents. The individual solvents detected in the personal samplers were all below ¼ TLV level of that recommended by the ACGIH (TLV–TWA) (ACGIH, 1990), while the 8-hr TWA hygienic effect of solvents ranged from 0 to 0.38; the median level was 0.12.

Exposure index 1: This is the short-term high exposure group, including 80 workers who were selected from 16 car-painting factories. They were exposed to a low level of organic solvents, the 8-hr TWA hygienic effects of solvents ranged from 0 to 3.38; the median level was 0.12. However, they must spend 0.5–1.5 hr in poorly ventilated painting booths every workday; the 15-min TWA hygienic effect of solvents while working in the painting booth ranged from 1.32 to 24.52 and the median level was 10.40. Because such a high level of exposure is usually limited to less than 1.5 hr among car painters, they are defined as the short-term high exposure group. While the kinds of major solvents detected inside and outside the painting booths were not different from each other, the concentration of toluene detected inside the booth is usually higher than that of xylenes (range, 11–948 vs 25–511 ppm; median, 354 vs 112 ppm).

Exposure index 2: This is the high exposure group, including 2 paint manufacturing (19 workers) and 1 trailer painting (39 workers) factories. Most of the workers are exposed to xylenes and toluene. The air concentrations of xylenes and toluene detected in the breathing zone are usually above the ½ TLV level recommended by the ACGIH. The 8-hr TWA hygienic effect of solvents ranged from 0.25 to 9.83; the median level was 1.66.

For comparison of each neurological symptom among different exposure indices, workers with or without a particular symptom were stratified by age and exposure indices and then analyzed by Mantel–Haenszel summary procedure (Mantel and Haenszel, 1959) and the Mantel extension for the test of trend (Mantel, 1963). Based on the 90th percentile for the numbers of the acute and chronic symptoms among workers of the low exposure group, a multiple logistic regression analysis and the Mantel–Haenszel procedure were performed to estimate the odds ratio and trends along the different exposure indices, simultaneously taking into consideration age and smoking effects.

RESULTS

A total of 196 workers were investigated. We divided workers into 3 groups according to their different exposure patterns and different categories of air concentrations as mentioned before. In general, workers with higher exposure were older and employed longer than the other 2 groups (Table 2).

All the workers showed no overt neurological signs such as ataxic gait, poor coordination, or muscle weakness. Workers who consumed more than 280 g of alcohol per week ($n = 8$), took antihypertensive medications ($n = 4$), or had a previous history of psychiatric disorder ($n = 1$) were excluded from the analysis to prevent any possible confounding effects.

We found that the severity of exposure was associated with acute symptoms of

TABLE 2
THE 8-hr TWA HYGIENIC EFFECT OF SOLVENTS, AGE, DURATION OF EMPLOYMENT, SMOKING, ALCOHOL CONSUMPTION, MEDICATION, AND PSYCHIATRIC DISORDER OF WORKERS WITH DIFFERENT EXPOSURE INDICES

	Index of exposure			
Term used in the text	0 (low)	1 (short-term high)	3 (high)	*P* of Kruskal–Wallis test
Total number of workers examined	58	80	58	
8-hr TWA hygienic effect of solvents				
Range	0–0.38	0–3.38 (1.3–24.5)[a]	0.25–9.83	$P < 0.0001$
Median	0.12	0.12	1.66	
Age (years)	29.7 ± 7.4	26.4 ± 8.7	41.0 ± 12.4	$P < 0.0001$
Duration of employment (years)	4.8 ± 6.4	6.4 ± 6.7	17.6 ± 13.3	$P < 0.0001$
No. of smokers (%)	26 (45.0)	52 (65.0)	40 (70.0)	
No. with alcohol consumption exceed 40 g/day (%)	0	4 (5.0)	4 (7.0)	
No. with antihypertensive in recent 2 weeks (%)[b]	0	0	4 (7.0)	
No. of workers with psychiatric disorder	0	0	1	

[a] The 15-min TWA hygienic effect of solvents in the painting booth.
[b] Taken antihypertensives during the past 2 weeks.

chest tightness and headaches and chronic symptoms of dizziness, easy fatigability, depressed mood, and palpitation. In addition, symptoms of irritability were only present in the high exposure group during the workday (Table 3). The number of symptoms in the peripheral nervous system showed no difference among different exposure categories. Table 4 shows that there was a trend toward an increased number of workers from the high exposure group who complained of two or more acute symptoms of the CNS. The analysis by multiple logistic regression also showed that the high exposure group had an increased risk (odds ratio = 2.7) of suffering from two or more acute symptoms of the CNS, while age, duration of employment, and smoking did not increase the frequency of acute symptoms of the CNS (Table 4).

The association of chronic symptoms of the CNS with the severity of exposure had results similar to the acute symptoms. Workers in the high exposure group were 3.9 times more likely to develop three or more chronic symptoms of the CNS than the low exposure group (Table 5). The multiple logistic model also showed a similar result with no effect found for age, duration of employment, and smoking on chronic symptoms of CNS.

DISCUSSION

Although CNS symptoms resulting from organic solvent exposures were well documented (Baker *et al.*, 1985; Spencer and Schaumburg, 1985; Waldon, 1986;

TABLE 3
NUMBERS OF WORKERS SUFFERING FROM ACUTE AND CHRONIC SYMPTOMS OF THE CENTRAL NERVOUS SYSTEM AT LEAST ONCE PER WEEK UNDER DIFFERENT EXPOSURE INDICES

	Exposure indices			
	0 (low, n = 58)	1 (short-term high, n = 76)	2 (high, n = 49)	P value of M–H test for trend
Acute symptoms				
1. Chest tightness or compressed feeling over upper chest	1	7	11	
M–H odds ratio	1	5.8	25.6	0.001
2. Headaches	1	4	8	
M–H odds ratio	1	3.2	13.1	0.018
3. Irritability	0	0	3	
M–H odds ratio	—	—	—	0.161
4. Soreness of knee joint[a]	1	3	5	
M–H odds ratio	1	2.1	8.3	0.066
Chronic symptoms				
1. Have you felt lightheaded or dizzy?	4	2	18	
M–H odds ratio	1	0.4	7.8	0.001
2. Have you tired more easily than expected for the amount of activity you do?	7	13	21	
M–H odds ratio	1	1.5	5.5	0.001
3. Have you felt depressed?	2	7	8	
M–H odds ratio	1	2.9	4.6	0.03
4. Have you had heart palpitation even when not exerting yourself	1	3	6	
M—H odds ratio	1	2.1	10.9	0.023
5. Have you felt "high" from the chemicals you use at work?	1	1	5	
M—H odds ratio	1	0.7	8.3	0.059
6. Have you found it hard to understand the meaning of magazine newspaper and books you have read?	1	5	5	
M–H odds ratio	1	3.7	7.0	0.109
7. Have you had difficulty concentrating?	5	2	11	
M–H odds ratio	1	0.3	2.6	0.129
8. Have you had an episode of diarrhea?[a]	3	1	3	
M–H odds ratio	1	0.2	2.0	0.741
9. Have you had an episode of tinnitus?[a]	6	5	5	
M–H odds ratio	1	0.6	1.1	0.897

Note. Frequencies for each symptom were stratified by age (>35 and ≤35 years old) and later summarized by Mantel–Haenszel procedure (M–H), which tested a linear trend along with the exposure severity among workers.

[a] Dummy symptom.

TABLE 4
NUMBERS OF WORKERS WITH TWO OR MORE ACUTE SYMPTOMS OF THE CENTRAL NERVOUS SYSTEM STRATIFIED BY AGE AND INDICES OF EXPOSURE (ANALYSIS BY MULTIPLE LOGISTIC REGRESSION IS ALSO SHOWN)

Age	No. of symptoms	Exposure indices		
		0 (n = 58)	1 (n = 76)	2 (n = 49)
≤35	≥2	9	8	9
	<2	39	59	16
>35	≥2	0	3	6
	<2	10	6	18
Total	≥2	9	11	15
	<2	49	65	34
Standardized odds ratio		1	0.93	3.33
Mantel extension for trend			χ^2 = 3.81 P = 0.055	

	Modeling by logistic regression		
	Odds ratio	95%CI	P value
Age (>35 vs ≤35)	0.77	0.30–1.98	0.586
Exposure index 1	1.03	0.40–2.68	0.955
Exposure index 2	2.72	1.00–7.41	0.050
Smoke 10–19 cig./day	0.80	0.31–2.05	0.645
Smoke ≥20 cig./day	0.89	0.34–2.34	0.807

NIOSH, 1987), and the issue was extensively reviewed by the expert committee organized by WHO (WHO, 1985), it has still been relatively difficult for an occupational physician to make a diagnosis based on symptoms alone owing to the nonspecificity of these symptoms. Similarly, our finding that there was an association between the increase of exposure indices and the presence of acute and chronic CNS symptoms did not necessarily indicate that the symptoms were caused by solvent exposure. However, we argue strongly for the causal association based on following reasons: first, workers who consumed alcohol in excess of 280 g per week, took antihypertensive medicine, or had a previous history of psychiatric disorder were excluded from the analysis. Therefore, the association could not be explained by alcohol abuse, medications, or psychiatric disorder. Second, we have considered age, duration of employment, and smoking by stratified and modeling analysis. All showed a consistent and independent association between exposure and CNS symptoms. Third, all three groups came from the same socioeconomic class with similar educations, incomes, and occupational skills. Thus, their work stress and social life were very similar and cannot explain the difference in prevalence of CNS symptoms among the three groups. Fourth, because we selected workers of low exposure instead of no exposure as the reference group, our estimates of the prevalence rates of CNS symptoms among workers could only underestimate the real figure. Last, the results of our three dummy questions (soreness of knee joint, diarrhea, and tinnitus in Table 3) did not show any statistical association to solvent exposure. Thus, we conclude that the association between increased exposure to organic solvents and a high prevalence of acute and chronic CNS symptoms in high exposure group was probably causal.

TABLE 5
NUMBERS OF WORKERS WITH THREE OR MORE CHRONIC SYMPTOMS OF THE CENTRAL NERVOUS SYSTEM STRATIFIED BY AGE, AND INDICES OF EXPOSURE (ANALYSIS BY MULTIPLE LOGISTIC REGRESSION IS ALSO SHOWN)

Age	No. of symptoms	Exposure indices		
		0 (n = 58)	1 (n = 76)	2 (n = 49)
≤35	≥3	6	7	9
	<3	42	60	16
<35	≥3	1	3	7
	<3	9	6	17
Total	≥3	7	10	16
	<3	51	66	33
Standardized odds ratio		1	1.15	3.87
Mantel extension for trend			χ^2 = 4.86 P = 0.027	

	Modeling by logistic regression		
	Odds ratio	95%CI	P value
Age (>35 vs ≤35)	1.17	0.48–2.94	0.715
Exposure index 1	1.06	0.40–3.14	0.387
Exposure index 2	3.27	1.19–9.39	0.022
Smoke 10–19 cig./day	0.63	0.22–1.80	0.388
Smoke ≥20 cig./day	1.30	0.50–3.39	0.588

Since organic solvents are widely used in various industrial processes, developing an inexpensive and simple method for early detection of adverse solvent-induced health effect, especially neurotoxicity, is an urgent priority. CNS symptoms were the earliest form of chronic toxicity resulting from organic solvent exposure (Axelson and Hogstedt, 1988). However, the nonspecific nature of these symptoms has prevented them from being widely used as a simple tool for the early recognition of occupational hazard. To solve this problem, a falsification attitude plus a multivariate analysis to exclude alternative explanations such as age, smoking, alcohol, and medication is necessary to successfully document the hazard. We, therefore, recommend that a constructive and standardized questionnaire followed by careful epidemiological study design and analysis be used to reach the goal of early detection of neurotoxicity caused by organic solvents. It can also be supplementary to environmental measurements. Moreover, an internationally standardized questionnaire such as the one by Hogstedt *et al.* might be needed to collect and compare the information of solvent-induced neurotoxicity from different exposure levels in different countries.

Our results showed that workers exposed to a medium level of 1.66 of TWA hygienic effects of solvents would be 2.7 times more likely to develop two or more acute symptoms and 3.3 times more likely to develop three or more chronic symptoms of CNS than workers with low exposure. We tentatively concluded that an exposure level exceeding 1.66 of TWA hygienic effect of solvents would increase the risk of solvent-induced neurotoxicity. A long-term followup of symp-

toms and/or batteries of objective neuropsychologic performance tests would also be useful to test the causal association. In addition, routine environmental or biological monitoring plus engineering controls should be performed to assure the safety of the exposure level and to prevent any possible adverse health effects caused by solvents.

Smoking was once proposed to be a confounder and/or cofactor to the CNS symptoms (Johnson, 1987) because it could increase the inhalation of solvents and elevate the level of carboxyhemoglobin, which in turn would result in CNS symptoms (Ferris, 1978). However, we could not document any effects due to smoking in our study even by multivariate logistic regression. The reason might be that smoking is generally prohibited at the workplace of spray painting, and few workers have enough time to smoke a great deal.

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Review of Air Pollution and Its Health Impact in Indonesia[1]

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Air quality monitoring is part of the initial strategy in the pollution prevention program in Indonesia. Since 1978, the government of Indonesia has had a commitment to the World Health Organization (WHO) to provide air quality data for the Global Environmental Monitoring System (GEMS Programme)—The WHO/UNEP Project, in which certain cities from all over the world have been selected. Air quality as part of the WHO/UNEP project is monitored with respect to pollutants like SPM, SO_2 and NO_x. The result of the monitoring indicates that SPM and NO_x are the predominant pollutants. Other pollutants such as O_x, H_2S, NH_3, and CO are also monitored in several big cities in Indonesia. The air pollution mainly comes from land transportation, industrial emissions, and a densely populated residential area where most people perform their activities. Review of the air pollution in Indonesia was based on the reports of the air quality monitoring in several large cities in Indonesia which covered air pollutants such as SPM, SO_2, NO_x, CO, O_x, and NH_3 from 1978 until the latest available data in 1989. This review also discusses health impact investigations conducted in the community, especially from the exposure to SPM, CO, and lead from motor vehicle exhaust.

INTRODUCTION

Like other developing countries, Indonesia is developing rapidly with growth in the economic sectors and increasing industrialization. In the past few decades, Indonesia has changed from a rural and agricultural society to urban and commercial–industrial one. There has been a massive influx of people into the major cities in search of jobs and better living conditions. These changes have placed a lot of pressure on society and the environment and natural resources such as air have deteriorated in quality.

The present air pollution problem in Indonesia is not considered to be serious. There has never been a case like the famous London smog and other episodes which killed hundreds of people. The concentration of photochemical oxidants is low, and acid rain has not been a problem. This may be due to favorable meteorological conditions as Indonesia is situated on tropical seacosts, and major cities are on the flatlands or in the coastal areas. There is also no need for heating. Weak or absence of temperature inversion also helps in dispersing pollutants.

However, this does not mean that Indonesia shall be as fortunate in the years to come. Monitoring data and studies on ambient air quality show that some of the air pollutants in several large cities are increasing with time and are not always at acceptable levels according to the national ambient air quality standards. Data on air pollution and case studies in Indonesia are very limited. This paper reviews the results of ambient air quality monitoring and studies related to air pollution in Indonesia.

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SOURCE OF AIR POLLUTION

Indonesia is an archipelago consisting of about 13,000 islands with an area of 2 million square km and a coastline of 81,000 km. The 1990 population was estimated about 180 million, with 60% living on the islands of Java and Madura (Central Bureau of Statistics, 1991). Java Island covers only 6% of the total land area of Indonesia. Problems arising from this unbalanced condition include not only socioeconomic activities, but also the urbanization of people from rural areas to the major cities in Java Island. In the near future Java Island will be totally an urban area, while other islands will become rural areas with all activity centered in the large cities.

An increase in motor vehicle use has been followed by an increase in fuel consumption. The rate of increase of motor vehicle use in Indonesia has averaged 8% per annum. Most of these vehicles are concentrated in major cities such as Jakarta, Surabaya, and Medan. Moreover, additional road space has grown at only 4% per year and has not kept pace with the high increase of vehicular traffic (Directorate General of Road Transport and Traffic, 1990). These conditions have caused severe congestion in almost all parts of the highway network and corridors, especially in the central business areas, and inevitably the environment in these areas has deteriorated due to exhaust gas emissions from the motor vehicles. Up to now, Indonesia has continued to use leaded gasoline. This, combined with the fact that many of the vehicles and roads are poorly maintained, has made air pollution from motor vehicles a critical problem. In some large cities like Jakarta, Surabaya, and Medan, the level of air pollutants caused by automobiles such as suspended particulate matter (SPM) and carbon monoxide is quite high due to a large population of motor vehicles.

Increased activity from the industrial sector has been accompanied also by increased use of energy and commodities traffic. There are about 14,600 industries in Indonesia classified as large and middle sized (Central Bureau of Statistics, 1987). Small industries and home industries are estimated to number about 113,000 and 1500, respectively. Most of the small- and middle-sized industries do not install pollution control equipment. This increases the emission of pollutants, especially in the industrial areas which in some cases contribute specific pollutants to the air. Moreover small industries are generally located in populated areas and emission control is more problematic. This is partly due to the fact that land use is not regulated in most of the country.

Other sources of pollutants include open burning of refuse, which is common at some poorly managed disposal sites and results in smoke and flyash problems. Smoke due to forest fires on Kalimantan Island and in South Sumatera sometimes contributes to the problem during the dry season. This condition has reduced visibility and disturbed airport areas for several days.

AIR QUALITY MONITORING IN INDONESIA

Initial ambient air quality monitoring began in 1978. The first station began operation in Jakarta City. The Meteorology and Geophysic Agency conducts air quality monitoring in cooperation with the World Meteorology Organization Programme (UNEP). The program measured particulates (SPM), NO_x, and SO_2, and rain acidity and composition. The National Institute of Health Research and Development has also set up two stations in Jakarta City under the World Health

Organization and supplies data for the UNEP Global Environmental Monitoring System (GEMS). The pollutants measured are SPM, SO_2, and NO_x. Since the development and growth of Jakarta City are very fast compared to other major cities, the Meteorology and Geophysic Agency set up more stations in other parts of the country, such as in Padang, Medan, Kupang, and Biak.

In the Jakarta metropolitan area, Jakarta Municipality has a monitoring network for air pollution which started in 1980, consisting of nine permanent stations. The area covered by the sampling stations is representative of residential, commercial, industrial, and mixed areas. The pollutants measured are CO, SO_2, NO_x, NH_3, O_x, hydrocarbons, and lead.

The monitoring results for 1986 to 1990 (Soedarmo, 1987; Urban and Environment Research and Development Center—Jakarta Municipality, 1986–1990; Tri-Tugaswati, 1991) obtained in Jakarta City indicated that generally the level of SPM in industrial (Pulogadung), mixed industrial and commercial areas such as Pasar Ikan and Bandengan Selatan, as well as North Jakarta and a bus terminal area (Cililitan) has increased continually since 1986. The level of SPM in those areas, in general, has exceeded the standard of 260 $\mu g/m^3$. On the other hand, stations at residential areas such as Tebet (South Jakarta), commercial areas such as Husada (West Jakarta), and mixed commercial and residential areas such as Rawasari are still below the standard.

A similar situation was observed for the level of NO_x in ambient air. The concentration pattern of NO_x suggests a strong influence of vehicle emissions at each site. However, the concentration at all sites does not exceed the air quality standard of 92.5 $\mu g/m^3$, and residential areas have the lowest value. Other major air pollutants such as oxidants and hydrocarbons have remained, in general, well below the standard.

AIR POLLUTION STUDIES IN INDONESIA

Studies on air pollution in Indonesia are very limited. Air pollution problems in Indonesia have been reported in some specific areas in large cities and in some industrial areas. Most of the air pollutants reported are related to local pollution, where the concentration is locally high. SPM and CO are the air pollutants most frequently studied because instruments that measure these are easy to obtain and relatively easy to operate. These instruments are not sensitive generally and the period of sampling is usually less than 24 hr. The results of these studies were variable and could not be compared due to the dissimilar nature of sampling (Rahardjani and Dharmoyo, 1978; Environmental Health Engineering Station—Ministry of Health, 1986; Soeprapto *et al.*, 1982; Soeparmo *et al.*, 1982; Anwar, 1986; Soesatyo *et al.*, 1983).

In 1976 one study of pollution concentration in heavily trafficked areas in Jakarta showed that a bimodal distribution of CO levels on weekdays occurred between 7:00 and 9:00 AM and from 6:00 to 10:00 PM. This situation occurred due to traffic moving very slowly and emitting more CO to the air. The level of CO in the atmosphere dropped significantly on weekends (Achmadi, 1981).

Studies done by Jakarta Municipality in 1979 reported that the highest level of air pollution is found in the commercial areas with heavy traffic (Urban and Environment Research and Development Center—Jakarta Municipality, 1986–1990). The lowest level of air pollution is found in residential areas. The same result is found in Surabaya City and Gresik City (Baktir *et al.*, 1988). Relatively

high levels of CO and NO_x are found in areas with high traffic in both cities; however, the levels are not yet high enough to harm human health.

In 1982–1984, surveys were carried out in 16 urban areas in Indonesia (State Ministry of Population and Environment, 1985). The cities chosen included major cities on the islands of Java, Bali, Sumatera, Kalimantan, and Sulawesi. Data collected indicated that generally SPM is a major problem for cities on Java Island, while data collected from cities outside Java Island, in general, indicated that the level of SPM is still in compliance with the standard. During the survey, many of the SPM data collected in major cities on Java Island exceeded the standard value of 260 $\mu g/m^3$. This situation may come from high traffic density and community activities which are concentrated in one certain location. The situation worsens with poor maintenance of the existing roads and old vehicles in these locations. Besides SPM, it was also found that the CO and NO_x levels were sometimes higher than the standards (20 ppm/8 hr and 0.05 ppm/m^3/24 hr), especially in the areas with heavy traffic, whether in residential, commercial, or industrial areas. Other major air pollutants such as NH_3, H_2S, O_x, hydrocarbons, and SO_2, in general, remained well below the maximum allowable concentrations of these pollutants.

An atmospheric lead survey in Bandung City was done by Djuangsih *et al.* (1988). It was found that the lead content in the atmosphere in Bandung City was in the range of 0.3 to 6.0 $\mu g/m^3$. The highest level found was in a mixed residential and industrial area, with a traffic volume of 2750 vehicles/hr, while the lowest level was found in a farming village situated far from traffic.

A similar study in Jakarta City done by Tri-Tugaswati *et al.* (1987) showed that lead concentrations in the air along streetsides were 3.6 and 1.7 $\mu g/m^3$ with respective traffic volumes of 5148 and 1284 vehicles/hr in one direction during the day. However, in the suburb of Jakarta, it was 0.3 $\mu g/m^3$ with 40 vehicles/hr. The study also measured the CO concentration, and it was found that the CO concentration downtown was in the range of 20 to 35 ppm, while in the suburb it was undetected. Carbon monoxide was measured by an instant test tube method (Kitagawa Tube). It is clear that the lead and CO levels in the downtown area are higher than those in the suburb, which has sparse traffic.

HEALTH IMPACTS

There are a very limited number of studies that relate air pollution to its health impact in Indonesia. The lack of data gathering from environmental epidemiology analysis health services makes it difficult to estimate the health impact of air pollution. The "Report on Household Survey" in 1986 stated that respiratory disease is still a prevalent cause of death as well as illness in seven provinces in Indonesia (Darmadi *et al.*,1987). Rais (1989) also stated that 48% of respiratory diseases in Jakarta City may due to the air pollution exposure. However, this statement is only a rough estimate because many factors can influence the disease. Because the main source of air pollution in the major cities in Indonesia is assumed to be motor vehicles, studies on the health impact of air pollution are limited to the concentration of CO and lead from human samples such as blood and urine.

Human exposure to CO can be estimated either by measuring the CO concentration in the air or by measuring of carboxyhemoglobin (HbCO) in the blood (WHO, 1979). Amsyari and Pariani (1984) stated that the HbCO of taxi drivers in

Surabaya increased from 0.75 to 1.30% during working hours. It is known that HbCO levels are dependent on a number of factors including time, length and quantity of CO exposure, smoking habits, ventilation, and blood volume. Work capacity is affected by CO exposure, and limitations may begin at HbCO levels of 4%. Although maximal work effort is not diminished at 2.5–4.0%, the length of time that such effort can be maintained is shortened (WHO, 1979).

The major source of lead in the atmosphere is the combustion product of leaded gasoline (Rabinowitz and Needleman, 1983; Ad Hoc Group to the Environment Committee of OECD, 1978). Organic leads, tetraethyl and tetramethyl lead, are added to gasoline because it is the most convenient and economic method of increasing the octane ratings of all grades of gasoline (Stubbs, 1972; Rosner and Markowitz, 1985). Since 1990, the gasoline available in Indonesia is leaded with 1.5 ml of tetraethyl lead per American gallon. It is known that continuous exposure to lead by inhalation can affect the hemopoietic system even under normal urban conditions (Hernberg *et al.*, 1970). Tri-Tugaswati *et al.* (1987) found that the average lead concentrations in blood and urine of public transportation drivers in Jakarta were 18.4 μg/dl (PbB) and 8.6 μg/liter (PbU), respectively. The normal range of lead in blood is approximately 10–25 μg/dl (WHO, 1977), which indicates that the average level of PhB in the drivers group is in the normal range. Other PbB reports in Indonesia have found levels of 14.7 μg/dl for 18 male students in Jakarta (Urban and Environment Research and Development Center—Jakarta Municipality, 1986–1990), 12.28 μg/dl for rural people in Bandung (Djuangsih *et al.*, 1988), and from 88 to 238 μg/dl for students living in the suburbs of Jakarta (Syamsuddin *et al.*, 1981). However, the last report could not be regarded as reliable. Other studies to detect lead poisoning were carried out among the population living around bus terminals (Directorate General of Communicable Diseases Control, 1989). Studies were carried out in three major cities, Jakarta, Bandung, and Yogyakarta. Data collected revealed that significantly higher levels of lead and COHb in blood were detected in the studied population group compared to those in the control group. This situation showed that those groups with high exposure to pollution are at the greatest risk.

Amsyari *et al.* (1987) studied the impact of industrial zone development in Gresik City on the population living close to the zone. It was found that the prevalence of chronic respiratory diseases in the exposed group was higher than that in control group.

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Studies on Neurolathyrism[1]

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Neurolathyrism is a neurological condition seen among people who eat the seeds of *Lathyrus sativus* (LS) as a principal source of food energy for 2 months or more. It is characterized by severe muscular rigidity and paralysis of the lower limbs. β-*N*-Oxalyl-L-α,β-diaminopropionic acid is the principal toxin found in the seed. No experimental animal model for neurolathyrism could be produced by feeding either the seeds or the toxin, although the condition has been known for centuries. We discovered that experimental neurolathyrism could be produced in guinea pigs and primates that needed an external supply of ascorbic acid by making them subclinically deficient in ascorbic acid and feeding them the seeds of LS or extracts thereof. Autoclaving the seeds of LS with lime removes the toxin.

INTRODUCTION

The crippling disease lathyrism, which has afflicted many thousands in Bangladesh and also in India, China, Ethiopia, and Nepal (Haimanot, 1990; Acherya and Pathak, 1990), is now well-known. The first definite mention of *Lathyrus* as a poisonous food is contained in an edict issued in 1671 by the Duke of Wuttemberg, in which the use of *Lathyrus* flour in making bread was prohibited because of its paralyzing effect on the legs (Dwivedi and Prashed, 1964). The word lathyrism was first introduced by Cantoni in Italy in 1973 (Cantoni, 1973); before that the disease was known under various names.

There are two types of lathyrism, osteolathyrism and neurolathyrism. Osteolathyrism has been observed in rats and other laboratory animals following the ingestion of seeds of *Lathyrus odoratus*, *Lathyrus hirsutus*, and *Lathyrus pusillus*. Neurolathyrism, caused by ingestion of Khesari (*Lathyrus sativus*), is known to cause great disability and distress in humans. The onset of neurolathyrism is sudden; the first symptoms are aching of the waist, rigidity of calf muscles, and a partial or total loss of control over the lower limbs. In some cases a scissor gait or crossed gait of the legs develops with a tendency to walk on the toes. Some patients have exaggerated knee and ankle jerks and ankle clonus.

Lathyrism may occur at any age, but the incidence is highest in young adults between 20 and 35 years of age. Males are afflicted more than females (Xiupyun, 1990). In humans the syndrome appears after continuous eating of Khesari as a staple for 2–3 months (Jahan, 1983). Many cases of neurolathyrism are seen in the districts of Rajshahi and Kushtia in Bangladesh, where the consumption of Khesari as a staple is also great.

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A full investigation of the problem has been difficult for want of a suitable experimental model with the same neurological manifestations and disability as those seen in humans, i.e., spastic paralysis of lower limbs (paraplegia). However, neural conditions of various descriptions were reported in number of animals fed a diet of *L. sativus* or extracts thereof. Some of the findings, however, could not be confirmed by other investigators (Bhagwat, 1964).

Among several compounds studied for their neuroactive action, β-*N*-oxalyl-L-α,β-diaminopropionic acid (ODAP) has attracted the most attention. It is believed to be responsible for human lathyrism (Rao *et al.*, 1964). However, this compound is nontoxic to adult laboratory animals such as rats and mice. It was observed that this compound was only toxic in very young avians (chicks) (Roy *et al.*, 1963).

From reading the literature cited above, it occurred to us that ascorbic acid deficiency could be a precipitating factor. Although one might regard this as a wild guess, the fact remains that most of the failures in producing experimental lathyrism were with the animals able to biosynthesize vitamin C; humans who suffer from the disability are unable to do so. Accordingly, guinea pigs, which depend on dietary ascorbic acid, were chosen as experimental animals.

EXPERIMENTAL METHODS

As summarized in Table 1, a group of adult male guinea pigs weighing 300–350 g was fed a diet of cooked *L. sativus* (Khesari) supplemented with all vitamins except ascorbic acid (Group A). Another group of animals, Group B similar in all respects, was given the same diet as Group A but was also given ascorbic acid (5 mg/animal/day). Two other sets of animals, Groups C and D, were fed diets in which *L. sativus* was substituted by another legume, namely *Phaseolus radiatus*. There was ascorbic acid in the diet of Group D but none in that of Group C. The latter two groups were included in the study in order to exclude or isolate any effect of ascorbic acid deficiency in the absence of *L. sativus*. As determined by chemical analysis, the diets were free of ascorbic acid except in the cases of Groups B and D which were supplemented with ascorbic acid as explained above.

Not a single animal in Group B, which received ascorbic acid, showed any neural symptoms. In contrast, 26 of 35 animals in Group A (which did not have ascorbic acid) developed progressively neurological symptoms, namely monoplegia, paraplegia, and finally hemiplegia after about 7 weeks. They showed various symptoms, such as tremor, ataxic gait, dragging of legs, rigidity of neck, pleurosthotonos, and emprosthotonos, etc.

At the stage of monoplegia an affected animal could be saved by including 5 mg of ascorbic acid in the diet. The reversal of the symptoms took place quickly. Complete or near complete recovery could be seen in 3–7 days. The amount of L-ascorbic acid thus administered was chosen arbitrarily. None of the animals developing hemiplegia could be saved.

We prepared another two sets of the animals, Groups E and F. Group E had the same diet as Group C (i.e., *P. radiatus* and not ascorbic acid) and Group F had the same diet but including ascorbic acid (5 mg/animal/day). After 3 weeks, each animal of these groups was administered intraperitoneally 2.5 ml of *L. sativus* extract freshly prepared according to Roy *et al.* (1963).

TABLE 1
EFFECT OF *Lathyrus sativus* ON YOUNG ADULT GUINEA PIGS (300–350 g): PROTECTIVE ACTION OF ASCORBIC ACID

Group	Diet	Observation	Remarks
A (35)	Cooked *L. sativus* and whole wheat (4:1) with supplements of all B vitamins and fat-soluble vitamins but no ascorbic acid	26 animals developed neurological symptoms of lathyrism between 3 and 7 weeks. The remaining 9 showed no neurological symptoms but died during the experiment.	Working formula: $P = \frac{X}{N}$, $Q = 1 - P$. $P \pm 3\sqrt{\frac{PQ}{N}} = 0.7428 \pm 0.2216$. The result is extremely significant.
B (35)	As above but with ascorbic acid, 5 mg per animal daily	None showed neurological symptoms.	
C (35)	Cooked *P. radiatus* and whole wheat (4:1) with supplement of all B vitamins and fat-soluble vitamins, but no ascorbic acid	Normal in all respects. None showed neurological symptoms. However, three died during the experimental period.	No neurological symptoms appeared in the experimental animals due to the absence of ascorbic acid in the diet when *L. sativus* was replaced by *P. radiatus*.
D (35)	As above but with added ascorbic acid, 5 mg daily per animal	None showed neurological symptoms.	

Note. The figures in parentheses indicate the number of animals in the respective groups.

All the animals of Group E developed neural symptoms within 1½ hr of administration. At first they started limping and then they developed the familiar spastic paralysis. None of the animals of Group F having ascorbic acid developed these symptoms. At the time of neural sickness, the serum vitamin C of the affected guinea pigs ranged between 0.2 and 0.4 mg/dl (Tables 2 and 3).

We repeated these experiments with monkey and found that adult monkeys fed a diet of *L. sativus* seeds deficient in vitamin C developed the same symptoms typical of neurolathyrism as guinea pigs (Table 4).

During visits to some villages of Rajshahi and Kushtia, we listed the families with one or more members afflicted by lathyrism and the neighboring families without the disease, and we took finger-prick blood samples to estimate the serum vitamin C level. We found that the ascorbic acid level in the serum of the families with the disease was significantly lower than that in the members of the families without the disease (Table 5).

Detoxification of L. sativus

The neural damage in humans could also be prevented by adding ascorbic acid to the lathyrogenic diet, as demonstrated in guinea pigs and monkeys. It is un-

TABLE 2
PLASMA VALUES OF VITAMIN C IN VITAMIN C-SUPPLEMENTED AND VITAMIN C-DEFICIENT GROUPS OF GUINEA PIGS

Number of weeks after start of diet	Groups supplemented with 5 mg vitamin C/animal/day (n = 35)[a]	Group without vitamin C (n = 35)[a]
0	0.60	0.62
1	0.60	0.62
2	0.64	0.56
3	0.68	0.44
4	0.72	0.38
5	0.76	0.16
6	0.80	0.20
7	0.84	0.20

Note. Plasma vitamin C values are expressed in mg/dl. These are the average values of the animals in each respective group.
[a] n, number of animals.

likely, however, that the population eating *L. sativus* as a staple would eat enough foods rich in ascorbic acid to prevent the condition. It is therefore necessary to find a simple procedure to detoxify the seeds.

Adiga *et al.* (1963) isolated and identified β-*N*-oxalyl-L-α,β-diaminopropionic acid from the toxic seeds. This neuroactive amino acid is considered responsible for most, if not all, of the toxicity. It was therefore thought that removal of this unusual amino acid would render the seeds nontoxic. Because this amino acid is water soluble, it was suggested (Acton, 1922) that the seeds be washed several times to remove the toxin. It was further suggested by Mohan *et al.* (1966) that

TABLE 3
WEIGHTS OF GUINEA PIGS FED VITAMIN C-DEFICIENT DIET AND THOSE OF CONTROL GROUP FOR A PERIOD OF 7 WEEKS

Weeks after the start of the diet	Weight of guinea pigs (in grams) supplemented with 5 mg vitamin C/animal/day (n = 35)[a]	Weight of guinea pigs (in grams) without any vitamin C (n = 35)[a]
0	300	350
1	300	350
2	320	340
3	350	320
4	360	310
5	370	300
6	385	270
7	400	270

Note. The table shows the gradual weight loss of the guinea pigs fed the vitamin C-deficient diet. These are the average weights of 35 animals of each respective group.
[a] n, number of animals.

TABLE 4
EFFECT OF *Lathyrus sativus* ON YOUNG ADULT MONKEYS WEIGHING 3500 TO 4000 g: PROTECTIVE ACTION OF ASCORBIC ACID

Group	Diet	Observation	Remarks
A (6)	Cooked *L. sativus* and whole wheat (4:1) with supplement of all B vitamins and fat-soluble vitamins, but no ascorbic acid	5 of 6 animals developed neurological symptoms within 3 months.	$P \pm 3\sqrt{\frac{PQ}{N}} = 0.8333 \pm 0.4564$ The result is extremely significant.
B (3)	As above but with ascorbic acid 20 mg/animal/day	No neurological symptoms were observed.	
C (3)	Cooked *P. radiatus* and whole wheat (4:1) with supplement of all the vitamins, except ascorbic acid	Normal in all respects. None showed any neurological symptoms.	No neurological symptom was observed in ascorbic acid-deficient condition when *L. sativus* was replaced by *P. radiatus*.
D (3)	As above but with added ascorbic acid 20 mg/animal/day.	None showed neurological symptoms	

Note. The figures in parentheses indicate the number of animals in the respective groups.

steeping the seeds for some time followed by boiling and discarding the cooking water would serve the purpose.

We cooked the decorticated seeds according to the method of Mohan *et al.* (1966) and found that they still retained ODAP when tested chromatographically. The water-soluble toxic amino acid might possibly be removed by repeated boiling and washing, but in the process a great deal of the water-soluble nutrients including free amino acids, vitamins, and minerals essential for good nutrition would also be lost, as was recognized by Mohan *et al.* (1966). A process is therefore needed in which washing would be unnecessary and the nutrient loss would be minimal.

We considered that ODAP, whether present as a free amino acid or in some combined form, should be labile under alkaline conditions when heated, preferably under pressure (autoclaved). The treatment of cereals and other foodstuffs with lime is a common practice in many cultures, so we treated the *L. sativus*

TABLE 5
SERUM ASCORBIC ACID LEVELS OF THE MEMBERS OF AFFECTED AND NORMAL FAMILIES OF THE VILLAGES OF RAJSHAHI

Families	No. of members	Ascorbic acid levels (serum mg/dl)	Remarks
Affected	116	0.430 ± 0.180	The result is significant.
Normal	84	0.647 ± 0.168	

seeds with lime and then cooked them. Autoclaving ensures complete removal of the toxin and at the same time eliminates the trypsin inhibitors in the seed (Roy and Rao, 1971).

Procedures

Two hundred grams of decorticated ground seeds was soaked in saturated lime water overnight and then cooked for 25 min. The amount of lime water was adjusted so that just enough was present to soak the seeds, with no excess to drain off. The treated seeds were then dried and further ground to make unleavened bread or other common preparations in which Khesari is used.

The lime-treated seeds were brought to pH 4 with hydrochloric acid and blended with 70% alcohol according to Mohan's method (Mohan *et al.*, 1966). Further processing was done to prepare the sample for chromatography to detect the presence of ODAP. The chromatogram was run on Whatman No. 1 filter paper using the solvent system butanol, acetic acid, and water (12:3:5). Control analysis was done on seeds treated similarly with water instead of lime water. A chromatogram was also made by adding a minute amount of ODAP to the lime-treated seeds after adjustment of the pH to 5.5 to confirm that it was ODAP that was lost during lime treatment.

Another lot was autoclaved for 10 min at 15 psi after overnight steeping in lime water. A small amount of ODAP was similarly added to lime water, autoclaved, brought to pH 4, and chromatographed. It was found that the ODAP was also split.

The patterns of the chromatograms can be seen in Fig. 1.

RESULTS

The use of *L. sativus* as a staple of the daily diet for a period of 2 months or so leads to human neurolathyrism.

Young adult guinea pigs and monkeys fed cooked *L. sativus* supplemented with all vitamins except L-ascorbic acid become paralyzed in 3–7 weeks, while those with the same diet supplemented daily with 5 mg of ascorbic acid stay well. When *L. sativus* is replaced by any other legume or cereal in an ascorbic acid-deficient diet, no symptoms of neurolathyrism appear.

When administered to guinea pigs and monkeys kept on ascorbic acid-deficient diet without *L. sativus,* Khesari toxin preparation produced neurolathyrism in these animals within 2 hr of administration. In control experiments, animals receiving ascorbic acid with the same diet remained unaffected by administration of toxin.

Ascorbic acid protects experimental animals from the neurotoxic effect of the toxin (ODAP) present in *L. sativus* seeds. When seeds were treated with lime water overnight and then cooked for 25 min or autoclaved, ODAP was completely removed.

DISCUSSION AND CONCLUSION

L. sativus has been a principal food item for many people on the subcontinent for centuries and has crippled many people for life. It is a disease of the Khesari-

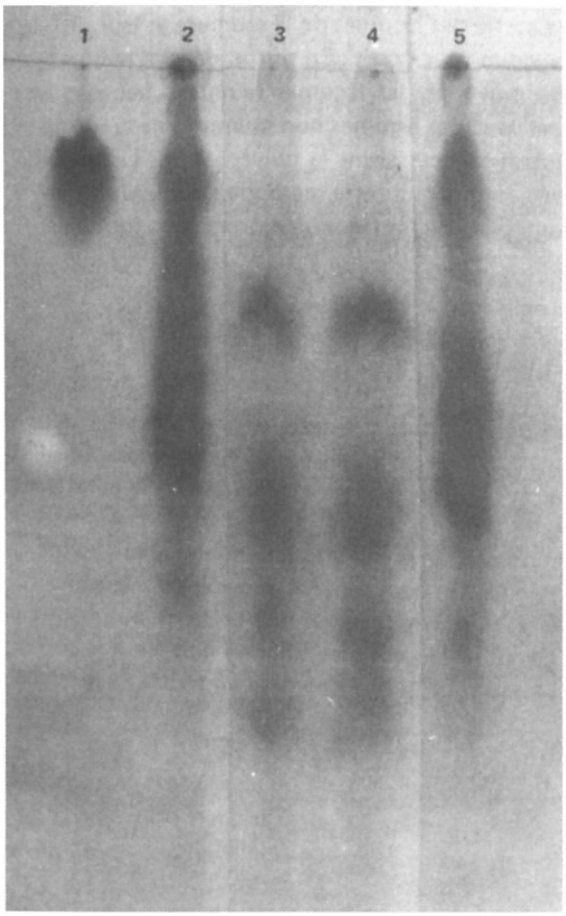

FIG. 1. Chromatograms. Lane 1, ODAP alone; lane 2, *Lathyrus sativus* boiled and washed with water; lane 3, *Lathyrus sativus* soaked in lime water and boiled; lane 4, *Lathyrus sativus* soaked in lime water and autoclaved; and lane 5, ODAP added to sample 4 just before chromatography.

eating population which is simultaneously deficient in vitamin C. The lathyrism may be prevented by the provision of ascorbic acid in the lathyrogenic diet, as demonstrated in guinea pigs and monkeys. It is not likely, however, that the poor population eating *L. sativus* as a staple food would eat enough ascorbic acid-rich food to prevent the condition. Thus, we need a simple procedure to detoxify the *Lathyrus* seeds. Simply soaking seeds in lime water overnight followed by boiling will destroy the toxin. This treatment also destroys trypsin inhibitors. Because lime is present in most households on the subcontinent for use with betel leaves, no costly ingredients need to be purchased for treating *L. sativus* seeds.

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Evaluation and Control of Mercury Vapor Exposure in the Cell House of Chlor Alkali Plants[1]

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A pilot study was carried out in the cell houses of three chlor alkali plants to assess level of exposure to mercury vapors among workers by air and biological monitoring. Overall airborne mercury concentrations (mg/m^3) were found to range from 0.05 to 0.42 (mean, 0.21, n = 68), 0.03 to 0.16 (mean, .08, n = 49), and 0.02 to 0.17 (mean, 0.04, n = 26), whereas urinary mercury levels (mg/liter) of the exposed workers of the respective plants ranged from 0.076 to 0.592 (mean, 0.207, SD, 0.107, n = 19), 0.015 to 0.220 (mean, 0.070, SD, 0.054, n = 16), and 0.013 to 0.275 (mean, 0.06, SD, 0.054, n = 23). Unattended mercury spillage on the floor and improper sealing of the lids of the end boxes of electrolysis cells were found to be main factors attributing to prevalence of mercury vapors in excess of the permissible exposure limit of 0.05 mg/m^3. Based on the deficiencies observed, appropriate control measures have been suggested to reduce airborne mercury vapor concentrations in the work environment.

INTRODUCTION

Prevalence of mercury vapors in the work environment of the cell house of a mercury cell electrolytic chlor alkali plant is a potential health hazard to the exposed workers. In a mercury cell electrolytic process mercury is used in a large quantity as a cathode at the bottom of the cell where sodium is liberated from brine solution to form a mercury amalgam. The amalgam is carried to a separate part of the cell (decomposer) where it causes sodium to return to a solution as sodium hydroxide while hydrogen is evolved from the cathode above. The regenerated mercury is pumped back into cell.

Repeated exposure to the levels exceeding the threshold limit value (TLV) (ACGIH, 1989) of mercury (0.05 mg/m^3) may result in the chronic manifestations predominating digestive and nervous symptoms known as mercurial parkinsonism. The early signs include slight digestive disorder, anorexia, intermittent tremor sometimes in a specific muscle group, and neurotic disorders varying in intensity depending on the individual mental and cultural level. The neuropsychiatric manifestation is known as erethism (loss of memory, insomnia, irritability, excessive shyness, emotional lability) (Parmeggiani, 1989).

In order to prevent incidence of chronic mercury poisoning among the exposed worker it is necessary to control the levels of exposure to mercury vapors in the work environment by incorporation of technical control measures. The TLV of a toxic substance serves as a reference value for air monitoring, whereas biological

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monitoring serves for the overall assessment of exposure through the measurement of the appropriate determinant(s) in the biological specimen collected from the exposed workers at a specified time. Biological exposure index (BEI) serves as a reference value for biological monitoring and is related to exposure to TLV. The biological threshold limit value of urinary mercury in relation to the TLV level of exposure has been reported to be in the range of 0.05–0.10 mg/liter (Smith *et al.*, 1970; Bell *et al.*, 1973).

The present paper entails the findings of a study carried out in the cell house of three chlor alkali plants using mercury for electrolytic production of caustic soda and chlorine from brine solution to

(i) evaluate the level of exposure to mercury vapors in the workroom environment by air monitoring and biological monitoring; and (ii) suggest remedial measures to control levels of mercury vapor exceeding the prescribed TLV in the work environment.

SUBJECTS AND METHODS

The three plants covered under the study are identified as A, B, and C. The amount of mercury used in the plants A, B, and C were 148, 43, and 31.5 tons distributed in 52, 33, and 50 electrolysis cells, respectively. The electrolysis cells were arranged in two rows with the provision of peripheral and central passage besides interspacing of about 2 ft between two cells in a row.

The cell houses of plants A and B were ventilated by openings on two opposite lengthwise sides, whereas plant C was provided with the openings on three sides. The electrolysis process is done under an enclosed system. The end boxes of the electrolysis cells of the plants A, B, and C were provided with a rubber flap, metallic lid without gasket, and with rubber linings, respectively. Epoxy coating was done partially on the floors of central and peripheral passage of only plants A and B.

Air Monitoring

Air samples were collected at the breathing zone of the workers at the locations covering the central peripheral and in between the two cells passages of the cell house. Mercury vapors were collected in a midget impinger tube containing aqueous solution of iodine (0.25%) and potassium iodide (3.0%) by personal samplers preset to a sampling rate of 2 liters/m (Jacobs, 1967). The mercury content of a sample was reduced to elemental state by alkaline sodium borohydride reagent and aerated into a mercury analyzer that was quantified by ultraviolet spectrophotometry (Baselt, 1980).

Biological Monitoring

Spot samples of urine were collected from the exposed workers at the end of the shift. The mercury content in a sample was analyzed by the procedure used for air samples except that in addition tributyl phosphate was used as an antifoaming agent. The urinary mercury concentration in a sample was computed for a specific gravity of 1.016.

RESULTS

The levels of airborne mercury concentrations prevalent in the workroom air of the plants and urinary mercury levels of the exposed workers are summarized in Table 1.

The mercury vapor concentrations were found to exceed the TLV in 100, 80, and 50% of the samples collected at the plants A, B, and C, respectively. The overall mean mercury concentration exceeded the TLV in plants A and B, whereas it was within the limit in plant C.

The urinary mercury levels were found to exceed the upper biological threshold limit value of 0.1 mg/liter in 85, 12, and 13% of the samples analyzed in respect to plants A, B, and C. The biological exposure index of mercury has not yet been established by American Conference of Governmental Industrial Hygienists and it is listed among the substances under study.

DISCUSSION

The evaluated airborne concentration of mercury vapor does not necessarily reflect time-weighted exposure of the workers because they only approach the electrolysis cell as and when required to attend a specific job. Usually they move freely or stay in a resting place designated in the cell house. The results of spot determination of urinary mercury levels at best reveal the cumulative long-term exposure of individuals.

From the industrial hygiene point of view the factors attributing to prevalence of mercury vapor in the cell houses in excess of its TLV were as follows:

(a) Mercury spillages on the floor;

(b) The floors had rough surfaces with cracks wherein spilled mercury was trapped;

(c) Inadequate frequency and drainage facilities for flushing of the spilled mercury;

(d) The lids of the end boxes of the electrolysis cells were not properly sealed and in some cells the lid was missing;

(e) Only locations facing the wall openings had the advantage of natural ventilation.

The comparative results show that mean airborne and urinary mercury levels in the cell house of plant C are within the suggested TLV and BTLV. This was

TABLE 1
MERCURY LEVELS IN WORKROOM AIR OF CELL HOUSES AND URINE OF EXPOSED WORKERS

	Airborne concentrations (mg/m^3)			Urinary levels (mg/liter)			
Plant	Mean	Range	*n*	Mean	SD	Range	*n*
A	0.21	0.05–0.42	68	0.207	0.107	0.076–0.592	29
B	0.08	0.03–0.16	49	0.07	0.054	0.015–0.220	16
C	0.04	0.02–0.17	26	0.06	0.054	0.013–0.275	23

achieved by incorporation of control measures such as the provision of rubber linings to the lids of the end boxes, better housekeeping by washing and drainage facilities, and the floor surface being not too rough.

Control of Exposure

Based on the deficiencies observed in controlling the mercury vapors in the work environment of cell houses covered under the study, the following remedial measures have been suggested:

- The floors of the cell house should be improved to have a smooth and impervious surface coating;
- The mercury spillage on the floor should be constantly supervised and steps should be taken to remove the spillage by a portable suction device or flushing with water through a proper drainage and collection system;
- To contain mercury vapors the sealing of the lids of the end boxes should be improved by using a suitable gasket. Care should be taken that the end boxes are always covered;
- The locations deprived of adequate natural ventilation should be provided with a forced draft ventilation system;
- The best solution is to eliminate the use of mercury by changing to newly developed membrane cell technology for electrolytic production of sodium hydroxide and chlorine.

CONCLUSION

Uncontrolled movements of workers in the cell house limits the computation of correct time-weighted average exposure of individuals to mercury vapors by mere air monitoring in the work environment. Biological monitoring carried out by the spot sampling method may not reflect the exposure of the same day because of long-term storage of mercury in the kidney region (Magos *et al.*, 1973). However, in conjunction air and biological monitoring provides better overall exposure indices. The spot study undertaken highlights the magnitude of the mercury hazard in the chlor alkali plants and the significance of simple control measures in achieving the objective of prevention of the chronic toxic manifestations.

In order to assess chronic exposure levels of mercury vapors in the cell houses of chlor alkali plants, prolonged in-depth air and biological monitoring is required. Such studies would also provide sufficient data for the establishment of an interrelationship between workers' TWA to mercury vapor per shift and their urinary excretion levels to arrive at an authentic BEI.

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A Clinical, Biochemical, Neurobehavioral, and Sociopsychological Study of 190 Patients Admitted to Hospital as a Result of Acute Organophosphorus Poisoning[1]

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To study acute organophosphorus (OP) poisoning cases, 190 OP-intoxicated cases admitted to Civil Hospital, Ahmedabad, were investigated in depth. The group consisted of subjects ranging from 11 to 60 years of age, with the maximum number of cases in the age group 21–30 years and a male-to-female ratio of 2.1:1. Most of the subjects (71.61%) were partially educated, 24.2% of the cases were illiterate, and only 4.2% of the cases were highly educated. Socioeconomically, 21.1% of the subjects were of low economic status, 52.6% were low middle class, 16.8% were upper middle class, and only 9.5% were upper class. With regard to marital status of the subjects, 98 cases were married and 92 were unmarried. About 67.4% of the cases had the intention of committing suicide, 16.8% of the cases were the result of occupational exposure, and 15.8% of the cases were from accidental poisoning. Social and domestic problems (37.5%), marital friction (15.6%), financial stress (15.6%), love affairs (14.1%), job problems (10.9%), chronic illness (4.7%), and failure in examination (1.6%) were observed as the precipitating factors. Muscarinic manifestations such as vomiting (96.8%), nausea (82.1%), miosis (64.2%), excessive salivation (61.1%), and blurred vision (54.7%) and CNS manifestations such as giddiness (93.7%), headache (84.2%), disturbances of consciousness (44.2%), and typical pungent odor from mouth and clothes (77.9%) were the main presenting symptoms. Cardiac manifestations such as sinus tachycardia (25.3%), sinus bradycardia (6.3%), and depression of ST segments with T-wave inversion (6.3%) were observed electrocardiographically, with hypertension (10.5%) and muscular twitching in some (2.1%) cases. Biochemical changes such as albuminuria (12.6%) and azotemia (18.9%) with inhibition of acetylcholinesterase enzyme activity in blood were recorded in 78.9% of the cases. About 89.5% of the cases recovered completely, 4.2% of the cases absconded after partial recovery, and 6.3% of the cases died. The mortality rate (6.3%) depended on various factors such as the organophosphorus compound consumed, the amount ingested, the time interval for hospitalization, and the general health of the patient. Chances of recovery were higher when the patient was hospitalized at the earliest indication.

INTRODUCTION

Organophosphorus compounds are used extensively in India to control malaria and to increase production of agricultural commodities. They not only are powerful inhibitors of cholinesterase, but also act directly on cholinergic nerve endings. This combination of properties helps to explain why some of them are among the most toxic synthetic compounds known. These chemicals are beneficial to mankind due to their toxic properties, but they also pose a risk to nontarget organisms, including humans.

Direct exposure to pesticides is encountered by persons engaged in manufacture, formulation, transport, and application of pesticides. In addition, because of

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rapid industrial development, the shifting of people from rural to urban areas is increasing daily and is leading to overpopulation in cities. Unemployment and social, domestic, and economic problems are also increasing, ultimately creating psychological disturbances. Various stresses of this kind may force a person to take such drastic steps as to consume easily available poisons such as organophosphorus insecticides. Since 1963 there has been a steady increase in the incidence of organophosphorus poisoning cases in India. This insecticide is consumed by individuals for suicidal purposes owing to its low price, high toxicity, easy availability, etc. Hence, organophosphorus compounds are used most often for suicidal purposes in our country.

A case–control study was therefore initiated to evaluate in-depth factors responsible for the incidence of acute organophosphorus poisoning cases and to determine the intention of poisoning, the socioeconomic status of victims, the precipitating factors, the clinical and biochemical alterations, and finally the outcome of the treatment of patients admitted to the local civil hospital.

MATERIALS AND METHODS

A total of 190 organophosphorus poisoning cases, ages 15–55 years (mean 35.5 years) with a male-to-female ratio of 2.1:1 admitted to Civil Hospital, Ahmedabad, were included in this investigation.

A detailed history regarding age, sex, address, occupation, socioeconomic status, education, marital life, psychological problems, major illness, past hospitalization, family disturbances, symptoms observed during hospitalization, etc., was recorded in precoded proforma. A detailed occupational history was recorded for cases coming from industries.

The medical examination consisted of a physical examination including neurological examination. ECG changes in each subject were recorded. Diagnosis of the cases depended on history or evidence of exposure to organophosphorus compounds, signs and symptoms of poisoning, improvement of signs and symptoms after administration of drug, and inhibition of cholinesterase activity in blood. The severity of the poisoning was graded in three categories according to the criteria described by Driesbach (1983). These were as follows:

(a) Mild cases—Anorexia, headache, dizziness, weakness, anxiety, tremors of the tongue and eyelids, miosis, and impairment of vision.

(b) Moderate cases—Nausea, salivation, lacrymation, abdominal cramps, vomiting, sweating, slow pulse, and muscular tremors or fasciculations.

(c) Severe cases—Diarrhea, pinpoint and nonreactive pupils, respiratory difficulty, pulmonary edema, cyanosis, loss of sphincter control, convulsions, coma, and heart block.

Laboratory investigations were carried out in all the cases. A sample of venous blood was obtained from each individual for estimating (a) hemoglobin (g%), (b) total and differential count, and (c) erythrocyte sedimentation rate (ESR) using the Wintrobe method. Renal function test included physicochemical and microscopic examination of urine. Blood urea (mg%) was estimated using the Natelson (1957) method. Serum creatinine (mg%) was measured according to the method of Bonsnes and Taussky (1945). Serum electrolytes such as Na^+, K^+, and Cl^- were evaluated using a flame photometer.

Blood cholinesterase levels were measured only on the day of admission in all

the cases. Plasma and red blood cell cholinesterase activities were determined by the ASCH/DTNB procedure of Voss and Sachsse (1970) using microsamples of blood.

A chest X ray (P.A. view) and ECG were done routinely in all the cases.

All cases were treated with supportive measures. A total of 110 cases received atropine as a specific antidote, while 80 cases were treated with atropine and pralidoxime chloride (PAM) as a specific antidote. Daily follow-up was continued until the patient was released from the hospital.

Student's *t* test was used for statistical evaluation whenever necessary.

Table 1 presents the age and sex distribution patterns of the patients. The group comprised 190 patients, of which the males dominated at a ratio of approximately 2.1:1. The majority of the cases (54.8%) fall within the third decade. The incidence in the second decade was 29.6:30.7% male:female. Further decreases were observed in the fourth, fifth, and six decades. The youngest case in present study was 15 years of age, and the oldest was 55. Areawise distribution patterns showed that 74.7% of the cases were from urban areas, while only 25.3% of the cases came from rural areas.

Table 2 presents the educational status of the patients. This indicates that 71.6% of the cases were educated up to the Eighth Standard, 24.2% of the cases were totally illiterate, and 4.2% of the cases had higher education.

In Table 3, the socioeconomic status of these patients is given. It is evident from the table that 21.1% of the cases were from the lower class, 52.6% of the cases were of lower middle class, and 16.9% of the cases came from the upper middle class. Only 9.5% of the cases were from the upper classes.

Table 4 shows the source of poisoning. The data indicated that poisoning occurred due to suicidal intention in a majority of the cases (67.4%). Occupational exposure to organophosphorus compounds was seen in 16.8% of the cases. Accidental ingestion of organophosphorus compounds was responsible for poisoning in only 15.8% of the cases. No cases of homicidal poisoning were recorded in the present study.

Table 5 lists the data from 128 suicidal cases. Social and domestic problems (joint family quarrel with mother-in-law, bad habits of the husband) were the main precipitating factors (37.5%) for the suicidal cases, followed by marital friction (15.6%), financial stress (15.6%), love affairs (14.1%), job problems (10.9%), chronic illness (4.7%), and failure in examination (1.6%).

Clinically, muscarinic manifestations such as vomiting (96.8%), nausea (82.1%),

TABLE 1
AGE AND SEX DISTRIBUTION

	Male		Female	
Age in years	No.	Percentage	No.	Percentage
0–10	—	—	—	—
11–20	38	29.6	19	30.7
21–30	70	54.7	34	54.8
31–40	15	11.7	7	11.3
41–50	4	3.1	2	3.2
51–60	1	0.8	—	—
Total	128		62	

TABLE 2
EDUCATIONAL STATUS OF THE STUDY GROUP

Education	No. of cases	Percentage
Illiterate	46	24.2
Educated up to middle class	136	71.6
Highly educated	8	4.2
Total	190	

miosis (64.2%), excessive salivation (61.1%), blurred vision (54.7%), abdominal cramps (33.7%) and CNS manifestations such as giddiness (93.7%), headache (84.2%), and disturbances of consciousness (44.2%) were the predominant clinical manifestations in the present study. The typical pungent odor (77.9%) and nicotinic manifestations such as sinus tachycardia (25.3%), hypertension (10.5%), and muscular twitching (2.1%) were also observed among the subjects. Respiratory findings were suggestive of bronchospasm and collection of bronchial secretion or pulmonary edema was noted in 20.0% of the cases.

Muscarinic manifestations such as perspiration (8.4%), bradycardia (4.2%), hypotension (3.2%), cyanosis (2.1%), and CNS manifestations such as coma with absent deep tendon reflexes or planter extensors (6.3%), restlessness (3.2%), tremor (1.1%), and convulsions (1.1%) were also observed in a number of cases. Burning sensations in upper GI tract due to local irritation from organophosphorus compounds were found in 23.2% of the cases.

Most of the cases (70.6%) had mild grade poisoning, and 14.7% was recorded for each of the categories of moderate and severe.

Electrocardiographic changes are presented in Table 6. Sinus bradycardia (6.3%), sinus tachycardia (25.3%), and depression of ST segment with inversion of T-wave in 6.3% of the cases were recorded. All ECG changes reverted to normal, except persistence of ST segment depression and T-wave inversion in one (1.1%) case even after total recovery from poisoning.

Behavioral Effects

Decrements in alertness and memory, increased irritability, memory deficit, lethargy, and lack of energy were observed in 5.6% of the cases. During their recovery, 10 cases showed delirium combativeness, hallucinations, depression, or psychosis.

Biochemical Investigations

No deviation from normal values was observed in hematological examination of all the patients. Urine analysis revealed albuminuria in 12.6% of the cases and azotemia in 18.9% of the cases.

TABLE 3
SOCIOECONOMIC STATUS OF THE STUDY GROUP

Socioeconomic status	No. of cases	Percentage
Lower class	40	21.1
Lower middle class	100	52.6
Upper middle class	32	16.8
Upper class	18	9.5

TABLE 4
CATEGORIES OF OP POISONING CASES

Category	No. of cases	Percentage
Suicidal	128	67.4
Occupational	32	16.8
Accidental	30	15.8

Cholinesterase activity in whole blood, plasma, and RBCs was estimated for all patients on the day of admission. It is well known that estimation of ChE activity is an index of extent and severity of exposure and its toxicity to organophosphorus insecticides. Inhibition of ChE activity in 78.9% of the cases was observed. The depression of ChE activity was more pronounced in plasma than in RBCs, especially in mild and moderately exposed cases. In severely exposed cases depression in plasma as well as in RBCs ChE activity was observed.

DISCUSSION AND CONCLUSION

Organophosphorus insecticides are highly toxic to humans. Poisoning due to OP insecticides is steadily increasing in India. These pesticides are preferred for the purpose of suicide, due to their easy availability and potent toxicity.

In a series of 312 cases of acute poisoning reported by Singh *et al.* (1984), organophosphorus compounds were recorded as the poisons used for suicidal purpose in 19.23% of cases. Diazinon, an OPC, seems to be the choice in the majority of the cases. It has replaced barbiturates (Limaye, 1966; Balani, 1968). In the present study, Tick-20 (2% fenitrothion) was the most common pesticide used for suicidal purposes. The peak incidence of suicide as reported by Quinby (1964), Balani (1968), and Gupta and Patel (1968) was in the third decade of life followed by the second decade, whereas the incidences described by Vishwanathan and Shrinivasan (1962) were similar in both decades. In the present study the peak incidence was in the third decade (54.7%), followed by the second decade. Our observations corroborate well with the earlier reports mentioned above. The age group 21–30 years is the most critical period; this is when one is likely to face various problems that may lead to psychological stress and ultimately force a person to take drastic steps to end his life by consuming available poisons.

In the present study, males were more likely to attempt suicide than females

TABLE 5
PRECIPITATING FACTORS AMONG SUICIDAL CASES

Factors	Cases	
	No.	Percentage
Social and domestic problems	48	37.5
Marital friction	20	15.6
Financial stress	20	15.6
Love affairs	18	14.1
Job problems	14	10.9
Chronic illness	6	4.7
Failure in examination	2	1.6
Total	128	

TABLE 6
RESULTS OF ECG CHANGES

ECG changes	No. of cases	Percentage
Sinus tachycardia	48	25.3
Sinus bradycardia	12	6.3
Depression of ST segment and inversion of T-wave in lead (II, III, aVF)	12	6.3

(2.1:1). Similar observations were reported by Mutalik (1962), Gupta and Patel (1968), Mehta (1971), and Balani (1968), while Vishwanathan and Shrinivasan (1962) reported higher numbers of suicidal cases among females than among males.

With regard to educational status, the present study is comparable with the study of De and Chattarjee (1967), who reported 75% of the suicidal incidences in the poor class and the remaining 25% in the middle class. In the present study the incidence of poisoning in the middle class (both upper and lower) was 69.4% followed by 21.1% in the lower class. The incidence in upper class society was only 9.5%. The psychological trauma faced by middle and lower class people in daily life is likely the cause which leads to suicidal tendency and to the consumption of easily available poison.

The incidence of organophosphorus poisoning in married people is higher than that in unmarried people, as evident from this study. Similar findings have been reported by Mutalik (1962), De and Chattarjee (1967), Gupta and Patel (1968), Chhabra *et al.* (1970), and Mehta (1971). The organophosphorus compound was consumed by 67.4% of the cases with the intention to commit suicide. Occupational exposure was the source in 16.8% of the cases. Only 15.8% of cases were associated with accidental consumption in the present study. Similar findings were recorded by Mutalik (1962), De and Chattarjee (1967), Gupta and Patel (1968), Chhabra *et al.* (1970), and Mehta (1971); whereas Quinby (1964) reported that 50% of the cases resulted from occupational exposure, followed by 45.4% from accidental exposure, and only 4.6% cases of intentional suicide.

Domestic problems (37.5%), marital friction (15.6%), financial stress (15.6%), love affairs (14.1%), and job problems were the main precipitating factors for consuming poison, while chronic illness and failure in examination were recorded for fewer cases. Similar findings have been presented by Desai (1983) in his study.

Clinically, vomiting, giddiness, headache, nausea, typical pungent odor from mouth and clothes, miosis, excessive salivation, and blurred vision were the most prominent symptoms observed in this study. Mutalik (1962), Balani (1968), and Gupta and Patel (1968) also observed similar symptoms in their study. Also, disturbed levels of consciousness, abdominal cramps, tachycardia, pulmonary edema, and burning sensations in the upper GI tract were recorded in 33% of the cases in the present study.

With regard to gradation of the poisoning and its correlation with symptoms, 70.6% of the cases were of mild grades, while 14.7% of the cases were recorded for each category—moderate and severe. In the Kabrawala study (1971) 76.8% of the cases were severe poisoning and the remaining 23.2% were the moderate grade of poisoning, suggesting that the pattern of poisoning may differ from region to region, depending on the quality of life.

ECG abnormalities recorded in this study showed sinus bradycardia (6.3%), sinus tachycardia (25.3%), and depression of ST segment with inversion of T-wave in 6.3% of the cases. These changes may be due to muscarine action of the organophosphorus compound, on the one hand, and nicotine action, on the other hand, depending on the variation in exposure. Such changes have been reported earlier by Ottevanger (1976). Chhabra *et al.* (1970) reported that the bradycardia observed in these cases may be due to the effect of ChE inhibitors acting either directly on the myocardium and the conducting tissues or through a neurogenic mechanism.

The behavioral effects of exposure to organophosphorus compounds have been investigated in humans (Metcalf and Holmes, 1969; Durham *et al.*, 1965; Bowers *et al.*, 1964). Motor defects including tremors, muscular twitching, and muscular fasciculations have been found to result from acute organophosphorus poisoning (Namba *et al.*, 1971). Similar changes were observed in this study, suggesting that behavioral changes cannot be ignored in intoxication from OP insecticides. These observations, however, need to be substantiated by means of objective parameters such as electrophysiological changes so that they can be used for early detection of health impairment from exposure to OP compounds.

Biochemical changes such as albuminuria (12.6%) and azotemia (18.9%) were observed and were reversible in all cases. However, these changes suggest that the metabolic products of OP compounds excreted through the kidneys have a deleterious effect on this organ.

The measurement of blood ChE activity gives useful information on the clinical state of the persons poisoned by the OP compound. Absorption of a significant amount of an OP compound is indicated by depression of ChE in plasma and RBC. Acute poisoning manifestations generally occur only when more than 50% of serum ChE is inhibited. In the present study, plasma and RBC ChE activities were investigated only on the day of admission. The results showed a significant depression in ChE activity, suggesting that it is a very useful criterion of confirmation of OPC poisoning.

The outcome of this study was that 89.5% of the cases recovered completely and 4.2% of the cases absconded after partial recovery, while 6.3% of the cases died due to OP poisoning. The present study suggests that mortality rate depends on various factors such as the type of OP compound consumed, the amount ingested, the time interval of hospitalization, and finally the general health of the patient. Chances of recovery are greater when the patient is hospitalized at the earliest indication of poisoning.

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Neurobehavioral Changes among Workers in Some Chemical Industries in Egypt[1]

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This study investigates the long-term neuropsychiatric manifestations of single or combined chemicals: manganese; zinc phosphide; lead, mercury, and TNT; and pesticides among exposed industrial workers. We found that 75% of the exposed subjects as a whole and 50% of those exposed to each of Zinc phosphide and pesticides presented with more than one neuropsychiatric symptoms or signs. The main signs were mask faces, hyporeflexia, hyperreflexia, peripheral neuropathy, static tremors, radiculopathy, muscle weakness, mental changes, fasciculations and tremors, wasting, hypotonia, abnormal deep reflexes, and sensory hyposthesia. Neurological manifestations were confirmed by electromyography and their severity was related to the duration of exposure and confirmed as well by electroencephalography. These results are discussed and their implications highlighted.

INTRODUCTION

The fact that human exposure to industrial chemicals is an unfortunate concomitant of industrialized growth has been a well-recognized disadvantage of our industrialized world. In fact this exposure to chemical substances is becoming more pervasive and extensive in both developed and developing countries (Alleyne, 1990).

It is now a well-known fact that several industrial chemicals are neurotoxic and are capable of producing adverse neuropsychiatric manifestations. Evidence of the neurotoxic effects of occupational exposure to these chemicals has been repeatedly observed by several investigators (Johnson, 1990).

Manganese appears to give rise mostly to chronic intoxication associated with severe extrapyramidal symptoms of the type seen in Parkinsonism. Psychological disturbances are often present at the same time: emotional tendency or disorder, uncontrollable laughing and crying, and impulsive effective outbursts preceded at an earlier stage by general apathy, languor, and sleepiness. Manganese can also produce chronic behavioral toxicity in the form of "manganese madness" that resembles schizophrenia (Slater and Roth, 1986; Lipowski, 1990). Chronic lead poisoning results in a variety of neuropsychiatric symptoms and signs including peripheral neuropathy, ataxia, irritability, nervousness, insomnia, depression, and cognitive impairment (Gross, 1987).

Neurological manifestations of chronic mercury poisoning include ataxia, tremors, unsteady gait, and parasthesia of the hands and feet. Psychological symptoms are frequent; early ones are fatigue and lassitude: Sudden attacks of anger, in-

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creasing irritability, loss of interest, and emotional withdrawal, a syndrome referred to as erethism, may occur later (Gossel and Bricker, 1990).

The major toxicological manifestation of trinitrotoluene is methemoglobinemia. However, evidence also shows that long-term exposure can result in cognitive impairment (dementia) (Lipowski, 1990).

Zinc phosphide has been reported to produce psychomotor stimulation. Furthermore, the mortality rate is approximately 75% when the first manifestations of poisoning are restlessness, irritability, drowsiness, and stupor compared to 50% when nausea and vomiting are also present (Gossel and Bricker, 1990).

The neuropsychiatric manifestations due to pesticide exposure are numerous and variable from headache, depression, dizziness, blurred vision, tremor, and, in coordination with peripheral neuropathy, memory impairment, generalized weakness, convulsions, and coma (Lipowski, 1990).

In Egypt, several reports have recently emerged which focused on neuropsychiatric aspects of occupational exposure to a variety of industrial chemicals (El-Samra *et al.*, 1984; El-Batanouni *et al.*, 1985; Amr *et al.*, 1986; Amr, 1990a,b). In this article we attempt to present an overview of these Egyptian studies analyzing their results and discussing their implications on further research.

MATERIALS AND METHODS

The population studied consisted of 479 subjects who were occupationally exposed to different industrial chemicals. The exposed groups were compared with nonexposed subjects who were studied as controls. Both groups were subjected to the following procedures:

1. Detailed history taking including personal, occupational and present and past medical histories.
2. Neurological and psychiatric clinical assessment.
3. Fundus examination.
4. Laboratory tests which differed according to the chemical nature of exposure as follows: (a) Manganese: Blood Mn levels using the atomic absorption spectroscopic methods. (b) Lead, mercury, and trinitrotoluene: Hemoglobin (HB) concentration, and red and white blood cell (RBC and WBC) counts. Furthermore, the atmospheric concentration of lead was determined and electroenephalography (EEG) was done. (c) Zinc phosphide: EEG and electromyography (EMG) in some cases. (d) Combination of pesticides: pesticide blood level, choline esterase enzyme levels, and EEG and EMG in some cases. The number, age, and duration of exposure of the subjects as well as other parameters related to each specific chemical substance are shown in Table 1.

RESULTS

The frequency of symptoms and signs among exposed groups was more than threefold that of the control groups. These manifestations were more frequent with the longer duration of exposure especially polyneuropathy (Table 2).

HB concentration and RBC and WBC counts were significantly decreased among subjects exposed to Pb, Hg, and TNT, their blood lead (Pb-B) was <50 g/dl (82 subjects) and >50 μg/dl (22 subjects).

EEG tracings were abnormal (borderline, mild, moderate, and marked) in 17.4% of those exposed to Zn and 15% of those exposed to combined pesticides, it was 1% among the control subjects. EMG studies showed evidence of partial

TABLE 1
STUDY POPULATION EXPOSED TO MN; COMBINED PB, HG, AND TNT; ZINC PHOSPHIDE, AND COMBINED PESTICIDES

Parameters	Manganese	Combined Pb, Hg, and TNT	Zinc phosphide	Combined pesticides
Number	47	104	46	300
Age	26–59	25–60	28–46	18–60
Range	36.8 ± 7.4	—	—	—
Mean	—	—	35.8	—
Duration of exposure (years)	2–30	5–30	5–21	2–35
Range	13.6 ± 6.8	—	11.3	—
Mean	—	—	—	—
Blood level	—	—	—	—
Range	7–15[a] μg/dl	Pb-B[b] 30.2–74.6 μg/dl	—	2/3[c]
			—	—
Mean	11.12 ± 2.16	43.14 + 10.14 Pb-A (μg/m^3)	—	—
Air of work place	6.82–30.21 mg/m^3	200 (in), 70–80 (out) (TWA 140)	—	—

[a] Mn blood level of controls = 3.05 + 0.89 μg/dl.
[b] Pb blood level of controls = 22.5 ± 7.2 μg/dl.
[c] 2/3 of the exposed subject's blood was contaminated with one or more pesticides.

denervation of the anterior tibial and flexor digiti minimi muscles in 2 of 30 workers exposed to Zp and in 25 of 100 workers exposed to combined pesticides.

Cholinesterase enzyme (ChE) was inhibited among 57% of rural subjects (group I) (levels: 51.3 ± 10.1%), 24% of urban subjects (group II) (level: 60.2 ± 11.2%), and only 7% among the controls (level: 65.2 ± 12%).

The level of ChE inhibition is related to the duration of exposure. For those subjects exposed more than 10 years it was 41.1% ± 10.2 in group I and 50.3 ± 11.1 in group II.

Depressive neurosis was significantly higher ($P < 0.05$) among exposed subjects and the commonest symptoms were irritability, insomnia, and erectile dysfunction.

Among subjects with long-term exposure (>20 years), the frequency of psychiatric disorders and symptoms was markedly higher and its significance varied between $P < 0.05$ for weeping to $P < 0.001$ for irritability.

DISCUSSION

In this work, we attempted to provide an overview of the research on clinical neuropsychiatric manifestations of some industrial chemicals in Egypt. We have presented evidence of the potential neurotoxic effects of a variety of chemical substances.

Workers exposed to Mn complained of headache, anxiety, numbness, impotence, and dizziness. Such vague poisoning symptoms are described as an early and prodromal stage of chronic Mn poisoning. Furthermore, in the intermediate and established stages of Mn poisoning, signs of extrapyramidal dysfunction such as mask faces, tremors, and exaggerated deep reflexes were observed, thus supporting the notion of Mn-induced Parkinsonian-like syndrome (Slater and Roth, 1986). The prevalence of Mn poisoning was correlated with both Mn concentration in air and the duration of exposure. Blood levels of Mn were poorly correlated

TABLE 2
FREQUENCY DISTRIBUTION OF THE NEUROPSYCHIATRIC SYMPTOMS AND SIGNS AMONG THE STUDIED POPULATION

Parameters	Mn $n = 41$	Pb, Hg, and TNT $n = 104$	Zp $n = 46$	Pesticides $n = 300$	Nonexposed $n = 300$
Symptoms					
Headache	29.8	15.4	24.0	10	8
Impotence	19.1	45.2	15.0	18.87	3
Numbness and parasthesia	12.8	60.5	2.2	30.33	11
Anxiety and irritability	12.8	4.8	19.6	30	10
Muscle weakness and easily fatigued	8.5	36.5	9.0	0	3
Tremors	8.5	7.7	0.0	15.67	2
Fear of poisoning	0.0	0.0	87.0	0	0
Other symptoms	10.6	15.3	9.0	2	6
Symptom free	27.7	21.2	13.0	30	80
Signs					
Abnormal reflexes	38.9	8.7	23.9	33.3	4
Hyposthesia (peripheral)	31.9	66.3	19.6	53	7
Mask faces	12.8	0.0	0.0	0	0
Muscle weakness and static tremors	10.6	40.4	6.5	15	2
Fundus changes	8.5	12.5	0.0	20	6
Psychiatric changes	0.0	0.0	13.0	50	12
Constricted pupil	0.0	0.0	0.0	9.3	0
EEG Change	0.0	0.0	17.4	15	1
Other	2.1	2.9	13.0	0.67	1
Sign free	38.3	28.8	54.4	40	76

with the clinical picture and are used only for supplementing the clinical diagnosis (World Health Organization, 1981).

The close relationship between Mn and Fe metabolism may explain the individual susceptibility in chronic Mn poisoning. Mn intestinal absorption in anemic subjects was found to be twice that of normals (mean *et al.*, 1974).

Regarding neurotoxic Pb, Hg, and TNT, our findings add further evidence of the potential and synergestic effects of these compounds. Workers exposed to them complained of symptoms and signs reminiscent of the syndrome produced by Hg and know as erethism. Furthermore, peripheral neuropathy (peripheral hyposthesia and distal muscle weakness) was also observed, a well-known manifestation of chronic lead toxicity. Hematological changes reported in this study are to be expected since Pb and TNT are well known to produce anemia and methemoglobinemia (Gossel and Bricker, 1990).

Our findings with zinc phosphide are particularly striking. The high frequency of fear of poisoning supports the hypothesis that occupational exposure poses a heavy psychological stress and is also associated with frequent signs of anxiety. Other manifestations observed were impaired attention and memory, easy fatiguability, and psychomotor hyperactivity. Results are similar to those of Zipf (1967).

TABLE 3
FREQUENCY DISTRIBUTION OF PSYCHIATRIC DISORDERS AND SYMPTOMS AMONG SUBJECTS EXPOSED TO COMBINED PESTICIDES AND THEIR CONTROLS

Parameter	Exposed $n = 208$	Control $n = 72$
Psychiatric disorders		
Depressive neurosis	19.2	6.9
Situational and reactive depression	19.2	16.7
Erectile dysfunction	5.3	2.7
Others	6.3	5.6
None	50.0	68.1
Symptoms		
Irritability	33.7	13.1
Depression	33.2	25.0
Insomnia	25.0	16.7
Erectile dysfunction	26.9	4.2
Weeping	16.3	18.1
Headache	10.8	8.3

In addition, peripheral neuropathy, both mixed and sensorimotor, was also detected.

The high incidence of polyneuropathy we reported in pesticide-exposed workers is consistent with the repeated reports of organophosphate-delayed neuropathy (Gossel and Bricker, 1990). Regarding the psychological aspects of pesticide exposure which have not been studied extensively (Otto *et al.*, 1990) we have demonstrated ample evidence of increased psychiatric morbidity among pesticide-exposed subjects in the form of chronic long-standing depression of moderate severity, with irritability, depression, headache, and erectile dysfunction as the most common symptoms.

Our results have demonstrated the serious and considerable magnitude of neurobehavioral changes associated with exposure to neurotoxic industrial chemicals in Egypt. This could be related to several factors among which are type and duration of exposure (our subjects were workers in manufacturing facilities with consequent chronic cumulative exposure) as well as safety measures whether general or individual (e.g., adequate ventilation and appropriate protective clothing) which were deficient or at least not fully applied in the case of our studied subjects. However, these results obviate the need for more extensive epidemiological studies on other high-risk populations, e.g., pesticide applicators.

Another important point worth considering is that of possible mechanisms underlying the neuropsychiatric changes observed in our studies. It is very difficult to distinguish between neuropsychiatric changes mainly due to direct toxic exposure and those due to indirect and more complex disturbances. For example impotence in the case of pesticides might be caused by pudendal nerve neuropathy or psychogenic due to associated depression. Headache in the case of zinc phosphide might be caused by central nervous system dysfunction as shown by EEG or a combination of anxiety and fear of poisoning. Lever injury due to endemic bilharzial infestation among Egyptian workers results in failure or delay in detoxification of these chemical agents with consequent earlier development of their cumulative effects.

Lipowoski (1990) stated that neuropsychiatric effects of potentially neurotoxic compounds are generally poorly defined in the literature. Nonspecific terms such as "mental effects" and behavioral "changes" are commonly used and mean little as no attempt is usually made to define them and to specify criteria for their application. This area needs more focused research to identify the exact nature of these neuropsychiatric manifestations particularly, because they are very common in early stages of intoxication, e.g., in Mn and pesticide exposure. By recruiting a joint team of psychiatrists and neurologists in our research on pesticide exposure we have been able to identify more clearly the nature of induced neurobehavioral changes. However, more precise diagnostic tools, e.g., neuropsychological tests to assess and accurately localize underlying cognitive impairment, are needed.

CONCLUSIONS

Our results have demonstrated the serious and considerable magnitude of the neurobehavioral changes associated with industrial neurotoxins in developing countries. This could be related to the inefficient preemployment and periodic medical examinations as well as lack of safety measures whether general or individual and the type and duration of exposure. However, those results obviate the need for more extensive epidemiological studies especially on the high-risk population exposed to chemicals.

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Occupational Exposure to Neurotoxicants: Preliminary Survey in Five Industries of the Camaçari Petrochemical Complex, Brazil[1]

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The Camaçari Petrochemical Complex (CPC) is the biggest and most important industrial complex of the northeastern region of Brazil. At present, its 54 companies employ directly and indirectly about 50,000 people. Used there as solvent and raw material, compounds such as benzene and its homologues *n*-hexane, haloalkanes, and some alcohols have as their prime targets the central and peripheral nervous systems. Despite widespread use of these chemicals, the workers are little aware of their toxicity, and the evaluation of exposure to them has only recently become a worrisome issue. This paper discusses the contamination of occupational environments in some industries of the CPC, as well as the neurobehavioral impairment that could be found among their workers.

INTRODUCTION

The Camaçari Petrochemical Complex (CPC) includes industries producing a range of intermediates for plastics, synthetic fibers, detergents, fertilizers or pharmaceutical goods, and end products synthesized from petroleum derivatives and natural gas. (Perfil das Empresas, 1988).

Construction of the biggest Brazilian petrochemical complex began in 1971 in the Bahia State in an area covering 243 km^2 in the municipalities of Camaçari and Dias d'Ávila, both within the metropolitan area of Salvador (Plano Diretor, 1974). The official statistics reveal that in 1988 there were about 50,000 directly and indirectly employed workers in the 54 companies then in operation, with 10 others in construction and the settlement of 4 more already approved. (Perfil das Empresas, 1988).

This paper presents a survey of work conditions within five petrochemical industries of CPC in order to provide basis for evaluation of workers exposed to neurotoxicants.

NEUROTOXICANTS

It is well known that a number of chemical agents are potentially neurotoxic, producing adverse effects on the central and peripheral nervous systems. The

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mechanism of their action is not yet well understood, although their effects are easily recognized in severe intoxication. Cases of poisoning due to carbon monoxide, carbon disulfide, lead, or aromatic hydrocarbons are documented in the literature (Blum and Manzo, 1985; Echeverria *et al.*, 1989; Encyclopedia of Occupational Health and Safety, 1985; Klaassen *et al.*, 1986; MacFarland, 1986) and are still part of the reality encountered by workers in this country.

The early phase of intoxication caused by these substances, especially when resulting from long-term exposure to a low concentration, is marked by vague and subjective symptoms such as anxiety, irritability, headaches, fatigue, lethargy, or depression. For this reason, there is considerable chance of these subtle effects remaining undetected or being incorrectly attributed to other causes. On the other hand, the brain has an extraordinary capacity to supplant deficiencies or to adapt functionally to new lesions. This means that when impairments finally become apparent compensatory brain mechanisms have already been damaged (Gilioli and Cassitto, 1983; Klaassen *et al.*, 1986; WHO, 1986).

Anger and Johnson (1985) listed more than 830 substances reported as altering behavior or neurological functions. Anger (1984) stated that 29% (167) of 588 chemicals found in the "Threshold Limit Values of Chemical Substances and Physical Agents in Workroom Environment," published by the ACGIH in 1982, have exposure limits based, at least in part, on direct neurobehavioral effects or on factors associated with the nervous systems (WHO, 1986).

Potentially neurotoxic substances handled in the CPC were selected for this paper from their list of raw materials and final products. Some secondary products, solvents, and catalysts were extracted from Bergamaschi *et al.*, (1983) and Candura. Actually, the majority of substances used in this complex exerts some kind of action on the central and peripheral nervous systems. Some of these chemicals, however, must be excluded because they do not prove to have accentuated neurotoxicity or, despite their toxicity, their physical and chemical properties would hardly cause noxious effects under the conditions of medium or long-term exposure at lower concentrations in open workplaces that are found in petrochemical plants (Blum and Manzo, 1985; Encyclopedia of Occupational Health and Safety, 1983; Gilioli and Cassitto, 1983; Gosselin *et al.*, 1984; Klaassen *et al.*, 1986; MacFarland, 1986; Windholz *et al.*, 1983). These include:

- Physical asphyxiant and anesthetic gases, such as nitrogen, methane, ethene, propene, or butene;
- Hydrocarbons and alcohols with high molecular weight, including glycols;
- Pharmaceutical products;
- Catalysts, generally inorganic or organometallic compounds, rarely in direct contact with workers;
- Sodium cyanide, despite its toxicity, given its solid state;
- Synthetic polymers, although they can eliminate toxic and additives under some circumstances;
- Nitriles that liberate cyanide *in vivo*, such as acrylonitrile, because of the probable predominancy of local irritant rather than systemic neurotoxic action in occupational exposures.

It has not been possible to analyze the chemical constitution of all pigments, stabilizers, and other additives because details of production processes are not available.

The neurotoxicants present in large amounts in the CPC are, obviously, organic compounds. Aromatic hydrocarbons with low molecular weight, plastic monomers such as vinyl chloride, and some alyphatic alcohols such as methanol and butanol are the most frequently used. Volatile alyphatic hydrocarbons are of more limited use (Perfil das Empresas, 1988). For the present topic *n*-hexane is the most interesting: it has a central depressive action and causes peripheral polyneuropathy through one of its metabolites, 2,5-hexanedione (Governa *et al.*, 1987; Klaassen *et al.*, 1986).

Several references correlating toluene with neurobehavioral alteration have been found (Echeverria *et al.*, 1989; Olson *et al.*, 1985; WHO, 1985), and also for styrene (Flodin *et al.*, 1989; WHO, 1983), largely used in the production of thermoplastics. Unfortunately, this correlation is not always evident for other substances. Neurobehavioral impairment is often attributed to exposure to mixtures of varying composition, referred to generically as "solvents" (Fidler *et al.*, 1987; Hagstadius *et al.*, 1989; Hawkes *et al.*, 1989; Maizlish *et al.*, 1987; Maroni and Barbieri, 1988; Mikkelsen *et al.*, 1988; Orbaek *et al.*, 1988; Spencer and Schaumburg, 1985; Stollery *et al.*, 1988; Van Vliet *et al.*, 1989). It is not clear, therefore, whether all components of these solvents have neurotoxic action, nor is the extent clear of each of their effects (Triebig *et al.*, 1989; Spencer and Schaumburg, 1985).

Twenty of the 54 industries in the CPC deal with the neurotoxicants already selected (Table 1). More rigorous screening would only be possible after evaluation *in loco* of the exposure conditions of workers. Regrettably, some companies did not allow research to be conducted in their plants.

TABLE 1
ORGANIC NEUROTOXICANTS HANDLED IN THE CAMAÇARI PETROCHEMICAL COMPLEX

	Basic petrochemical	Chemical and petrochemical intermediate											Final petrochemical			Fine chemical			
Chemical	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Benzene	X			X		X		X	X		X					X			
Toluene	X	X		X		X	X											X	
Xylenes	X						X		X										
Cumene	X																		
Ethylbenzene				X															
Styrene				X										X					
Nitrochloro-benzene																X			
Cyclohexane						X													
o-Hexane														X					
Chlorinated hydrocarbons												X			X	X			
Methanol	X		X		X		X			X						X		X	
Ethanol																			X
Isopropanol																X			
Butanols		X									X								

In addition to chemical and petrochemical companies, the CPC has a copper metallurgy, where inorganic toxicants such as lead, selenium, or arsenic oxides and salts are handled (Blum and Manzo, 1985; Gilioli and Cassitto, 1983). In addition, there is a center for the treatment of effluents (CETREL) and seven more companies are responsible for maintenance, services, and industrial construction (Perfil das Empresas, 1988). The exposure of their workers depends upon workplace atmosphere, effluent, and residue contamination levels of producing industries.

OCCUPATIONAL RISK SURVEY

This preliminary risk survey was conducted within five companies through direct observation and standard questionnaire applied to supervisors and engineers. A is the only basic petrochemical industry of CPC; E produces methanol from natural gas and carbon dioxide; F transforms benzene or cyclohexane into caprolactam; at G, the plant producing dimethyl phthalate was studied; and O is a high density polyethylene industry (Perfil de Empresas, 1989). Only at F were contamination levels of benzene, cyclohexane, and toluene determined in some areas (Carvalho *et al.*, 1991).

Situations in which the workplace exposure was observed were focused as follows:

(a) Unloading liquid products from trucks—The trucks are connected manually to equipment with a hose. After unloading, the permanence of chemicals inside the hose was observed. Residues, at E and O, were poured into open buckets left nearby.

(b) Loading trucks—At E, methanol was loaded using manually controlled pumps, and the filling level was checked looking inside the tank of the truck. A similar situation was observed at F: the tank remained open to equilibrate the inner pressure during a benzene transference. Unlike in an unloading operation, the tank opening was, in this case, an emission source. The level of benzene found at a single point approximately 2 m from the truck during the unloading process was 10 ppm. None of the companies visited have vapor collecting systems coupled to the tanks during these activities.

(c) Gauging storage vessel levels—At E and G, some storage vessels were checked by immersing a tape measure through an opening at the top.

(d) Collecting samples—Lab technicians as well as operators collected samples manually for quality control.

(e) Maintenance in equipment containing chemicals—This activity usually involves longer permanence of workers in proximity to the emission source. At F, up to 44 ppm of benzene and 125 ppm of ciclohexane in the air were detected by personal sampling during the changing of packs. Measurement conducted inside or in front of manholes opened after inadequate washing of equipment were identified as another source of contamination.

Only some workers wore respirator masks during these activities. The use of an inorganic chemical filter instead of the correct one for organic vapors was detected during the survey.

Small leakages and fugitive emissions, although not relevant to total production, represent important risk factors. At F, the make-up of benzene used as a solvent was estimated to be about 14 kg/ton of final product, corresponding to 675 liters per day of benzene. This loss resulted from the sum of chemical incineration, discharge into the liquid organic effluent, or liberation into the workplace atmosphere. Benzene levels in the air between 1.5 and 6.5 ppm were found at the unit where this solvent was utilized. It seemed that its loss is more under control where it is used as raw material: much lower levels, from 0.05 to 2.00 ppm, were found over same period as above (Carvalho *et al.*, 1991).

The odor of hexane was observed around the storage vessel at O and, likewise, of toluene near opened ducts receiving discharged water from equipment at F. Leakage of methanol vapors or dripping of this liquid was observed at E. The supervisor that guided the visit to this company stated that such losses were irrelevant.

At E and F no corrective intervention was taken when strong odors of nonprimary irritant chemicals contaminated the air.

No chemical risk survey has been conducted by the industries visited, although noise and illumination mapping were available. Environmental monitoring data were scattered, activities with increased exposure risks were not described, and high exposure events were not registered.

Results from biological monitoring have been occasionally used to detect some cases of occupational disease or acute intoxication, but have not contributed to workplace hygiene.

DISCUSSION

There are not sufficient data available for an evaluation of past and present exposure in the companies visited. There is also no indication that different situations would be found in other industries. For this reason, the direct observations made during the visits to the CPC companies are extremely important.

Industrial hygiene is seldom practiced within the CPC. Not only are experts in this field rarely part of the crew—some of their activity is executed by safety engineers—but also there is a real lack of specialized knowledge needed for the monitoring and control of workplaces.

Available data suggest that, at present, contamination of occupational environments in the CPC is commonly below threshold limit values on normal production days. However, weather conditions unfavorable for pollutant dispersion and accidents involving chemicals concur to raise frequently the exposure of all workers. Some activities identified in this survey periodically cause higher exposures. Maintenance personnel are especially subject to this risk.

Presumed neurobehavioral impairment among workers within the complex would therefore be caused not only by medium or long-term exposure to lower concentrations of chemicals, but also to single or multiple episodes of high-level contamination. The chemicals involved can act either predominantly alone, as at E, or in the presence of other neurotoxicants, such as at F. Noise, always present, can be a concurrent cause.

The evidence presented in this study makes clear the urgent necessity of monitoring programs and corrective hygiene in industry and of further research into diverse risk factors which impair health in the occupational environment.

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Comparison of the Neurotoxicity of Several Chemicals Estimated by the Peripheral Nerve Conduction Velocity in Rats[1]

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INTRODUCTION

The peripheral nerve neurotoxicities of lead, manganese, 2-propanol, and styrene, commonly used in industry, were studied. Lead affects both the central and peripheral nervous systems. When peripheral nerves are seriously damaged by lead, paralysis of the forearm extensors results because of radial nerve palsy, and dropped wrist occurs (Committee on Biologic Effects of Atmospheric Pollutants, 1972). Although severe cases are rarely observed these days (Horiuchi, 1965), a change in peripheral nerve conduction velocity is once more drawing attention as a measure of the subclinical effects of lead (Bergamini and Sibour, 1960; Catton *et al.*, 1970; Zielhuis, 1977; Araki, 1980). Manganese damages the central nervous system; a major symptom of chronic manganese intoxication is Parkinson syndrome (Rodier, 1955; Horiguchi *et al.*, 1966; Horiuchi *et al.*, 1970). Effects on the peripheral nervous system have also been reported (Sano *et al.*, 1982; Matsuura *et al.*, 1980). 2-Propanol and styrene inhibit the central nervous system, with a toxicity that is common to organic solvents (Rowe and McCollister, 1982; Hamilton and Hardy, 1983). Reportedly, they also influence the peripheral nervous system (Nakaseko *et al.*, 1991; Ito *et al.*, 1979; Seppalainen, 1978b).

Although these four chemicals are widely used in industry, their effects on the peripheral nervous system have not been fully elucidated. In the present study, to clarify their effects on the peripheral nervous system, peripheral nerve conduction velocity was measured in the caudal nerves of rats, and the influences of these chemicals on conduction velocity were compared.

METHODS

Lead

Forty male Jcl-Wistar rats 8 weeks of age were divided into three groups of 12–13 rats each. Twelve rats in the 1% lead group were given solid food containing sufficient lead acetate to give a 1% lead concentration. Twelve rats in the 5% lead group were fed solid food containing enough lead acetate for a 5% lead concentration. Thirteen rats in the control group were given ordinary solid food. The rats

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were given these feeds from 8 to 36 weeks of age and then fed ordinary solid food from the end of lead administration to 56 weeks of age. Water was freely available.

The conduction velocity of the caudal nerve was measured by the method of Ono *et al.* (1979). The motor nerve conduction velocity (MCV) and sensory nerve conduction velocity (SCV) were measured when the rats were 8, 12, 20, 28, 36, 40, 44, 48, 52, and 56 weeks old.

Manganese

Forty Jcl-Wistar rats at 8 weeks of age were divided into four groups, each containing 10 rats. The three manganese administration groups were given 0.5, 2, or 8 mg/kg of manganese chloride ($MnCl_2 \cdot 4H_2O$) dissolved in physiological saline by the intraperitoneal route twice weekly for 24 weeks. The control group was given only physiological saline in the same way. All animals had free access to food and water.

Caudal nerve conduction velocity was measured every week for 8 weeks following the start of manganese administration, then every 2 weeks until the end of administration, and also at 1, 2, and 4 weeks after the end of administration.

2-Propanol

Thirty Jcl-Wistar rats at 8 weeks of age were divided into three groups of 10 rats each. The two experimental groups were exposed to 1000 and 8000 ppm of 2-propanol for 8 hr/day 5 days/week for 20 weeks in an exposure chamber for small animals (Teramoto and Horiguchi, 1985). The control group was treated with air in the same way. During exposure, the rats were provided with water freely but were given no food; the rest of the time, they had free access to food and water. Caudal nerve conduction velocity was measured at 8, 10, and 12 weeks of age and thereafter every 4 weeks to 40 weeks of age.

Styrene

Thirty Jcl-Wistar rats at 8 weeks of age were divided into three groups, each containing 10 rats. Rats in two of the groups were exposed to 200 or 2000 ppm of styrene for 8 hr/day 5 days/week for 32 weeks, in an exposure chamber for small animals (Teramoto and Horiguchi, 1985). Rats in the control group were treated with air in the same way. During exposure, rats were provided with water freely but were given no food; the rest of the time, they had free access to food and water. The caudal nerve conduction velocity was measured at 8, 10, and 12 weeks of age and thereafter every 4 weeks to 40 weeks of age.

All rats were weighed three times a week throughout the experimental period.

Data obtained in these studies were analyzed by Student's t test.

RESULTS

Lead

Changes in mean body weight, MCV, and SCV are shown in Table 1. Body weight decreased with lead administration. The body weight of rats in the 1% lead group was significantly lower than that in the control group up to 20 weeks after the end of lead administration ($P < 0.05$). Body weight of rats in the 5% group was also lower than that in the control group ($P < 0.01$). In the control group, the conduction velocities rose with age but reached a plateau at about 24–26 weeks of

TABLE 1
CHANGES IN BODY WEIGHT, MOTOR NERVE CONDUCTION VELOCITY (MCV), AND SENSORY NERVE CONDUCTION VELOCITY (SCV) DURING AND AFTER LEAD ADMINISTRATION ORALLY

Group	Lead (%)	Body weight				MCV				SCV			
		B	M	E	C	B	M	E	C	B	M	E	C
1%	1	↘	↘	→	→	→	→	→	→	→	→	→	→
5%	5	↓	↓	↓	↓	↓	↓	↘	→	↓	↓	↓	→

Note. B, beginning of administration (8–16 weeks of age); M, middle of administration (20–28 weeks of age); E, end of administration (28–36 weeks of age); C, cessation of administration; →, the same value as the control group; ↓, value decreased vs the control group; ↘, value decreased slightly vs the control group.

age. There were no significant differences in the conduction velocities between the 1% lead and the control groups throughout the experimental period. MCV and SCV for the 5% lead group were significantly lower than those for the control group ($P < 0.01$). The decrease in SCV was greater than the decrease in MCV. The nerve conduction velocities returned to the control levels 4 weeks after the end of lead administration.

Manganese

Changes in mean body weight, MCV, and SCV are shown in Table 2. There was no significant difference in body weight between the 2-mg and control groups. Body weight of rats in the 8-mg group was 10% lower than that of the control group. MCV levels of rats in the 2-mg and control groups were comparable throughout the experimental period. In the 8-mg group, MCV rose significantly 3 weeks after the start of manganese administration. SCV decreased slightly during manganese administration in the 8-mg group ($P < 0.01$), but returned to the control level after the end of the administration period.

2-Propanol

Changes in mean body weight, MCV, and SCV are shown in Table 3. There was no significant difference in body weight between rats in the 1000-ppm and the control groups. Body weight of rats in the 8000-ppm group was lower than that for rats in the control group in the early stage ($P < 0.01$) and middle stage ($P < 0.05$)

TABLE 2
CHANGES IN BODY WEIGHT, MOTOR NERVE CONDUCTION VELOCITY (MCV), AND SENSORY NERVE CONDUCTION VELOCITY (SCV) DURING AND AFTER MANGANESE ADMINISTRATION INTRAPERITONEALLY

Group	Mn (mg/kg)	Body weight				MCV				SCV			
		B	M	E	C	B	M	E	C	B	M	E	C
0.5 mg/kg	0.5	→	→	→	→	→	→	→	→	→	→	→	→
2 mg/kg	2	→	→	→	→	→	→	→	→	→	↘	↘	→
8 mg/kg	8	↓	↓	↓	→	↗	→	→	→	→	↘	↘	→

Note. B, beginning of administration (8–12 weeks of age); M, middle of administration (14–24 weeks of age); E, end of administration (26–32 weeks of age); C, cessation of administration; →, the same value as the control group; ↓, value decreased vs the control group; ↘, value decreased slightly and or sometimes vs the control group; ↗, value increased sometimes and slightly vs the control group.

TABLE 3
CHANGES IN BODY WEIGHT, MOTOR NERVE CONDUCTION VELOCITY (MCV), AND SENSORY NERVE CONDUCTION VELOCITY (SCV) DURING AND AFTER 2-PROPANOL EXPOSURE

Group	2-Propanol (ppm)	Body weight				MCV				SCV			
		B	M	E	C	B	M	E	C	B	M	E	C
1000 ppm	1000	→	→	→	→	→	→	→	→	→	→	→	→
8000 ppm	8000	↓	↘	→	→	→	→	↗	→	→	↗	↗	→

Note. B, beginning of administration (8–12 weeks of age); M, middle of administration (16–20 weeks of age); E, end of administration (24–28 weeks of age); C, cessation of administration; →, the same value as the control group; ↓, value decreased vs the control group; ↘, value decreased slightly vs the control group; ↗, value increased slightly vs the control group.

of the exposure period. MCV levels in the 1000-ppm group and the control group were comparable throughout the experimental period. In the 8000-ppm group, MCV increased slightly but significantly during exposure only ($P < 0.05$). SCV also increased significantly in the 8000-ppm group ($P < 0.01$) during exposure. The conduction velocities both became normal again after the end of the administration period.

Styrene

Changes in mean body weight, MCV, and SCV are shown in Table 4. There was no significant difference in body weight between rats in the 200-ppm and the control groups. Body weight of rats in the 2000-ppm group was lower than that in the control group in the middle stage ($P < 0.05$) and last stage ($P < 0.05$) of the exposure period. MCV levels of rats in the 200- and 2000-ppm groups were comparable with the MCV levels of rats in the control group throughout the experimental period. SCV decreased slightly but significantly during exposure in the 2000-ppm group ($P < 0.05$). The conduction velocity became normal after the end of the administration period.

DISCUSSION

Changes in Mean Body Weight and Caudal Nerve Conduction Velocity

The nerve conduction velocity increased with age in the control groups in all four experiments and reached a plateau at 24–26 weeks of age. There is a possi-

TABLE 4
CHANGES IN BODY WEIGHT, MOTOR NERVE CONDUCTION VELOCITY (MCV), AND SENSORY NERVE CONDUCTION VELOCITY (SCV) DURING AND AFTER STYRENE EXPOSURE

Group	Styrene (ppm)	Body weight				MCV				SCV			
		B	M	E	C	B	M	E	C	B	M	E	C
200 ppm	202	→	→	→	→	→	→	→	→	→	→	→	→
2000 ppm	1829	↓	↓	↓	→	→	→	→	→	→	↘	→	→

Note. B, beginning of administration (8–16 weeks of age); M, middle of administration (19–30 weeks of age); E, end of administration (31–40 weeks of age); C, cessation of administration; →, the same value as the control group; ↓, value decreased vs the control group; ↘, value decreased slightly vs the control group.

bility that the nerve conduction velocity changes with changes in body weight. However, Misumi *et al.* (1979) reported that the caudal nerve conduction velocity was not affected even in animals with two-thirds the body weight of the control animals in a diet restriction experiment using 13-week-old Sprague–Donryu rats. Similarly, no correlation was found between body weight and the caudal nerve conduction velocity in rats of the same age in any of our experiments.

Heavy Metals (Lead and Manganese)

Little work has been done on the effects of lead on peripheral nerve conduction velocity in animals, other than Fullerton's study (1966) of guinea pigs, Hopkins's report (1970) on baboons, and a study by Ohnishi *et al.* (1977) using rats. Fullerton and Ohnishi *et al.* observed decreases in conduction velocity, but Hopkins did not. No work has been done to identify the behavior of conduction velocity with the passage of time. In our study, there were no significant differences in the nerve conduction velocity between the 1% lead and the control groups during the lead administration period or after the end of administration. In the 5% lead group, however, MCV and SCV decreased significantly in comparison with the control group at the fourth week of lead administration. The significant decreases in MCV and SCV persisted during the administration period. The decrease in SCV was more marked than the decrease in MCV. This finding agrees with the reports of Seppäläinen and Hernberg (1972) and Seppäläinen (1974) that the dose–response relationship between lead and SCV is stronger than that between lead and MCV. In the manganese experiment, the nerve conduction velocity rose significantly at the third week of manganese administration in the 8-mg manganese group. This change may have been due to either the manganese or a temporary rise because of growth. When a similar experiment was performed with 27-week-old rats in which the nerve conduction velocity had reached its plateau, MCV rose temporarily 4 weeks after the commencement of manganese administration. Therefore, the rise in nerve conduction velocity seems to be an effect of manganese. Meiri (1972) reported that the manganese ion inhibited release of neurotransmitters in the neuromuscular junction and inhibited synaptic transmission reversibly. Our results may be explained if inhibition of synaptic transmission in the neuromuscular junction is more potent than the increase in nerve conduction velocity.

Organic Solvents (2-Propanol and Styrene)

Little is known about the mechanisms of organic solvent effects on the peripheral nervous system, except in the cases of *n*-hexane (Takeuchi *et al.*, 1980) and methyl-*n*-buthyl ketone (MBK) (Mendell *et al.*, 1974). Metabolites of *n*-hexane and MBK inhibit the transport of substances in the nerve fibers. 2-Propanol has not been implicated in peripheral nerve injury except for the case of polyneuropathy reported by Ito *et al.* (1979). Effects of styrene on the peripheral nervous system have been described in many papers after examinations of workers handling styrene (Spencer *et al.*, 1942; Ikeda, 1985; Oltrare *et al.*, 1974; Parkki, 1985). Seppäläinen (1978a) and Misumi *et al.* (1986) reported finding no effects of these solvents on rats. Decreases in the caudal nerve conduction velocity caused by *n*-hexane and MBK appeared at 8–16 weeks after the start of exposure in almost all cases. In our study, 2-propanol affected SCV at the exposure dose of 8000 ppm during the 20-week exposure period. Although the dose was high, the possibility does exist that 2-propanol affects the peripheral nervous system. Styrene affected

SCV at the exposure dose of 2000 ppm at 10–12 weeks after the start of exposure. The effects of styrene on the peripheral nerves thus seem to be greater than those of 2-propanol.

CONCLUSIONS

All of the chemicals that we examined, lead, manganese, 2-propanol, and styrene, affected the peripheral nerve conduction velocity. Their effects on SCV were greater than their effects on MCV, and changes caused by the chemicals disappeared when administration or exposure ended. The effects on rat caudal nerve conduction velocity were greatest for lead, followed in decreasing order of severity by styrene, 2-propanol, and manganese.

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Effects of Methylmercury on Protein Kinase A and Protein Kinase C in the Mouse Brain[1]

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The effects of methylmercury administration on adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent protein kinase (protein kinase A) and protein kinase C were investigated by determining their second messenger bindings ([^{3}H]cAMP binding for protein kinase A and [^{3}H]PDBu for protein kinase C) and enzymatic activities in the brains of methylmercury-treated mice. After single administrations of methylmercury (10 mgHg/kg, sc), no neurological symptoms were observed, while the mercury concentration in the brain reached 5.6 ppm. Neither second messenger bindings nor enzymatic activities of either protein kinase displayed significant changes. When methylmercury was administered repeatedly (10 mg Hg/kg × 5), the mercury concentration was 11.7 ppm and the enzymatic activity of protein kinase C was reduced to 75% of the control level without significant change in [^{3}H]PDBu binding. Significant change has not been observed in either [^{3}H]cAMP binding or enzymatic activity of protein kinase A. The reduction of enzymatic activity of protein kinase C was reversed by the simultaneous administration of selenite (0.5 mgSe/kg × 5). However, the fact that selenite administration alone displayed not a significant but about a 20% increase in [^{3}H]PDBu binding suggested that selenite itself could affect the level of protein kinase C despite having no apparent effects on protein kinase C *in vitro*. Further investigation is necessary to assess whether protein kinase C is involved in the detoxication mechanism of selenite with respect to methylmercury. Since the mercury concentration in the brain was higher than the IC_{50}s for both protein kinase A and protein kinase C observed *in vitro* even after single administration, methylmercury might inhibit both protein kinases, which might impair intracellular signal transduction. This might in part conceal the symptoms during the early stages of methylmercury toxicity.

INTRODUCTION

The mechanism underlying methylmercury toxicity is apparently related to its extremely high affinity with the SH groups of proteins (Hughes, 1957; Rabenstein, 1978). This high affinity, however, also causes methylmercury to bind easily with any cell structure; hence its tissue concentrations do not always reflect the actual available concentration, which makes it difficult to demonstrate the primary site of its toxic action. Even when the primary site of action is limited to the neural tissues (Chang, 1977), a variety of biochemical or physiological changes (Chang, 1977; Omata and Sugano, 1986; Saijoh *et al.*, 1987a,b, 1988a) has been reported both *in vivo* and *in vitro*. Since the complexity of neuron networks or intracellular signal transduction cascades, in which protein kinases play an important role, makes interpretation of data more difficult, causal relationships with neurological symptoms are still controversial.

When extracellular signals, such as those of neurotransmitters, are received by

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receptors on membrane surfaces, the signals are amplified and converted to intracellular signals, i.e., those of second messengers. Protein kinases are usually activated by second messengers, such as cAMP for protein kinase A (Taylor, 1989) and diacylglycerol and phorbol ester for protein kinase C (Nishizuka, 1986), and then phosphorylated to so-called third messengers. These intracellular signal transduction cascades will consequently induce many cellular events. In order to fully assess the effects of methylmercury, it is necessary to determine the effects on each step in these cascades. In the present study, the effects of methylmercury administration on protein kinases were examined and compared with the effects observed in *in vitro* experiments in order to clarify dose–response relationships.

MATERIALS AND METHODS

Chemicals. The following compounds were used in this study: [^{3}H]adenosine 3′,5′-cyclic monophosphate ([^{3}H]cAMP) (sp act 1217.3 GBq/mmole) [^{3}H]phorbol 12,13-dibutylate ([^{3}H]PDBu) (sp act 580 GBq/mmole), and [γ-^{32}P]ATP (sp act 111 TBq/mmole) obtained from New England Nuclear; EDTA, 3-isobutyl-1-methylxanthine (IBMX), phenylmethylsulfonyl fluoride, leupeptin, 2-mercaptoethanol, dithiothreitol, sodium selenite, and polyethyleneimine from Nacalai tesque; phosphatidylserine and methylmercury chloride from Tokyo Kasei; ATP, cAMP, PDBu, kemptide, and protein kinase inhibitor from Sigma; and diolein from Serdary. H1 histone was provided by Dr. U. Kikkawa, Department of Biochemistry, Kobe University School of Medicine. All other compounds used were of analytical grade from commercial sources.

Administration of methylmercury. Female mice (Jcl:ICR, 4-week-old) were housed in cages in a temperature-controlled room with a fixed lighting schedule and with food and water available *ad lib.* Methylmercury was administered as described elsewhere (Katsuyama *et al.*, 1989a). That is, for single-dose experiments, methylmercury chloride (10 mg Hg/kg body wt) dissolved in physiological saline was administered by subcutaneous injection. In repeated dose experiments, methylmercury chloride and/or sodium selenite (0.5 mgSe/kg body wt) were administered five times on every third day. Mice were sacrificed 72 hr after the last injection and the brains were quickly removed and homogenized with 10 vol of homogenate buffer [20 mM Tris/HCl (pH 7.4), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.01% leupeptin, and 10 mM dithiothreitol for protein kinase A or 10 mM 2-mercaptoethanol for protein kinase C]. Dithiothreitol or 2-mercaptoethanol was used throughout the experiments in order to prevent methylmercury in the preparation from inhibiting the reaction in the assay mixture. The use of these reagent during preparation removed methylmercury which bound to kinases *in vivo* (Inoue *et al.*, 1988) and permitted measurement of the total number of second messenger binding sites and maximum available enzymatic activity, whether or not they were inhibited *in vivo*. The homogenate was centrifuged at 100,000*g* for 1 hr. The supernatant was further centrifuged at 120,000*g* for 45 min. The membrane fraction was solubilized with 0.1% Triton X-100. For protein kinase C analysis, the preparations were further purified over a DE52 column in accordance with the method of Kikkawa *et al.* (1986).

Second messenger binding and enzymatic activity. Second messenger binding was quantified using [^{3}H]cAMP for protein kinase A and [^{3}H]PDBu for protein kinase C. Enzymatic activity was measured by the incorporation of ^{32}P into kemptide for protein kinase A and H1 histone for protein kinase C from [γ-^{32}P]ATP.

Protein kinase A. [^{3}H]cAMP binding in the supernatant was measured as described elsewhere (Saijoh *et al.*, 1991) except that dithiothreitol was used. Briefly, 10 μl of the preparation was added to 90 μl of an assay mixture containing 20 mM Tris/HCl (pH 7.4), 30 nM [^{3}H]cAMP, 2 mM dimethylsulfoxide, and 2 mM IBMX. After incubation at 30°C for 30 min, the mixture was rapidly filtered and radioactivity on the filter was measured as described above. Nonspecific binding was measured in the presence of 10 mM nonradioactive cAMP. Enzymatic activity was measured in accordance with a modification of the method of Nestler and Tallman (1987). Briefly, duplicate 5 μl aliquots of the supernatant were incubated for 3 min at room temperature in a final volume of 50 μl containing 20 mM Tris/HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, 10 mM dithiothreitol, 2 mM IBMX, 1.3 mM kemptide, and 100 μM [γ-^{32}P]ATP (5×10^6 cpm/nmole, New England Nuclear) in the absence or presence of 5 μM cAMP or protein kinase inhibitor. Following the incubation period, the mixture was blotted on 1.5 × 1.5-cm squares of P81 phosphocellulose filter paper (Whatmann). The filter paper squares were washed three times with 0.5% phosphoric acid for 10 min. The ^{32}P contained in the filter paper was quantitated by liquid scintillation spectrophotometry. ^{32}P incorporation into kemptide in the absence of cAMP and the presence of protein kinase inhibitor served as a blank.

Protein kinase C. [^{3}H]PDBu binding and enzymatic activity were determined as described previously (Inoue *et al.*, 1988; Katsuyama *et al.*, 1989b). That is, [^{3}H]-PDBu binding of the supernatant and purified enzyme preparation was assayed in a 300-μl mixture compounded to a final concentration of 20 mM Tris/HCl (pH 7.4), 10 nM [^{3}H]PDBu, 1 mM EDTA, 0.2 mM $CaCl_2$, 30 mg/ml phosphatidylserine, 3 mM 2-mercaptoethanol, and 4 mg of bovine serum albumin. A total of 5 to 50 nM [^{3}H]PDBu was used for kinetic analysis. After 30 min incubation at 30°C, the mixture was rapidly filtered and washed on a Whatman GF/B glass filter, which had been soaked for 1 hr prior to use in a fresh 0.5% polyethyleneimine solution. Nonspecific binding was measured in the presence of 30 mM nonradioactive PDBu. Radioactivity was determined using a scintillation spectrophotometer. About 75% of the total [^{3}H]PDBu binding was recovered in the purified preparation. Enzymatic activity in the purified preparation was assayed by adding 70 μl of purified preparation to the reaction mixture to form a total volume of 180 μl, containing 20 mM Tris/HCl (pH 7.4), 10 μM [γ-^{32}P]ATP (30,000 cpm/mmol), 10 μg/ml H1 histone, 5 mM magnesium acetate, 0.5 mM $CaCl_2$, 3 mM 2-mercaptoethanol, 8 μg/ml phosphatidylserine, and 0.8 μg/ml diolein. For kinetic analysis, 1 to 100 μM [γ-^{32}P]ATP was used. The mixture in the absence of diolein served as a blank. The mixture was incubated at 30°C for 5 min. The reaction was terminated by addition of 1 ml of 25% trichloroacetic acid and the precipitate was collected and washed by filtration over a membrane filter. The ^{32}P contained in the filter was quantitated by liquid scintillation spectrophotometry. Total activity was calculated using the recovery rate measured by [^{3}H]PDBu binding.

Other methods. The concentration of total mercury in the preparations was measured as described elsewhere (Katsuyama *et al.*, 1989a). Protein was measured using a Bio-Rad protein assay kit (Bio-Rad).

RESULTS

General Toxicity and Mercury Concentration in the Brain

No signs of toxicity were observed 3 days after administration of a single dose

of methylmercury (10 mgHg/kg, sc), while methylmercury accumulated in the brain at the concentration of 5.6 ppm (=28 μM) (Table 1). The methylmercury concentration reached higher than the IC_{50}s for enzymatic activities of both protein kinases and second messenger binding of protein kinase C (Table 2). In the brain, 11.7 ppm of mercury accumulated after a cumulative dose of 50 mg/kg, causing a decrease in involuntary movement, piloerection, and a mild staggering gait but no hind limb paralysis. The body weight of the mice decreased by about 8% after the fifth dose. The simultaneous injection of selenite reduced the symptoms but increased the accumulation of methylmercury in the brain. These concentrations were almost as high as the IC_{50} for second messenger binding of protein kinases A.

Administration of Methylmercury and Protein Kinase A

cAMP binding was not changed in either the soluble or the membrane fractions, either after single administration or after repeated administration (Fig. 1A). Enzymatic activity of protein kinase A also displayed no significant changes, although that in the soluble fraction increased slightly after repeated administration.

Administration of Methylmercury and/or Selenite and Protein Kinase C

Prior to administration of methylmercury, about 60% of [^{3}H]PDBu binding to protein kinase C existed in the soluble fraction and 40% in the membrane fraction, while almost the same enzymatic activity was detected in both fractions (Fig. 1B). After single administration, neither [^{3}H]PDBu binding nor enzymatic activity of protein kinase C displayed significant changes. Even after repeated administration, [^{3}H]PDBu binding did not change in either the soluble or the membrane fractions, while enzymatic activity in the soluble fraction was significantly reduced to about 75% of the control level. By contrast, the enzymatic activity in the membrane fraction increased to 110% of the control level, although this was not significant.

Administration of selenite with methylmercury reversed the decrease in enzymatic activity in the soluble fraction (Table 3). Furthermore, it increased [^{3}H]-PDBu binding, although not significantly, to about 120% of the control level in both the soluble and membrane fractions. Selenite administration alone also induced apparent but not significant increases in B_{max} for [^{3}H]PDBu binding in the membrane fraction. All of these increases were associated with slight increases in K_d or K_m values, while selenite did not seem to affect protein kinase C directly

TABLE 1
MERCURY CONCENTRATIONS IN THE BRAIN

	ppm	μM
Human, no history of unusual exposure[a]	0.10 ± 0.04	0.5
Human, Minamata disease[b] (within 100 days after onset)	3.6–21.4	18–107
Mice, control	0.10 ± 0.03	0.5
Mice, single dose	5.6 ± 0.8	28
Mice, repeated doses	11.7 ± 1.9	58.5
Mice, repeated doses and selenite	13.6 ± 1.6	68

[a] Sumino *et al.*, 1975.
[b] Takizawa, 1986.

TABLE 2
IC_{50} VALUES OF METHYLMERCURY ON PROTEIN KINASE A AND PROTEIN KINASE C *IN VITRO*

	Protein kinase A (μM)[a]	Protein kinase C (μM)[b]
Second messenger binding	70 ± 10 (14 ppm)	20.9 ± 2.5 (4.18 ppm)
Enzymatic activity	1.5 ± 0.8 (0.3 ppm)	1.3 ± 0.3 (0.26 ppm)

[a] Saijoh *et al.*, 1991.
[b] Inoue *et al.*, 1988.

since the IC_{50} values of selenite for PDBu binding and enzymatic activity were more than 2 mM (data not shown).

DISCUSSION

Although the mercury concentration in the brain was higher than that reported previously (Katsuyama *et al.*, 1989a), the symptoms observed in the present study were very similar to those previously reported. The symptoms seemed to be less severe than those reported in rats (Komulainen and Tuomisto, 1985), which were treated with the same doses of methylmercury by gavage. Gavage administration to mice provided a brain mercury concentration of 22.5 ppm ($= 1.1 \times 10^{-4}$ M) and created symptoms as severe as those reported by Komulainen and Tuomisto (1985). However, more than 20% body wt loss was observed in these mice, which could make the comparison of data difficult.

On the other hand, as shown in Table 2, these concentrations seemed to be sufficiently high to inhibit not only enzymatic activities but also second messenger bindings of both protein kinases A and protein kinase C. In some cases, mercury levels in human brains with no history of unusual exposure to mercurials are high enough to inhibit enzymatic activities of both protein kinase A and protein kinase C, while the mercury levels of even Minamata disease patients are apparently not always sufficient to inhibit second messenger bindings (Sumino *et al.*, 1975). Methylmercury, however, is transformed to inorganic mercury in tissues, and when tissue mercury concentrations reach 2 μM, 3–6% of methylmercury is transformed to inorganic mercury (0.3–0.6 μM) (Norseth and Clarkson, 1970). Inorganic mercury can inhibit second messenger bindings and enzymatic activities of both protein kinases at 10–200 times lower concentrations (Inoue *et al.*, 1988; Saijoh *et al.*, 1988b). Not only methylmercury accumulated in the brain but also transferred inorganic mercury can inhibit protein kinases. Since methylmercury at such concentrations caused Minamata disease, results obtained from excessive-dose experiments do not seem to reflect its etiology. Thus, subcutaneous injection, rather than gavage, was used in the present study.

Protein kinase A consists of regulatory subunits and catalytic subunits. A regulatory subunit dimer is bound to two catalytic subunits in the absence of the second messenger, cAMP, forming an inactive holoenzyme. The binding of cAMP to the regulatory subunit dissociates active catalytic units (Taylor, 1989), in which two cysteine residues exist. It has been suggested that catalytic subunits are labile to SH blocking when active (Bechtel *et al.*, 1977; Armstrong and Kaiser, 1978) and that the binding of regulatory subunits to catalytic subunits not only inacti-

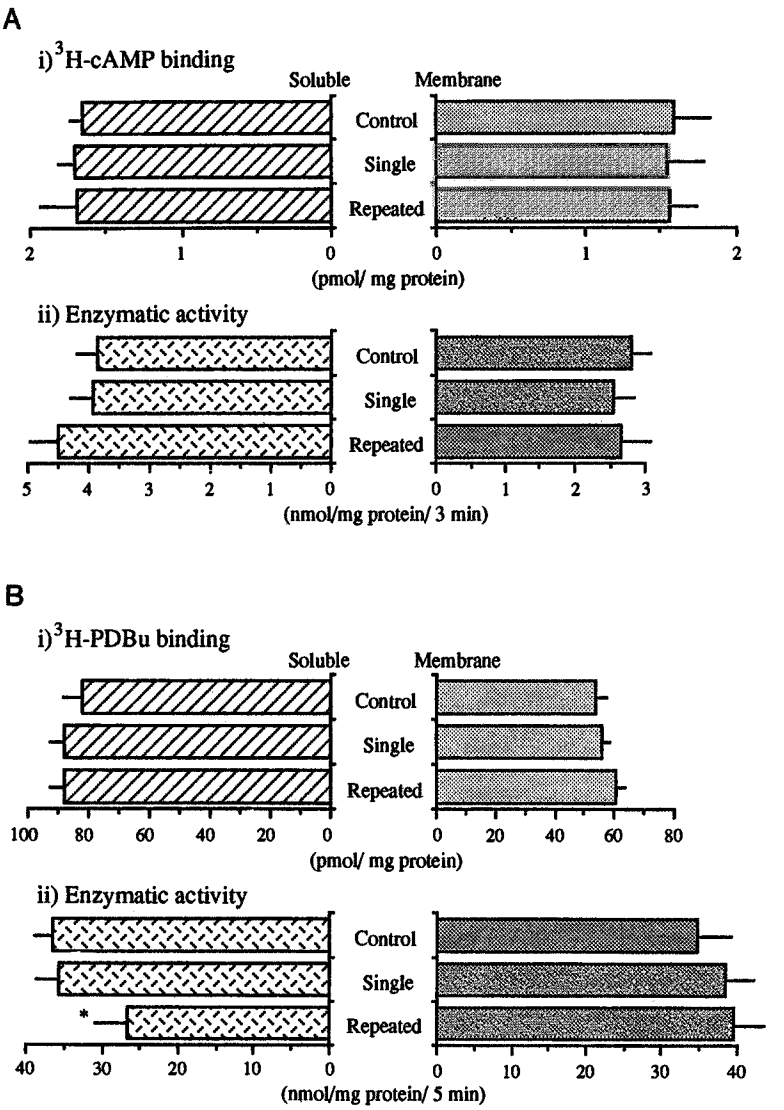

FIG. 1. Changes in second messenger binding and enzymatic activity of (A) protein kinase A and (B) protein kinase C after administration of methylmercury to mice. Each column represents the mean ± SE for eight animals. $^*P < 0.05$. Significantly different from control and single administration (one-way ANOVA and post hoc test using Tukey's HSD).

vates the latter but also protects it from degeneration (Armstrong and Kaiser, 1977; Nelson and Taylor, 1983).

After methylmercury administration, active catalytic subunits may be inhibited. However, accumulated methylmercury seemed to be sufficient to inhibit cAMP binding to regulatory subunits, which might prevent dissociation of holoenzyme, i.e., might preserve catalytic units intact. The slight increment in enzymatic activity in the soluble fraction after repeated administration was apparently only the result of preservation of catalytic subunits. Methylmercury did not induce an increase in either the number of catalytic subunits or the number of regulatory

TABLE 3

EFFECTS OF METHYLMERCURY AND/OR SELENITE ADMINISTRATION ON PDBu BINDING AND ENZYMATIC ACTIVITY OF PROTEIN KINASE C

	Soluble fraction				Membrane fraction			
	PDBu binding		Enzymatic activity		PDBu binding		Enzymatic activity	
	K_d (nM)	B_{max} (pmole/mg protein)	K_m (μM)	V_{max} (nmole/mg protein/5 min)	K_d (nM)	B_{max} (pmole/mg protein)	K_m (μM)	V_{max} (nmole/mg protein/5 min)
Control	6.1 ± 1.0	136.1 ± 13.7	8.1 ± 1.2	53.3 ± 2.0	7.4 ± 0.8	81.1 ± 11.1	10.4 ± 1.7	50.9 ± 5.4
Methyl-mercury	6.5 ± 1.1	139.0 ± 10.0	8.0 ± 1.5	39.1 ± 6.4*	9.4 ± 1.4	88.7 ± 5.2	10.5 ± 1.9	56.3 ± 2.9
Selenite	7.2 ± 0.5	131.0 ± 21.2	10.2 ± 1.5	53.4 ± 2.5	8.2 ± 1.6	98.1 ± 5.8	10.1 ± 1.7	57.8 ± 2.9
Methyl-mercury + selenite	7.7 ± 0.8	168.4 ± 11.1	11.7 ± 2.1	51.7 ± 3.4	9.2 ± 1.0	105.5 ± 6.8	10.6 ± 1.5	54.3 ± 6.8

Note. Methylmercury (10 mgHg/kg body wt) and/or selenite (0.5 mgSe/kg body wt), or saline was administered to mice five times every 3 days by subcutaneous injection. Each value is the mean ± SE for eight different animals. *$P < 0.05$. Statistically significant from control, selenite, and methylmercury + selenite (one-way ANOVA and post hoc test using Tukey's HSD).

subunits. Consequently, signal transduction via protein kinase A seemed to be impaired by methylmercury either through direct inhibition of active catalytic subunits or through preventing their dissociation from the holoenzyme.

Protein kinase C consists of at least eight subspecies, which display different sensitivities to phospholipids and second messengers (Ono *et al.*, 1988) and can translocate between the soluble and the membrane fractions (Niedel *et al.*, 1983). Second messenger binding triggers the activation of protein kinase C (Nishizuka, 1986) as well as protein kinase A (Taylor, 1989). In contrast with protein kinase A, protein kinase C is a single-chain peptide and consists of a regulatory domain, to which second messengers bind, and a catalytic domain. Both the regulatory and catalytic domain are rich in cysteine (Parker *et al.*, 1986; Coussens *et al.*, 1986; Ohno *et al.*, 1987; Ono *et al.*, 1987). SH residues in the catalytic domain are more labile to SH-blocking agents than those in the regulatory domain. SH-blocking agents, however, impair enzymatic activity of protein kinase C through direct inhibition of catalytic domain and through prevention of activation by second messenger binding (Inoue *et al.*, 1988; Saijoh *et al.*, 1988b). Methylmercury appeared to display an inhibitory effect alone on protein kinase C unlike the effects on protein kinase A.

Inhibition of enzymatic activity by methylmercury is reversible *in vitro* (Inoue *et al.*, 1988). The reduced enzymatic activity after repeated administration indicated that accumulation of methylmercury to more than 10 ppm caused irreversible damage to protein kinase C, which did not induce *de novo* synthesis of protein kinase C. Even when the mercury concentration was less than 10 ppm, both enzymatic activity in the catalytic domain and activation by PDBu binding seemed to be inhibited. To the extent that methylmercury did not alter the number of PDBu binding sites, that is, the quantity of protein kinase C, this inhibition might result in the prevention of intracellular signal transduction, similar to the case of protein kinase A. This might partially conceal the symptoms during the early stage of methylmercury toxicity.

Selenite is known to reduce the toxicity of methylmercury (Parizek and Ostadalova, 1967), but the mechanism of this effect is still unknown. Sumino *et al.* (1977) reported that selenite released methylmercury from tissue homogenates, which might be involved in the detoxication mechanism of selenite. It appears attractive to conjecture that the protective effect of selenite against methylmercury toxicity involved release of methylmercury from protein kinase C. Accordingly, the effects of selenite on methylmercury inhibition of protein kinase C were investigated.

Although the simultaneous injection of selenite with methylmercury recovered the enzymatic activity of protein kinase C, it is still unclear whether selenite causes recovery of protein kinase C from irreversible inhibition, prevents methylmercury from irreversibly inhibiting protein kinase C, or enhances induction of protein kinase C independent of methylmercury toxicity. Because administration of selenite with methylmercury increased the accumulation of methylmercury in the brain (Table 2), selenite itself might induce an increase in PDBu binding sites, that is, might increase the quantity of protein kinase C, etc. Moreover, methylmercury increases intracellular calcium concentration (Kauppinen *et al.*, 1988), which might cause translocation of protein kinase C (Niedel *et al.*, 1983). Hence the indirect effects of selenite through changes in intracellular calcium levels must also be taken into account. Further investigation is necessary in order to clarify

whether the recovery in enzymatic activity of protein kinase C caused by selenite corresponds to its detoxication effect with respect to methylmercury.

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Effects of Methyl Mercury in Postnatal Developing Rats[1]

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Rats on Postnatal Days 1 (PD 1), 14 (PD 14), and 35 (PD 35) were orally administered 0, 2.60, 3.64, 5.10, 7.14, and 10 mg/kg/day of methyl mercury chloride (MMC) for 10 consecutive days. Mercury (Hg) accumulation in the brain of the rats treated with 10 mg/kg/day of MMC for 10 consecutive days was highest in PD-14 rats, followed by PD-35 and PD-1 rats. Hg accumulations in the liver and kidney were lowest in PD-1 rats and increased markedly with development in postnatal phase. The effect of MMC treatment on body weight change was most severe in PD-35 rats. The body weight loss began on Day 5 in PD-35 rats and on Day 10 in PD-14 rats treated with 10 mg/kg/day of MMC, but not in PD-1 rates under the same treatment. The phenomenon of hindlimb-crossing was induced on Day 11 in PD-14 rats and on Day 14 in PD-35 rats treated with 10 mg/kg/day of MMC, but was not observed in PD-1 rats. The deficit of rotarod performance was apparent only at the dose of 7.14 mg/kg/day of MMC in PD-35 rats, whereas rotarod performance was dose-dependently inhibited by MMC treatment in PD-14 rats, and lowered even at the dose of 2.6 mg/kg/day of MMC. However, the performance was gradually restored to the control level by 1 month except in rats given 7.14 mg/kg/day of MMC. These findings indicated that the Hg distribution and the effects of MMC treatment on body weight gain and motor coordination were different among the rat postnatal developing phases.

INTRODUCTION

Many animal studies have indicated that a developing organism in the prenatal and early postnatal stages may be at higher risk in toxic metal exposure than adult (Kostial *et al.*, 1978; Jugo, 1979; Kostial, 1983). Many infants were congenitally affected by methyl mercury in the epidemics in Minamata and Niigata, Japan, and Iraq (WHO, 1990). The offspring were more affected by methyl mercury than their mothers and showed severe neurological disturbance (Harada, 1978). In methyl mercury poisoning, damage to the human central nervous system is an important phenomenon (WHO, 1990). Rapid brain growth occurs primarily during the third trimester in humans, whereas in rats it occurs after parturition (Dobbing and Sands, 1979; West *et al.*, 1984). Accordingly, determining the effects of methyl mercury on neonatal rats might be helpful for understanding its effects on the developing central nervous system in the human fetus. The effects of MMC in the different growth phases of postnatal rats were examined in the present study.

MATERIALS AND METHODS

Adult female and male Wistar rats were mated and females were maintained on

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a 12-hr light/12-hr dark cycle at 23°C with free access to rat chow and tap water. Within 24 hr of birth, a litter was randomly reduced to eight neonates, which were then maintained by a dam until weaning on Postnatal Day 30. After Postnatal Day 31, rats were maintained with free access to rat chow and tap water.

The rats were then used for the experiment at Postnatal Days 1 (PD 1), 14 (PD 14), and 35 (PD 35). Each group was divided into six subgroups (eight rats from different litters) and treated with 0, 2.60, 3.64, 5.10, 7.14, and 10 mg/kg/day of MMC (Merck), respectively, for 10 consecutive days. MMC and cysteine (molecular ratio 1:1) were dissolved in 10% condensed milk was orally administered with a microman pipet (Gilson) for PD-1 and PD-14 rats and a stainless catheter for PD-35 rats. Weight changes and the hindlimb-crossing phenomenon were observed throughout the experimental period. A rotarod test was examined in the surviving rats on the day after the final MMC treatment and every week thereafter. The rotarod test could not given to PD-1 rats until 2 weeks after the final treatment because until then they were too small for the test. The rotarod test (8 cm in diameter, 5 rpm, Natume Co., Ltd.) was given in the form of six trials for each rat. Intervals between the mounting on the rod and falling from it in 60 sec were recorded as the performance time. The result was expressed as the percentage of rats which met a criterion of at least one complete trial (more than 60 sec on the rod) in six trials.

For determining the Hg contents in the brain, liver, and kidney, five rats treated with 10 mg/kg/day of MMC for 10 days were dissected under pentobarbital anesthesia on the day after final treatment. To remove blood from the tissues, each rat was perfused via the heart with physiological saline. The brain was removed and separated from the spinal cord at the decussation of pyramids, and the liver and kidney were also removed. Total Hg determination was performed according to the oxygen combustion–gold amalgamation method with a Sugiyamagen Mercury Analyzer, following procedures described by Jacobs (Jacobs *et al.*, 1960).

Statistical significance was tested by one-way analysis of variance (ANOVA). The level of significance was put at $P < 0.01$.

RESULTS

Figure 1 shows Hg concentration in the brain, liver, and kidney of PD-1, -14, and -35 rats on the day after final treatment with 10 mg/kg/day of MMC for 10 consecutive days. There were significant differences of Hg concentrations in the brain, liver, and kidney among PD-1, -14, and -35 rats. The mean mercury concentration in the brain was highest in PD-14 rats (30.4 μg/g), followed by PD-35 and PD-1 rats (2.43 and 20.3 μg/g, respectively). The mean mercury concentrations in the liver and kidney were lowest in PD-1 rats and increased markedly with development in postnatal phase.

Figures 2a, 2b, and 2c show the changes in mean body weight of the rats administered MMC for 10 consecutive days from Postnatal Days 1, 14, and 35. A striking dose-dependent decline in body weight gain appeared in PD-35 rats. The gain was lowered dose dependently by MMC treatment in PD-1 rats, but no body weight loss was observed even in the rats treated with 10 mg/kg/day of MMC. All of the PD-1 rats treated with 10 mg/kg/day of MMC died by Day 11, without the

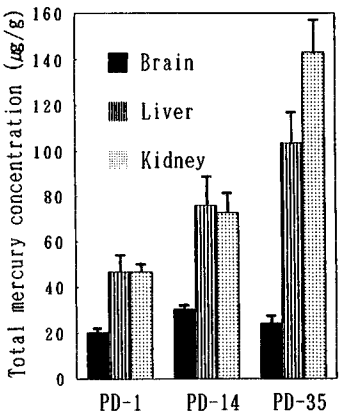

FIG. 1. Mean total mercury concentrations in the brain, liver, and kidney of five rats administered 10 mg/kg/day of methyl mercury for 10 consecutive days from Postnatal Days 1 (PD 1), 14 (PD 14), and 35 (PD 35), respectively. There were significant differences ($P < 0.01$) in Hg concentrations of the brain, liver, and kidney among PD-1, -14, and -35 rats by ANOVA.

phenomenon of hindlimb-crossing and with their stomachs full of milk. Sudden body weight loss began at Day 10 in PD-14 rats treated with 10 mg/kg/day of MMC and severe hindlimb-crossing appeared in all of them on Day 11. Three of them showed backward bending of their hindlimbs, and none could walk on the floor. All of them died on the next day. The hindlimb-crossing appeared in five of the PD-14 rats treated with 7.14 mg/kg/day of MMC, and the phenomenon continued permanently in one of them. The body weight loss began on Day 5 in PD-35 rats treated with 10 mg/kg/day of MMC. The hindlimb-crossing in PD-35 rats appeared from Day 12 and all of them showed it on Day 14, but died by Day 16. PD-35 rats treated with 7.14 mg/kg/day of MMC also showed the weight loss from Day 8, but the hindlimb-crossing was not observed.

Figure 3 shows the changes in the percentage of rats reaching the rotarod test criterion on the day after final MMC treatment and every week thereafter in PD-1, -14, and -35 rats. The data in PD-1 rats were available only at 3 weeks after the final MMC administration, and the deficit of rotarod performance was observed only at the dose of 10 mg/kg/day of MMC at that time. All surviving PD-14 and -35 rats treated with 10 mg/kg/day of MMC fell from the rod immediately after being mounted on it on the final day of MMC treatment. The rotarod performance in PD-14 rats was dose-dependently inhibited by MMC treatment. The lowered motor coordination in the rats was observed even at the dose of 2.6 mg/kg/day of MMC. However, the performance was gradually restored to the control level by 1 month except for the rats treated with 7.14 mg/kg/day of MMC. On the other hand, the deficit of rotarod performance was apparent at the treatment level of 7.14 mg/kg/day of MMC in PD-35 rats.

DISCUSSION

Several animal experiments have indicated that prenatal exposure to methyl mercury causes developmental or behavioral alterations in the postnatal phase

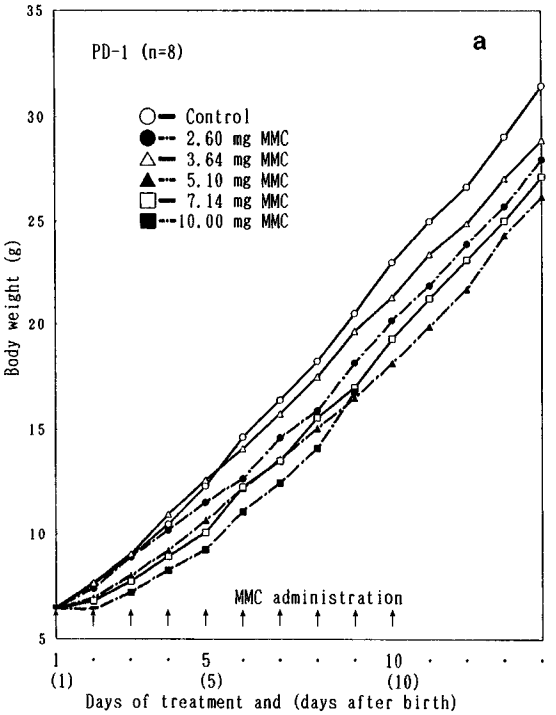

FIG. 2. (a) Changes in body weight of rats administered methyl mercury for 10 consecutive days from Postnatal Day 1. Each point represents a mean of eight rats. (b) Changes in body weight of rats administered methyl mercury for 10 consecutive days from Postnatal Day 14. Asterisk indicates the onset of hindlimb-crossing. Each point represents a mean of eight rats. (c) Changes in body weight of rats administered methyl mercury for 10 consecutive days from Postnatal Day 35. Asterisk indicates the onset of hindlimb-crossing. Each point represents a mean of eight rats.

(Eccles and Annau, 1982; Cuomo *et al.*, 1984; Elsner *et al.*, 1988). However, the effects of methyl mercury on neonatal animals are not well established. In the present study, the dose-dependent effects of MMC in several growth phases of postnatal rats were investigated.

Accumulations of Hg in the kidney and liver were lowest in PD-1 rats and increased markedly with development in postnatal phase. On the other hand, the Hg accumulation in the brain was highest in PD-14 rats followed by PD-35 and PD-1 rats. Hg accumulation in the brain was not apparently changed with development in postnatal phase, compared with the accumulations in the liver and kidney. From the practical point of view, one of the most important and distinctive features of rats in an earlier developmental stage was relatively high Hg accumulation in the brain, in contrast to its low accumulations in the liver and kidney. Thus, the brain–kidney or brain–liver ratios of Hg concentration are higher in earlier postnatal rats than that in later postnatal ones. The growth period is characterized by functional immaturity of organs, which could be the main reason for the different Hg accumulation. For example, the glomerular filtration rate is known to be very low in immature kidneys (Spitzer, 1985). The slow blood

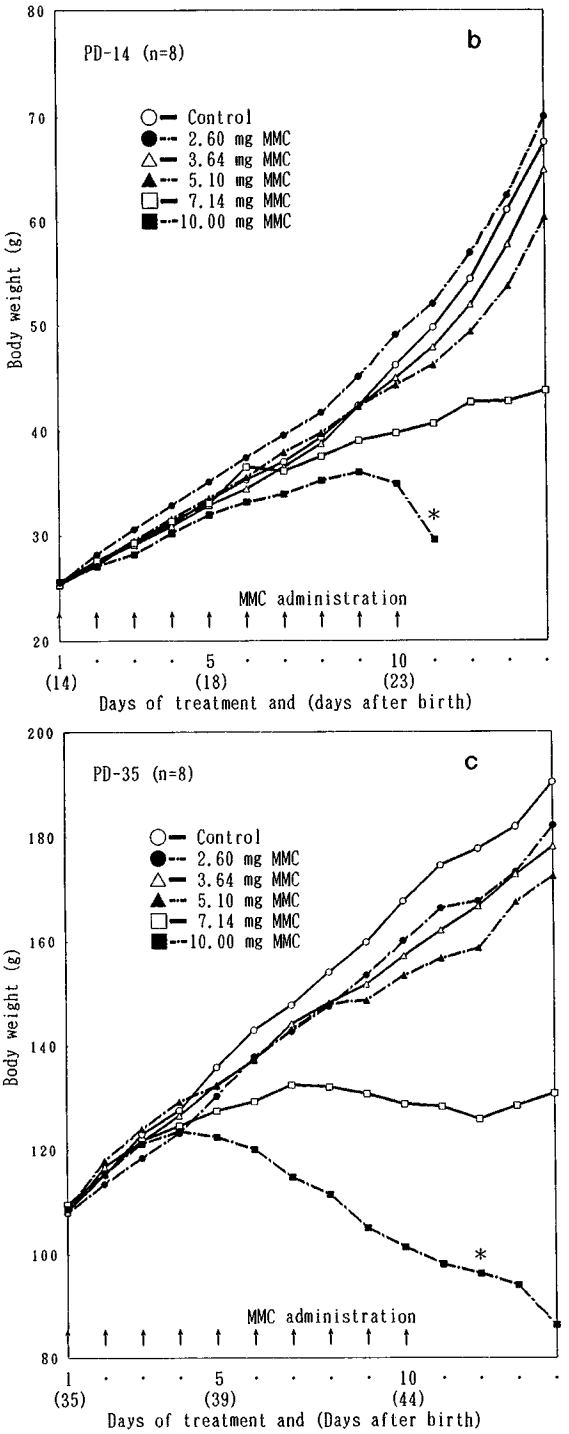

FIG. 2—*Continued*

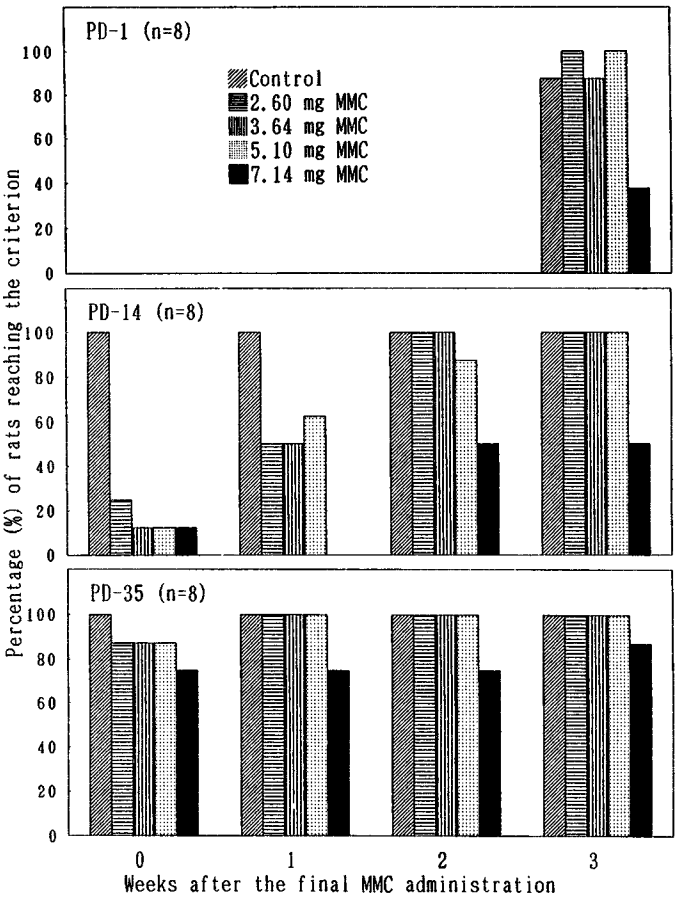

FIG. 3. Percentage of rats (eight rats per group) reaching the criterion of at least one complete trial (60 sec) at six trials after the final administration of methyl mercury. Methyl mercury was administered for 10 consecutive days from Postnatal Days 1 (PD 1), 14 (PD 14), and 35 (PD 35), respectively. Data until 2 weeks after the final treatment were not available for PD-1 rats.

flow into the renal tissue may cause less accumulation of MMC in the kidney of neonatal rats. Hg accumulation in the kidney of mice following MMC exposure was lower in the fetus than in the dam (Inouye *et al.*, 1986).

The body weight loss at a toxic level of MMC is a typical phenomenon in adult rats and mice (Magos and Webb, 1983; WHO, 1990) and is caused by anorexia (WHO, 1990). However, in the present experiment the rats in an earlier developmental stage seemed to be resistant to the anorexic effect of methyl mercury and no body weight loss was induced. As indicated in Fig. 1, very low Hg accumulations in the kidney and liver in the rats may explain the phenomenon. The high sensitivity to the weight loss in the later postnatal rats, in contrast, may be partly due to the increased Hg accumulations in their kidney and liver. Lin *et al.* (1975) reported that LD_{50} values for oral methyl mercury in rats decreased with age, indicating that an immature organism could be more resistant to the lethal action

of methyl mercury. The striking hindlimb-crossing that was observed in PD-14 rats treated with 10 mg/kg/day of MMC presumably was due to the high Hg concentrations in their brains (mean 30.4 μg/g). However, PD-1 rats given the same treatment died without showing this phenomenon. Further research is necessary on the mechanism of the hindlimb-crossing which did not appear in the earlier neonatal rats.

In every species of animal, the main target of methyl mercury toxicity is the nervous system and one of the earliest signs is ataxia (WHO, 1990). In the present experiment, the rotarod test was adopted to examine the deficit of motor coordination. Impaired development of the cerebellum accompanied by deficit of motor coordination was reported in rats neonatally exposed to alcohol (Kelly *et al.*, 1987; Meyer *et al.*, 1990; Goodlett *et al.*, 1991). Data in PD-1 rats were not available, but the deficit of motor coordination was observed at a much lower dose of MMC in PD-14 rats than that in PD-35 rats. These deficits of the motor coordination would be related to damage or impaired development of the cerebellum, which was significant during the MMC treatment period. Golgi preparations of the cerebellum of MMC-treated neonatal rats revealed a significant reduction in dendritic arborization of Purkinje cells (Choi *et al.*, 1981). Therefore, we consider that the neonatal period is a "critical" or "vulnerable" stage in terms of neurotoxicity of methyl mercury. Further pathological and neurochemical studies are needed to reveal the mechanism of the lowered motor coordination.

In summary, Hg accumulations in the kidney and liver (but not in the brain) were very low in the rats in an earlier developmental stage, and they evidenced no anorexic effect of methyl mercury. However, the deficit of rotarod performance was induced at a much lower dose of methyl mercury in the earlier postnatal rats than in the later ones. Thus, neurological dysfunction must be a predominant effect of methyl mercury poisoning in the earlier developmental phase of animals.

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REVIEW

Behavioral Approaches to Toluene Intoxication[1]

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Toluene is a chemical that is very useful in our lives but harmful to our health. Behavioral toxicology has the merit of providing an accurate indication of functional toxicity to the CNS through the analysis of learned behavior and use of behavioral analysis techniques that give us various learning paradigms for investigating the effects of chemicals on memory, stimulus discrimination, attention, time perception, etc. Learning is a common ability among various species and it is possible to predict toxicity to human health from animals. Behavioral toxicology is assumed to play an important role in occupational and environmental health. Using typical test batteries such as shuttle, Sidman, and pole-climb avoidance, and FI, FR, DRL, and DMS tasks, the effects of toluene were investigated and the results were reviewed. One important objective of a test battery is to be able to detect already-known toxicity. Behavioral toxicology research indicated such effects of toluene toxicity as hyperactivity, ataxia, addiction, insomnia, and memory disturbances. Some excellent results which might indicate clinically unknown effects of toluene such as hearing loss, impairments of time discrimination, and improvements of STM were also demonstrated. Introduction of blood and brain toluene levels as an index of toluene exposure and more sophisticated learning tasks which reflect specific higher nervous functions of the CNS has been proposed.

INTRODUCTION

Toluene, a volatile organic solvent, is an important and applicable chemical. It has been used extensively in the painting, presswork, and chemical industries as a solvent in lacquer, an additive in motor fuels, and a raw material to produce dyes, synthetic fibers, and other chemicals. However, toluene is highly soluble to lipid tissues and easily absorbed into the central nervous system (CNS). It is well known to have great variety of harmful effects on CNS functions of those who have worked in these industries and received occupational exposure to toluene vapor. Clinical and epidemiological studies have reported that workers frequently complained of CNS symptoms such as headache, dizziness, euphoria, unstable mood, memory disturbances, attention deficits, and poor intelligence (Benignus, 1981; Foo *et al.*, 1988; Fornazzari *et al.*, 1983; Hänninen *et al.*, 1976; Juntunen *et al.*, 1985; Stollery and Flindt, 1988). Furthermore, toluene has been frequently abused and long-term voluntary sniffing has induced marked brain atrophy together with dysfunctions of the CNS (Fornazzari *et al.*, 1983; Lazar *et al.*, 1983).

Physiological, biochemical, and pathological studies of toluene toxicity have been continuing, but its effects are still unclear. It is urgent to clarify the effects

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of toluene toxicity on CNS functions so that legal controls can be imposed for occupational and environmental health reasons.

In the present paper, a new methodology, behavioral toxicology, is discussed and its merits and advantages are described. The results of behavioral toxicology studies on toluene toxicity are reviewed and further developments are suggested.

BEHAVIORAL TOXICOLOGY

One of the various methodologies able to answer these urgent demands is behavioral toxicology, which evaluates the harmful effects of chemical substances by means of behavioral indices. Behavioral toxicology has the merit of providing an accurate indication of functional toxicity to the CNS through the analysis of behavior, especially learned behavior. Since learned behavior is realized by the integrated works of higher nervous functions of the CNS and malfunctions of the CNS induce behavioral disorders, for instance, learning impairments, it is possible to speculate on damages to the CNS functions through behavioral analyses.

Impairments in learned behavior inevitably create great disadvantages to organisms with regard to survival. We can easily interpret, describe, and classify the effects of chemicals and clearly conclude that the chemicals are harmful.

Behavioral analysis techniques based on operant conditioning methods give us enormous amount of data about the learned behavior of rodents, birds, and primates including humans. Learning is a common ability among these species, and it is said that the structures and functions of their CNS surprisingly resemble each other. We can perform comparative studies among species and predict toxicity of chemicals to human health from experiments with animals (Evans and Daniel, 1984).

Behavioral analysis techniques have also resulted in various types of learning paradigms, and a wide range of applications of the paradigms makes it possible to evaluate the effects of chemicals on specific higher nervous functions of the CNS such as memory, attention, time perception, and inference (Paule, 1990; Pearce, 1987; Wenger, 1990).

Furthermore, certain workers receive repeated exposure to toxic chemicals, and the long-term stability of indices for evaluating toxicity is necessary. Animals indicate high steady-state baseline performance of the learned behavior after the acquisition of learning has once been established. Learned behavior has merits of studying chronic effects of chemicals.

Thus, behavioral toxicology is assumed to play an important role in occupational and environmental health.

BEHAVIORAL APPROACHES TO TOLUENE TOXICITY

Operant learning which is used in behavioral toxicology research is divided into two types, according to the reinforcements used. Negative reinforcements are such aversive things as electrical shock and strong light, while positive reinforcements are food and water. In the former case, for example, animals can postpone the presentation of electrical shock for a fixed interval (such as 30 sec) by performing a specific operant response (lever-pressing, pole-climbing, and spatial locomotion). The animals which are trained under these conditions learn to perform operant responses to avoid electrical shock. In the latter case, animals can gain food or water by performing a specific operant response. The animals frequently respond to obtain reinforcement stimuli.

In behavioral approaches to toluene toxicity which use an aversive stimulus such as electrical shock, Sidman avoidance (Mullin and Krivanek, 1982; Shigeta *et al.*, 1978, 1979, 1980), pole-climbing avoidance (Pryor *et al.*, 1983a,b, 1984), and shuttlebox avoidance (Battig and Grandjean, 1964; Wada *et al.*, 1988, 1989) schedules have been used.

Animals can postpone electrical shock for a fixed interval by lever-pressing responses in the Sidman avoidance task, and they learn to press a lever frequently approaching the presentation of shock. This means that the animals learn to evaluate (or discriminate) a fixed time interval (Reynolds, 1975) and learn to predict the onset of shock.

Shigeta *et al.* (1978, 1979) and Mullin and Krivanek (1982) reported that a single exposure to toluene vapor at concentrations of about 300 ppm impaired Sidman avoidance learning in the rats, but there was no effect at concentrations ranging up to 1000 ppm. The rats exposed to 3000 ppm toluene vapor for 4 hr had shortened lever-pressing interresponse intervals (IRTs) (Shigeta *et al.*, 1978). However, the effects of toluene on shock avoidance learning were short-term and transient in all cases. Repeated exposure to 800 ppm toluene vapor for 7 hr per day for 12 weeks resulted in no impairments of Sidman avoidance learning in the rats, but caused shortened IRTs at concentrations of 2000 ppm (Shigeta *et al.*, 1979). This result was interpreted to mean that individual differences in sensitivity to toluene appeared in the shortening of IRTs.

In a shuttlebox avoidance task, in which animals can avoid electrical shock by means of locomotor responses, repeated exposure to toluene vapor at concentrations of 550 and 750 ppm for 4 hr per day for 3 weeks did not impair shock avoidance learning (Battig and Grandjean, 1964). Toluene exposure at concentrations of 1000 and 2000 ppm for 4 hr daily for 1, 3, and 6 weeks did not influence shock avoidance learning and activity levels. However, in an analysis of response latency (RL) of avoidance responses, toluene-exposed rats showed a wide range of RLs or short RLs, while control rats gradually increased RLs and responded at a short range of RLs (Wada *et al.*, 1988). Single toluene exposure impaired shock avoidance performance at concentrations of 4000, 6000, and 8000 ppm, but did not affect performance at 2000 ppm. RLs were prolonged at 4000 ppm toluene exposure (Wada *et al.*, 1989). These changes in RLs were interpreted as suggesting that certain higher nervous functions of the CNS which control timing behavior were confused.

Pryor *et al.* (1983a,b, 1984) used a pole-climbing avoidance task, in which the rats could avoid electrical shock by climbing a pole fixed to the ceiling of the experimental box, and investigated effects of repeated exposure to toluene vapor on learning using weanling rats. As a result, repeated exposure to toluene vapor at concentrations of 900, 1200, and 1400 ppm for 14 hr daily impaired pole-climbing avoidance. This impairment did not occur when the warning stimulus was light or nonaversive footshock. In cases where a high-frequency tone served as a warning stimulus, learning impairments were extremely severe. Pryor *et al.* pointed out that toluene caused cognitive deficits in high-frequency hearing loss. The morphological examination of these rats revealed loss of, and damage to, hair cells in the basal turn of the cochlea (Pryor *et al.*, 1984). Hearing loss induced by toluene in the rats was evidenced by means of auditory-evoked response in the brain stem, and repeated exposure to toluene vapor attenuated the amplitude of the evoked potential and elevated the latency and the threshold for the appearance

of evoked responses (Rebert *et al.*, 1983). As far as the authors know, there is no clinical report that toluene induces hearing loss.

Behavioral toxicology researchers have been also adopting appetitive schedules other than these shock-avoidance schedules. In the appetitive task animals could receive reinforcements (food, milk, and water) by performing a specific operant response (for example, lever-pressing).

In a fixed-interval (FI) schedule, in which a fixed interval had to pass before it became possible to obtain reinforcement by lever-pressing, subacute exposure to toluene vapor increased responses at concentrations of 1000 ppm for 4 hr daily for 5 days, and decreased responses at concentrations of 2000 ppm. Subacute exposure to 500 ppm toluene vapor had no effects on FI responses (Glowa, 1981).

A schedule in which an interval must pass before a reinforcement becomes available as a result of lever-pressing, and in which the intervals vary every time, is called a variable interval (VI) schedule. The responses in a VI schedule increased with exposure to toluene vapor at concentrations of 1000 and 2000 ppm for 4 hr (Miyagawa *et al.*, 1984).

In a fixed-ratio (FR) schedule, animals can receive a reinforcement after pressing a lever a certain number of times. For example, rats can get 1 food pellet for every 10 lever-pressing responses in a FR10 schedule. Colotla *et al.* (1979) reported that rats exposed to 574 ppm toluene vapor had a decreased number of responses with a FR schedule. Moser and Balster (1985) also indicated a concentration-dependent decrease of response rates in a FR schedule. The effective concentration 50% (EC_{50}), which is expected to decrease performance to 50% of control levels, was determined to be 1853 ppm of toluene vapor (Moser and Balster, 1985).

Wood *et al.* (1983) used a fixed consecutive number (FCN) 8 schedule of reinforcement; rats were trained to respond using the right lever after pressing the left lever at least 8 consecutive times. Toluene-exposed rats switched, using the right lever before pressing the left lever 8 times consecutively. Marked changes in accuracy of pressing the left lever 8 times were induced at concentrations of 1700 and 3000 ppm. The EC_{50} was 1081 ppm.

A differential reinforcement of low rates (DRL) schedule demands that animals inhibit lever-pressing responses for a specific interval and then press a lever. Rats learn to respond frequently after the specific interval. The DRL schedule is also used to investigate toluene toxicity (Colotla *et al.*, 1979; Ikeda and Miyake, 1978; Miyake *et al.*, 1983; Moser and Balster, 1981). Moser and Balster (1981) indicated a decrease in reinforcements in acute exposure to toluene vapor at concentrations over 1600 ppm for 30 min, and a decrease in responses at concentrations of 6400 ppm. The IRTs of lever-pressing responses were shortened with exposure to toluene vapor at concentrations of 1600 and 3200 ppm. Repeated exposure to 6000 ppm toluene vapor for 30 min per day for 7 weeks decreased the percentage of reinforcements. Ikeda and Miyake (1978) exposed rats to 4000 ppm toluene vapor for 2 hr per day for 60 days. The rats showed poor acquisition of DRL learning. Since there was no difference between toluene-exposed rats and control rats in the open field test and the wheel activity test, repeated exposure had little effect on spontaneous locomotor activities and emotionality. Body weights did not show significant differences either. The DRL learning was retarded in repeated exposure to toluene vapor at concentrations of 1000, 4000, and 7000 ppm for 1 hr per day for 154 days, but activities, emotionality, and body weights did not show

significant differences. Furthermore, no macroscopic or microscopic abnormalities could be detected by means of histological examination of the CNS, lung, liver, and kidney (Miyake *et al.*, 1983). Ikeda and Miyake (1978) and Miyake *et al.* (1983) suggested that the cognitive processes in the CNS were impaired by toluene inhalation, so that the DRL learning was retarded.

Toluene-induced cognitive dysfunctions have also been investigated in recent years (Evans *et al.*, 1985; Geller *et al.*, 1985; Taylor and Evans, 1985; Wada *et al.*, 1991). Animals must memorize the physical features of a sample stimulus and after a delay interval they are required to respond on the same choice stimulus as the sample to be reinforced. This is called a delayed matching to sample (DMS) task. Using this task, the effects of toxic substances on short-term memory (STM) can be studied. Evans *et al.* (1985) trained pigeons and monkeys in a DMS task. The matching accuracy in the pigeons was reduced after 1 to 2 weeks of daily exposure to 3000 ppm toluene vapor, and the effects of toluene were greater at longer delay intervals between the sample and choice stimuli. The monkeys showed no observable impairments at toluene concentrations up to 1000 ppm.

Toluene inhalation at concentrations of 2000, 3000, and 4500 ppm impaired the DMS performance in monkeys (Taylor and Evans, 1985). The monkeys showed performance impairments even in the delay interval 0 sec, and the observation to response keys were decreased. These results were interpreted as indicating attention deficits rather than STM deficits. Baboons were also used as subjects and showed decreases in responding and increases in response time at concentrations of 750 and 1000 ppm toluene vapor; however, the matching errors were few (Geller *et al.*, 1985).

In contrast, the pigeons injected with toluene exhibited an increase in correct responses in the DMS task and the performance was improved (Fig. 1). It was reported that toluene administration at relatively low dosages seemed to have excitatory effects on the CNS in pigeons and presumably its arousal actions activated STM processes (Wada *et al.*, 1991). No one had reported toluene-induced improvements of the DMS performance and it is a notable result.

There is one study in which toluene vapor was used as a reinforcement for operant responses (Weiss *et al.*, 1979). A monkey could sniff toluene vapor for 15 sec by pushing a button. As a result, the monkey established self-administration behavior with toluene vapor at concentrations of 1000 and 3000 ppm. This means that toluene vapor functions as a reinforcement and it has the possibility of inducing addiction.

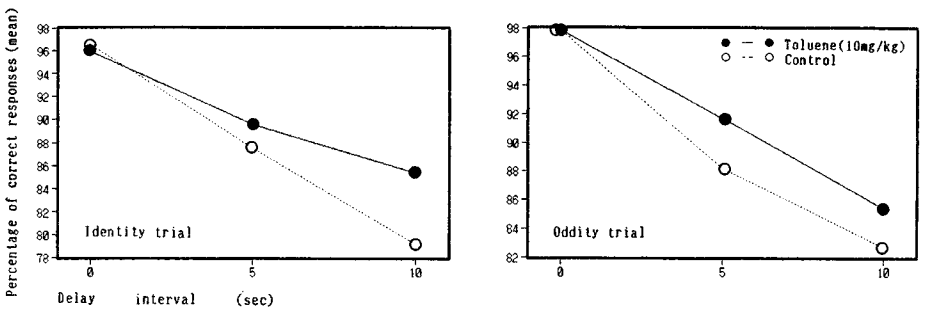

FIG. 1. Effects of toluene administration on the percentage of correct responses. Values are means (n = 4) and data of control (olive oil) injections are pooled for 3 days.

CONCLUSIONS AND FURTHER DEVELOPMENTS

One of the more important requirements for the test batteries of toxicity is to be able to detect already-known toxicity. Clinical and epidemiological studies reported several symptoms of the CNS induced by toluene inhalation, and some of them were detected by means of behavioral approaches (Table 1).

Toluene is reported to induce hilarity and exhilaration at low concentrations and unconsciousness and coma at high concentrations (Benignus, 1981). The same biphasic effects were observed in toluene-exposed rats. The rats exhibited increases in locomotor activity after low-level toluene exposure and decreases in locomotor activity after high-level toluene exposure accompanied by ataxia and paralysis (Hinman, 1987; Wada *et al.*, 1989).

Toluene self-administration behavior was established in monkeys (Weiss *et al.*, 1979). Toluene is easily vaporized at room temperature and is usually inhaled as vapor by organisms. These chemical properties make it difficult to control constant concentrations and accurate sniffing durations of toluene vapor and to present it as a reinforcement immediately after operant responses. However, toluene addiction was experimentally demonstrated by the report and further research will contribute to the elucidation and prevention of toluene sniffing.

Insomnia is a frequent complaint of workers exposed to toluene vapor for a long time. Arito *et al.* (1984, 1985) investigated the effects of toluene administration on the circadian rhythms of sleep–wakefulness. Rats administered toluene and housed under a 12-hr light/dark cycle exhibited reduced sleeping and increased locomotor activity during light periods and their brain monoamine metabolisms were related with disturbances in circadian rhythms.

Memory disturbances and attention deficits were also indicated using a DMS task. Repeated exposure to toluene vapor impaired the DMS performance in the pigeons, and matching accuracy declined as the delay intervals became longer (Evans *et al.*, 1985). The results were interpreted as STM impairments. On the other hand, monkeys exposed to toluene vapor showed performance impairments even in the 0-sec delay interval, although they need not memorize the sample stimulus. Since the observations to response keys were decreased, attention deficits rather than STM deficits were suggested (Taylor and Evans, 1985).

The results of behavioral analysis were almost consistent with clinical and

TABLE 1
COMPARISON OF ALREADY-KNOWN TOXICITY OF TOLUENE AND RESULTS OF BEHAVIORAL TOXICOLOGY RESEARCHES

Already-known toxicity	Behavioral toxicology research
Hilarity and exhilaration	Increase of activity (low concentration)
Unconsciousness and coma	Decrease of activity (high concentration)
Addiction	Establishment of self-administration
Insomnia	Disturbance of circadian rhythm
Memory disturbance	Impairment of DMS performance
Attention deficit	Decrease of observation
Unstable mood	?
Poor intelligence	?
?	Impairment of sensory discrimination
?	Impairment of time discrimination
?	Improvement of DMS performance

epidemiological studies, which means that the techniques could detect already-known toxicity of toluene to the CNS. In addition to the results mentioned above, behavioral analysis techniques were used to evaluate the influences of various chemicals, for example, psychotropic drugs, other organic solvents, and heavy metals. Consequently, behavioral toxicology seems to be a useful and valid methodology for detecting and evaluating harmful effects of chemicals on the CNS.

However, behavioral toxicology is a new and developing research area and has not yet been adequately formulated. The effects of each chemical were evaluated under various exposure conditions and learning tasks as we reviewed above. The results include many different factors and were exceedingly complicated. For the further development of behavioral toxicology, it is absolutely necessary to discover a law which can describe and can be widely applied to the influences of chemicals, for instance, concentration (dose)–response relationships.

The inhalation methods are very popular in experimental studies of toluene toxicity, but it is difficult to maintain toluene vapor at constant levels for a long period. In addition, they let us describe exposure conditions with two parameters, namely concentrations of toluene vapor and exposure durations. The descriptions with two parameters cause us a great deal of trouble when comparing studies. Moreover, the descriptions indicate only procedures of toluene exposure: how high the concentrations and how long the durations of exposure to toluene vapor. The real volumes absorbed by the organisms are obscure.

Some reports introduced blood toluene levels and brain toluene levels as indices (Kishi *et al.*, 1988; Miyagawa *et al.*, 1984, 1986). Toluene increased responding at low brain toluene levels and decreased responding at high brain toluene levels, so that inverted U-shaped relationships were observed between brain toluene levels and lever-pressing responses. It was suggested that the behavioral effects of toluene could be described in a simple manner if the brain toluene levels were introduced (Miyagawa *et al.*, 1984, 1986). Blood and brain toluene levels express with one parameter the real volumes of toluene absorbed by organisms. Attempts introducing blood and brain toluene levels as an index of toluene exposure would produce good results to compare with each study of toluene intoxication and to discover a law widely applied to behavioral toxicology.

Learned behavior is regulated by various factors, some of which, such as activity, emotionality, motivation, sensitivity to electrical shock, and other peripheral effects, affect behavior nonspecifically. Learned behavior is apparently impaired by nonspecific effects of chemicals. Sometimes it results in misunderstanding of conclusions. Since toluene increases activity levels at relatively low concentrations and decreases activity levels at relatively high concentrations, the changes in responding in learning tasks as we reviewed in the previous sections could be explained by and attributed to one factor, namely the effects of toluene on activity levels. If we reconsider the results of behavioral toxicology research, some of behavioral impairments can be considered such nonspecific effects rather than real learning impairments.

Understanding the real factors that regulate each schedule-controlled behavior is absolutely important to interpreting, describing, and simplifying the toxicity of chemicals. Hence, more reliable concentration–response (activity levels) relationships of toluene, which are widely applicable to various schedule-controlled behavior, will be established.

However, all behavioral impairments cannot be attributed to nonspecific ef-

fects. Several impairments were induced by toluene exposure, unless other nonspecific effects were observed.

Weanling rats exposed repeatedly to toluene vapor could acquire pole-climbing avoidance learning when a light or nonaversive footshock was presented as a warning stimulus. Nevertheless, they indicated impairments in avoidance learning when a tone was used as a warning stimulus (Pryor *et al.*, 1983a,b). IRTs in Sidman avoidance and DRL schedules were shortened (Moser and Balster, 1981; Shigeta *et al.*, 1978, 1979) and time discrimination learning was retarded (Miyake *et al.*, 1983) in toluene-exposed animals, although there were no influences of toluene on activity, emotionality, body weights, and even internal organs (Miyake *et al.*, 1983). In a shuttlebox avoidance task, toluene-exposed rats exhibited a wide range of RLs or short RLs, while control rats gradually increased RLs and finally responded at a short range of RLs (Wada *et al.*, 1988). There were no differences in activity levels between toluene-exposed rats and control rats. Furthermore, pigeons injected with toluene gave the same high performance as control-injected birds in a DMS task at the 0-sec delay interval, in which the pigeons were not required to memorize the sample stimulus. This means that nonspecific actions such as physical pains, inflammations of organs, motor dysfunctions, reduced motivation, and stimulus discrimination deficits were not induced by toluene injections. However, the pigeons exhibited higher performance levels than the controls at the 5- and 10-sec delay intervals (Wada *et al.*, 1991). Accordingly, the results could be attributed to the effects of toluene on specific functions of the CNS such as stimulus discrimination, time discrimination, and STM, primarily cognitive functions of the CNS.

As far as the authors know, results such as these behavioral toxicology data have not been reported in clinical and epidemiological studies (Table 1); there might be unknown toxicity of toluene that behavioral toxicology research could predict. It is a great advantage for behavioral toxicology to be able to evaluate toxicity of chemicals on specific higher functions of the CNS, and these are excellent examples that adequately exhibit the merits of behavioral toxicology and elucidated the toluene-induced functional toxicity to the CNS.

Workers are usually exposed to toxic chemicals at low levels for a long time, and serious dysfunctions of the CNS are reportedly induced. Repeated exposure to toluene vapor impaired various cognitive functions of the CNS in animals, although activity, emotionality, and body weights were not affected. This suggests that the higher functions of the CNS such as cognitive functions are extremely sensitive to toxic effects of chemicals, especially at low levels and long-term exposures. Therefore, the studies of cognitive functions become a powerful measure for detecting and evaluating the influences of chemicals at low levels and chronic exposures. Hereafter, the techniques of behavioral toxicology will be widely used and the studies of functional toxicity to the CNS will advance if we use the highly sophisticated learning tasks which reflect specific higher nervous functions.

The effects of toxic chemicals are often irreversible and cause lethal damage to organisms. The acute effects of toluene exposure were reversible and transient even at the extremely high concentrations which induce coma and paralysis; however, it is reported that chronically exposed workers and abusers complain of memory loss, poor intelligence, and insomnia. Brain atrophy is also observed in these patients, so that toluene may possibly have irreversible effects on organ-

isms. Therefore, it is absolutely necessary to establish the methodologies as quickly as possible to detect, evaluate, and predict the toxicity of chemicals.

Behavioral toxicology is a relatively new research area; it has been no more than 20 or 30 years since results began to be reported. It is our important work to develop behavioral toxicology into more sensitive and reliable methodologies to be able to detect and predict unknown toxicity of newly synthesized chemicals in the future.

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Effects of Toluene Administration on Delayed Matching-to-Sample Performance in the Pigeon[1]

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Four pigeons were trained using a delayed matching-to-sample task. This task was composed of identity matching-to-sample trials and oddity from sample trials. In the case of identity trials, pigeons were required to choose the same stimulus as a sample to reach a food reward, and in the case of oddity trials, they had to choose a different stimulus from a sample to gain a food reward. After the performance became stable, toluene was administered intramuscularly at dosages of 10, 20, 40, and 80 mg/kg and tests were executed. The dosage of 10 mg/kg toluene caused increases in the percentage of correct responses in the 5- and 10-sec delay intervals, and 20 and 40 mg/kg toluene injections also increased the correct responses to some extent. At the dosage of 80 mg/kg toluene, the correct responses decreased and the performance was impaired. Toluene administration at relatively low dosages, especially 10 mg/kg, seemed to have excitatory effects on the CNS in pigeons, and presumably its arousal actions activated memory processes.

INTRODUCTION

Toluene is one of the main organic solvents and is used extensively in the painting, printing, and chemical industries as a solvent in paint and a raw material in the manufacturing of chemicals. Toluene is an important and useful material; however, it is widely known that people working in these industries complain of headache, dizziness, euphoria, and unstable mood after occupational exposure to toluene vapor. Epidemiological studies of toluene intoxication have reported various cognitive dysfunctions of the central nervous system (CNS) such as memory loss, impairment of attention and concentration, and poor abstracting ability (Foo *et al.*, 1988; Hänninen *et al.*, 1976; Juntunen *et al.*, 1985; Stollery and Flindt, 1988). There is an urgent need to clarify toluene toxicity in order to make it possible to impose legal controls for the health of workers.

One of the various methodologies used to answer these demands is behavioral toxicology, which evaluates the effects of toxic substances on behavior. Behavioral toxicology has the merits of providing an accurate grasp of functional toxicity in the CNS through the analysis of learned behavior, because learned behavior reflects the integrated higher nervous functions of the CNS. Since behavioral analysis techniques based on operant conditioning methods give us various

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learning paradigms, we can also investigate the effects of chemicals on cognitive functions of the CNS such as memory, stimulus discrimination, attention, and time perception (Paule, 1990; Wenger, 1990). Furthermore, learning is a common ability among various species. It is possible to predict toxicity in humans by using experimental animals. Therefore, behavioral toxicology is assumed to play an important role in occupational and environmental health.

Indeed, behavioral toxicology investigations using an animal exposed to toluene vapor demonstrated almost the same impairments of the CNS cognitive functions as have been clinically reported. Weanling rats repeatedly exposed to toluene vapor could not acquire pole jump avoidance learning when a tone was presented as a warning stimulus (Pryor *et al.*, 1983). The hearing loss of high-frequency tone was indicated in a sensory discrimination task (Pryor *et al.*, 1984). Interresponse times (IRTs) in Sidman avoidance and differential reinforcement of low rate (DRL) schedules, which were used for time discrimination learning, were shortened (Moser and Balster, 1981; Shigeta *et al.*, 1978, 1979) and time discrimination learning was retarded (Miyake *et al.*, 1983) in toluene-exposed animals. This shortening of IRTs and the retardation of time discrimination learning might indicate impairment of time estimation (Wada *et al.*, 1988, 1989).

A delayed matching-to-sample (DMS) task has been widely applied to the study of memory processes, especially for short-term memory (STM). In this task, animals are required to memorize the physical features of a sample stimulus until the choice stimuli are presented, and after a delay interval, they must choose one of the choice stimuli which was the same as the sample to gain rewards.

One of the main symptoms of CNS dysfunction of which people exposed to toluene vapor frequently complain is memory disturbance. Studies of toluene toxicity on memory processes are urgently needed. In the present study, therefore, the effects of toluene administration on delayed matching-to-sample performance in the pigeon were investigated, and the effects of toluene toxicity on short-term memory and dose–response relationships are discussed.

MATERIALS AND METHODS

Subjects

The subjects were four experimentally naive pigeons and were housed in individual cages with water continuously available. All subjects were maintained at about 80% of their free-feeding weights by additional feeding, if necessary, after the experimental sessions.

Apparatus

Two identical operant chambers for pigeons (SEC-002, BRS/LVE, U.S.A.), which also served as a sound and light attenuation box, were used as an experimental apparatus. The chamber was 37 cm high, 35 cm wide, and 31 cm long, and three circular keys, a food hopper, and a house light were installed on the front panel. Three keys 2.5 cm in diameter were mounted 27 cm above a wire-mesh floor and were spaced 6 cm apart, center to center. Each key could be transilluminated from the rear side of the front panel with an in-line projector (IC-900-696

Extra Film Patterns, BRS/LVE, U.S.A.). The food hopper, 5 cm wide and 6 cm long, was located 11 cm above the floor. The house light was also mounted 33 cm above the floor. A reinforcement schedule and data recording were controlled by a personal computer (PC9801-VX, NEC, Japan) installed in an adjacent room.

Procedure

Training stage 1. All pigeons were first trained to eat from the food hopper. Then using a successive approximation method, they were trained to peck one of three circular keys, which was transilluminated with a white light, under a fixed ratio 1 (FR1) schedule. If the pigeons responded on the transilluminated key, they could have 5 sec access to the food hopper. The FR value was progressively increased and finally became 10 (FR10). The FR10 training procedure confirmed that pigeons pecked the transilluminated key. The house light was on throughout the experimental sessions.

Training stage 2. In this stage, two pattern stimuli (circle and triangle) and two color stimuli (green and red) were introduced. A trial began when the house light presentation and one of four stimuli was randomly projected on one of the three keys. The pigeons were required to make 10 peckings on the transilluminated key to approach the food hopper for 3.5 sec. After 10 peckings, the house light was turned off and a 10-sec intertrial interval (ITI) followed. The peckings on incorrect keys were not counted.

Training stage 3. The pigeons were divided into two groups (A and B) of equal size and were trained under a delayed matching-to-sample (DMS) task composed of identity matching-to-sample trials (identity trials) and oddity from sample trials (oddity trials). The pigeons were required to choose the same comparison stimulus as a sample stimulus in the identity trials and a different comparison stimulus from a sample stimulus in the oddity trials to reach the food hopper. Group A was trained with a triangle–green pair in the identity trials and a circle–red pair in the oddity trials. Hence, when a triangle or a green sample was presented, the pigeons in group A had to respond on the same comparison stimulus as the sample, because it was an identity trial. When a circle or a red sample was presented, they had to respond to a comparison stimulus different from the sample, because it was an oddity trial. Group B was trained with the opposite pairs of stimuli, namely, circle–red in identity trials and triangle–green in oddity trials to counterbalance the stimulus preference. The details are summarized in Table 1.

Each trial was initiated with the house light presentation. After 3 sec, the sample stimulus was projected onto one of the three keys. If the pigeons responded 10 times on the key, the sample was turned off and two comparison stimuli were immediately presented on the remaining two keys (delay interval 0 sec). If the pigeons pecked the key 10 times that was transilluminated with the correct comparison stimulus, the two comparisons went out and they could approach the food hopper for 3.5 sec. After a 10-sec ITI with the house light off, the next trial began. If the pigeons responded on the incorrect key 10 times, two comparisons and the house light were terminated simultaneously and a 15-sec

TABLE 1
PAIRS OF STIMULI IN GROUP A AND GROUP B

Group	Trial	Identity		Oddity	
A	Sample	△	G	○	R
	Comparison	△–G	△–G	○–R	○–R
	Correct	△	G	R	○
B	Sample	○	R	△	G
	Comparison	○–R	○–R	△–G	△–G
	Correct	○	R	G	△

Note. △, triangle; ○, circle; G, green, R, red.

blackout was initiated. Then the same trial began again until the pigeons responded on the correct key 10 times (correction method). The flow chart of the procedure is shown in Fig. 1.

Since the sample stimulus and the transilluminated key were chosen according to the pseudorandom sequences, the same sample was never presented for two consecutive trials and the same key was never transilluminated for three consecutive trials. Furthermore, the four stimuli (triangle, circle, green, and red) were used as samples with equal probability. One session was composed of 72 trials, and the training was carried out 1 session a day, 7 sessions a week until the pigeons attained about 95% correct responses in both identity trials and oddity trials for 5 consecutive days.

Training stage 4. Following training stage 3, three delay intervals of 0, 5, and 10 sec were introduced to the DMS task. All pigeons were required to memorize the physical features of the sample stimulus during the delay intervals and then to respond on an appropriate key to be reinforced. Each delay interval was presented with equal probability and the same delay interval was never used for three consecutive trials. The training continued until the pigeons maintained about 95% correct responses in identity trials and oddity trials, respectively.

Toluene administration. After the performance in training stage 4 became stable, toluene administration was begun. Toluene was dissolved in olive oil to prevent inflammation of internal organs and 10% (v/v) toluene in olive oil solution was administered into the breast muscle at dosages of 10, 20, 40, and 80 mg/kg. Before each toluene administration day, all pigeons were intramuscularly injected with 0.15 ml olive oil for 3 consecutive days to serve as control injections. Each injection was carried out 30 min prior to the sessions. At least 1 week passed before the next toluene administration was given. The training sessions were continuously performed during nontoluene days.

RESULTS

The effects of toluene administration on the percentage of correct responses in the DMS task are shown in Figs. 2–5. Regardless of identity trials or oddity trials, the pigeons injected with olive oil (control injection) maintained extremely high

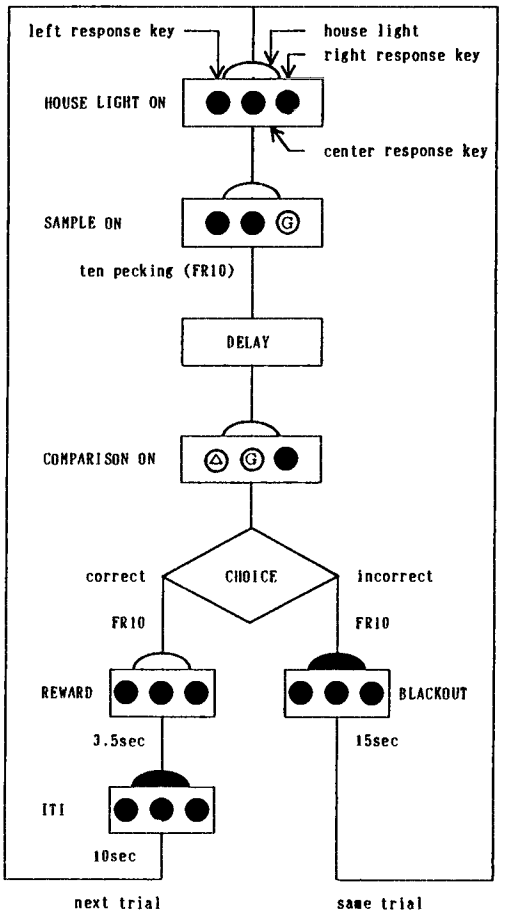

FIG. 1. Flow chart of a delayed matching-to-sample task composed of identity matching-to-sample trials and oddity from sample trials. A trial began when a house light was turned on. After 3 sec, the right response key was transilluminated with green as the sample in this chart. The pigeons pecked the right key 10 times (FR10) and then the sample was turned off. Following the delay interval, two comparison stimuli, a triangle and a green, were projected on the left and the center response keys, respectively. If it was an identity trial (group A), the pigeons were required to peck the center key, and if it was an oddity trial (group B), the pigeons were required to peck the left key. After pecking 10 times on the correct key, the comparisons went out and the pigeons were reinforced with 3.5 sec access to the food hopper. A 10-sec ITI which turned off the house light was inserted and then the next trial was started. If the pigeons pecked the incorrect key 10 times, both the house light and the comparisons were terminated and a 15-sec blackout was started. Then the same trial began again until the pigeons responded on the correct key 10 times (correction method).

percentages of correct responses in the delay interval of 0 sec, and the performance declined as a function of the delay intervals. Even in the delay interval of 10 sec, the pigeons showed much higher percentages of correct responses than a chance level (50%). The performances in oddity trials were generally better than those in identity trials.

At the dosage of 10 mg/kg toluene (Fig. 2), the pigeons showed the same good

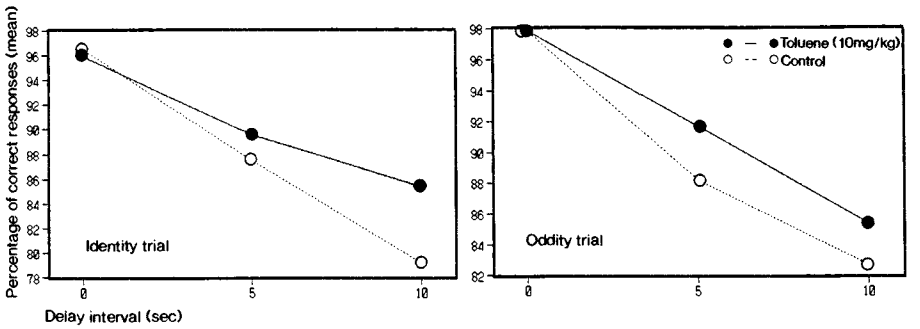

FIG. 2. Effects of toluene administration on the percentage of correct responses. Values are means (n = 4), and data of control (olive oil) injections are pooled for 3 days.

performance in the delay interval of 0 sec as the control injections, and the percentage of correct responses also decreased as the delay intervals became longer. However, the pigeons given 10 mg/kg toluene injections had higher percentages of correct responses than those given control injections in both identity trials and oddity trials. The performance was improved in the delay intervals of 5 and 10 sec.

Figure 3 shows the effects of 20 mg/kg toluene administration on the percentage of correct responses. The performances with toluene injections were almost same as those with control injections, although slight increases in correct responses were observable in the delay intervals of 10 sec (identity trials) and 5 sec (oddity trials).

The effects of 40 mg/kg toluene administration on the DMS performance are shown in Fig. 4. Toluene administration at the dosage of 40 mg/kg increased the percentage of correct responses in the oddity trials in the delay intervals of 5 and 10 sec, and the performance was improved. In the identity trials, correct responses were increased in the delay interval of 10 sec but decreased in that of 5 sec.

Figure 5 indicates the effects of 80 mg/kg toluene administration on the DMS performance. Toluene administration decreased the percentage of correct re-

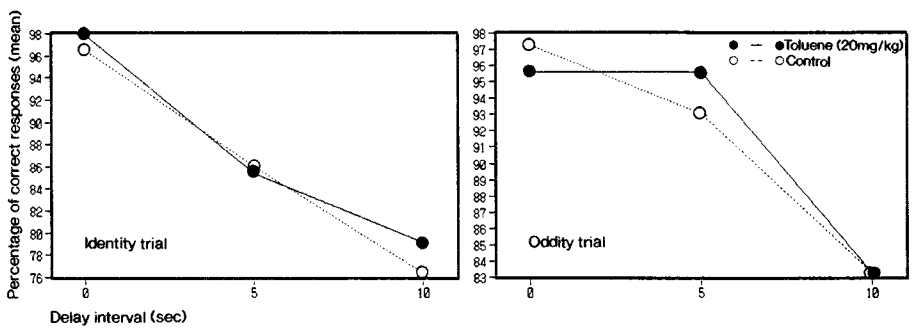

FIG. 3. Effects of toluene administration on the percentage of correct responses. Values are means (n = 4), and data of control (olive oil) injections are pooled for 3 days.

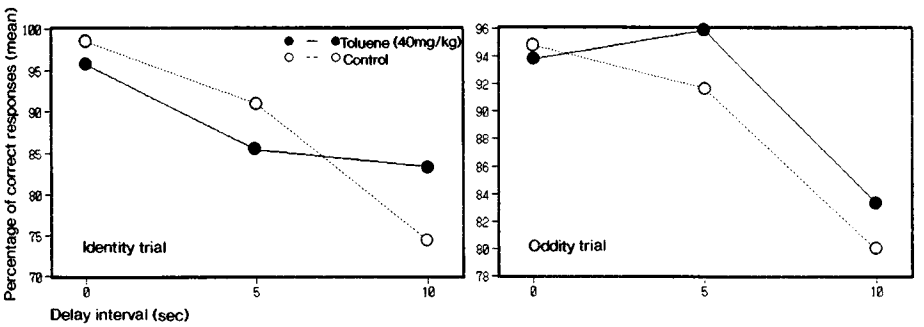

FIG. 4. Effects of toluene administration on the percentage of correct responses. Values are means (n = 4), and data of control (olive oil) injections are pooled for 3 days.

sponses in both identity trials and oddity trials in the delay intervals of 0 and 10 sec, and the performance was impaired. However, the performance was higher than chance level and the impairments in oddity trials were more moderate than those in identity trials.

Prior to toluene administration tests, pilot injections of 100, 200, and 400 mg/kg were given to two other naive pigeons. The pigeons injected with 100 mg/kg toluene exhibited wing strokes and then crouching. Dosages of 200 and 400 mg/kg toluene caused the wing strokes, and about 1 hr later, vomiting.

DISCUSSION

The organic solvent toluene is an important and useful chemical, and it has been widely used in painting, presswork, and chemical industries. Nevertheless, those who have worked in these industries have inhaled vaporized toluene for long periods and it is well known that such occupational exposure to toluene causes various dysfunctions of the CNS. Epidemiological studies have reported that workers frequently complained of such CNS symptoms as memory disturbances, impairments of attention, poor intelligence, and unstable mood (Foo *et al.*, 1988; Hänninen *et al.*, 1976; Juntunen *et al.*, 1985; Stollery and Flindt, 1988). Moreover, long-term toluene inhalation is also known to cause brain atrophy (Fornazzari *et*

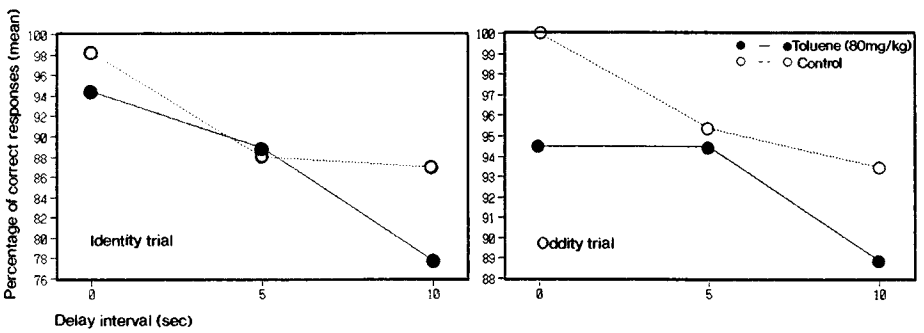

FIG. 5. Effects of toluene administration on the percentage of correct responses. Values are means (n = 3), and data of control (olive oil) injections are pooled for 3 days.

al., 1983; Lazar *et al.*, 1983). Therefore, it is urgent to investigate the effects of toluene toxicity on cognitive functions of the CNS and to prevent occupational diseases.

One of the serious dysfunctions of the CNS cognitive processes observed most frequently in toluene-exposed workers was memory impairment (Foo *et al.*, 1988; Hänninen *et al.*, 1976; Juntunen *et al.*, 1985). However, the effects of toluene on memory processes are still unclear and experimental investigations are necessary to be able to establish exposure parameters. Only a few previous studies reported using a DMS task which has been widely applied to the studies of memory processes, especially of STM (Evans *et al.*, 1985; Geller *et al.*, 1985; Taylor and Evans, 1985). The animals in the DMS task were required to memorize physical features of a sample stimulus, and after a delay interval they had to respond to one of the choice stimuli which was the same as the previous sample to gain a reward.

Pigeons trained in the DMS task received daily exposure to toluene and the influences on the performance were evaluated (Evans *et al.*, 1985). Inhalation of 3000 ppm toluene vapor for 1 to 2 weeks decreased the matching accuracy and its effects on performance were greater as the delay intervals became longer. Toluene inhalation at concentrations of 2000, 3000, and 4500 ppm impaired the DMS performance in monkeys (Taylor and Evans, 1985). The monkeys showed performance impairments even in the 0-sec delay interval and the attention to the correct response keys was decreased. These results were interpreted to indicate attention deficits rather than STM deficits. Baboons were also used as subjects and showed decreases in responding and increases in response time at concentrations of 750 and 1000 ppm toluene vapor; however, the matching errors were fewer (Geller *et al.*, 1985).

In the present studies, the pigeons were intramuscularly injected with toluene and the effects of toluene on the percentage of correct responses were investigated. If toluene administration decreased the percentage of correct responses in the 0-sec delay interval, it could be suggested that nonspecific actions such as physical pain, inflammation of organs, motor dysfunctions, reduced motivation, and stimulus discrimination deficits were induced by toluene rather than impairments of STM, because the pigeons were not required to memorize the sample stimulus in the 0-sec delay interval. However, the pigeons injected with toluene at dosages of 10, 20, and 40 mg/kg maintained an extremely high performance level in the 0-sec delay interval, almost the same level as that with control injections. This means that toluene administration had no influence on the performance in the 0-sec delay interval and the nonspecific effects mentioned above were not induced.

The dosage of 10 mg/kg toluene increased the percentage of correct responses in the 5- and 10-sec delay intervals. This indicated that toluene administration improved the performance of DMS task and it is possible that toluene activated STM processes. Dosages of 20 and 40 mg/kg toluene also increased correct responses in the longest delay interval of 10 sec. It has been said that toluene has excitatory effects on the CNS at low doses and inhibitory effects at high doses. Toluene administration ranging from 10 to 40 mg/kg, but especially at 10 mg/kg,

might have excitatory effects on the CNS in the pigeons and might activate memory processes.

Injections of 80 mg/kg toluene caused decreases in the percentage of correct responses and reduced the DMS performance. The pigeons exhibited impairment in performance even in the 0-sec delay interval, in which they did not need to memorize the sample stimulus. The effect of 80 mg/kg toluene seemed to be nonspecific rather than only on memory impairment.

In the present studies the DMS task was composed of two different types of learning, namely identity trials and oddity trials. The pigeons showed a trend of higher baseline performance in the oddity trials than in the identity trials, and toluene was effective in improving the performance in the oddity trials. The reduction in performance level at a dosage of 80 mg/kg was more moderate in the oddity trials than in the identity trials. The influences of toluene could depend partly on the baseline performance and the structure of the learning task.

Toluene was administered using injection methods, which have the advantages of simplicity, convenience, and accurate control of volumes absorbed into organisms, compared with inhalation methods, which require complex techniques and expensive equipment and are difficult to control. Injection methods are not popular and only a few neurobehavioral studies have been reported; hence, the present results cannot be compared with those of other studies.

The effects of toluene on memory processes, important cognitive functions, have been recently investigated using a DMS task, but no one reported improvements in performance. It is notable that toluene administration at low dosages improved the DMS performance in the present studies and probably activated STM processes.

Needless to say, toluene is a toxic chemical and has harmful effects on organisms. Further studies of long-term toluene administration are also necessary to confirm its influences on memory processes.

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Acute Neurobehavioral Effects of Co-inhalation of Toluene and *n*-Hexane on Schedule-Controlled Behavior in Rats[1]

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Whether coexposure to toluene and *n*-hexane had any combined effects on the shock avoidance performance in rats was studied. Eighteen Wistar male rats with an avoidance rate of over 80% were selected and divided to three groups based on performance and body weight: (1) toluene, (2) *n*-hexane, and (3) toluene + *n*-hexane. Each group was exposed alternately first to air and then to a particular organic solvent for 4 hr at various concentrations (50, 100, 200, 400, or 800 ppm, in ascending order). The effects of each organic solvent were evaluated by comparing the performance of rats during and after exposure with their own performance under the sham exposure to air by three-way ANOVA. The main results were that (1) 200, 400, or 800 ppm toluene exposures increased lever press rates, (2) 50 ppm *n*-hexane exposure decreased lever press and avoidance rates in a transitory manner and 800 ppm *n*-hexane exposure increased the lever press rate, (3) the 50 ppm mixture (25 ppm toluene + 25 ppm *n*-hexane) decreased lever press and avoidance rates persistently during and after the 4-hr exposure and the 800 ppm mixture (400 ppm toluene + 400 ppm *n*-hexane) decreased lever press and avoidance rates unpredictably when compared to the results of 400 or 800 ppm of toluene or *n*-hexane alone. In conclusion, *n*-hexane showed narcotic effects at 800 ppm and modified the acute neurobehavioral effects of toluene in rats at 400 ppm toward unpredictable results.

INTRODUCTION

Studies on the psychological test performance of workers exposed to a single solvent (toluene) and to a mixture of organic solvents suggested that the greater effects in workers exposed to solvent mixtures are partly the result of potentiating interaction in connection with simultaneous exposure to several solvents (Iregren, 1982). Even though exposure to a mixture of organic solvents is common in industry and other environments, few human experiments have been reported with regard to possible combined neurobehavioral effects produced by simultaneous exposure to two different kinds of organic solvents, except for studies concerning alcohol intake such as those on trichloroethylene and alcohol (Windemuller and Ettema, 1978), xylene and alcohol (Savolainen, 1980), *m*-xylene and 1,1,1-trichloroethane (Savolainen *et al.*, 1981), toluene and alcohol (Cherry *et al.*, 1983), toluene and methyl ethyl ketone (Dick *et al.*, 1984), toluene and *p*-xylene (Olson *et al.*, 1985), and toluene and ethanol (Iregren *et al.*, 1986).

As yet, no interactional effects of the nonadditive type on behavioral performance have been demonstrated for combined exposure to two solvents or for the combination of exposure to one solvent and the intake of alcohol at levels close to threshold limit values.

Toluene and *n*-hexane are among the most ubiquitous organic solvents. They, frequently in combination, are major constituents of common household products

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(Fukabori *et al.*, 1983) and are used in many industrial and laboratory processes as glue, paints, thinners, inks, or degreasers (Inoue *et al.*, 1984; Lehmann *et al.*, 1986).

But little information is available concerning the combined effects on central nervous system function of simultaneous exposure to toluene and *n*-hexane; however it is well known that toluene greatly reduces peripheral nerve toxicity in rats caused by chronic exposure to *n*-hexane (Takeuchi *et al.*, 1981) by disturbing the metabolism of *n*-hexane to 2-hexanol and 2,5-hexanedione in rats (Perbellini *et al.*, 1982). Some neurochemical evidence showed the synergistic interaction between toluene and *n*-hexane in the central nervous system in rats (Honma, 1983; Ikeda *et al.*, 1986).

From this neurochemical evidence, it was supposed that co-inhalation of toluene and *n*-hexane might cause synergistic neurobehavioral effects in rats. Thus, the present study was designed to investigate the acute neurobehavioral effects of exposure to toluene and *n*-hexane separately and in combination in rats.

MATERIALS AND METHODS

Animals and Apparatus

Experimentally naive Wistar male rats (Shizuoka Laboratories, Japan) obtained at 6 weeks of age were housed four per cage at a temperature of 23 ± 2°C with a 12-hr dark/light cycle (the dark period beginning at 7 AM) and had free access to laboratory diet (MF diet blocks, Oriental Yeast Co., Ltd, Japan) and tap water. For the behavioral test three Skinner boxes (BRS/LVE), each of which was installed in a separate gas chamber, were used with a microcomputer for programming and recording. A shock generator and scrambler (BRS/LVE) was used to deliver a 250-V, 2.5-mA DC shock to the grid floor of each Skinner box for 0.3 sec.

Chemicals and Exposure

Toluene was of a special grade (purity over 99%, Wako Chemical Co., Japan), *n*-hexane was a special grade (purity over 99%, for assay of residuary pesticides, Wako Chemical Co., Japan).

Static exposure was carried out using three identical stainless steel gas chambers (70 × 70 × 120 cm) for three parallel groups (group TL, group NH, and group TL + NH), each type of exposure being carried out in a separate exposure chamber.

A calculated volume of toluene, *n*-hexane, or a mixture of toluene and *n*-hexane was introduced from an upper inlet by using a vaporizer. Rats were exposed to fresh air or various concentrations (50, 100, 200, 400, 800 ppm, in ascending order) of (1) toluene, (2) *n*-hexane, or (3) a mixture (half concentration of toluene + half concentration of *n*-hexane).

The concentration of toluene or *n*-hexane in the gas chamber was measured several times during every exposure period by flame ionization gas chromatography. The concentration of O_2 in the gas chamber at the end of every exposure period was 20–21% in every case, as measured by an Edmont Oxygen Analyzer (made in the United States). The concentration of CO_2 in the gas chamber at the end of every exposure period was 0.4–0.45% in every case, as measured using a Kitagawa gas detector.

Behavioral Procedure

The shock avoidance response (Ferster and Skinner, 1957) (negative fixed-interval schedule with a light signal) was used to observe the changes from the behavioral baseline during and after the exposure to organic solvents. Rats, 2 months of age at the beginning of the experiment, were trained with a reinforcement schedule having a 10-sec shock-to-shock interval, a light signal being presented for 5 sec before every electric shock. Under this schedule, rats could avoid an electric shock if they pressed the lever during the warning period when the light signal was on. The behavioral baseline was established in the animals following about 25 training sessions. Eighteen rats that had an avoidance rate of over 80% were selected and divided to three groups based on performance and body weight (groups TL, NH, TL + NH, respectively, 427 ± 22 g, 424 ± 29 g, 432 ± 29 g, mean ± SD of body weight, $n = 6$). Each group was exposed alternately first to air, as an internal control, and then to particular organic solvent. Performance tests under the above avoidance schedule began at 10:00 every day, and the level of performance of the pretest (1 hr immediately before exposure) was used as a behavioral baseline for that day. Rats were exposed to air or organic solvents for 4 hr from 11:00 to 15:00 and continued to be tested up to 1 hr after the 4-hr exposure. The interval between exposures to the organic solvent for each rat was set at 14 days to prevent any buildup of effects. Sham exposure to air for internal control was carried out every 7th day following exposure to the organic solvent. The number of lever presses and avoidances per 20 min during these 6-hr test periods were adopted as behavioral parameters.

Statistical Analysis

The effects of each organic solvent were evaluated by comparing the performance of rats during and after exposure with their own performance under the sham exposure to air. The statistical significance of the results was determined for every 1-hr period by three-way analysis of variance, in which (1) exposure effects (two levels: exposure to solvent and to air), (2) time effects (three levels: effects every 20 min in a 1-hr period) and (3) the six individual rats were included as the three factors.

RESULTS

Behavioral changes are shown as mean lever press rate (Figs. 1, 3, and 5) and mean avoidance rate (Figs. 2, 4, and 6), respectively, expressed as the percentage of baseline performance of preexposure. In all figures shading expresses the variation range (mean ± 2 SE, $n = 30$) of the performance under the sham exposures to air. Table 1 shows significant differences comparing the performance of each rat during and after exposure with its own performance under a sham exposure to air. The numbers of rats in which behavioral changes induced by exposure to organic solvents per six rats in each group are shown in parentheses.

As shown in Figs. 1 and 2 and Table 1, exposure to 50 ppm toluene induced considerable, but transitory, decreases of the lever press (4/6) and avoidance rates (5/6) only during the initial 20 min of the exposure. No behavioral change in the lever press or avoidance rate could be detected in rats exposed to 100 ppm toluene. At 200, 400, and 800 ppm toluene exposure concentration-related and time-related behavioral changes were observed. Exposure to 200 ppm toluene induced a transitory but appreciable increase of lever press rate during the initial 60 min of

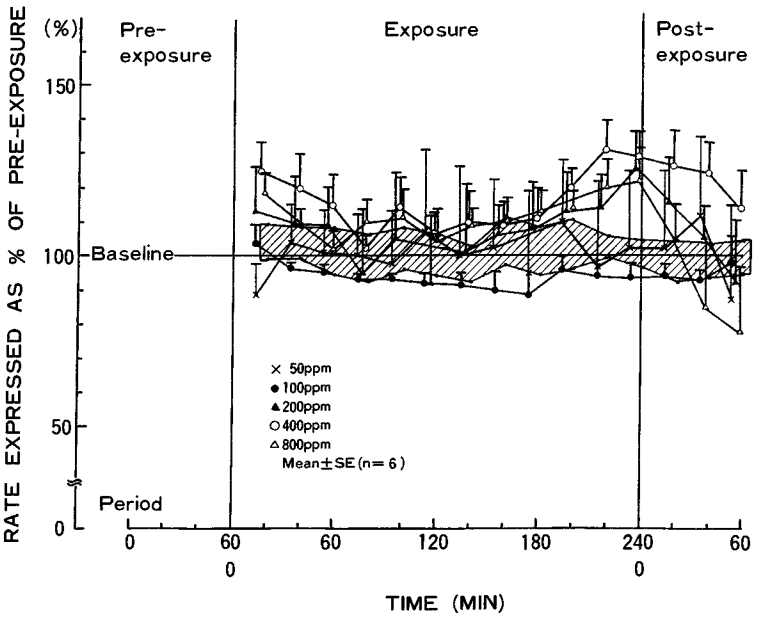

FIG. 1. Lever press rate during and after exposure to toluene. Shading expresses the variation range (mean ± 2 SE, n = 30) of the performance under the sham exposures to air.

exposure compared with own performance under a sham exposure to air ($F(1,10) = 17.04$, $P < 0.01$). Although this behavioral change reverted to the performance level of the sham exposure to air after 60 min of exposure, a time-related considerable increase of the lever press rate ($F(1,10) = 92.12$, $P < 0.01$) was observed again during exposure following 180 min of exposure, but then quickly returned to

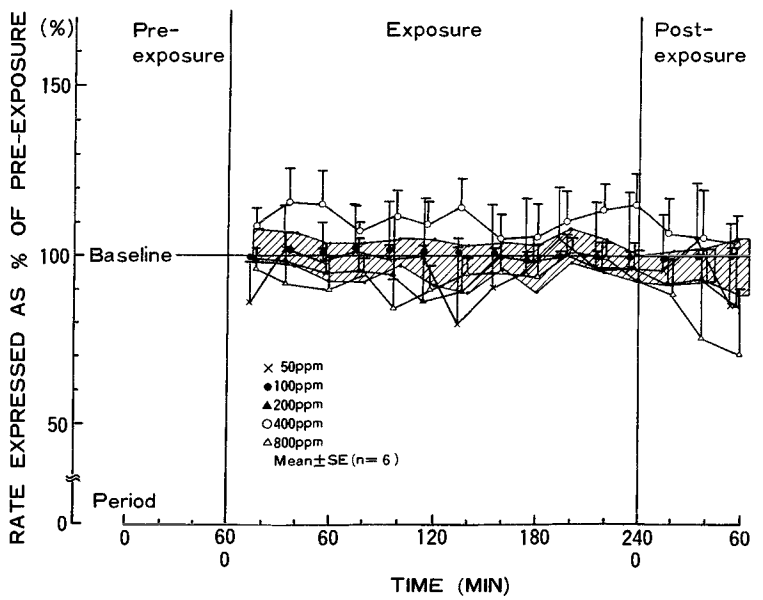

FIG. 2. Avoidance rate during and after exposure to toluene. Shading expresses the variation range (mean ± 2 SE, n = 30) of the performance under the sham exposures to air.

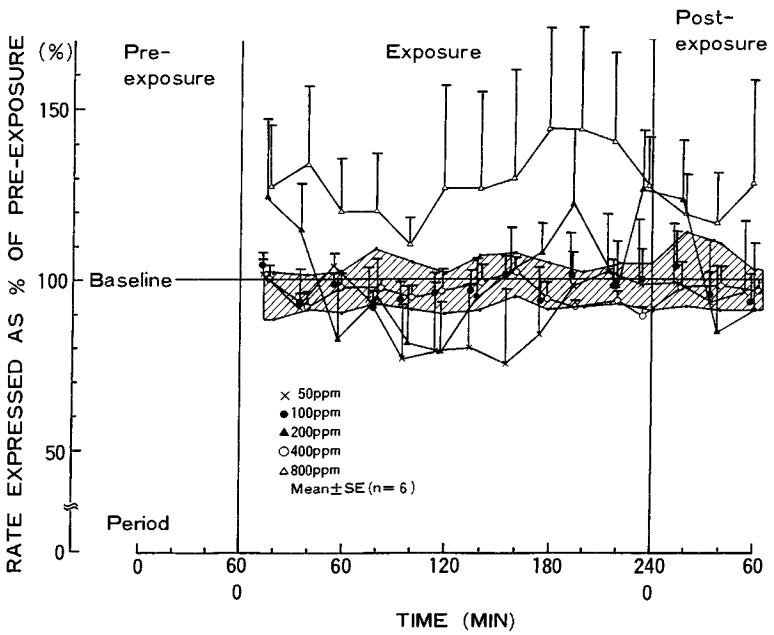

FIG. 3. Lever press rate during and after exposure to *n*-hexane. Shading expresses the variation range (mean ± 2 SE, $n = 30$) of the performance under the sham exposures to air.

the performance level of the sham exposure to air. Exposure to 400 ppm toluene induced a marked increase of lever press rate during the initial 60 min of the exposure ($F(1,10) = 9.78$, $P < 0.05$). However, the rats gradually recovered from this behavioral change during the next 60 min of exposure, a time-related marked

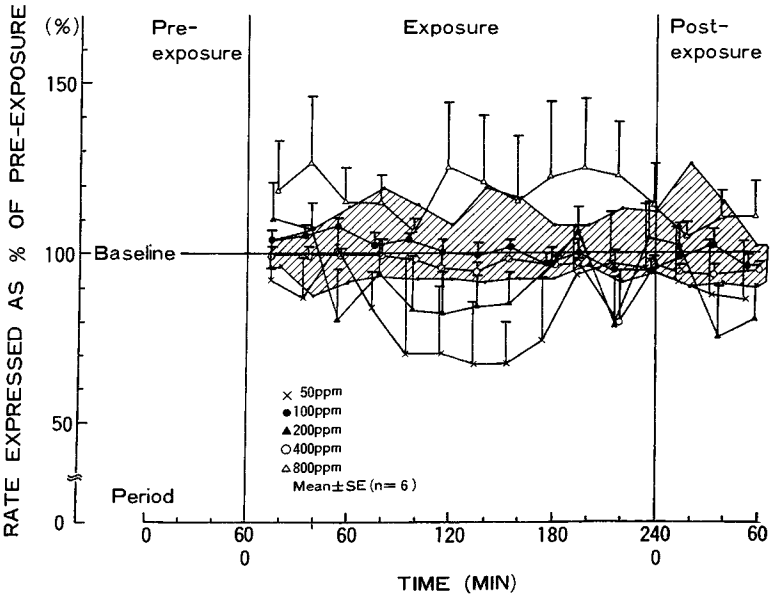

FIG. 4. Avoidance rate during and after exposure to *n*-hexane. Shading expresses the variation range (mean ± 2 SE, $n = 30$) of the performance under the sham exposures to air.

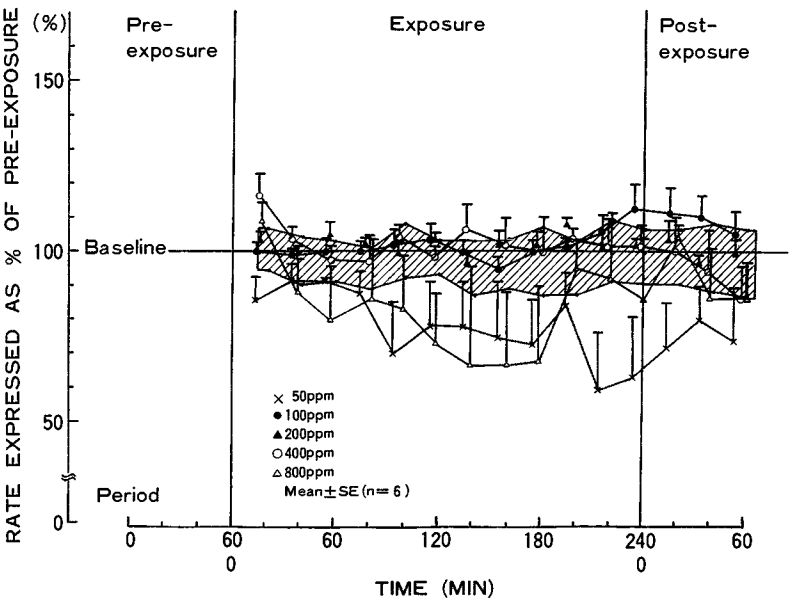

FIG. 5. Lever press rate during and after exposure to mixture of toluene and *n*-hexane. Shading expresses the variation range (mean ± 2 SE, $n = 30$) of the performance under the sham exposures to air.

increase of lever press rate was again observed following 180 min of exposure ($F(1,10) = 58.87$, $P < 0.01$). Increased lever press rate continued for at least 1 hr after the exposure ($F(1,10) = 18.88$, $P < 0.01$). Exposure to 800 ppm toluene also induced a considerable increase of lever press rate during the initial 120 min of

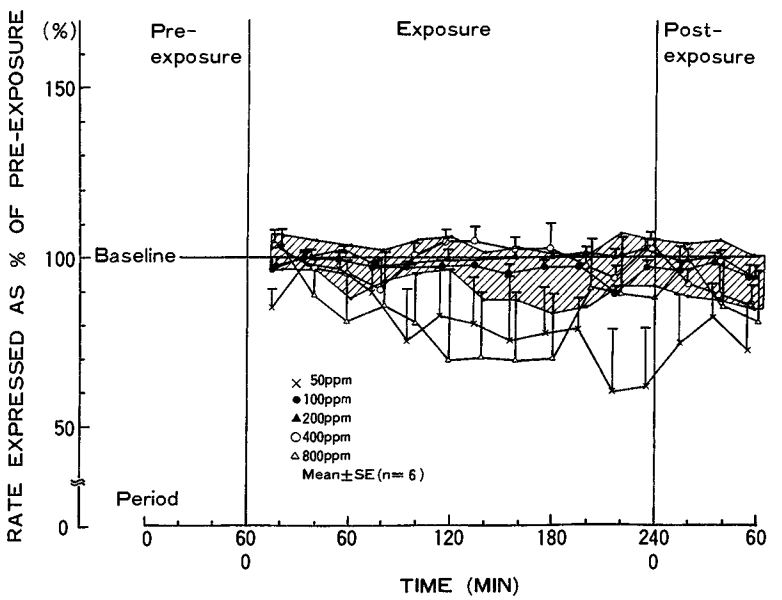

FIG. 6. Avoidance rate during and after exposure to mixture of toluene and *n*-hexane. Shading expresses the variation range (mean ± 2 SE, $n = 30$) of the performance under the sham exposures to air.

TABLE 1

SIGNIFICANT DIFFERENCES BETWEEN OWN PERFORMANCES UNDER SHAM EXPOSURE TO AIR AND UNDER EXPOSURE TO ORGANIC SOLVENT

	Toluene					*n*-Hexane					Toluene + *n*-hexane				
	Exposure (min)				Post-exposure (min)	Exposure (min)				Post-exposure (min)	Exposure (min)				Post-exposure (min)
Concn. (ppm)	0 ~ 60	60 ~ 120	120 ~ 180	180 ~ 240	0 ~ 60	0 ~ 60	60 ~ 120	120 ~ 180	180 ~ 240	0 ~ 60	0 ~ 60	60 ~ 120	120 ~ 180	180 ~ 240	0 ~ 60
Lever press															
50	—	—	—	—	—	—	⇩ (3/6)	↓ (2/6)	—	—	↓ (4/6)	↓ (5/6)	↓ (4/6)	↓ (5/6)	↓ (5/6)
100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
200	↓ (4/6)	—	—	↓ (5/6)	—	—	⇩ (3/6)	—	—	—	—	—	—	—	—
400	⇩ (4/6)	—	—	↓ (5/6)	↓ (5/6)	—	—	—	—	—	—	—	—	—	—
800	⇩ (3/6)	⇩ (3/6)	—	↓ (5/6)	⇩ (3/6)	↓ (5/6)	—	↓ (4/6)	↓ (5/6)	—	—	⇩ (3/6)	↓ (4/6)	—	—
Avoidance															
50	—	—	—	—	—	⇩ (4/6)	↓ (4/6)	↓ (3/6)	—	↓ (4/6)	↓ (6/6)	↓ (5/6)	↓ (4/6)	↓ (6/6)	↓ (6/6)
100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
200	—	—	—	—	—	—	⇩ (3/6)	—	—	—	—	—	—	—	—
400	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
800	—	—	—	—	↓ (3/6)	—	—	—	—	—	—	↓ (4/6)	↓ (2/6)	—	—

Note. Arrows show statistically significant levels and directions: $P < 0.05$, ⇧ (increase) or ⇩ (decrease); $P < 0.01$ ↑ or ↓; not significant (—). Numbers of rats in which behavioral changes induced by exposure to organic solvents per six rats in each group are shown in parentheses.

exposure ($F(1,10) = 7.82$, $P < 0.05$, $F(1,10) = 5.10$, $P < 0.05$ as shown in Table 1). The rats reverted to the performance level of the sham exposure to air after 120 min of exposure. A time-related marked increase in lever press rate was once more observed during exposure following 180 min of exposure ($F(1,10) = 22.69$, $P < 0.01$), while the lever press rate slightly decreased ($F(1,10) = 5.11$, $P < 0.05$) and the avoidance rate decreased considerably ($F(1,10) = 10.13$, $P < 0.01$) after the exposure.

As shown in Figs. 3 and 4 and Table 1, exposure to 50 ppm *n*-hexane induced a considerable decrease of avoidance rate ($F(1,10) = 7.14$, $P < 0.05$) during the initial 60 min of the exposure, while no significant difference could be detected in the lever press rate. Marked decreases of the lever press rate ($F(1,10) = 8.06$, $P < 0.05$; $F(1,10) = 51.18$, $P < 0.01$ as shown in Table 1) and the avoidance rate ($F(1,10) = 46.73$, $P < 0.01$; $F(1,10) = 112.49$, $P < 0.01$ as shown in Table 1) were observed during Minutes 60–180 of the exposure. However these marked decreases of lever press and avoidance rates returned to the performance level of the sham exposure to air during Minutes 180–240 of the exposure, and a considerable decrease of the avoidance rate ($F(1,10) = 62.06$, $P < 0.01$) was once again observed after the exposure. No behavioral changes in the lever press and avoidance rates could be detected in rats exposed to 100 ppm *n*-hexane. Exposure to 200 ppm *n*-hexane induced large, variable behavioral changes. Although no behavioral change in the lever press and avoidance rates could be detected in rats exposed to 400 ppm *n*-hexane, exposure to 800 ppm *n*-hexane induced a considerable increase in the lever press rate ($F(1,10) = 12.30$, $P < 0.01$) during the initial 60 min of exposure. Though the rats reverted once from this behavioral change after 60 min of exposure, a considerable increase of lever press rate ($F(1,10) = 21.74$, $P < 0.01$; $F(1,10) = 20.31$, $P < 0.01$, as shown in Table 1) was persistently observed again during Minutes 120–240 of the exposure, then the rats reverted to the performance level of the sham exposure to air after the exposure.

As shown in Figs. 5 and 6 and Table 1, exposure to 50 ppm mixture of toluene and *n*-hexane (25 ppm toluene + 25 ppm *n*-hexane) consistently induced a marked decrease of the lever press rate ($F(1,10) = 31.28$, $P < 0.01$; $F(1,10) = 50.27$, $P < 0.01$; $F(1,10) = 23.4$, $P < 0.01$; $F(1,10) = 31.55$, $P < 0.01$; $F(1,10) = 226.38$, $P < 0.01$ as shown in Table 1) and the avoidance rate ($F(1,10) = 97.15$, $P < 0.01$; $F(1,10) = 110.67$, $P < 0.01$; $F(1,10) = 20.97$, $P < 0.01$; $F(1,10) = 77.46$, $P < 0.01$; $F(1,10) = 156.56$, $P < 0.01$ as shown in Table 1) during and after the exposure. Although no behavioral change could be detected with 100, 200, and 400 ppm mixtures of toluene and *n*-hexane (50 ppm toluene + 50 ppm *n*-hexane, 100 ppm toluene + 100 ppm *n*-hexane, and 200 ppm toluene + 200 ppm *n*-hexane, respectively), exposure to 800 ppm mixture of toluene and *n*-hexane (400 ppm toluene + 400 ppm *n*-hexane) induced marked decreases of the lever press rate ($F(1,10) = 7.35$, $P < 0.05$; $F(1,10) = 39.58$, $P < 0.01$ as shown in Table 1) and the avoidance rate ($F(1,10) = 19.25$, $P < 0.01$; $F(1,10) = 73.36$, $P < 0.01$ as shown in Table 1) during Minutes 60–180 of the exposure, lever pressing occurring sporadically as the rats wandered aimlessly around the cage. This behavioral change disappeared following 180 min of the exposure and after the exposure.

DISCUSSION

From the standpoint of the prevention of "narcosis of sufficient degree to increase the likelihood of accidental injury, impair self-rescue or materially reduce

work efficiency," our present study focused on acute effects on learned behavior during and after exposure to toluene, *n*-hexane, or a mixture of toluene and *n*-hexane.

Concerning the relationship between concentration and behavioral response to toluene exposure, the present results were in good agreement with our previous study (Kishi *et al.*, 1988), in which the effects of a single 4-hr exposure to toluene (125, 250, 500, 1000, 2000, and 4000 ppm) on signaled lever press shock avoidance responses in rats were tested. In our present study of toluene exposures (50, 100, 200, 400, and 800 ppm), lever press rates were the highest in the first 20 min, after which they gradually returned to the performance level of the sham exposure, while lever press rates increased concentration- and time-relatedly at the latter stage (180–240 min) of 4-hr exposures to toluene. The behavioral changes induced at the early stage of exposures may have been mainly due to irritable effects of toluene, such as eye, throat, and nose irritation in human (Carpenter *et al.*, 1976), and those induced at latter stage (180–240 minutes) of the exposures may have been mainly due to the narcotic effects of toluene such as exhilaration, dizziness, sleepiness, fatigue, or incoordination in human (Oettingen *et al.*, 1942; Carpenter *et al.*, 1944) as well as impairments of reaction time and perception in human (Gamberale and Hultengren, 1972).

There are few animal studies of the acute neurobehavioral effects of solvents during and after exposure, especially at levels close to threshold limit values.

Our present results show that exposure to 100 ppm toluene had no effect on signaled lever press shock avoidance responses. Exposure to 200, 400, and 800 ppm toluene induced a transitory but appreciable increase of lever press rate during the initial 60 min of exposure compared with own performance under a sham exposure to air.

Wood and Colotla (1990) carefully studied the acute effects of 1-hr exposure to various concentrations of toluene (300, 560, 1000, 1780, and 3000 ppm) on the locomotor activity of mice. Toluene exposure had no effect on activity at the lowest concentration studied (300 ppm), it increased activity at intermediate concentrations (560–1780 ppm), and it decreased activity at high exposure level (3000 ppm).

The "signaled lever press shock avoidance schedule" used in our present study seems to have a high sensitivity to the acute neurobehavioral effects of solvents and good reproducibility.

Little information is available on the acute effects of *n*-hexane on the central nervous system. *n*-Hexane is an anesthetic (Haydon *et al.*, 1977). Swann *et al.* (1974) reported that (1) concentrations up to 8000 ppm of *n*-hexane produced no anesthesia during exposure in mice, (2) at 32,000 ppm of *n*-hexane the mice went directly into anesthesia with occasional sporadic body movements, and (3) at 64,000 ppm of *n*-hexane, during the first minutes of exposure, excitation was generally followed by light anesthesia. In studies on human volunteers, inhalation of 5000 ppm for 10 min resulted in vertigo and giddiness but there were no symptoms with exposure to 2000 ppm for the same duration (Patty and Yant, 1929). Occupational exposures to hexane at concentrations of 1000 to 2500 ppm for periods of 30–60 min caused drowsiness (Yamada, 1967).

In our present experiments, exposure to 50 ppm *n*-hexane induced a decrease of the lever press rate during Minutes 60–180 of exposure and a decrease of the avoidance rate that continued during and after the exposure except during Min-

utes 180–240 of exposure. On the other hand exposure to 50 ppm toluene induced considerable, but transitory, decreases of the lever press and avoidance rates only during the initial 20 min of exposure, after which rats quickly returned to the performance level of the sham exposure. Our results suggest that aversive or irritable effects may be stronger in the exposure to *n*-hexane than in that to toluene.

Exposure to a 50 ppm mixture of toluene and *n*-hexane (25 ppm toluene + 25 ppm *n*-hexane) induced marked and persistent decreases of lever press and avoidance rates during and after exposure. Performance decrements were observed immediately after the beginning of exposure to 50 ppm mixture. Special attention should be paid to the fact that these behavioral changes were not predictable from the findings of the exposure to either toluene or *n*-hexane alone at a concentration of 50 ppm. These rapid decreases of lever press and avoidance rates in these rats may be a "sensory uncomfortable response" (Nelson *et al.*, 1943), which, although it is not necessarily injurious to health, may have some influence on efficiency in human working conditions and may be large enough to decrease the safety margin of industrial tasks.

Regarding concentrations higher than 100 ppm, the behavioral changes induced by exposure to 50 ppm *n*-hexane or to a 50-ppm mixture (25 ppm toluene + 25 ppm *n*-hexane) disappeared. The reason performance decrements with 50 ppm *n*-hexane or 50 ppm of the mixture exposures were greater than those with 100, 200, or 400 ppm exposure might be explained by the so-called first exposure effect. This suggests that there may be adaptation at levels close to threshold-limit values.

Exposure to 800 ppm *n*-hexane induced an increase of lever press rate during exposure resembling those with 200, 400, or 800 ppm toluene, although individual differences of the lever press rate were larger in the rats exposed to *n*-hexane than in the rats exposed to toluene.

Exposure to the 800-ppm mixture (400 ppm toluene + 400 ppm *n*-hexane) induced transitory decreases of lever press and avoidance rates during Minutes 60–180 of the exposure, but these were not associated with analogous changes in the rats exposed to toluene or *n*-hexane alone at 400 or 800 ppm.

Ikeda *et al.* (1986) reported that 30 days of continuous exposure to a mixture of *n*-hexane and toluene, both at 200 ppm, produced changes in noradrenaline and dopamine levels, although the changes in dopamine levels such as elevation in the hippocampus and reduction in the midbrain were not predictable from the findings with exposure to either solvent alone at 200 or 400 ppm. Honma (1983) also reported that in a single exposure, the acetylcholine level in the hippocampus was lower in rats exposed to *n*-hexane at 2000, 4000, and 8000 ppm in combination with 4000 ppm toluene than in rats exposed to 4000 ppm toluene alone, while the acetylcholine level in hippocampus increased with exposure to 2000 ppm or did not change with exposure to 4000 ppm *n*-hexane alone. These neurochemical results showed evidence of synergistic interaction between toluene and *n*-hexane in the central nervous system in rats, although these experimental conditions differed from our present neurobehavioral experiment.

In conclusion, it should be emphasized that *n*-hexane showed narcotic effects at 800 ppm and modified the acute neurobehavioral effects of toluene in rats at 400 ppm toward unpredictable results. The present findings suggest that these two most popular constituents of commercial organic solvents may interact in the

central nervous system when given simultaneously, especially at concentrations higher than threshold-limit values.

Further behavioral study of long-term exposure should be carried out to examine the possible chronic combined effects which result from specific toxic actions of toluene and *n*-hexane on the central nervous system. To understand the mechanism underlying unpredictable behavioral changes, pharmacokinetic (absorption, distribution, metabolism, and excretion) and pharmacodynamic (transmitters and receptors) approaches will be needed.

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ENVIRONMENTAL RESEARCH 63, 287–294 (1993)

Neurotoxic Effects of 2,5-Hexanedione on Rapidly Growing Unmyelinated Peripheral Nerve Axons of a Rat Fetus: Dose–Effect Relationship[1]

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To investigate the potential neurotoxicity of 2,5-hexanedione (2,5HD) on developing axons we examined peripheral nerves of rat fetuses. Pregnant female Sprague–Dawley rats were injected subcutaneously with 680 mg/kg of 2,5HD once a day from Day 12 of gestation (GD12) to GD16 in one group and with 340 mg/kg of 2,5HD once a day from GD12 to GD20 in the other group. On GD20 live fetuses were removed from the uteri and their sciatic nerves were examined morphologically. By electron microscopical observations, affected nerves revealed axons which were aggregated and fused together, but there were no axons aggregated with neurofilaments. The diameter distributions of axons revealed an increase in the number of small-size axons in the nerves of the 340 mg/kg group and showed an enlargement of part of the axons in the nerves of the 680 mg/kg group, suggested by the appearance of a second peak at a diameter larger than that of the first peak.

INTRODUCTION

The solvent *n*-hexane is widely used in industry. Its neurotoxicity to peripheral nerves is due much to one of its metabolites 2,5-hexanedione (2,5HD) (Spencer *et al.*, 1980). Neuropathy of 2,5HD is characterized by causing distal axonopathy mainly of large myelinated fibers. Early degenerative changes in the peripheral nervous system are marked by the development of axonal swellings on the proximal side of the nodes of Ranvier in nonterminal distal regions of affected fibers (Spencer and Schaumberg, 1975). Axonal transport of the neurofilament is accelerated by this substance (Monaco *et al.*, 1989) and this may explain the distal swelling.

The axons of fetal peripheral nerves vigorously elongate, enlarging their diameters, and are not yet myelinated so that they have no nodes of Ranvier (Peters and Muir, 1959). Effects of 2,5HD on the developing axon might offer a new insight into the neurotoxicity of 2,5HD. We found that the sciatic nerves of fetuses of mother rats exposed to 2,5HD (680 mg/kg/day sc for 5 days during gestation) showed as a characteristic feature fusion of the axon (Ogawa *et al.*, 1991). In this paper we evaluate the dose dependency of the effect and analyze the morphometric measurement of the fetal axons.

MATERIALS AND METHODS

Male and female Sprague–Dawley rats were purchased from Charles River Japan, Inc. Animals were individually housed in cages with stainless-steel wire

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bottoms and provided with pelleted rodent chow (CRF-1; Oriental Yeast Co., Ltd., Japan) and tap water *ad libitum* throughout the study. Temperature of the animal room was kept at 22 ± 2°C, with humidity at 55 ± 10% and with a 12-hr light/dark cycle.

Eighteen 12-week-old virgin female rats weighing 240–293 g were bred by housing each with one adult male overnight. When a copulation plug was observed the following morning, the day was designated as Day 0 of gestation (GD0). Mated female animals were randomly divided into three groups of the same size. The first group (H) was injected with 2,5HD (purified to >97% supplied by Tokyo-Kasei-Kogyo, Tokyo, Japan), 680 mg/kg body wt subcutaneously once a day from GD12 to GD16. The second group (L) was injected with 2,5HD 340 mg/kg body wt subcutaneously once a day from GD12 to GD19. Animals in the third group (C) were injected with 0.9% saline, 0.68 ml/kg body wt subcutaneously over the same gestational period as group L. All animals were weighed and their clinical conditions were observed daily during and after the treatment period. One animal from group H was identified as nonpregnant.

The rats were sacrificed by CO_2 asphyxiation on GD20 and laparotomized to determine their reproductive status. Live fetuses were removed from the uteri, weighed, sexed, and examined for external abnormalities. Four male fetuses from different litters were randomly selected from each group and were perfused through the umbilical vein with 2.5% glutaraldehyde in 0.2 *M* cacodylate buffer (pH 7.4). The right sciatic nerve was sampled from each fetus (one nerve sampling from group L was unsuccessful). The tissues were postfixed in 2.5% glutaraldehyde solution at 4°C and in 1% osmium tetroxide for 1 hr at 4°C, dehydrated in graded alcohols and propylene oxide, and embedded in Epon 812.

Transverse ultrathin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy (LEM2000, Akashi Beam Technology, Tokyo). Electron photomicrographs of 10 randomly selected areas (each covering an area of 205 μm^2) from each specimen were taken. Morphological changes such as fusion, irregularity, and the number of vacuoles in the axons were evaluated. We randomly selected two nerves from each group and the diameters of all the axons on three electron photomicrographs from each nerve were measured with an image analyzer system (SP500, Olympus, Japan). Diameters were calculated from the areas of the transverse sections of axons, assuming that the sections were all circles.

STATISTICAL ANALYSIS

Mean values of the variables from three groups were compared first by ANOVA and then by Scheffé's multiple-comparison test. To quantify the morphological changes, three electron photomicrographs from each nerve were mixed thoroughly and two researchers who did not know which photos were from which animals classified them into three classes: negative, positive, and extremely positive. A 3 × 3 contingency table for each pathologist was constructed and was tested by the χ^2 test.

RESULTS

Intoxication with 2,5HD led to rapid impairment of weight gain of dams in the two exposed groups (Fig. 1). The mean maternal body weight of group H de-

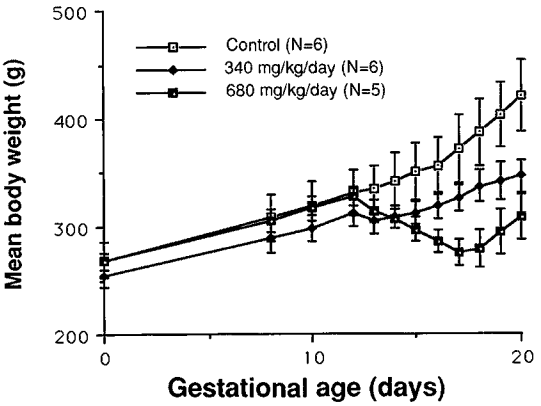

FIG. 1. Changes in mean body weights of dams from three groups. One group was exposed to 680 mg/kg/day of 2,5HD from GD12 to GD16 and another group was exposed to 340 mg/kg/day of 2,5HD from GD12 to GD19. Error bar indicates standard deviation.

creased 53 g during the exposure, while those of group L and group C increased during the same period. But the weight gain in group L was less than that in group C. At GD15 there was a significant difference ($P < 0.05$) between group L and group C and a highly significant difference ($P < 0.01$) between group H and group C, but no significant difference between group L and group H. At GD17 there were highly significant differences ($P < 0.01$) between the groups. The body weight of group H increased after the cessation of the exposure and the difference of mean body weights between group H and group L became not significant at GD20, but the difference between the exposed groups and group C remained highly significant ($P < 0.01$). No abnormal behavior was observed among the dams. The results of reproductive parameters examined are shown in Table 1. There were no differences among the three groups in reproductive status such as the number of resorptions per pregnant female and the number of live fetuses per pregnant female except for the significant decrease ($P < 0.05$) in the number of implants per pregnant female in group L compared with that in the others. There were highly significant ($P < 0.01$) differences among the mean live fetal weights of three groups. The decrease had dose dependency. No external abnormalities were observed in any live fetuses.

In transverse sections of the nerves treated by 2,5HD and examined by electron

TABLE 1
REPRODUCTIVE AND FETAL INDICES[a]

		2,5-Hexanedione (mg/kg/day)	
	Control	340	680
No. of pregnant rats	6	6	5
Mean No. of implantations/litter	16.0 ± 1.3	14.2 ± 1.0*	16.4 ± 1.1
Mean No. of resorptions/litter	0.5 ± 0.8	0.3 ± 0.5	0.8 ± 1.1
Mean No. of live fetuses/litter	15.5 ± 1.6	13.8 ± 0.8	15.6 ± 1.1
Mean fetal weight (g/fetus)**	3.66 ± 0.26	3.38 ± 0.27	2.71 ± 0.43

[a] Mean ± SD.
* $P < 0.05$ compared with control and 680 group (Scheffé's S test).
** $P < 0.01$ compared with each other (Scheffé's S test).

microscopical observations, the axons of the affected nerves showed several morphological abnormalities. The most remarkable feature was irregularly shaped large axons. It seems that several axons had aggregated and fused together (Fig. 2B). Rather smaller axons were affected in group H than in group L. In the affected nerves, irregularly shaped vacuoles and irregularly distributed neurofilaments were frequently observed in the large axons but aggregation of neurofilaments was not observed. Vacuolated mitochondrial remnants were seen in some axons. Schwann cells appeared intact. Classification of the electron photomicrographs by two researchers showed a significant difference between group C and exposed groups and one researcher detected a relation between dose and severity of morphological changes (Table 2).

Morphometric measurements of axons indicated dose-dependent changes (Fig. 3). The distribution curve of axon diameters was unimodal and asymmetrical with a positive skewness (ranging from 0.07 to 2.35 μm) in group C, with a peak at 0.2–0.25 μm. The distribution curve of group H was bimodal (ranging from 0.08 to 2.82 μm), with two peaks at 0.2–0.25 and 0.45–0.5 μm. The distribution curve of group L was unimodal and asymmetrical with a positive skewness (ranging from 0.07 to 2.87 μm) and irregular deformation on the right side of the foot, suggesting the appearance of a small peak. The unimodal peak was at 0.15–0.2 μm and the deformity was at 0.4–0.6 μm. There were abundant small-size axons less than 0.3 μm in diameter in the nerves of this group.

DISCUSSION

The present study shows that 2,5HD injection into pregnant rats produces fetal peripheral nerve lesions which are a fusion of axons and an axonal enlargement without aggregation of neurofilaments. The former lesion has not been observed in adult rats exposed to 2,5HD. The severity of morphological changes was dose related. The changes in the diameter distribution of axons were also dose related. The diameter distribution of axons of group L nerves showed a generalized shift to the left compared with that of group C nerves. This suggests that the increased number of immature axons was produced by regeneration. The diameter distributions also showed a sprout at the second peak on the right side of the first peak. Nerves of group H showed no shift of the first peak, which suggests no or very little regeneration. The second peak was at the right side of the first peak. This may suggest axonal enlargement of some axons.

The lesions are considered to be specific in developing rat fetuses but they are not determined to be due to 2,5HD-specific toxicity given the following unresolved issues. The first problem is placental transfer of 2,5HD. There is no direct evidence of placental transfer of 2,5HD. However, an experiment in pregnant rats exposed to *n*-hexane showed that the concentration of 2,5HD in the maternal blood was comparable to that of the whole fetus; that is, 1.73 μg/ml in maternal blood and 1.67 μg/ml in the fetus (Bus *et al.*, 1978). The role of the placenta in xenobiotic metabolism is considered to be minor (Mihaly and Morgan, 1984) and only a part of the blood volume of placental circulation perfuses the liver (Edel-

FIG. 2. (A) Electron micrograph of the sciatic nerve from a rat fetus of control group: transverse section. Scale bar, 1 μm. (B) Electron micrograph of the sciatic nerve from a rat fetus exposed to 680 mg/kg/day of 2,5HD: transverse section. Scale bar, 1 μm. Arrowheads, aggregated and fused axons; SN, Schwann cell nucleus.

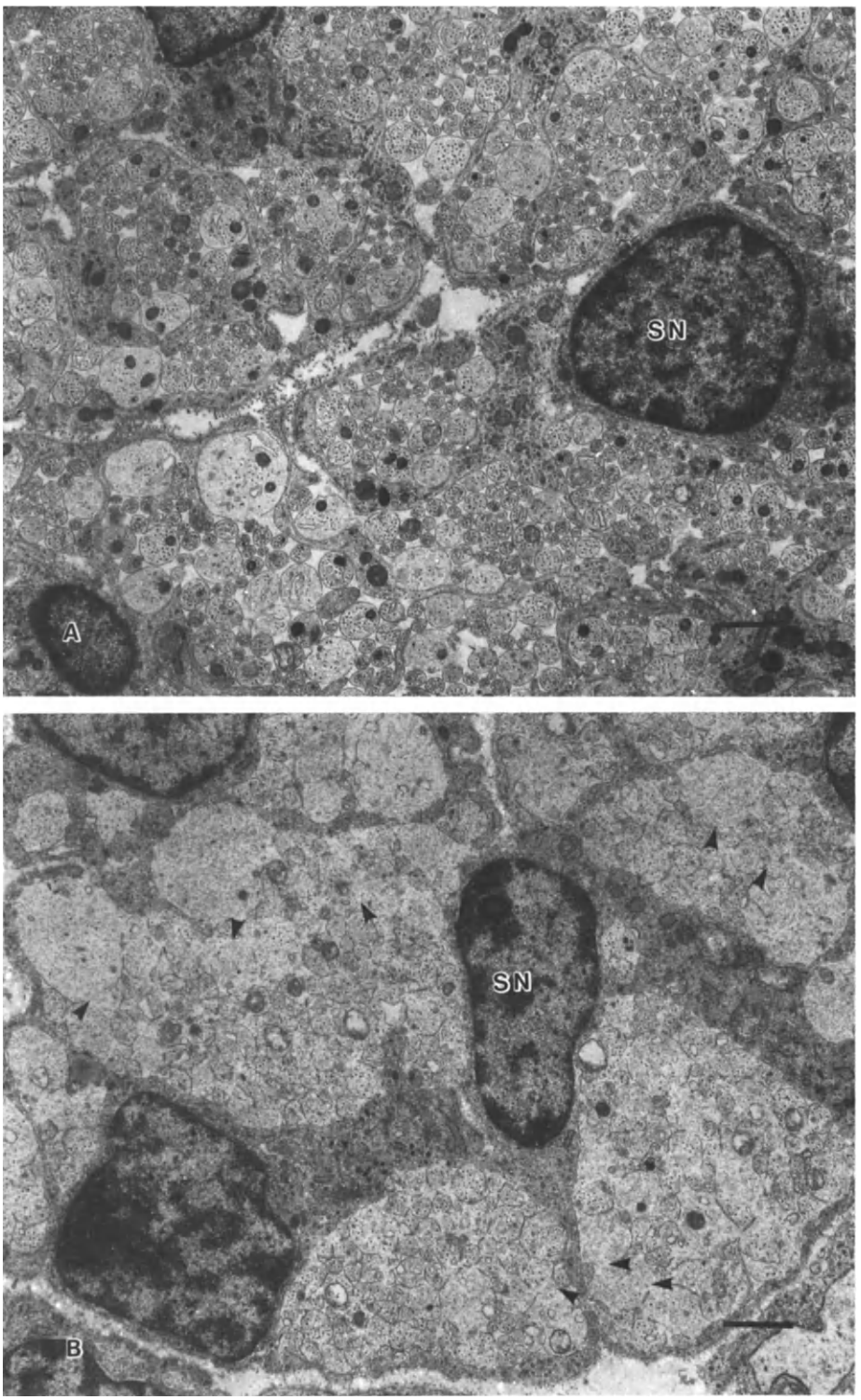
SN
A
SN
B

TABLE 2
CLASSIFICATION OF 33 ELECTRON PHOTOMICROGRAMS ON THE BASIS OF SEVERITY OF MORPHOLOGICAL CHANGE BY TWO RESEARCHERS A AND B

Morphological change	(−)	(+)	(H)
	A**		
Control	9	1	2
340 mg/kg/day	0	6	3
680 mg/kg/day	3	4	5
	B*		
Control	9	3	0
340 mg/kg/day	1	4	4
680 mg/kg/day	3	4	5

* $P < 0.05$ by the χ^2 test.
** $P < 0.01$ by the χ^2 test.

stone *et al.*, 1977). These results suggest that 2,5HD metabolized from *n*-hexane in the maternal tissue transfers to the fetus across the placenta. The second problem is the significant loss in body weight found in the exposed pregnant rats. This might produce nutritional neuropathy. There have been no studies showing that low-calorie diets lead to peripheral nerve degeneration of adults or fetuses. The mean body weights of dams and fetuses were dose related and correlate with the severity of morphological changes. The effects might depend on growth retardation, but we cannot explain the deviation of the first peak of the axon diameter distribution of group L nerves toward the smaller diameter. If this phenomenon occurs due to growth retardation then the first peak of group H nerves should also deviate toward the smaller diameter, but there was no such deviation evident.

Although possible causes of the neuropathy are vitamin deficiencies, hypothyroidism, (Manson, 1986) or some cause other than 2,5HD, there is a possibility that the specific axonal lesions indicated as a form of axonal fusion were caused by 2,5HD. There are reports that sterologenesis of peripheral nerve was selectively inhibited by 2,5HD in the rat (Gillies *et al.*, 1981). A decrease in the cholesterol content of the membrane causes its instability (Demel and Kruyff, 1976) and this might lead to dysfunction of the axonal membrane. Moreover, axons of a fetus are not separated individually by the tongues of Schwann cells. Many axons in contact with each other are packed in a common compartment limited by enveloping Schwann cell tongues and their contours are mutually adapted (Peters and Muir, 1959).

CONCLUSION

Exposure of pregnant rats to 2,5HD caused degeneration in the sciatic nerves of their fetuses. The most remarkable morphological feature was expressed as a fusion of axons without axonal enlargement by aggregation of neurofilaments. The diameter distributions of axons revealed an increase in the number of small-size axons in the nerves of group L and showed an enlargement of a part of the axons in the nerves of group H, suggested by the appearance of second peak at a diameter larger than that of first peak. Axonal fusion may be due to the selective decrease in the cholesterol content of the axolemma.

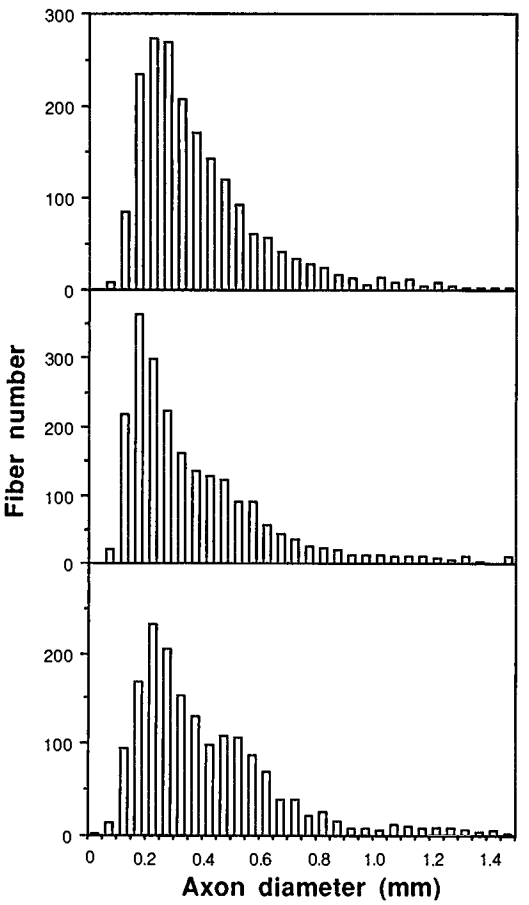

FIG. 3. Comparison of frequency distributions of the axonal diameters of fetal sciatic nerves from three groups: control group (top), a group exposed to 340 mg 2,5HD/kg/day (middle), and a group exposed to 680 mg 2,5HD/kg/day (bottom). Each histogram was constructed from the electron photomicrographs of two nerves which include 1952 fibers in the control group, 2188 fibers in the 340 mg/kg/day group, and 1707 fibers in the 680 mg/kg/day group.

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Effect of Carbon Tetrachloride on Allylnitrile-Induced Head Twitching[1]

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Allylnitrile is known to induce head twitching in rats and mice. Carbon tetrachloride (CCl_4) impairs the hepatic mixed function oxidase system and lowers acute toxicity of nitriles. In the present study we examined the effect of CCl_4 on the allylnitrile-induced head twitching to elucidate the mechanism of the abnormal behavior. In rats, CCl_4 pretreatment inhibited the head twitching induced by allylnitrile (1.49 mmole/kg, po), the maximal and dose-dependent inhibition occurring when CCl_4 was given just prior to the nitrile administration, while CCl_4 post-treatment had no effect on the head twitching. A dose-dependent inhibition of cyanide formation arising from allylnitrile in the liver and a dose-dependent attenuation of acute toxicity of allylnitrile were observed when CCl_4 was given just prior to the nitrile administration in rats and mice. Intracerebroventricular injection of allylnitrile (2.0 to 18 μmole/brain) induced no head twitching in rats. The results suggest that active metabolites of allylnitrile are responsible for the head twitching, and that CCl_4 prevents the metabolic process in the liver by forming conjugates with allylnitrile, resulting in the inhibition of the head twitching.

INTRODUCTION

There are a number of chemicals which induce behavioral abnormalities in experimental animals (Selye, 1957; Handley and Singh, 1986). As for nitriles, 3,3′-iminodipropionitrile ($NH(CH_2CH_2CN)_2$) has long been known to have such an effect (Selye, 1957; Schneider *et al.*, 1981). We have recently shown in comparative toxicological studies on certain nitriles which are widely used in chemical industries that allylnitrile ($CH_2=CHCH_2CN$), crotononitrile ($CH_3CH=CHCN$), and 2-pentenenitrile ($CH_3CH_2CH=CHCN$) induce behavioral abnormalities such as circling, hyperactivity, head twitching, and occasional backward running in mice pretreated with carbon tetrachloride (CCl_4) (Tanii *et al.*, 1989a,b). We have also found that allyl- and crotononitrile induce behavioral abnormalities in mice untreated with CCl_4 and all three nitriles do so in rats without CCl_4 pretreatment (Tanii *et al.*, 1991), and that CCl_4 even inhibits the activity of these nitriles when given 1 day prior to the nitrile in rats (unpublished observation).

The inhibitory action of CCl_4 in rats suggests that this compound modifies the mechanism underlying the induction of behavioral abnormalities caused by nitriles, probably affecting their metabolism. The objective of the present study is to examine further the effect of time and dose of CCl_4 treatment on allylnitrile-

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induced head twitching and on cyanide formation in the liver in rats and mice to better understand the mechanism of the behavioral abnormality. The effect of direct injection of allylnitrile into the rat brain was also examined.

MATERIALS AND METHODS

Chemicals

Allylnitrile was obtained from Tokyo Kasei Co. (Tokyo, Japan); CCl_4, sodium *p*-toluenesulfonchloramide trihydrate (Chloramine T), bis(1-phenyl-3-methyl-5-pyrazolone), 1-phenyl-3-methyl-5-pyrazolone, and other chemicals (reagent grade) were from Wako Pure Chemical Industries (Osaka, Japan).

Animals

Male rats of Wistar strain weighing about 250 g and male mice of ddY strain weighing about 25 g at the beginning of experiment were maintained on a 12-hr light/12-hr dark cycle with free access to laboratory chow and water.

Effect of Carbon Tetrachloride

As previously described (Tanii *et al.*, 1989b, 1991) allylnitrile was given orally to rats (1.49 mmole/kg) and mice (1.67 mmole/kg) using olive oil as vehicle, in a volume of 0.5 ml/200 g for the rats and 0.1 ml/25 g for the mice. In order to study the pretreatment time of CCl_4, rats were given 0.32 ml of a 10% CCl_4 solution in olive oil intraperitoneally or the same volume of olive oil per 200 g body wt, 0, 6, 12, 24, or 48 hr prior to the administration of allylnitrile. To study the effect of the dose dependency of CCl_4, rats and mice were given either 0.32 ml of 0, 1, 2, 5, or 10% CCl_4 intraperitoneally (0, 0.166, 0.332, 0.83, or 1.66 mmole/kg) or 0.12 ml of 0, 0.1, 0.5, 1.0, or 5% CCl_4 (0, 0.0498, 0.249, 0.498, or 2.49 mmole/kg) in olive oil per 25 g body wt, just prior to allylnitrile. To study the effect of post-treatment on the head twitching, rats were given 0.32 ml of a CCl_4 solution intraperitoneally (0, 5, 10, or 20%) in olive oil per 200 g body wt 7 days after an oral injection of allylnitrile (1.49 mmole/kg), when they had displayed the head twitching. In each study, the animals were placed individually in a cage between 0900 and 1500 while their behavior was observed by one of the authors who was unaware of the treatments. The number of head twitches were counted for 5 min per day over a period of 30 days as in our previous report (Tanii *et al.*, 1989a).

Cyanide Determination

Cyanide in the liver was assayed as described previously (Tanii and Hashimoto, 1984a). Briefly, the aeration apparatus consisted of three serial tubes containing 20% NaOH, 20% trichloroacetic acid, and 0.1 N NaOH, respectively. Immediately following, a 1.0-ml aliquot of liver homogenate in 50 mM phosphate buffer, pH 7.4, was added to the tube containing trichloroacetic acid and then aeration was started. After 10 min aeration an aliquot from the tube containing 0.1 N NaOH was removed, neutralized, and subjected to cyanide analysis.

LD_{50} Determination

The animals received an intraperitoneal injection of CCl_4 at various dose levels, followed immediately by oral allylnitrile at four different dose levels using four animals per dose level. LD_{50} was calculated according to Weil (1952).

Intracerebroventricular Injection of Allylnitrile

The rats were anesthetized with chloral hydrate (500 mg/kg) and were given an intracerebroventricular injection of allylnitrile. For these injections, the skull was exposed with a midline incision and a microliter syringe was stereotaxically inserted into the lateral ventricles (A–P, 1.0 mm behind the bregma; M–L, 1.5 and −1.5 mm; D–V, −4.00 mm from the skull surface), the midbrain raphe (A–P, 0.1 mm behind λ; M–L, 0 mm; D–V, −6.0 mm from the skull surface), or the dorsal raphe (A–P, 0.1 mm behind λ; M–L, 0 mm; D–V, −5.0 mm from the skull surface). In another experiment, a microliter syringe was inserted into the cisterna magna. The rats received 2.0 to 18 μmole allylnitrile dissolved in saline.

RESULTS

Effect of CCl_4 on Allylnitrile-Induced Head Twitching

Figure 1 shows the frequency and time course of the head twitching induced by 1.49 mmole/kg of allylnitrile in rats after pretreatment with a fixed amount of CCl_4 (1.66 mmole/kg) at different time schedules (0, 6, 12, 24, or 48 hr before allylnitrile). The control rats and those pretreated with CCl_4 24 and 48 hr before the administration of allylnitrile showed frequent head twitching after 2 days and over a period of 30 days, while those pretreated with CCl_4 6 and 12 hr before the administration showed less frequent twitching, lasting a shorter period. Rats pretreated with CCl_4 just before the allylnitrile administration exhibited no head twitching at all.

Figure 2 shows the frequency and time course of the head twitching induced by 1.49 mmole/kg of allylnitrile in rats after pretreatment with different amounts of CCl_4 (0 to 1.66 mmole/kg) just prior to the allylnitrile administration. The frequency and time course of head twitching were inhibited by CCl_4 dose dependently; in particular, the rats given 1.66 mmole/kg of CCl_4, the dose being more than the equivalent of allylnitrile (1.49 mmole/kg), exhibited no head twitching.

Figure 3 shows the frequency and time course of the head twitching induced by 1.67 mmole/kg allylnitrile in mice after treatment with different amounts of CCl_4 (0 to 2.49 mmole/kg) just prior to allylnitrile. As with rats, the frequency and time course of head twitching were inhibited by CCl_4 dose dependently, and in the mice given 2.49 mmole/kg CCl_4, the molar being larger than that of allylnitrile (1.67 mmole/kg), the head twitching was completely blocked.

We examined further whether CCl_4 post-treatment could affect the head twitching. Rats were given allylnitrile (1.49 mmole/kg) orally. Seven days later, when the rats were displaying head twitching, they received CCl_4 (0.83 to 3.32 mmole/kg) intraperitoneally and their behavior was observed over the subsequent 30 days. CCl_4 had no effect on the allylnitrile-induced head twitching (data not shown).

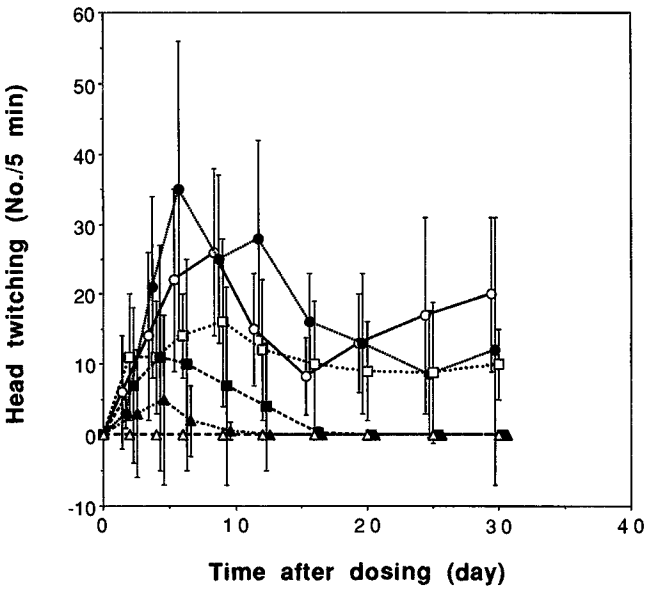

FIG. 1. Effect of pretreatment with CCl_4 on allylnitrile-induced head twitching. Rats were given 1.66 mmole/kg CCl_4 intraperitoneally. Zero (△), 6 (▲), 12 (■), 24 (□), or 48 (●) hr later, they received an oral injection of 1.49 mmole/kg allylnitrile, and their behavior was observed over a period of 30 days. Control animals (○) were given olive oil intraperitoneally, and 48 hr later they received allylnitrile as above. Each value represents the mean ± SD of 4 to 6 rats.

Effect of CCl_4 on Cyanide Formation in Liver after Allylnitrile Administration

Cyanide formation was examined as an indicator of the metabolism of allylnitrile in the liver of rats and mice. Pretreatment with CCl_4 48 hr prior to allylnitrile administration had no effect on cyanide formation in the rat liver, when compared to the rats pretreated with vehicle only (Fig. 4), while pretreatment with CCl_4 (0.332 to 1.66 mmole/kg), immediately prior to allylnitrile administration, greatly inhibited the formation of cyanide in the liver (Fig. 5). In mice, pretreatment with CCl_4, immediately prior to allylnitrile administration, inhibited the formation of cyanide in a dose-dependent manner (Fig. 6). It should be noted that using 2.49 mmole/kg CCl_4, the molar being larger than that of allylnitrile (1.67 mmole), produced a complete inhibition of cyanide formation.

Effect of CCl_4 on Acute Toxicity of Allylnitrile

In order to examine the effect of CCl_4 on the acute toxicity of allylnitrile, rats and mice were given vehicle or CCl_4 at various dose levels, followed immediately by allylnitrile at various dose levels. Results are shown in Table 1. A statistically significant correlation between the CCl_4 dose level and the acute toxicity of allylnitrile was obtained ($r = 0.9919$, $P < 0.05$ for rats; $r = 0.983$, $P < 0.05$ for mice).

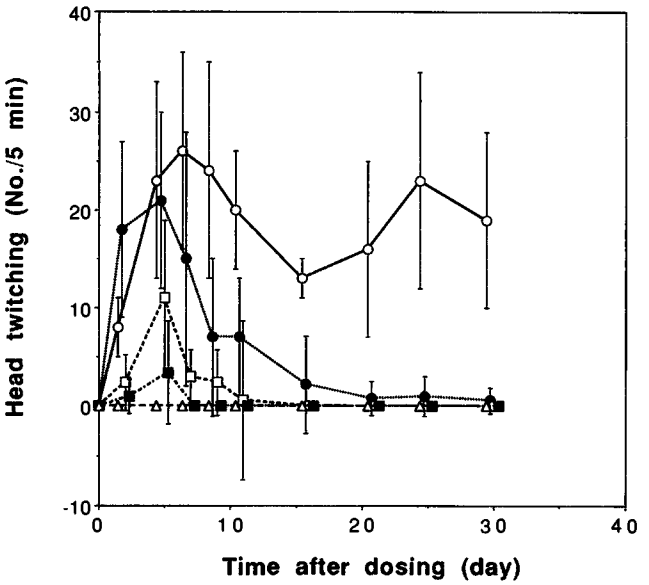

FIG. 2. Dose-dependent inhibition by CCl_4 of allylnitrile-induced head twitching in rats. Rats were given 0 (○), 0.166 (●), 0.332 (□), 0.830 (■), 1.66 (△) mmole/kg CCl_4 intraperitoneally, followed immediately by allylnitrile (1.49 mmole/kg, po). Each value represents the mean ± SD of 4 to 6 rats.

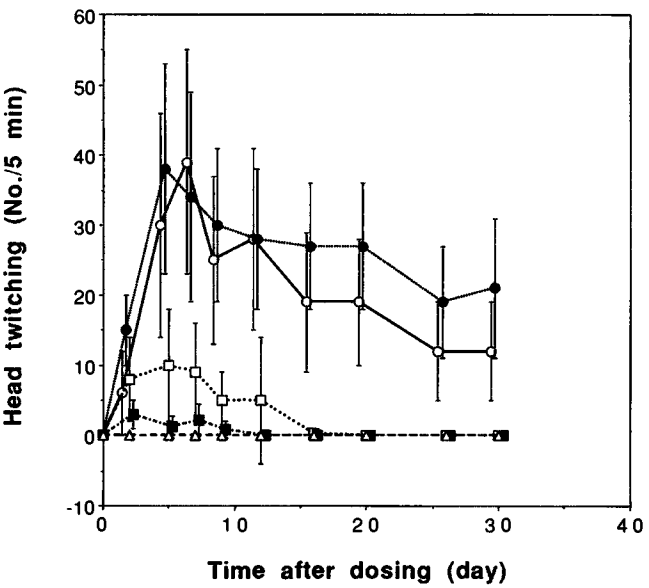

FIG. 3. Dose-dependent inhibition by CCl_4 of allylnitrile-induced head twitching in mice. Mice were given 0 (○), 0.050 (●), 0.249 (□), 0.498 (■), or 2.49 (△) mmole/kg CCl_4 intraperitoneally, followed immediately by allylnitrile (1.67 mmole/kg, po). Each value represents the mean ± SD of 4 to 5 mice.

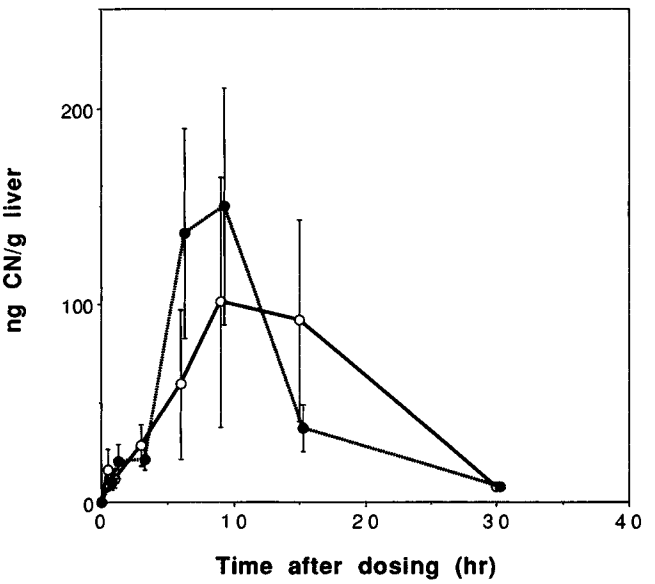

FIG. 4. Effect of pretreatment with CCl_4 on cyanide formation in rat liver after allylnitrile administration. Rats were given 1.66 mmole/kg CCl_4 (○) or olive oil (●) intraperitoneally, and 48 hr later they received an oral dose of 1.49 mmole/kg allylnitrile. They were killed by decapitation at indicated times, and cyanide in the liver was analyzed as mentioned under Materials and Methods. Each value represents the mean ± SD of 3 rats.

Intracerebroventricular Injection of Allylnitrile

Neither the injection of allylnitrile (2.0 to 18 μmole) into the lateral ventricles nor the injections into the midbrain raphe, the dorsal raphe, and the cisterna magna, caused behavioral abnormalities over the observation period of 30 days (data not shown).

DISCUSSION

CCl_4 is well known to impair the hepatic mixed function oxidase system (Sasame *et al.*, 1968; Castro *et al.*, 1972; Glende, 1972; Glende *et al.*, 1976) and to cause a selective loss of polypeptide in liver microsomes in rats (Noguchi *et al.*, 1982). CCl_4 has also been shown to lower acute toxicity of nitriles in mice, provided that CCl_4 is given 24 hr before nitrile administration (Tanii and Hashimoto, 1984b).

The present study was done to examine further the inhibitory effect of CCl_4 on the allylnitrile-induced head twitching under various time and dose schedules. The results revealed that the inhibitory effect was maximum when CCl_4 was given just prior to allylnitrile administration and was dose dependent and total at a dose level greater than that of allylnitrile. The effects of CCl_4 were associated with the inhibition of the release of cyanide from the nitrile in the liver, the inhibition being total only at the molarity of CCl_4, which was larger than that of allylnitrile.

Certain nitriles, especially alkyl nitriles, are known to be biotransformed by the hepatic microsomal mixed function oxidase system to a cyanohydrin intermedi-

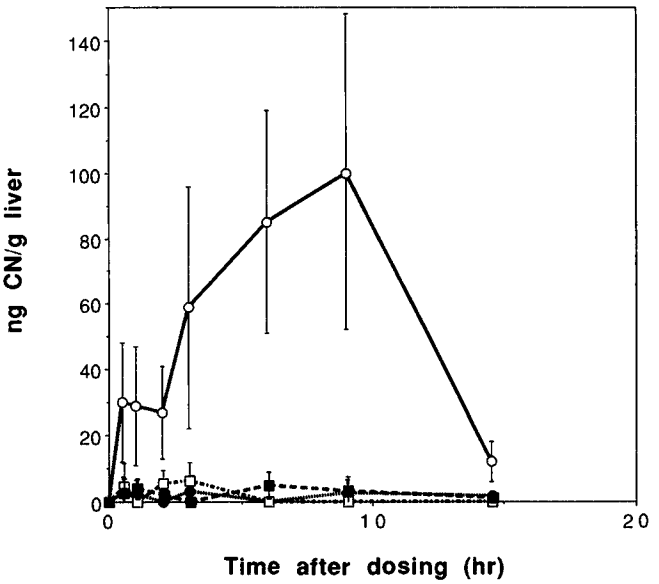

FIG. 5. Effect of treatment with CCl_4 on cyanide formation in rat liver after allylnitrile administration. Rats were given 0 (○), 0.332 (●), 0.830 (□), or 1.66 (■) mmole/kg CCl_4 intraperitoneally, followed immediately by allylnitrile (1.49 mmole/kg, po). They were sacrificed by decapitation at the indicated times, and their livers were subjected to cyanide analysis as mentioned under Materials and Methods. Each value represents the mean ± SD of 3 rats.

ate, which is then spontaneously decomposed to an aldehyde and hydrogen cyanide (Parke, 1968; Ohkawa *et al.*, 1972). Allylnitrile could also follow the same metabolic process to give allylaldehyde and cyanide, and CCl_4 pretreatment may inhibit the process as already shown in various other nitriles (Tanii and Hashimoto, 1984a). As for the time course of the inhibitory effect of CCl_4 on the hepatic mixed function oxidase system, Mannering *et al.* (1981) have reported that the effect is most evident 24 hr after the administration. In the present study, CCl_4 completely blocked both head twitching and cyanide formation in the liver when it was given just prior to allylnitrile, suggesting that CCl_4 induced the inhibitory effects by acting not on the oxidase system but on allylnitrile itself.

Based on the present results and previous information, it is possible to deduce that active metabolites produced in the liver from allylnitrile may induce the behavioral abnormalities and that CCl_4 prevents the metabolic process, by forming a conjugate with allylnitrile, resulting in the inhibition of the abnormalities. The real character of the active metabolites, however, is not yet clear, although we postulate allylaldehyde as one of the candidates. Hydrogen cyanide is unlikely to be responsible for the behavioral abnormalities because no such evidence has yet been reported and because there are many other nitriles which liberate cyanide in the liver without producing any behavioral disturbances (Tanii and Hashimoto, 1984a).

Another possibility is that allylnitrile itself is active in inducing the behavioral abnormalities, and CCl_4 inhibits the effects by binding with allylnitrile. Head

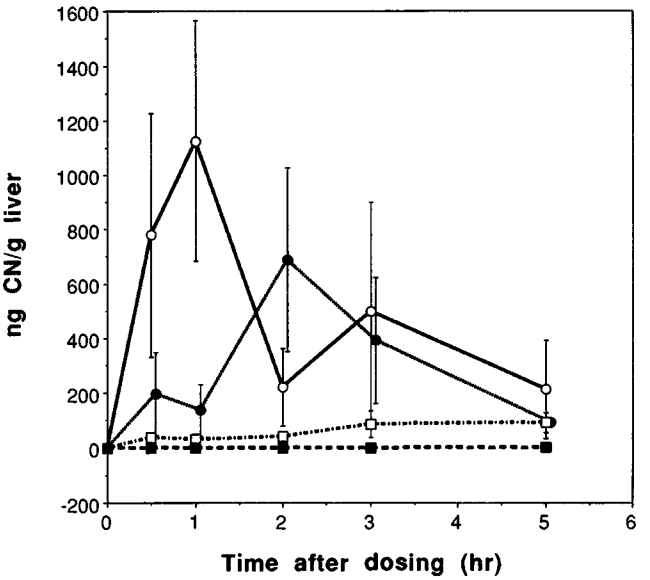

FIG. 6. Effect of treatment with CCl_4 on cyanide formation in mouse liver after allylnitrile administration. Mice were given 0 (○), 0.050 (●), 0.249 (□), or 2.49 (■) mmole/kg CCl_4 intraperitoneally, followed immediately by allylnitrile (1.67 mmole/kg, po). They were killed by dislocation at the indicated times, and their livers were subjected to cyanide analysis as mentioned under Materials and Methods. Each value represents the mean ± SD of 3 mice.

twitching has been presumed to be mediated through serotonin 2 receptors (Green, 1984), and serotonin antagonists for the receptors block the head twitching (Peroutka *et al.*, 1981). It has also been shown that intracerebroventricular injection of serotonin induces the head twitching in mice (Handley and Braun, 1982). In the present study, however, the injection of allylnitrile into the raphe region, which is abundant in serotonin neurons (Cooper *et al.*, 1986), did not induce any behavioral abnormality, suggesting that allylnitrile itself is not responsible for inducing the head twitching.

In our previous study, allylnitrile exhibited acute toxicity mainly through re-

TABLE 1
DECREASE IN ACUTE TOXICITY OF ALLYLNITRILE BY CCl_4

CCl_4 (mmole/kg)	LD_{50} (mmole/kg)	
	Rat	Mouse
0 (olive oil)	1.67 (1.38–2.02)	2.40 (2.16–2.67)
0.207	1.70 (1.55–1.87)	3.82 (3.32–4.39)
1.04	2.35 (2.02–2.72)	5.50 (4.79–6.32)
2.08	3.24 (2.81–3.72)	7.58 (6.66–8.62)

Note. Rats and mice were given olive oil or CCl_4 intraperitoneally at indicated dose levels, followed immediately by oral allylnitrile. LD_{50} was determined by the method of Weil (1952) and is expressed as the mean (95% confidence interval).

leasing cyanide in the liver and the toxicity was lowered by CCl_4 pretreatment (Tanii and Hashimoto, 1984a,b). In these studies, however, CCl_4 was given to the animals 24 hr prior to allylnitrile, but in the present study it was given just prior to allylnitrile. Although both treatments with CCl_4 inhibited the release of cyanide from the nitrile, the mechanisms involved would be different. As previously mentioned, CCl_4 administered just prior to allylnitrile would interfere with the metabolic process through forming a conjugate with it, while that administered 24 hr prior to the nitrile, through an impairment of the hepatic mixed function oxidase system.

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ENVIRONMENTAL RESEARCH 60, 233–241 (1993)

Axonal Sprouting of Motor Nerve in Acrylamide-Intoxicated Rats with Progressive Weakness[1]

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Quantitative morphologic studies on the motor axons in endplates of extensor digitorum longus muscle (EDL M) and soleus muscle, and on the myelinated fibers of the nerve to EDL M and other lower limb nerves were made on rats showing progressive weakness, intoxicated with acrylamide for 4 weeks (test), using silver staining. Terminal sproutings were significantly greater in frequencies in test compared with control in both muscles. In addition, morphologic changes in test consisted of a significant increase in the number of axon terminal branches and in the frequency of swellings of the preterminal, terminal, and ultraterminal axons and in an increase of myelinated fibers showing axonal degeneration in the nerve to EDL M. Such findings were in contrast with the previous report that cumulative doses of acrylamide inhibit sprouting of motor axons in endplates.

INTRODUCTION

The acrylamide monomer has been shown to induce pathologic changes in the central and peripheral nervous system. Some studies have looked into its effects on motor nerve terminals (Cavanagh, 1982; Jennekens *et al.*, 1979; Kemplay and Cavanagh, 1984a,b; Tsujihata *et al.*, 1974). Kemplay and Cavanagh (1984b) showed that the cumulative doses of acrylamide inhibit sprouting of motor axons in endplates following partial denervation and botulinum toxin injection. Their findings support the conclusions of Morgan-Hughes *et al.* (1974) and Griffin *et al.* (1977) on impaired axonal regeneration after nerve injury in acrylamide intoxication. However, other workers (Cavanagh, 1982; Fullerton and Barnes, 1966; Shaumburg *et al.*, 1974; Suzuki and Pfaff, 1973) have noted the presence of axonal regeneration in the peripheral nerves of acrylamide-treated animals. In this study we aimed to reveal morphometrically the presence or absence of axonal regeneration and the changes produced on the motor axon in endplates by a dosing schedule of acrylamide described previously.

MATERIALS AND METHODS

One-month-old male Wistar rats weighing 90–100 g were separated into two groups. One group of rats was injected with 50 mg acrylamide (Eastman Kodak Co., USA)/kg body wt intraperitoneally three times a week for 4 weeks as test. Another group of rats was given saline intraperitoneally as control. After the

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4-week regimen, the sural nerve was harvested at midthigh (proximal) and ankle (distal) level, together with the tibial and peroneal nerves above the knee. The nerve to the EDL M was dissected out under the light microscope. All the nerves were divided into two portions. One portion was fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) in immersion, postfixed in 1% osmium tetroxide, dehydrated, and embedded into epoxy. Semithin sections (1.0 μm) were stained with toluidine blue and were observed. The other portion was processed for teased-fiber preparations. About 100 teased myelinated fibers were classified into conditions described by Dyck *et al.* (1984) for each specimen.

The extensor digitorum longus muscle (EDL M) and soleus muscle (SOL M), injected with 4% buffered paraformaldehyde *in vivo,* were removed and then fixed in 4% paraformaldehyde for 1 hr and kept in the buffer at 4°C. Longitudinal sections of 120–150 μm in thickness were cut using a vibratome. For silver impregnation, the method described by Beerman and Cassens (1976) was used. The impregnated sections were examined under higher magnification. All measurements were done using a Zeiss micrometer eyepiece. Six test and six control rats were systematically studied in the same manner as that described below.

In quantitative evaluation, 60 endplates per muscle, where preterminal, terminal, and ultraterminal axons seem to be included in the longitudinal plane of section of the muscle, were analyzed in both EDL M and SOL M in each of six test and six control rats.

Endplates were classified into four types based on the number of branches of the terminal axon and the presence of collateral branching (Tuffery, 1971) (see footnotes in Table 1 for definition). The frequency of each endplate type was obtained as percentage of 60 endplates in each muscle and the mean and standard deviation (SD) of the frequencies were calculated in six muscles of SOL M and EDL M of control and test.

The following morphometric data were obtained in 60 endplates in each muscle and expressed as mean ± SD of six muscles in each of SOL M and EDL M of control and test: (1) Number of terminal axon branches per endplate; (2) frequency of the endplate showing each of terminal and nodal sproutings which are longer than 8 μm in length; (3) frequency of endplates with axonal swellings, and their sizes (μm) measured as the largest diameter perpendicular to the length of the axon in each of preterminal, terminal, and ultraterminal axons. The statistical evaluation of the means between test and control was carried out using Wilcoxon rank-sum test.

RESULTS

Findings in teased fiber preparations. The frequencies (%, mean ± SD in 6 nerves) of myelinated fibers showing linear rows of myelin ovoids in the peroneal, tibial, and proximal and distal sural nerves, and nerve to EDL M in test were 6.9 ± 7.0, 11.3 ± 7.2, 4.0 ± 1.9, 8.2 ± 9.5, and 28.0 ± 13.8, respectively. They were all significantly higher ($P < 0.05$) than those in control. Such frequency was significantly higher ($P < 0.05$) in the nerve to EDL M than in any other nerves.

Findings in Epon-embedded sections of nerves. Myelin ovoids were more fre-

quently seen in the nerve to the EDL M (Fig. 1) than in any nerves in test. No definite abnormalities were found in the nerves in control rats.

Morphometric findings in motor axons of SOL M. The frequency of endplate types with two or three branches of terminal axon and with collateral branching (Fig. 2, top) was higher ($P < 0.01$) in test than in control (Table 1). The number of terminal axon branches per endplate was greater in test ($P < 0.01$) than in control. The frequencies of endplates with terminal and nodal sprouting (Fig. 2,

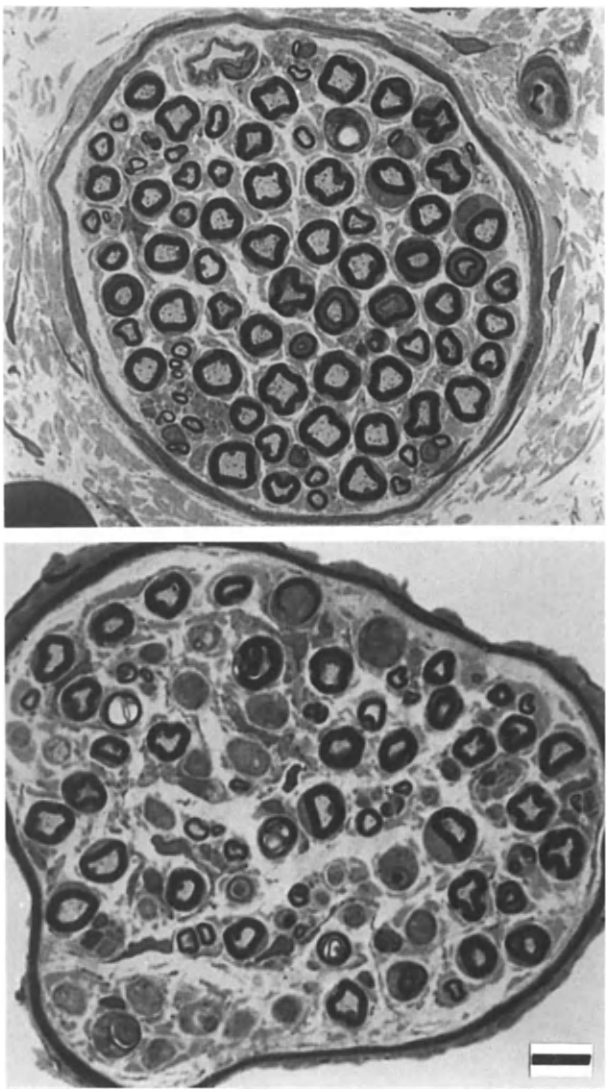

FIG. 1. Transverse section of nerve to the extensor digitorum longus muscle in control (top) and in test (bottom). Note the presence of myelin ovoids and loosely packed myelinated axons suggestive of endoneurial edema and loss of myelinated fibers in test (bottom). Epon-embedded semithin sections stained with toluidine blue. Bar represents 10 μm.

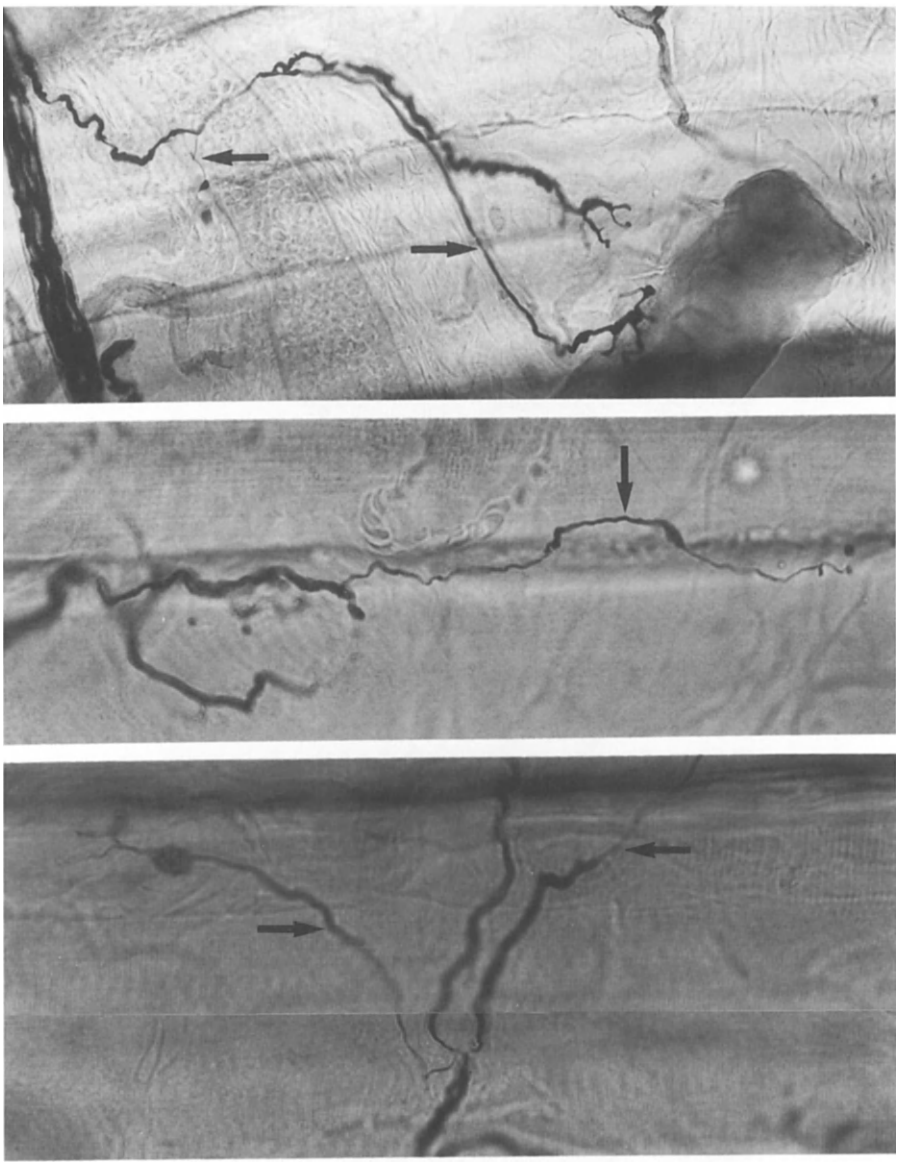

FIG. 2. Collateral branchings (arrows) with thickening of the ultraterminal axons are seen (top). A terminal sprouting (arrow) is seen arising from the terminal axon (center). Nodal sproutings (arrows) ending on different muscle fibers are seen (bottom). Silver staining of test soleus muscle. Bar represents 20 μm.

center and bottom, and Fig. 3) were greater ($P < 0.01$) in test than in control (Table 2). Degenerating axons were occasionally identified (Fig. 3). The frequency of endplates with swellings in the preterminal, terminal, and ultraterminal axons (Fig. 4) was significantly greater ($P < 0.01$) in test than in control. Their mean diameter of axonal swellings in test was in the range of 3 to 6 μm and greater (P

TABLE 1

FREQUENCY (%)[a] OF ENDPLATE TYPES IN SOLEUS MUSCLE (SOL M) AND EXTENSOR DIGITORUM LONGUS MUSCLE (EDL M) IN CONTROL AND ACRYLAMIDE-INTOXICATED RATS (TEST)

	SOL M		EDL M	
Endplate types[b]	Control	Test	Control	Test
T1	87.7 ± 9.6	31.0 ± 3.9*	94.6 ± 4.6	45.9 ± 9.4*
T2	11.4 ± 10.0	52.6 ± 9.4*	5.4 ± 4.2	44.6 ± 8.9*
T3	0.28 ± 0.7	9.7 ± 5.7*	0	5.7 ± 4.1*
Collateral branching	0.6 ± 0.9	6.2 ± 1.9*	0	3.4 ± 2.9*

[a] Mean ± SD in six muscles in each of SOL M and EDL M of control and test rats.

[b] Endplate types (Tuffery, 1971): T1, formed from one unbranched terminal axon; T2, formed from two branches of terminal axon; T3, formed from three branches of terminal axon; Collateral branching, formed from two or more branches of terminal axon ending on separate sole plates on separate muscle fibers.

* Significantly different ($P < 0.01$) in test compared with control.

< 0.01) than those in control for each axon (Table 2). Bulbous axonal swellings reaching as large as 13.5 μm in diameter were occasionally observed in ultraterminal axons.

Morphometric findings in motor axon of EDL M. The frequency of endplate types with two or three branches of terminal axon and with collateral branching was higher ($P < 0.01$) in test than in control (Table 1). The number of terminal axon branches per endplate was greater ($P < 0.01$) in test than in control. Fur-

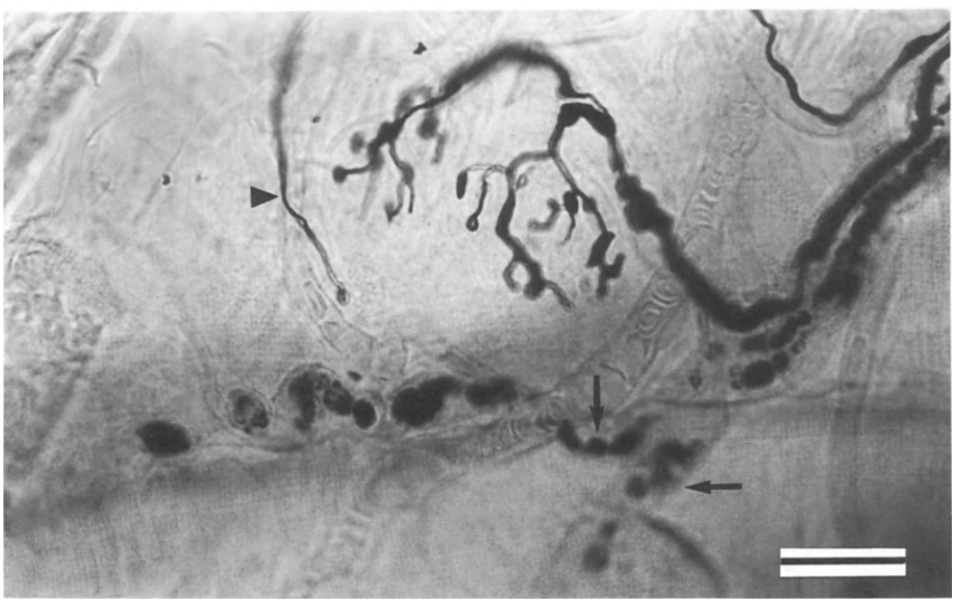

FIG. 3. An endplate showing focal axonal thickening in the preterminal and ultraterminal axons. Note the degeneration of two axons in the foreground (arrows) and the evidence of regeneration in the form of nodal sprout (arrowhead). Silver staining of test soleus muscle. Bar represents 20 μm.

TABLE 2
MORPHOMETRIC DATA[a] OF MOTOR AXONS IN SOLEUS MUSCLE IN CONTROL AND ACRYLAMIDE-INTOXICATED RATS (TEST)

Findings of axon	Control (N = 6)	Test (N = 6)
Number of terminal axon branches per endplate	4.4 ± 1.8	8.0 ± 1.4*
Frequency (%) of endplates with		
terminal sprouting	1.9 ± 0.7	13.4 ± 5.1*
nodal sprouting	4.2 ± 3.5	15.6 ± 6.9*
Frequency (%) of endplates with swelling in		
preterminal axon	0	18.6 ± 6.0*
terminal axon	0.6 ± 1.3	20.3 ± 6.3*
ultraterminal axon	6.7 ± 4.0	83.9 ± 8.6*
Diameter (μm) of axonal swelling of		
preterminal axon	0	4.7 ± 0.5*
terminal axon	0.6 ± 0.8	5.5 ± 0.7*
ultraterminal axon	1.7 ± 0.3	3.5 ± 0.2*

[a] Mean ± SD.
* Significantly greater ($P < 0.01$) in test than in control.

thermore, the frequency of terminal sprouting was greater ($P < 0.01$) in test than in control (Table 3). The frequency of endplates with axonal swellings in the preterminal, terminal, and ultraterminal axons was greater ($P < 0.01$) in test than in control. The mean diameter of axonal swellings in test was in the range of 3 to 6 μm and greater ($P < 0.01$) than those in control for each axon (Table 3).

Comparison of motor axons of EDL M and SOL M in test (Tables 1, 2, and 3). Morphometric findings of motor axons in SOL M and EDL M described are similar except for higher ($P < 0.01$) frequency of endplates with nodal sprouting and with ultraterminal axonal swellings, but lower ($P < 0.01$) frequency of endplates with preterminal and terminal axonal swellings in SOL M than in EDL M.

DISCUSSION

Our study has clearly demonstrated that endplate remodeling and axonal sproutings occur in both SOL M and EDL M of rats showing progressive hindlimb weakness with a cumulative dose of 600 mg acrylamide/kg body wt. We conclude that both regeneration and axonal degeneration exist in the motor terminal axons in acrylamide intoxication.

Evidences of axonal regeneration have been seen during acrylamide intoxication (Cavanagh, 1982; Fullerton and Barnes, 1966; Shaumburg *et al.*, 1974; Suzuki and Pfaff, 1973). Although Cavanagh (1982) noted occasional ultraterminal sprouting in both forelimb and hindlimb muscles of rats given a cumulative dose of 450 mg acrylamide/kg body wt, quantitative analysis with adequate control was not shown. No systematic morphometric studies as ours in acrylamide-intoxicated rats have been reported.

On the other hand, our conclusion is sharply in contrast with that of Kemplay and Cavanagh (1984b) who reported the inhibition of terminal sprouting and endplate staining with cumulative doses of acrylamide using a zinc-osmium staining technique. However, the axonal sprouting in their study may have been under-

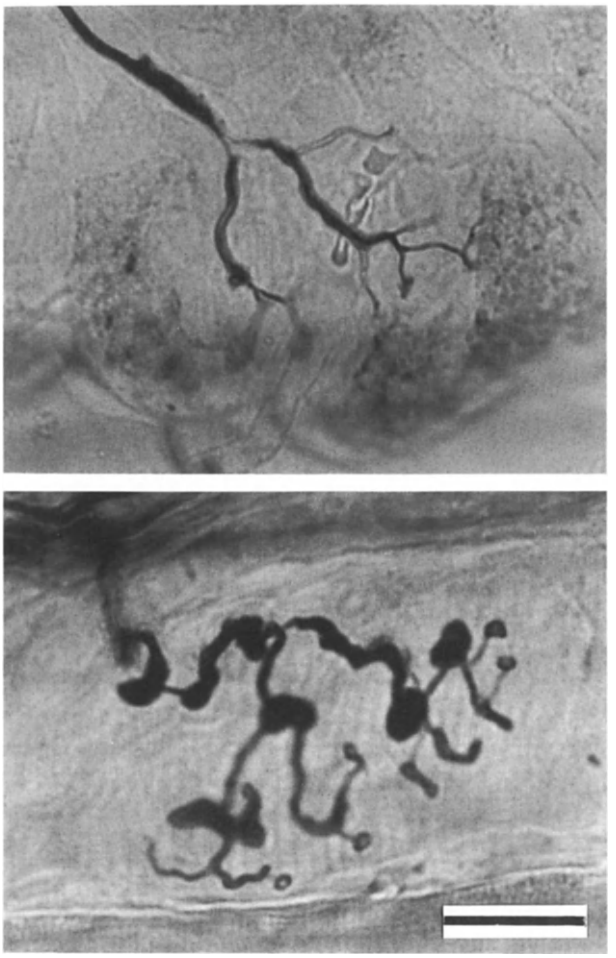

FIG. 4. Focal swelling and thickening of preterminal, terminal, and ultraterminal axons are frequently seen and ultraterminal branches are greater in number in test (bottom) compared with control (top). Silver staining of test soleus muscle. Bar represents 20 μm.

estimated and the nodal sprouting undetected because zinc-iodide osmium is not ideal for the staining of collateral and nodal sprouts as experienced by several workers (Brown *et al.*, 1980; Kemplay and Stolkin, 1980).

In our study, sprouts shorter than 8 μm were deliberately not included. However, more than 10% of endplates in test showed terminal sprouting. Although the present study cannot address the question of whether such regenerative findings are functionally significant or not, further systematic morphometric studies in the recovery stage of the muscle weakness may provide some clues. The presence of denervated muscle fibers and denervated endplates, and impaired axonal transport as evidenced by axonal swelling, may have played a significant role in producing endplate remodeling and regeneration in the motor axons (Brown *et al.*, 1980; Brown and Ironton, 1978; Keynes *et al.*, 1983).

TABLE 3
MORPHOMETRIC DATA[a] OF MOTOR AXONS IN EXTENSOR DIGITORUM LONGUS MUSCLE IN CONTROL AND ACRYLAMIDE-INTOXICATED RATS (TEST)

Findings of axon	Control (N = 6)	Test (N = 6)
Number of terminal axon branches per endplate	3.6 ± 0.5	7.0 ± 0.7*
Frequency (%) of endplates with		
terminal sprouting	4.2 ± 2.3	14.7 ± 6.4*
nodal sprouting	1.7 ± 2.6	1.9 ± 3.0
Frequency (%) of endplates with axonal swelling in		
preterminal axon	0	38.2 ± 13.0*
terminal axon	0	50.6 ± 17.3*
ultraterminal axon	0	57.7 ± 10.7*
Diameter (μm) of axonal swelling of		
preterminal axon	0	4.6 ± 0.4*
terminal axon	0	5.7 ± 0.8*
ultraterminal axon	0	3.5 ± 0.2*

[a] Mean ± SD.
* Significantly greater ($P < 0.01$) in test than in control.

The finding of significantly higher frequency of nodal sprouting in SOL M than in EDL M in test may be indicative of more severe muscle fiber denervation in the SOL M (K. Hachisuka *et al.*: Soleus muscle is more vulnerable than EDL M in acrylamide neuropathy in rats, in preparation). In fact, the differential endplate changes in the normal aging process and pathologic conditions between EDL M and SOL M have been described (Brown and Ironton, 1978; Duchen, 1970; Keynes *et al.*, 1983). Also such a finding may be related to the lesser frequency of endplates with axonal swellings in both preterminal and terminal axons in the SOL M than in the EDL M. These findings may be indicative of the greater degree of acrylamide-induced changes in the endplates and nerve to the EDL M. Such differential vulnerability of nerves to acrylamide has been reported (Shaumburg *et al.*, 1974).

ACKNOWLEDGMENTS

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Comparative Study of Modification and Degradation of Neurofilament Proteins in Rats Subchronically Treated with Allyl Chloride, Acrylamide, or 2,5-Hexanedione[1]

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Allyl chloride (ALL), acrylamide (ACR), and 2,5-hexanedione (2,5-HD) are all industrial neurotoxicants and known to produce accumulation of neurofilament (NF) proteins in both the central and peripheral nervous systems. To clarify whether any common mechanisms underlie these neurofilamentous axonopathies, the ability of ALL, ACR, and 2,5-HD to cross-link the NFs and the effects on NF degradation by Ca^{2+}-activated neutral protease were investigated in spinal cords from rats subchronically treated with these chemicals. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by immunoblot analysis revealed the appearance of high-molecular-weight species of NF triplets immunoreactive to each anti-68K, anti-160K, and anti-200K NF antibody in the 2,5-HD-treated rats, whereas it was not found in those treated with ALL or ACR. A time course study on the degradation of NF proteins conducted by the co-incubation with Ca^{2+} showed degradation resistance in all three NF subunits from animals treated with 2,5-HD, while no significant alterations in the rate of NF degradation were observed in the ALL- or ACR-treated group. The present results suggest that neurofilament-filled axonopathy induced by ALL or ACR and axonopathy induced by 2,5-HD may not share a common mechanism, though the initial step for the pathogenesis of this chemically induced neurotoxicity is not fully understood at present.

INTRODUCTION

Allyl chloride (ALL), monomeric acrylamide (ACR), and *n*-hexane are all widely used in industries and have been reported to produce occupational peripheral neuropathies in man chronically exposed to these chemicals (Fujita *et al.*, 1960; Yamamura, 1969; Schaumburg *et al.*, 1974; He *et al.*, 1980; He and Zang, 1985). After outbreaks of neuropathies, the neurotoxicity of these chemicals, including 2,5-hexanedione (2,5-HD), a potent metabolite of *n*-hexane (Couri *et al.*, 1978), has been confirmed through many experimental studies using a variety of animal species. Pathologically, it has been known that administration of both 2,5-HD and ACR cause neurofilament-filled swelling proximal to the nodes of Ranvier in the distal, preterminal axon of both the peripheral and central nervous system (Prineas, 1969; Schaumburg and Spencer, 1976; Spencer and Schaumburg, 1977a,b), though it has also been suggested that the manner of development of the morphological changes by the two chemicals follows quite a different pattern (Cavanagh, 1982; Sayre *et al.*, 1985). As for ALL, He *et al.* (1981, 1985) reported that neurofilament (NF) accumulations were also observed at an early stage of intoxication with this chemical. However, it was indicated that the accumulations

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of NFs were never as large as those seen in hexacarbon neuropathies, neither did they tend to aggregate at paranodal regions, and that the degree of filament increase caused by ALL more closely resembled that found in ACR neuropathies.

The mechanisms by which these chemicals produce neurofilamentous axonopathy are not fully understood. Recently, increased studies on the mechanisms for 2,5-HD (hexacarbon) neurotoxicity indicated that 2,5-HD covalently binds to proteins at lysyl ϵ-amino groups *in vitro* and *in vivo* (DeCaprio *et al.*, 1982, 1983, 1988; Graham *et al.*, 1982, 1984; Anthony *et al.*, 1983b; DeCaprio and O'Neill, 1985) to form pyrrole adducts. The pyrrole formation in the cytoskeletal protein followed by a second cross-linking reaction of NFs (Genter *et al.*, 1987; Rosenberg *et al.*, 1987), or the pyrrole-induced hydrophobicity followed by lowered solubility (DeCaprio *et al.*, 1988) have been suggested as the initial steps of the pathogenesis of hexacarbon neurotoxicity. As for the mechanism of acrylamide neuropathy, it has been shown that degradations of NF triplet proteins were inhibited in ACR-treated animals, which suggested that Ca^{2+}-activated neutral protease (CANP), thiol protease thought to be responsible for normal degradation of NF proteins in axon, might be inhibited by ACR leading to the accumulation of NF proteins (Tanii *et al.*, 1988). On the other hand, there has been no data on the mechanisms for ALL-induced neurotoxicity. It is meaningful, therefore, to elucidate the molecular basis of the mechanisms for ALL neurotoxicity in comparison with 2,5-HD and ACR.

The purpose of the present study was to compare, using the same methods of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblot analysis, the effects of all three chemicals on the modification and degradation of NF proteins in spinal cord from rats subchronically intoxicated with these chemicals.

MATERIALS AND METHODS

Chemicals

Allyl chloride (CH_2CHCH_2Cl, >95%) and 2,5-hexanedione ($CH_3COCH_2CH_2COCH_3$, >95%) were obtained from Tokyo Kasei Co. (Tokyo, Japan). Acrylamide ($CH_2CHCONH_2$, >99.9%) and other chemicals used for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories (Richmond, CA). Triton X-100, phenylmethylsulfonyl fluoride (PMSF), 3,3′-diaminobenzidine, and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, MO). Leupeptin was purchased from Chemicon International Inc. (Temecula, CA). Mouse monoclonal antibodies to the neurofilament triplet proteins (anti-68K, anti-160K, and anti-200K) were obtained from Amersham International Plc (Buckinghamshire, England). A Vectastain ABC (peroxidase mouse IgG) kit was obtained from Vector Laboratories Inc. (Burlingame, CA). All other chemicals were of reagent grade.

Animals and Treatment

Twenty-four male Donryu rats (7 weeks old) were housed in plastic cages (2 or 3 rats/cage) containing wood flake bedding. After 1 week of acclimation, the animals were divided equally into three treatment groups and a control. The treatment groups received 2 mmole/kg ALL, 0.25 mmole/kg ACR, or 2.5 mmole/kg 2,5-HD by sc injections 5 days a week for 3 months. The dosage levels and

treatment periods for developing clinical neuropathies in rats were chosen after preliminary range-finding studies. Animals were provided with pellet food (Nippon Clea CE-2) and water *ad libitum*. Room temperature was maintained at 25 ± 2°C and humidity at 55 ± 5%. Body weight and neurological signs were monitored weekly.

Evaluation of Neurotoxicity

To estimate the effects of ALL, ACR, or 2,5-HD administrations on peripheral nerve functions of the rats, measurements of the maximum conduction velocities of motor and sensory fibers (MCV and SCV) in the tail nerves were conducted after 3 months treatment. The electrophysiological techniques used have been described in detail in previous reports (Misumi, 1979; Misumi and Nagano, 1984).

Preparation of Spinal Cord NFs

Spinal cords were removed from both treated and control rats by an injection of ice-cold saline according to the method of De Sousa and Horrocks (1979). NF-rich cytoskeletal proteins from the spinal cords were prepared according to Chiu and Norton (1982). In brief, the spinal cord from each animal was homogenized in 10 ml of Buffer A consisting of 50 mM Tris–HCl, pH 6.8, 2 mM EDTA, 2 mM PMSF, and 0.5% v/v Triton X-100 in a glass Teflon homogenizer. The homogenate was centrifuged at 13,000*g* for 15 min at 4°C. The pellet (P_1) was similarly homogenized in 10 ml of 0.9 M sucrose in Buffer A and centrifuged as before. The resulting pellet (P_2), enriched in NFs, represented the final cytoskeletal preparation and was suspended in 2.5 ml of 0.1 M phosphate-buffered saline.

SDS–PAGE

SDS–PAGE was performed by the method of Laemmli (1970) with 7% separating and 5% stacking gels. All samples for PAGE were prepared with a final concentration of 62.5 mM Tris–HCl, pH 6.8, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue, followed by heating at 100°C for 5 min. Equal amount of the solubilized proteins per lane were electrophoresed, and then the proteins on the gels were stained with Coomassie brilliant blue (CBB) R250. The destained gels were scanned at 555 nm with a chromatoscanner (Shimazu CS-910, Kyoto, Japan). Protein concentrations of samples were determined by Bradford's method (1976) using BSA as the standard.

Immunoblotting

PAGE-separated proteins were electrophoretically transferred to a nitrocellulose paper by the method of Towbin *et al.* (1979). Transfer was carried out overnight at 4°C, 50 V. The paper was then removed and soaked for 60 min in a blocking solution of 2.5% BSA in Tris-buffered saline (TBS, 0.1 M Tris–HCl, pH 7.6, 0.9% NaCl). Then, a 1:5 dilution of anti-68K, anti-160K, or anti-200K NF mouse monoclonal antibody in 0.1% serum in TTBS (0.1% Tween in TBS) was applied for 60 min at room temperature. After five washes with TTBS, the second antibody (biotynylated horse anti-mouse IgG antibody) was applied for 30 min. The paper was then washed three times with TTBS followed by incubation with avidin–biotin–peroxidase complex (ABC) in TTBS for 30 min. After three washes in TBS, the bound peroxidase was developed in a 1:1 mixture of 60 mg 3,3′-

diaminobenzidine in 60 ml TBS and 40 μl H_2O_2 in 60 ml TBS. The reaction was stopped by washing with water.

Degradation of NF Proteins

In another experiment, degradation patterns of NF spinal cord from both treated and control rats were investigated. The three treatment groups (n = 3–4) received 2.5 mmole/kg ALL, 0.25 mmole/kg ACR, or 2.0 mmole/kg HD by sc injection 5 days a week for up to 3 months. The control group (n = 5) received 0.2 ml of saline on the same schedule as the treated groups. The spinal cord from each animal was removed 24 hr after the final treatment. The axonal cytoskeleton preparations (P_2 pellets) from the spinal cords were prepared as described above and suspended in 2.5 ml of 50 mM Tris–HCl, pH 7.4, 25 mM KCl, and 10 mM $MgCl_2$ (sample preparation). The proteolytic activity of CANP in the spinal cord NF preparations was assessed by the method of Ishizaki *et al.* (1983) with some modifications. The standard mixture (1 ml) contained 0.2 ml of the sample preparation, 0.7 ml of 20 mM Tris–HCl, pH 7.4, 5 mM 2-mercaptoethanol, and 0.1 ml of 50 mM $CaCl_2$ or 50 mM EDTA. After incubation at 37°C for various times, the reaction was stopped by the addition of the SDS buffer (final concentration consisting of 2% SDS, 62.5 mM Tris–HCl, pH 6.8, 10% (v/v) glycerol, and 5% (v/v) 2-mercaptoethanol), and the samples were boiled at 100°C for 5 min. Aliquots were subjected to SDS–PAGE and proteolytic activity was assessed by the amount of breakdown of NF proteins.

Statistics

Statistical evaluation between the control and treated groups was performed using one-way analysis of variance with Scheffe's multiple comparisons.

RESULTS

Toxicological Evaluation

As shown in Table 1, clinical signs of neurotoxicity were apparent in all the treated groups at the end of the treatment schedule. A significant decrease of body

TABLE 1
EFFECTS OF SUBCHRONIC TREATMENT WITH ALL, ACR, OR 2,5-HD ON BODY WEIGHT AND CLINICAL SIGNS IN RATS

Treatment	Dose (mmole/kg/day)	Final body weight (g)	Clinical sign
Control		479 ± 47 (100)	–
ALL	2.0	414 ± 34 (86)	+[a]
ACR	0.25	377 ± 39 (79)*	+[a]
2,5-HD	3.0	254 ± 30 (53)**	+[a]

Note. Rats received sc injections of each chemical for 3 months. ALL, allyl chloride; ACR, acrylamide; 2,5-HD, 2,5-hexanedione.

[a] By the end of treatment, all animals in the ALL or ACR-treated group showed spreading of the hindlimbs, and all 2,5-HD treated animals exhibited dragging of the hindlimbs as well as urinary incontinence.

Each value represents the means ± SD of six rats. Figures in parentheses indicate the % of control.

* $P < 0.05$.

** $P < 0.01$.

weight gain compared with the controls was observed in the ACR- and 2,5-HD-treated groups. Nerve conduction studies also provided evidence of neuropathy in all the treated groups (Table 2). That is, the SCV and MCV of the tail nerve in the 2,5-HD-treated groups decreased by 67 and 56% of the control, respectively. The ALL- and ACR-treated groups also showed significant decreases of both the SCV and the MCV at the end of treatment compared with the control group.

When intact spinal cords were obtained by the De Sousa and Horrocks method, the weight of the spinal cords from the 2,5-HD- or ALL-treated animals was significantly reduced compared with those from the controls (Table 3). In addition, the protein content of the cytoskeletal preparations (mg/g of spinal cord) from the 2,5-HD-treated group was markedly reduced. In contrast, there were no significant reductions of protein concentrations in the ALL- or ACR-treated groups.

SDS–PAGE of Cytoskeletal Preparation from Rat Spinal Cord

Figure 1 shows the SDS–PAGE patterns of the cytoskeletal preparations from both control and treated animals. Subchronic treatment of rats with 2,5-HD produced substantial reductions in all three subunits of NF proteins (200K, 160K, and 68K) in the spinal cords, although the amounts of proteins applied per lane were the same in all four groups. The decrease of neurofilament subunits in this group was also confirmed by quantitative assessment of cytoskeletal proteins using a chromatoscanner (Table 4). In this evaluation, the 200K protein in the ALL- or ACR-treated groups was significantly reduced compared with that in the control. Glial fibrillary acidic proteins (GFAP) in the preparations from all the treated animals seemed to increase in amount, but were not significant when compared with those from the control group (Table 4).

Chemical Modification of NF Triplet Proteins

Immunoblot analysis of the spinal cord cytoskeletal preparations is shown in Fig. 2. Immunoreactive antigens to anti-200K, anti-160K, and anti-68K NF antibodies appeared as several or diffused stains of high-molecular-weight species in

TABLE 2
ELECTROPHYSIOLOGICAL FINDINGS OBSERVED IN ALL, ACR, OR 2,5-HD-TREATED RATS

Treatment group	SCV (m/sec)	MCV (m/sec)	RL (msec)	DL (msec)
Control	56.4 ± 1.5 (100)	46.0 ± 1.0 (100)	0.60 ± 0.11 (100)	1.86 ± 0.09 (100)
ALL	50.6 ± 0.7* (90)	40.6 ± 4.5* (88)	0.72 ± 0.18 (120)	2.20 ± 0.04* (118)
ACR	43.0 ± 3.6** (76)	36.5 ± 4.5** (79)	0.83 ± 0.09 (138)	2.45 ± 0.12** (132)
2,5-HD	37.8 ± 2.5** (67)	25.8 ± 0.4** (56)	1.54 ± 0.42** (257)	3.75 ± 0.19** (202)

Note. Rats received sc injection of each chemical for 3 months. Electrophysiological measurements were conducted 1 week prior to sacrifice. Each value represent the means ± SD of six rats. Figures in parentheses indicate the % of control. SCV, sensory nerve conduction velocity; MCV, motor nerve conduction velocity; RL, residual latency; DL, motor distal latency.

* $P < 0.05$.

** $P < 0.01$.

TABLE 3
PROTEIN CONTENTS IN THE CYTOSKELETAL PREPARATIONS FROM RAT SPINAL CORDS

Treatment	Spinal cord (g)	Cytoskeletal protein (mg/g spinal cord)
Control	0.729 ± 0.065	11.79 ± 1.11
ALL	0.615 ± 0.058*	10.40 ± 1.66
ACR	0.668 ± 0.038	11.35 ± 1.72
2,5-HD	0.528 ± 0.045**	6.49 ± 1.66**

Note. After 3 months of treatment with each chemical, the animals were decapitated and the intact spinal cords were removed by de Sousa and Horrocks' methods. The cytoskeletal proteins were prepared according to Chiu and Norton as described. Each value represents the means ± SD of six rats.

* $P < 0.05$.

** $P < 0.01$.

the 2,5-HD-treatment group, but not in the ALL- or ACR-treated animals (Figs. 2a–2c). Immunodetection with anti-GFAP antibody showed a faint stain of a high-molecular-weight GFAP band in the preparations from 2,5-HD-treated animals (Fig. 2d). Although an immunoreactive breakdown product of NF triplets was observed in all the treated animals (Figs. 2a–2c), no bands characteristic of each treated group were detected. In the 2,5-HD-treated animals, the amount of degraded product diminished compared with that of the controls (Figs. 2b and 2c). In comparison to the NF triplet proteins, GFAP from animals in the control and treated groups were not degraded when 4 μg of protein was loaded on each lane.

Time Course of Degradation of NF Proteins

The time course of the degradation of NF proteins in the spinal cords is shown in Table 5. After 6 hr of incubation with Ca^{2+}, the 68K, 160K, and 200K NF subunits in the control were degraded 81, 75, and 60% from 0 hr of incubation, respectively. No degradation of NF proteins was observed when 5 mM EDTA was added in incubation mixtures in place of Ca^{2+}. Resistance to degradation of all the NF triplet proteins was observed in the spinal cords from the 2,5-HD-treated

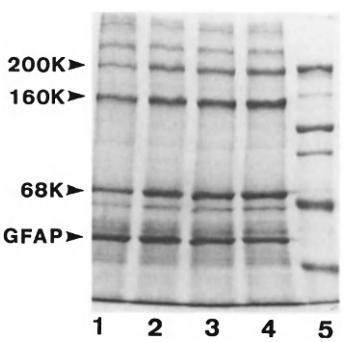

FIG. 1. SDS–PAGE of cytoskeletal proteins in spinal cords from 2,5-HD (lane 1)-, ACR (lane 2)-, ALL (lane 3 0-treated and control (lane 4) rats. Eight micrograms of protein were loaded per well. Arrowheads indicate the 200, 160, and 68K of NF subunits and GFAP (51K). Bio-Rad molecular weight standards (lane 5 and arrows) used were, from top to bottom, myosin, 200K; b-galactosidase, 116K; phosphorylase b, 97K; bovine serum albumin, 66K; ovalbumin, 43K.

TABLE 4
RELATIVE CHANGES IN CONTENTS OF CYTOSKELETAL PROTEINS IN SPINAL CORDS FROM RATS TREATED WITH ALL, ACR, OR 2,5-HD

Treatment	GFAP	NF68K	NF160K	NF200K
Control	100 ± 15	100 ± 21	100 ± 29	100 ± 25
ALL	125 ± 17	85 ± 13	83 ± 12	64 ± 23*
ACR	152 ± 42	80 ± 36	82 ± 25	63 ± 10*
2,5-HD	146 ± 61	38 ± 7**	40 ± 11**	29 ± 8**

Note. After SDS–PAGE separations of cytoskeletal proteins of spinal cords from control and treated animals, a quantitative evaluation of the CBBR 250-stained proteins on gels was conducted using a chromatoscanner with a recording integrator. Identical amounts of proteins (15 μg) were introduced to each lane. Data are represented as % of each band of control and the means ± SD of six rats.

* $P < 0.05$.

** $P < 0.01$.

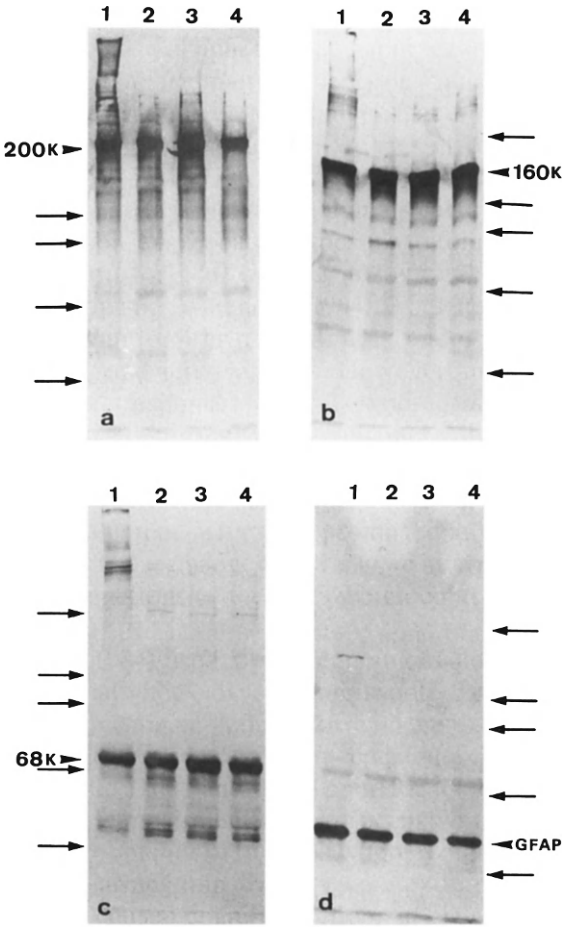

FIG. 2. Immunoblots of the cytoskeletal proteins from 2,5-HD (lane 1)-, ACR (lane 2)- ALL (lane 3)-treated and control (lane 4) animals to anti-200K (a), anti-160K (b), and anti-68K (c) NF antibodies and anti-GFAP (d) antibody. Four micrograms of proteins were loaded on each well. Arrowheads indicate the positions of original bands of each NF protein. Molecular weight standards (arrows) are the same as in Fig. 1. Note the appearance of high-molecular-weight species proteins immunoreactive to anti-200K, anti-160K, and anti-68K NF antibodies and also to anti-GFAP antibody on the lanes from 2,5-HD-treated animals.

TABLE 5
TIME COURSES IN THE DEGRADATION OF NEUROFILAMENT PROTEINS FROM SPINAL CORDS

	% Degradation of neurofilament								
	68K			160K			200K		
Treatment	1 hr	3 hr	6 hr	1 hr	3 hr	6 hr	1 hr	3 hr	6 hr
Control	38 ± 19	65 ± 17	81 ± 10	29 ± 26	63 ± 13	75 ± 9	29 ± 16	47 ± 18	60 ± 11
ALL	48 ± 23	61 ± 22	74 ± 19	41 ± 23	67 ± 15	84 ± 13	30 ± 24	46 ± 17	66 ± 17
ACR	28 ± 11	48 ± 14	72 ± 12	40 ± 16	67 ± 15	79 ± 13	40 ± 12	46 ± 8	56 ± 15
2,5-HD	27 ± 17	28 ± 20	29 ± 20*	21 ± 6	30 ± 13*	40 ± 4**	5 ± 4	34 ± 24	35 ± 11*

Note. The NF-enriched cytoskeletal preparations from spinal cords were incubated at 37°C for 0, 1, 3, and 6 hr in a medium containing 5 mM Ca^{2+}. After incubation, the sample was solubilized by SDS, then subjected to SDS–PAGE. A quantitative evaluation of NF proteins on gel was performed using a chromatoscanner with integrator. Each value represents % degradation compared with respective values of 0 hr incubation, and the means ± SD of three to five rats.

* $P < 0.05$.

** $P < 0.01$.

group. While, no significant alteration in the degree of degradation was observed in the NF proteins from the ALL- or ACR-treated group.

DISCUSSION

It has been recognized that several chemicals including β, β′-iminodipropionitrile (IDPN) (Clark *et al.*, 1980; Griffin and Price, 1980), carbon disulfide (Seppäläinen and Haltia, 1980), and aluminium salts (Wisniewski, 1984) as well as ALL, ACR, and 2,5-HD produce an accumulation of 10-nm neurofilaments, regardless of differences in the locations of lesions observed along the axon and in the kinds of affected neurons. However, it is not well known whether the common molecular mechanism(s) for the pathogenesis underlie the neurotoxicity induced by these chemically unrelated compounds. In the present study, the ability of ALL, ACR, and 2,5-HD to cross-link the NF proteins and the Ca^{2+}-dependent proteolysis of NFs were evaluated as conceivable mechanisms for the accumulation of NF proteins relevant to the appearance of neurotoxicity.

Regarding 2,5-HD, Lapadula *et al.* (1986) showed evidence of cross-linking of NF proteins *in vivo* using immunoblotting analysis. We also confirmed the many bands of high-molecular-weight species of NF proteins which react with each of anti-200K, anti-160K, and anti-68K NF antibodies in the spinal cords from 2,5-HD-treated animals. As has been suggested, the cross-linked NF proteins might be blocked from passing the contracted node of Ranvier and/or might be resistant to normal degradation of NFs by CANP, leading to an accumulation of NFs above the node of Ranvier in the axon (DeCaprio *et al.*, 1982; Graham *et al.*, 1982, 1984; Anthony, 1983a,b).

In our study, it was an interesting but not unexpected observation that non-neural GFAP was also cross-linked, because it has been demonstrated that 2,5-HD has cross-linking reactions with many sorts of proteins *in vivo* and *in vitro*, i.e., BSA, ovalbumin, spectrin (Anthony *et al.*, 1983b). The susceptibility of NF proteins to chemical modification by 2,5-HD relative to other proteins might be explained by its high stability and low turnover rate (Sayre *et al.*, 1985).

We anticipated that allyl chloride might have the potency to cross-link with NF proteins covalently because it has a chemically reactive double bond and halogen in its molecular structure. Nevertheless, we could not get evidence of the cross-

linking of NF proteins from animals treated with ALL and with ACR. These results suggest that the accumulations of NF proteins observed in 2,5-HD- and ALL- or ACR-treated animals may not share a common mechanism. However, the possibility that the differences in experimental designs, including the levels and periods of treatment, the amount of NF antigen from intoxicated animals, and the amount of anti-NF antibodies applied, might produce different results cannot be discarded at present.

In contrast to previous observations of ACR neurotoxicity by others (Tanii *et al.*, 1988), no significant reduction in the degradation of all the NF triplet proteins of spinal cords from the ACR-treated animals was observed in our study, even though the time course study of the degradation was conducted by employing considerably longer incubation periods (1 to 6 hr). In addition, the degree of percentage degradation of NF proteins from the control animals in our study was retarded compared to that obtained by Tanii *et al.;* the former study showed 38, 29, and 29%, and the latter, 65, 85, and 63% of degradation of the 68K, 160K, and 200K NF proteins, respectively, after 1 hr of incubation. The reasons for the discrepancy between the two results are unclear. One explanation of these differences may relate to preparation of the cytoskeletal proteins. Schlaepfer and Freeman (1980) reported that Ca^{2+}-mediated disruption of NFs was entirely dependent on soluble tissue factor(s). Furthermore, Ishizaki *et al.* (1983) showed that protease activity remained associated with the cytoskeleton in the physiological ionic condition, when a NF-rich fraction was prepared from a Triton-insoluble fraction. Tanii *et al.* and we also prepared the cytoskeletal proteins from a Triton-insoluble fraction in accordance with Chiu and Norton (1982). A slight incorporation of soluble fraction into cytoskeletal pellet, therefore, may lead to the differences in the degradation rates. We are now conducting further investigations of this problem.

In the present investigation, we employed a subchronic intoxication schedule (3 months) and selected a dose level to produce apparent clinical signs of neurotoxicity after this treatment period. The reason is as follows: (1) occupational neuropathies in man develop after chronic exposure to these chemicals, and (2) evidence of irreversible covalent binding of chemicals with NF proteins, proposed as one of the mechanisms of the neurofilamentous axonopathy, might be more detectable at the point at which animals exhibit marked neurotoxicological signs. However, it seems to be complicated to elucidate the mechanism(s) for the chronic effects of these chemicals on neurotoxicity. Pathologically, it has been reported that chronic exposure to these chemicals also produced Wallerian-like degeneration in the distal axon (Schaumburg *et al.*, 1974) as a secondary change of neurofilamentous swelling followed by axonal nutrient flow loss along the axon (DeCaprio *et al.*, 1983). CANP activity is known to increase in nerves displaying Wallerian-like degeneration, by the influx of Ca^{2+} into the axon (Schlaepfer, 1987; El-Fawal *et al.*, 1990). Therefore, to understand the mechanisms it seems important to clarify the relationships among time- and dose-dependent biochemical, neuropathological and clinical changes.

Finally, from the present study on the effects of ALL, ACR, and 2,5-HD administrations on cross-linking abilities and on the degradation of NF proteins *in vivo,* it is indicated that different mechanisms from 2,5-HD may be related to pathogenesis of ALL- and ACR-induced neurofilamentous axonopathy. Moreover, several other mechanisms, such as alteration of phosphorylation state of

NF, microtubles, and microtuble-associated proteins, have also been suggested for neurotoxicities of these compounds (Howland and Alli, 1986; Gold *et al.*, 1988; Horan *et al.*, 1989; Berti-Mattera *et al.*, 1990). However, the initial event which determines the NF accumulations remains to be elucidated. Further studies must be conducted to clarify the mechanism(s).

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Effect of Ethanol on the Development and Maturation of Synapses in the Rat Hippocampus: A Quantitative Electron-Microscopic Study[1]

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The effects of chronic ethanol administration on the development and maturation of synapses in the strata radiatum and lacunosum-moleculare of CA1 in the hippocampus were quantitatively examined in rats exposed to ethanol for the entire period of fetal life as well as the whole period of postnatal life. Synapse densities in the strata radiatum and lacunosum-moleculare of the ethanol-treated group were significantly lower than those of the control group at 2, 14, 21, and 70 days of age. However, the rates of density reduction did not change between either of the strata that receive different groups of afferent fibers. The ratio of axospinous to axoshaftic synapses also did not change between control and ethanol-treated groups. These data suggest that chronic administration of ethanol reduces the density of synapses in this area and that this effect is not specific to neither the type of afferent fibers nor the type of synapses.

INTRODUCTION

Chronic ethanol exposure in developing and adult brains results in mental retardation and disorder of memory. One part of the central nervous system associated with learning and memory is the hippocampal formation (Streissguth, 1986; Dudai, 1989). Morphological studies have demonstrated the susceptibility of the hippocampal formation to the toxic effects of ethanol (for review see West and Pierce, 1986; Jones, 1988): reduction of the number of hippocampal pyramidal cells and dentate granule cells due to prenatal and postantal exposure to ethanol (Walker *et al.*, 1980; Barnes and Walker, 1981); alteration of neuronal circuits in the hippocampus after heavy ethanol exposure in prenatal and early postnatal periods (West *et al.*, 1981; West and Hamre, 1985); and reduction in the size of dendritic arbors and in the number of dendritic spines of the pyramidal cell after prenatal and postnatal exposure (Riley and Walker, 1978; Davies and Smith, 1981; McMullen *et al.*, 1984; Lopez-Tejero *et al.*, 1986). However, ethanol exposure has been reported to have a very limited effect on the density of synapses in the hippocampal formation: no significant decrease in the density of synapses in the molecular layer of the dentate gyrus after prenatal exposure (Hoff, 1985, 1988); no significant decrease in the density of synapses in the strata radiatum and oriens of the adult rats and mice after long-term exposures (Lee *et al.*, 1981; Phillips and

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Cragg, 1983); no significant change in the length of synaptic appositions and in the shape of dendritic spines in the molecular layer of the dentate gyrus of adult mice after chronic ethanol consumption (Markham *et al.*, 1987); and a significant decrease in the density of synapses in the stratum oriens of CA1 of the hippocampus of long-sleep mice after 3 months ethanol exposure and no significant decrease in that of short-sleep mice (Scheetz *et al.*, 1987).

To examine the effect of ethanol on the density of synapses in the rat hippocampus, this study is designed to quantitatively analyze the density of synapses in the strata radiatum and lacunosum-moleculare of CA1, where a highly organized structure of afferent fibers exists. Afferent fibers from the nucleus reuniens of the thalamus and from the entorhinal cortex terminate in the stratum lacunosum-moleculare (Steward and Scoville, 1976; Herkenham, 1978); association and commissural afferents terminate in the stratum radiatum (Gottlieb and Cowan, 1973; Swanson *et al.*, 1978), and septal afferents terminate in the both strata (Meibach and Siegel, 1977).

MATERIALS AND METHODS

Treatment of animals. At 28 days of age, 47 female Fisher-strain rats (Nippon Crea Co. LTD) were divided into two groups. In the ethanol-treated group, the animals were fed by free access to drinking water 10% ethanol–aqueous solution and MF pellets (Oriental Yeast Co., Ltd). The rats in the ethanol-treated group were mated with ethanol-free male rats at 98 days of age. The dams freely ingested the ethanol solution during the entire pregnancy and lactation periods. After delivery, pups also freely ingested the ethanol solution until sacrifice on Postnatal Days 2, 7, 14, 21, and 70. Tap water for drinking water and MF pellets for food were given to the control groups over periods described above.

Tissue preparation. A minimum of three animals was used for each stage in the control and ethanol-treated groups. On Postnatal Days 2, 7, 14, 21, and 70, three to six pups of both groups were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and perfused through the heart with the following solutions: 4% paraformaldehyde, 0.5% glutaraldehyde, 0.54% dextrose in 0.1 *M* sodium phosphate buffer (pH 7.4) for 2- and 7-day-old rats (Crain *et al.*, 1973) and 1% paraformaldehyde, 1% glutaraldehyde in 0.1 *M* sodium phosphate buffer (pH 7.4) for 14-, 21-, and 70-day-old rats. Brains were exposed, and coronal slices were cut at the middle portions of the rostrocaudal extent of the hippocampus. The slices were postfixed in 1% OsO_4, stained *en bloc* with aqueous uranyl acetate, and embedded in Epon. Ultrathin sections of the CA1 region were collected on Formvar-coated 0.5 × 2-mm slot grids and stained with uranyl acetate. For each sample a photomontage was made of the entire strata radiatum and lacunosum-moleculare of CA1, i.e., the area immediately above somata of pyramidal cells up to the hippocampal fissure, where blood vessels were frequently found (Fig. 1A). The density of synapses was analyzed from the photomontages (final magnification, ×12,600).

Analysis of electron micrographs. A synapse was defined as a structure consisting of pre- and postsynaptic densities and of more than one synaptic vesicle closely apposed to the presynaptic density. Every synaptic profile was counted on each montage and assigned to the following categories: (1) Axospinous synapse, i.e., synapse on dendritic spine. Dendritic spine was identified as a postsynaptic ovoid profile that had a floccular appearance without any organelles except ribo-

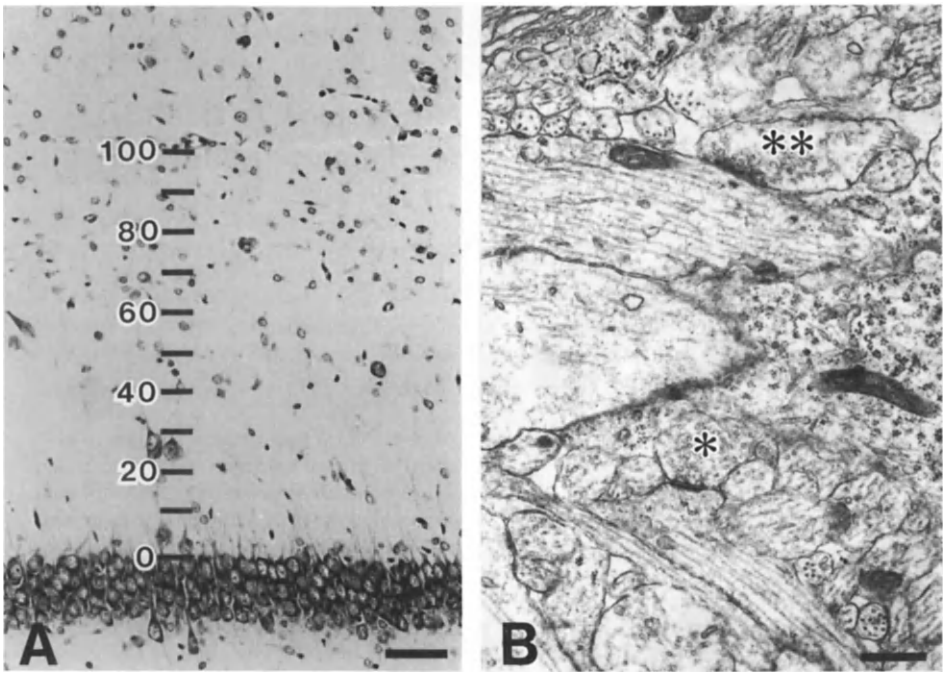

FIG. 1. Photomicrograph of a Nissl-stained section of CA1 in the rat hippocampus (A). Numbers in the strata radiatum and lacunosum-moleculare indicate 10 percentile intervals from the level (0%) immediately above the stratum pyramidale to the level (100%) directly under the hippocampal fissure. Scale bar, 50 μm. (B) Electron micrograph of stratum radiatum of a 7-day-old control rat. Note the axon terminal (asterisk) forming an axospinous synapse and a terminal (double asterisk) forming an axoshaftic synapse. Scale bar, 1 μm.

somes and spine apparatus (Peters *et al.*, 1976; Steward, 1983). (2) Axoshaftic synapse, i.e., synapse on dendritic shaft. Dendritic shaft was identified by an abundance of microtubules and some other organelles (Peters *et al.*, 1976). (3) Unclassified synapse, of which the postsynaptic structure could not be positively classified as a spine or a shaft (Fig. 1B). Densities of synapses were plotted as a function of 10% width of the entire strata radiatum and lacunosum-moleculare and expressed as number of synapses per 1000 μm^2. The density of synapses in the stratum radiatum was estimated from the proximal 70% width (neighboring region of somata of the pyramidal cells) of the photomontage. The density in the stratum lacunosum-moleculare was also estimated from the distal 30% width of the photomontage, because the stratum lacunosum-moleculare has been reported to occupy the distal 30% zone between the hippocampal fissure and the stratum pyramidale in developing and adult rats (Ritter *et al.*, 1971; Gottlieb and Cowan, 1973; Gerbrandt *et al.*, 1978). Student's *t* test with analysis of the difference in maternal population was used to compare the mean values of the density of synapses between control and ethanol-treated groups on each day studied.

RESULTS

Effects of Ethanol on the Density of all Synapses in the Strata Radiatum and Lacunosum-Moleculare

Figure 2 shows the effect of ethanol on all synapses in the strata radiatum and lacunosum-moleculare. In the control group, the density of synapses was low

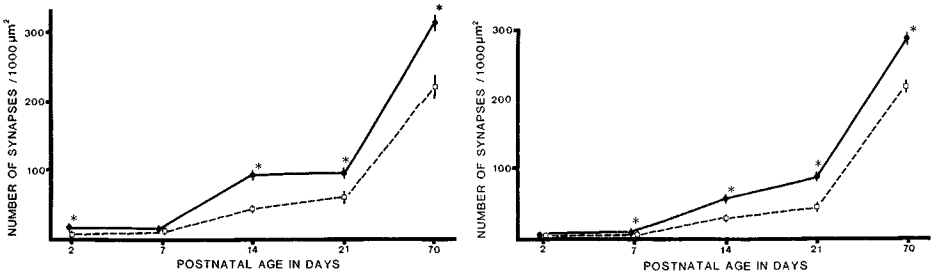

FIG. 2. Effect of ethanol on the density of all synapses in the strata radiatum and lacunosum-moleculare. Control group, solid line; ethanol-treated group, broken line. $*P < 0.05$.

FIG. 3. Effect of ethanol on the density of axospinous synapses in the strata radiatum and lacunosum-moleculare. Control group, solid line; and ethanol-treated group, broken line. $*P < 0.05$.

(19.0 ± 1.8/1000 μm^2, mean ± SEM) on Day 2, 16.2 ± 1.2 on Day 7, 93.9 ± 6.9 on Day 14, 99.4 ± 13.4 on Day 21, and 317.7 ± 18.6 on Day 70. Densities of synapses in the ethanol-treated group are significantly lower than those in the control group on Day 2 (5.8 ± 0.6/1000 μm^2), Day 14 (49.0 ± 1.2), Day 21 (60.5 ± 6.5), and Day 70 (228.6 ± 12.7).

Effects of Ethanol on Each Type of Synapse

Figure 3 shows the effect of ethanol on the development of axospinous synapses in the strata radiatum and lacunosum-moleculare. In the control group, densities of axospinous synapses are very low during the first postnatal week (0.7 ± 0.2 on Day 2 and 5.0 ± 0.9 on Day 7), and increase to 57.8 ± 4.8 on Day 14 to 85.6 ± 5.8 on Day 21 and to 282.6 ± 9.4 on Day 70. Densities of axospinous synapses in the ethanol-treated group are significantly lower than those in the control group on Day 7 (1.0 ± 0.2), Day 14 (29.0 ± 3.0), Day 21 (44.6 ± 7.2), and Day 70 (219.8 ± 9.8). Values of densities of axoshaftic synapses in the control group remain roughly similar during the period studied: 18.0 ± 1.6, 13.0 ± 1.1, 31.1 ± 3.5, 19.7 ± 1.0, and 19.6 ± 0.8, respectively, on Days 2, 7, 14, 21, and 70 (Fig. 4). Densities of axoshaftic synapses in the ethanol-treated group are significantly lower than those in the control group on Days 2, 14, 21, and 70 (5.4 ± 0.2, 14.8 ± 0.6, 11.1 ± 1.0, and 11.1 ± 1.0, respectively). However, the ratio of densities of spine to shaft synapses in control animals are similar to those in the ethanol-treated group: 1:18.2, 1:2.6, 1:0.5, 1:0.2, and 1:0.1, respectively, on Days 2, 7, 14, 21, and 70 in control animals and 1:10.9, 1:11, 1:0.5, 1:0.2, and 1:0.1 on Days 2, 7, 14, 21, and 70 days in the ethanol-treated group.

Effect of Ethanol on Synapses of Different Kinds of Afferent Fibers

The association and commissural fibers of the hippocampus are distributed mainly in the stratum radiatum of CA1 in developing and adult brains (Ritter *et al.*, 1971; Gottlieb and Cowan, 1973; Swanson *et al.*, 1978). Figure 5 shows the effect of ethanol on the development of all synapses in the stratum radiatum. In the control group, densities of synapses are 16.6 ± 1.9, 14.9 ± 0.2, 90.8 ± 3.6, 109.6 ± 15.5, and 314.2 ± 21.1 on Days 2, 7, 14, 21, and 70, respectively. Densities of synapses in the ethanol-treated group are significantly lower on Days 2, 14, 21, and 70 (6.0 ± 0.1, 52.2 ± 0.7, 56.6 ± 6.3, and 228.6 ± 12.7, respectively). The reuniens nucleus of the thalamus and the entorhinal cortex project to the stratum

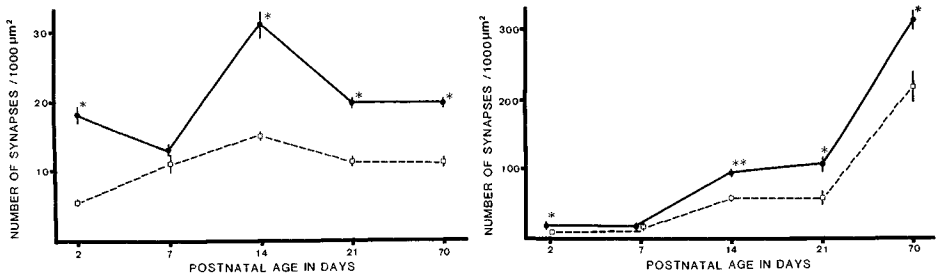

FIG. 4. Effect of ethanol on the density of axoshaftic synapses in the strata radiatum and lacunosum-moleculare. Control group, solid line; ethanol-treated group, broken line. $*P < 0.05$.

FIG. 5. Effect of ethanol on the density of all synapses in the stratum radiatum. Control group, solid line; ethanol-treated group, broken line. $*P < 0.05$, $**P < 0.01$.

lacunosum-moleculare of CA1 (Ritter *et al.*, 1971; Steward and Scoville, 1976; Herkenham, 1978). In the control group, densities are 24.6 ± 1.5, 19.2 ± 0.2, 101.1 ± 14.6, 75.5 ± 8.3, and 326.1 ± 12.7 on Days 2, 7, 14, 21, and 70, respectively (Fig. 6). Densities of synapses in the ethanol-treated group are significantly lower than those of control group on Days 2 and 70 (5.4 ± 1.8 and 254.2 ± 13.1, respectively). However, the ratio of synaptic densities in control to ethanol-treated groups are similar between both strata: 1:0.4, 1:1, 1:0.6, 1:0.5, and 1:0.7 in the stratum radiatum on Days 2, 7, 14, 21, and 70; 1:0.2, 1:0.7, 1:0.4, 1:0.9, and 1:0.8 in the stratum lacunosum-moleculare on Days 2, 7, 14, 21, and 70.

DISCUSSION

The principal finding of this study is that long-term exposure to ethanol during the entire fetal as well as the postnatal periods of life significantly reduces the density of synapses in the strata radiatum and lacunosum-moleculare of the hippocampus in rats 2, 14, 21, and 70 days of age. In clear contrast with this study, a number of earlier reports described limited effects of ethanol on the density of synapses in the hippocampal formation either after prenatal ethanol exposure followed by an ethanol-free lactation (Hoff, 1985, 1988) or after ethanol exposure in the adult alone (Lee *et al.*, 1981; Phillips and Cragg, 1983; Markham *et al.*, 1987; Scheetz *et al.*, 1987). The different effects of ethanol on the synaptic densities between the present and earlier studies could largely be explained by the difference in time of the ethanol exposure, because the present administration of eth-

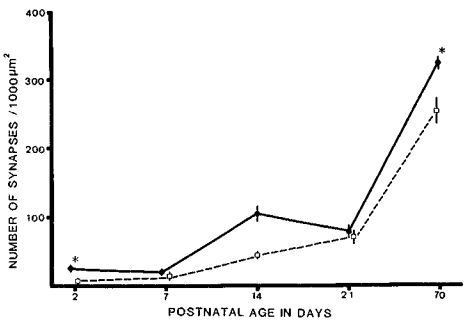

FIG. 6. Effect of ethanol on the density of all synapses in the stratum lacunosum-moleculare. Control group, solid line; ethanol-treated group, broken line. $*P < 0.05$.

anol resulted in moderate levels of blood alcohol according to our data (Omoto *et al.*, 1981) as well as those of Borges and Lewis (1982). In contrast to the reports of Hoff (1985, 1988), the reduction of synaptic density in this study might be attributed to the effect of ethanol exposure during the neonatal period, a time of exceptional susceptibility to ethanol (Bauer-Moffet and Altman, 1977; Phillips and Cragg, 1982; West and Pierce, 1986; Jones, 1988), and accords with an ultrastructural study reporting a delayed synaptogenesis in the cerebellum of the rat exposed to ethanol during pre- and neonatal periods (Volk, 1984). The ethanol administrations described in many reports (Lee *et al.*, 1981; Phillips and Cragg, 1983; Markham *et al.*, 1987; Scheetz *et al.*, 1987) started in the adult long after the neonatal period of exceptional vulnerability to ethanol. Thus, the combination of pre- and postnatal ethanol exposure reported in this study leads to more extensive damage to nerve cells than the exposure in the adult alone (Phillips and Cragg, 1982; West and Pierce, 1986; Jones, 1988).

The time course of the development of axoshaftic and axospinous synapses in the strata radiatum and lacunosum-moleculare in control rats is very similar to that reported in other brain areas (for review see Jacobson, 1978; Cowan *et al.*, 1980). Densities of synapses of both types in ethanol-treated rats are significantly lower than those of controls on Days 14, 21, and 70. However, the ratio of densities of spine to shaft synapses in control animals is similar to that in the ethanol-treated group. In consideration of the difference of afferent fibers between stratum radiatum and stratum lacunosum-moleculare (Ritter *et al.*, 1971; Gottlieb and Cowan, 1973; Steward and Scoville, 1976; Gerbrandt *et al.*, 1978; Herkenham, 1978), we also compared the rate of synaptic reduction after ethanol exposure in stratum radiatum with that in stratum lacunosum-moleculare. However, the rates are similar between both strata. These results suggest that the effect of ethanol is neither specific to the type of synapse nor to the difference of afferent fibers in this region.

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The Effects of Ethanol Exposure on Radial Arm Maze Learning and Behavior of Offspring Rats[1]

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The effects of maternal drinking on offspring have been studied epidemiologically, in human beings, and experimentally, in rats. The physical growth of offspring of female alcoholic rats, including histological growth of brain, lung, thymus gland, liver, and kidney, was previously reported by us. In the present study, we observed the effect of ethanol intake by the mother rat on learning ability and behavior of offspring rats using an eight radical arm maze. At the same time histological observations of the cerebrum were carried out. The mother rat was exposed to ethanol from a young age to delivery (P-DEL) or to weaning (P-NURS). After weaning, the offspring was exposed to ethanol until the tests began (P-WEAN). Experimental groups, classified by length of ethanol exposure, as mentioned above, disclosed the following: (1) Number of trials required for fulfilling learning criterion was significantly large in P-DEL and P-NURS rat groups relative to the controls; that is, P-DEL and P-NURS rats were slow in learning. (2) Numbers of rats which did not fulfill the learning criterion were: Group P-DEL, one male of eight; Group P-NURS, three males of seven. The behavior of the rats in Group P-WEAN differed from those in other groups; while they were receiving acclimation training, they were, unlike ordinary rats, not watchful of the device, slow to find the feed, and indifferent. They seemed to lack carefulness and sometimes failed to eat the feed even though they succeeded in selecting correct arms. Their motion was abrupt and they ran at extraordinarily high speeds. (3) In the observations of correct choices in the first eight choices, groups P-DEL and P-NURS showed significantly low values. This suggested the lowering of their learning ability. (4) In the observations of continuous correct choices, Group P-DEL showed a significantly low value. This suggested the rats did not learn thoroughly enough to retain their acquisition long. (5) Body weight, learning ability, and hippocampal neurons were affected by ethanol exposure more severely in Group P-NURS than in Group P-DEL. An even more severe effect was observed in Group P-WEAN.

INTRODUCTION

Abnormality in offspring due to maternal alcohol consumption during pregnancy has been noted since ancient Greek times. In 1973, Jones and Smith designated the following three peculiarities to indicate Fetal Alcohol Syndrome (FAS): Facial disorder, retarded growth, and insufficiency of the functions of the central nervous system. In the 1980s, however, the word Fetal Alcohol Effects (FAE), which was proposed by Abel (1984), was coined for cases in which all three peculiarities were not detected, but in whom some effect of the mother's drinking were observed. There are quite a few reports of human FAS and FAE in Europe, but only about 50 cases of FAS, including Tanaka's (1981) 26 cases, have been reported in Japan and even those cases could come under the FAE category.

Body weight, height, and circumference of the head of a growing FAS child can be recorded rather easily, but it is hard to measure the development of a child's

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activities and intellect. In his retrospective investigation, Aronson (1985) closely observed emotional instability, hyperactivity, distractability, short attention span, and other symptoms other than IQ scores. Most of these observations, however, were obtained through IQ scores. For many cases retrospective observation is difficult. In particular, it is very hard to estimate retardation of mental activity and hyperactivity which are due to insufficiency of the central nervous system. This may be included in the concept of behavioral anomaly.

In the field of experimental animal studies, we find many reports of pregnancy rate decrease, retardation of body weight and height, etc. (Yamamoto *et al.*, 1980; Iwase *et al.*, 1980; Omoto, 1982b; Endo *et al.*, 1983; and many others). Characteristics similar to human FAS, such as ossification, neural anomalies, and cardiac and eyelid dysmorphology, were reported in animals (Chernoff, 1977). On the other hand we scarcely find studies on mental and kinetic development of animals and it is obvious that evaluation of this subject through animal experiments is difficult.

We have noted that birth rates and numbers of offspring of mother animals exposed to ethanol are comparatively low, and such offspring tend to weigh less and grow more slowly than normal offspring (Omoto *et al.*, 1981; Imai and Omoto, 1983). Our epidemiological study on birth weight, height, and head circumference of offspring whose mothers had chronic exposure to alcohol found all three values to be low (Omoto 1982a). Data on behavioral anomalies of those offspring, however, were not obtained.

In order to collect data on the extent to which intellect and behavior are affected by ethanol exposure, we experimented on rats which, like human beings, are slow in maturing; these rats also have a growing period longer than that of human beings (Dobbing and Sands, 1979). Ethanol was given to all mothers in the prenatal period, to some mothers during lactation, and to a few offspring after weaning.

SUBJECTS AND METHODS

Animals

Littermates of Fisher strain rats (Nihon Crea Company) were used as basic experimental animals, and rats born in the laboratory among these animals served as parental rats in the experiment.

Treatment

At 4 weeks of age, female littermates rats were allowed free access to a 10% aqueous solution of ethanol as their sole fluid source and to food pellets CE-7 (Nihon Crea Company). At 5 or 6 months of age, they were allowed to mate. The offspring were classified in three groups as follows:

Group P-DEL—The mother rat was exposed to ethanol from the first day of pregnancy to delivery.

Group P-NURS—The mother rat was exposed to ethanol from the first day of pregnancy through nursing (until 28 days of age).

Group P-WEAN—The mother rat was exposed to ethanol from the first day of pregnancy to weaning. Offspring were exposed thereafter until 12 weeks of age, at which time learning experiments began.

There were 6 to 10 rats in each group.

Throughout the experimental period room temperature was kept at 22 ± 2°C and humidity at 45 ± 3%. The room was light from 8 AM to 8 PM.

Learning Experiment

Treatment prior to the experiment. At 12 weeks of age, offspring were put on a special diet for a week to reduce their weight by 20%. The diet was carefully designed so that no significant differences among groups would occur in the reduced weight percentage.

Apparatus. A radial eight arm maze was used. As shown in Fig. 1, this is an elevated (50 cm high) maze which has a central platform (37 cm in diameter) and eight radially extended arms (60 cm long, 12 cm wide). At the end of each arm there is a round dent (3.5 cm in diameter, 0.5 cm deep) which holds a pellet (about 30 mg) in reward for the rat's learning.

Task and evaluation. Each rat was placed in the center of the platform after the pellets were put in the dents, and was given free access to the pellets. A trial was completed when all eight pellets were eaten or 10 min elapsed, whichever was first. No more than one test was given each day per rat. A correct choice was defined as a rat reaching an untouched pellet and eating it. The employed criterion of success in learning was seven or eight correct choices in the first eight choices for 5 days in a row. We carried out 30 trials on 30 consecutive days and the time necessary for each procedure during the experiment was recorded. At the same time, we observed the rat's walking manner and behavior.

Items studied were number of trials before a rat fulfills the learning criterion; number of correct choices in the first eight choices; number of continued correct choices together with time required; number of correct choices in 30 trials; body weight and feed intake from birth until learning test began and in the postlearning period, with rats normally fed *ad lib,* until the rat reached the age of 180 days; histological observation of brain at 180 days of age.

Details of histopathological studies were as follows:

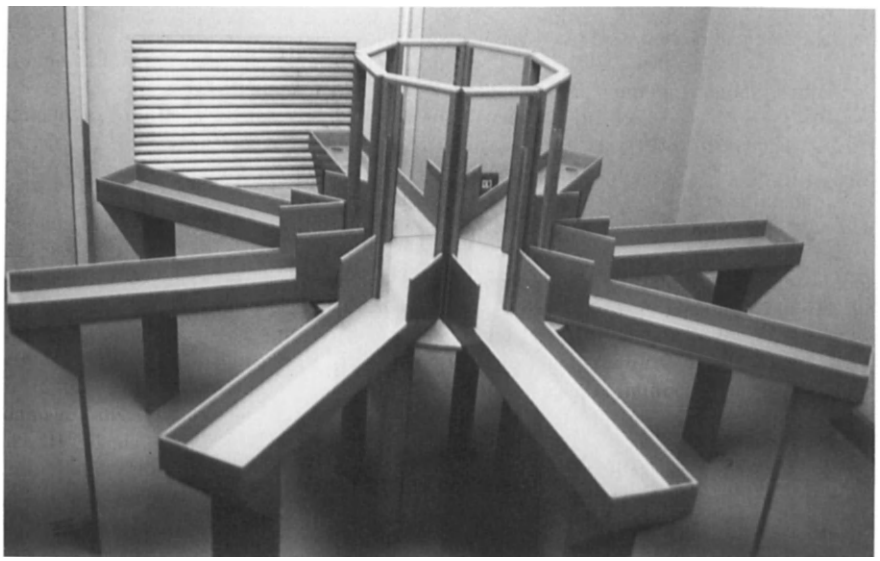

FIG. 1. Apparatus for the radial eight-arm maze learning.

At 18 days of age, the rats were sacrificed and the brain was dissected out. Then it was fixed with 10% neutral formalin, embedded in paraffin, sectioned 4 μm thick, stained with hematoxylin eosin (HE), and examined with optical microscope.

Statistical treatment of the results was made using the Student *t* test.

Statistical Analysis

Data were expressed by means ± SE. Analysis of variance with Student's *t* test was used comparing the control and P-DEL, the control and P-NURS, and the control and P-WEAN.

RESULTS

Body weight after 1 week on the diet was 80.1 ± 1.3% of prediet weight.

Number of Trials Required for Fulfilling Learning Criterion (Fig. 2 and Table 1)

As shown in Fig. 2, males in the groups exposed to ethanol had significantly high values ($P < 0.01$), relative to the controls.

There were some subjects which did not reach the learning standard, as follows:

Group P-DEL: one of eight males;

Group P-NURS: three of seven males.

The number of trials for these rats was plotted at 30, as shown in Fig. 2. This means that the number of trials needed to succeed was assumed to be 30; however, for groups in which some rats required more than 30 days to succeed, the mean value is higher.

The numbers of trials Group P-WEAN required to reach the standard were 8.4 ± 3.3 for males and 8.0 ± 4.0 for females. There were no differences between the values for the controls and these values. The behavior of Group P-WEAN differed from other groups; for instance, while Group P-WEAN rats were receiving training prior to the test in order to become accustomed to the device, they were not scared by the device at all and walked to the end of the arm quickly, they were slow to find the pellet, though. In addition, they sometimes, even on the latest days of the trials, did not eat the pellet they had found. They lacked carefulness; also running into arms at high speeds indicates a behavioral anomaly. (P-WEAN required an even smaller number of trials than required by P-NURS to reach the learning standard.)

Correct Choices in the First Eight Choices (Table 2)

Group P-DEL. Values recorded in the first, middle, and last parts of the trials were significantly lower than those of the control.

Group P-NURS. Males showed significantly lower values than the controls in the period from the latter half of the first trials to the middle of the trials and again in the latter period of the trials. Females' values were significantly low in the first period of trials.

Group P-WEAN. No significant differences were observed.

Number of Continuous Correct Choices (Table 3)

Only males in Group P-DEL showed significantly lower values than the controls in the early and middle periods.

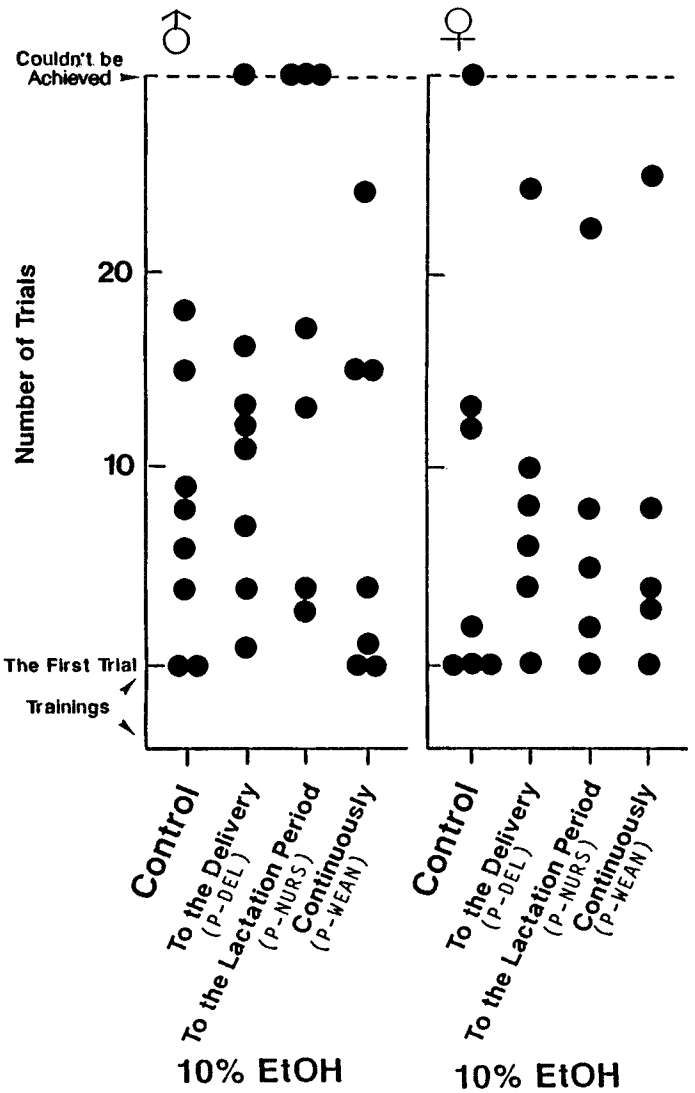

FIG. 2. Number of trials needed to fulfill the criterion of eight radial arm maze learning. Control: Offspring of rats exposed to no ethanol (drank plain water). Ethanol-exposed rats (ethanol—10% aqueous solution): Mother rat was exposed to ethanol from 28 days of age until delivery (Group P-DEL) and until weaning (Group P-NURS). After weaning, offspring succeeded mother in ethanol exposure until maze tests began (Group P-WEAN). Number of trials before reaching learning standard: If more than seven correct choices were made in the first eight choices on 5 consecutive days, the first of these 5 days is taken as this number.

Body Weight (Table 4)

22-day-old rats. All groups and both sexes showed significantly lower body weights than the controls.

56-day-old rats. Males in groups P-DEL, P-NURS, and P-WEAN and females in groups P-NURS and P-WEAN had significantly lower weights than the controls.

84-day-old rats. Males in groups P-DEL, P-NURS, and P-WEAN and females in P-WEAN had significantly lower weights than the controls.

TABLE 1
NUMBER OF TRIALS REQUIRED FOR FULFILLING LEARNING CRITERION

		10% EtOH free intake from 8 weeks before pregnancy		
	Control (no EtOH)	To delivery (P-DEL)	To lactation period (P-NURS)	Continuous intake until the maze experimental period (P-WEAN)
♂	7.5 ± 2.2 0[b]/8	11.8 ± 2.9** 1[b]/8	18.1 ± 4.2** 3[b]/7	8.4 ± 3.3[a] 0[b]/7
♀	8.1 ± 3.9 1[b]/7	8.7 ± 2.8 0[b]/6	7.4 ± 3.5 0[b]/5	8.0 ± 4.0[a] 0[b]/5

Note. Values are means ± SE.

[a] Subjects with abnormal behavior (hyperactivity, short attention span, distracted).

[b] Number of Subjects that did not reach learning standard. Number of trials for these rats was plotted at 30 as shown in Fig. 1. This means that number of trials needed to succeed was assumed to be 30; however, for groups in which some rats required more than 30 days (30 trials) to succeed, the mean value is higher.

** Significant difference between each EtOH-treated group and control, $P < 0.01$ by t test.

180-day-old rats. Only males in groups P-NURS and P-WEAN showed significantly lower values than the controls.

No female rats weighed significantly different.

Although all male subjects were lighter than the controls until 84 days of age, the average weight of males in Group P-DEL on the 180th day was not different from that of the controls.

As for female subjects, the weight of Group P-DEL was not different from the controls at 56 days of age; Group P-NURS overtook the controls at 84 days of age, and Group P-WEAN at 180 days of age. This implies that female rats can catch up with normally growing rats quickly, as compared with male rats.

Amount of Feed Eaten (Table 5)

Before undergoing the diet in preparation for the learning tests, males in groups P-NURS and P-WEAN and females in P-WEAN did not eat as much feed as the controls did.

TABLE 2
NUMBER OF CORRECT CHOICES IN THE FIRST EIGHT CHOICES IN RADIAL ARM MAZE LEARNING TESTS

			10% EtOH free intake from 8 weeks before pregnancy					
Number of trials	Control (no EtOH)		To delivery (P-DEL)		To lactation period (P-NURS)		Continuous intake until the maze experimental period (P-WEAN)	
	♂	♀	♂	♀	♂	♀	♂	♀
1 ~ 5	6.8 ± 0.3	7.0 ± 0.3	6.4 ± 0.3*	6.7 ± 0.3	6.7 ± 0.3	6.5 ± 0.3*	6.8 ± 0.3	6.9 ± 0.4*
6 ~ 10	6.8 ± 0.2	6.9 ± 0.3	6.6 ± 0.2	6.8 ± 0.2	6.5 ± 0.3*	6.6 ± 0.3	6.9 ± 0.3*	6.8 ± 0.3
11 ~ 15	7.0 ± 0.2	6.7 ± 0.2	6.7 ± 0.2**	6.9 ± 0.2	6.7 ± 0.3**	6.7 ± 0.3	6.9 ± 0.3	7.0 ± 0.4
16 ~ 20	7.1 ± 0.2	6.7 ± 0.3	6.9 ± 0.2	6.9 ± 0.3	6.9 ± 0.3	6.7 ± 0.3	7.2 ± 0.3	6.5 ± 0.3*
21 ~ 25	7.2 ± 0.2	6.8 ± 0.3	7.0 ± 0.2	7.1 ± 0.2	7.0 ± 0.3	6.8 ± 0.3	7.2 ± 0.2	7.0 ± 0.3
26 ~ 30	7.2 ± 0.2	6.9 ± 0.3	6.7 ± 0.2**	7.1 ± 0.2	6.6 ± 0.3*	6.8 ± 0.2	7.4 ± 0.2	7.2 ± 0.2

Note. The controls and ethanol-exposed rats are the same as those used for Fig. 1. Figures in the first column, Number of trials, show the days of tests; for example, 1–5 (first trial to fifth trial) means tests were done on the first to the fifth day of the trials. Values in the tables are means ± SE. Significant difference between the controls and ethanol-exposed subjects by t test $^*P < .05$; $^{**}P < .01$.

TABLE 3
NUMBER OF CONTINUOUSLY CORRECT CHOICES IN EIGHT RADIAL ARM MAZE LEARNING TESTS

Number of trials	Control (no EtOH)		10% EtOH free intake from 8 weeks before pregnancy					
			To delivery (P-DEL)		To lactation period (P-NURS)		Continuous intake until the maze experimental period (P-WEAN)	
	♂	♀	♂	♀	♂	♀	♂	♀
1 ~ 5	6.0 ± 0.6	6.1 ± 0.7	5.3 ± 0.5*	5.5 ± 0.6	5.9 ± 0.5	5.4 ± 0.6	5.7 ± 0.6	5.6 ± 0.9
6 ~ 10	5.4 ± 0.5	5.7 ± 0.6	5.5 ± 0.5	5.7 ± 0.5	5.1 ± 0.6	5.6 ± 0.7	5.6 ± 0.6	5.9 ± 0.7
11 ~ 15	5.8 ± 0.6	5.6 ± 0.5	5.6 ± 0.5	5.9 ± 0.5	5.6 ± 0.6	5.9 ± 0.5	6.3 ± 0.5	5.8 ± 0.8
16 ~ 20	6.2 ± 0.5	5.6 ± 0.5	5.6 ± 0.5*	5.9 ± 0.6	5.9 ± 0.6	5.6 ± 0.6	6.2 ± 0.6	5.3 ± 0.8
21 ~ 25	6.4 ± 0.6	5.6 ± 0.6	6.0 ± 0.5	5.9 ± 0.6	6.2 ± 0.6	5.7 ± 0.6	6.0 ± 0.7	5.4 ± 0.8
26 ~ 30	6.0 ± 0.6	5.5 ± 0.7	5.7 ± 0.5	6.4 ± 0.5*	5.7 ± 0.6	4.7 ± 0.7	6.7 ± 0.5	6.3 ± 0.7

Note. Values are means ± SE. Significant difference between each EtOH-treated group and control, $*P < 0.05$ by *t* test.

Considering calorie intake through ethanol, there was no difference between amounts eaten by females and the control.

After restricted feeding for 37 days, including one pretest week and 30 days during the test, to keep appropriate body weight, normal feed was given *ad lib* to both the control and all ethanol-exposed groups. The amounts eaten during 160–180 days of age were significantly small for both sexes in groups P-NURS and P-WEAN relative to the controls.

Histological Findings of the Cerebrum at 180 Days of Age (Fig. 3)

Compared with the controls, males in groups P-DEL and P-NURS showed greater atrophy of pyramidal cells of the cerebrum; no such difference was observed among females. Although the axis cylinder was not wound in the controls or members of Group P-DEL, it was slightly wound in P-NURS rats and even more clearly so among P-WEAN rats. Females showed extremely slight winding.

Atrophy of hippocampal neurons of males was observed to a great extent in Group P-WEAN, compared with the controls; that of females was as slight as the controls.

DISCUSSION

In rats exposed to ethanol in the prenatal period, it has been reported that the number of hippocampal pyramidal cells decreases (Imai and Omoto, 1983) and considerable loss is observed in the production of axis cylinders of dentate granule cells (Barness and Walker, 1981); moreover, formation of dendritic spines of hippocampal pyramidal cells is considerably reduced (Davies and Smith, 1981; West *et al.*, 1984). Hippocampus has been noted as a part in the central cerebrum presiding over memory and learning. These anomalies are thought to be related to retardation of differentiation and proliferation of embryonic cells which are basically found in FAS. Brown *et al.* (1979) have pointed out that this retardation is caused by the direct action of ethanol. It has been noted that ethanol exposure in the nursing period also causes retardation of brain development (Bauer-Moffett and Altman, 1977; Phillips and Gragg, 1982; Imai and Omoto, 1989; Kuge *et al.*, 1993). Through our former experiment, it was suggested that prenatal and postnatal ethanol exposure resulted in the significant reduction in density of synapses in the rat hippocampus CA1 (Kuge *et al.*, 1993). Our present experiment similarly

TABLE 4
OFFSPRING'S BODY WEIGHT AT 21, 56, 84, AND 180 DAYS OF AGE

			10% EtOH free intake from 8 weeks before pregnancy					
	Control (no EtOH)		To delivery (P-DEL)		To lactation period (P-NURS)		Continuous intake until the maze experimental period (P-WEAN)	
Age (days)	♂ (8)	♀ (7)	♂ (8)	♀ (6)	♂ (7)	♀ (4)	♂ (7)	♀ (5)
21	28.1 ± 1.6	26.2 ± 1.7	23.8 ± 1.2**	22.6 ± 1.8**	22.2 ± 3.5**	19.5 ± 2.8**	22.1 ± 2.7**	22.3 ± 3.6* (g)
56	185.2 ± 7.5	127.9 ± 5.7	167.0 ± 17.7*	127.0 ± 10.2	130.4 ± 12.3**	107.4 ± 4.3**	126.1 ± 15.7**	108.6 ± 4.3**
84	241.2 ± 7.6	147.1 ± 7.3	212.9 ± 29.9*	143.8 ± 16.4	182.8 ± 24.4**	133.7 ± 11.6	168.0 ± 27.6**	134.8 ± 3.8**
180	269.4 ± 13.4	165.7 ± 6.8	241.1 ± 44.4	169.2 ± 7.4	243.3 ± 16.8**	157.0 ± 7.7	210.7 ± 43.7*	162.8 ± 4.9

Note. Numbers in parentheses indicate number of subjects. Values are means ± SD in grams. Significant difference between each EtOH-treated group and control, $*P < 0.05$, $**P < 0.01$ by t test.

TABLE 5
OFFSPRING'S FEED INTAKE AT 70–80 AND 160–180 DAYS OF AGE

			10% EtOH free intake from 8 weeks before pregnancy					
	Control (no EtOH)		To delivery (P-DEL)		To lactation period (P-NURS)		Continous intake until the maze experimental period (P-WEAN)	
	♂	♀	♂	♀	♂	♀	♂	♀
70 ~ 80 days	(8)	(7)	(8)	(6)	(4)	(3)	(7)	(5)
Diet volume (g)	16.80 ± 1.24	11.03 ± 0.77	15.59 ± 2.01	10.67 ± 1.43	11.50 ± 1.16**	10.47 ± 0.12	11.21 ± 1.52**	9.42 ± 0.61**
Calorie intake (kcal)	57.98 ± 4.30	38.04 ± 2.66	53.04 ± 6.65	36.97 ± 4.85	39.68 ± 4.02**	36.13 ± 0.41	44.36 ± 5.55**	37.46 ± 2.50
160 ~ 180 days	(8)	(7)	(8)	(6)	(7)	(4)	(7)	(5)
Diet volume (g)	16.96 ± 0.85	10.81 ± 0.81	16.14 ± 2.19	10.52 ± 1.18	13.54 ± 0.79**	9.47 ± 0.28**	10.31 ± 1.42**	9.00 ± 0.73**
Calorie intake (kcal)	58.44 ± 3.01	37.27 ± 2.83	54.48 ± 7.61	36.28 ± 4.03	46.71 ± 2.73**	33.18 ± 0.52**	40.56 ± 5.28**	36.00 ± 2.80

Note. Effective calories of ethanol were assumed to be 70% of calories of ethanol taken by mouth. Numbers in parentheses indicate number of subjects. Values are means ± SD. Significant difference between each EtOH-treated group and control, $*P < 0.05$, $**P < 0.01$ by t test.

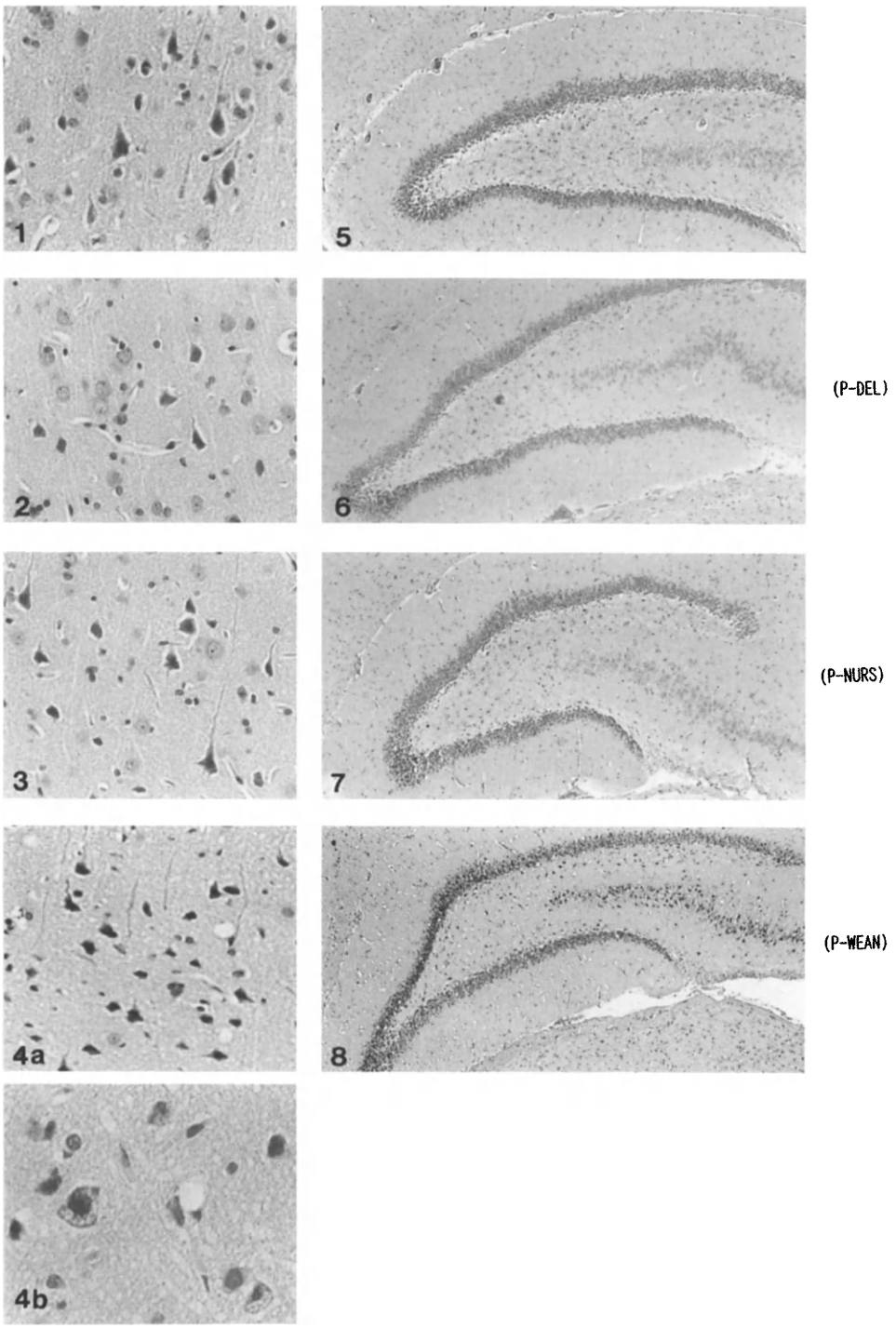

FIG. 3. Histopathological findings at 180 days of age. (1,2,3,4a) Atrophy of pyramidal cells of the cerebrum cortical layer V and wound of axis cylinder. Distinctness: 4a > 3 > 2 > 1. ×100. (4b) Vacuolar degeneration of pyramidal cells. ×200. (5,6,7,8) Atrophy of hippocampal neurons. Distinctness: 8 > 7 > 6 > 5. ×20.

found that atrophy of neurons in hippocampus and its circumferential tissues, as well as winding of axis cylinders, was not remedied, even among 180-day-old rats. An accelerating trend of aging was observed, too.

Histological changes in the brain due to ethanol exposure reflect the effect upon learning of ethanol; the effects were observed to be more severe in males than in females.

It is widely known that the hippocampus and the limbic system of the cerebrum play an important role in selecting motions, such as those necessary to negotiate a radial arm maze (Olton *et al.*, 1979). We have seen a report that ethanol exposure in prenatal and preweaning periods retards differentiation of cerebrum and cerebellum from the nerve histological standpoint (Jarrard, 1978). Rosman (1979) reported a delay in myelinogenesis and we pointed out that different periods of ethanol exposure result in different effects on development of neurons of hippocampus (Imai and Omoto, 1989). It has also been reported that ethanol readily accumulates in the hippocampus when it is drunk; that under normal physical conditions, much zinc accumulates in the hippocampus; and that the amount of zinc in the plasma and umbilical cord of pregnant alcoholic women is low and this amount of zinc has a negative correlation with the birth of malformed babies (Flynn, 1981). Tanaka (1983) reported that synthesis of RNA and protein, which are needed for formation of hippocampal neurons, is restricted by ethanol but this restriction is relieved to some extent when zinc is supplied. No precise reports, however, on these issues have as yet been published.

Administration of ethanol to a mother rat decreases her feed intake and inhibits normal absorption and metabolism of feed; accordingly malnutrition of offspring occurs (Bond, 1981). It has been reported that such malnutrition is the cause of retardation of behavioral development and learning (Bartley *et al.*, 1983). As for obstruction of radial arm maze learning due to malnutrition, however, both supporting (Jordan *et al.*, 1981) and nonsupporting (Hall, 1983) data have been reported. The rate of body weight increase of offspring is low when alcohol is administered in the lactation period, as compared to that when administration is in the prenatal period. Iwasaki reported that, as far as body weight is concerned, offspring malnutrition is caused effectively by alcohol administration in the lactation period compared with administration in the prenatal period (Iwasaki, 1986). Our present study found that neuronal development in the cerebrum, particularly in the hippocampus, and physical growth reflected learning ability; in other words, ethanol exposure in the prenatal and lactation periods affected learning and physical growth severely.

Differences between the effects on males and females were clear. Effects of ethanol on learning ability, physical growth, and cerebral neurons were deep for males but slight for females. According to various reports, rats aged 90–120 days showed sex differences in learning ability (Beatty and Beatty, 1970); rats younger than 50 days showed no sex difference (Bauer-Moffet and Altman, 1978); rats 4–9 weeks showed no sex difference (Mizutani, 1982). Our present study used rats which were in an appropriate age bracket for observing learning ability through the radial arm maze experiment. Thus, rats 84–120 days old showed, as stated above, clear sex differences in learning ability, cerebrum parameters, and body weight. Effects of ethanol exposure on all items studied were stronger in males than in females. We are interested in these differences between males and females and perhaps will study them further.

Severe atrophy, in comparison to other groups, in the cerebrum of Group P-WEAN members suggested that aging was accelerating at the time that cerebral organization was observed, that is, at 180 days of age.

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Change in Hen Sciatic Nerve Calcium after a Single Oral Dose of Tri-o-tolyl Phosphate[1,2]

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Six trace elements were monitored in neural tissue homogenates from White Leghorn hens orally dosed with tri-*o*-tolyl phosphate (TOTP) or tri-*m*-tolyl phosphate (TMTP) (200 mg/kg). Treated birds were monitored daily for development of delayed neurotoxicity, and concentrations of calcium, copper, iron, magnesium, manganese, and zinc were measured with atomic absorption spectroscopy at the time of maximal locomotor impairment (27–35 days postdosing). TOTP-treated birds manifested motor deficit by 15 days postdosing, while hens administered TMTP exhibited no signs of delayed neurotoxicity. Total calcium content in the sciatic nerve homogenates from TOTP-dosed hens was significantly less ($P < 0.05$) at the time of maximal locomotor impairment, while no shifts in the other trace elements were found. Therefore, the ortho isomer of tritolylphosphate ilicited symptoms of delayed neurotoxicity in the hen (i.e., organophosphorus ester-induced delayed neurotoxicity or OPIDN) and caused a decrease in total calcium content in the sciatic nerve homogenates, in contrast to effects of the meta isomer. Analysis of neural homogenates at time of maximal locomotor impairment reflected secondary events in the degradative processes, since the initial assault of TOTP happens early after administration. Therefore, at fully developed OPIDN alteration of calcium balance in sciatic nerves is an indicator of axonopathy in a degenerated nerve following chemical injury.

INTRODUCTION

Organophosphate compounds as a group of toxic chemicals are known to cause acute effects in animals due to the inhibition of acetylcholinesterase. Several organophosphate compounds are also known to cause delayed effects, organophosphorus ester-induced delayed neurotoxicity, by the phosphorylation of a target protein called neuropathy target esterase (NTE) (Johnson, 1987). One such organophosphate, tri-ortho-tolyl phosphate (TOTP) is a common component isomer of tritolyl phosphate fire-retardant preparations used in plasticizers, oil additives, lubricants, and solvents (Schaumburg and Spencer, 1984). Investigations concerning the biochemical mechanisms of the delayed neurotoxic actions of organophosphates have been undertaken; these efforts have been most recently

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reviewed (Zech and Chemnitius, 1987). With the exception of copper, the possible role of trace elements in the mechanism of organophosphate poisoning has not yet been investigated. One study showed that treatment of hens with single doses of dihexyl-2,2-dichlorovinyl phosphate and mono-*o*-cresyl diphenyl phosphate does not affect total and plasma-free copper when measured during the development of polyneuropathy (Lotti *et al.*, 1988). This is contrary to what was found in an earlier study, which found that triorthocresyl phosphate and a dichlorovinyl phosphate analog cause an increase in serum copper (Kimmerle and Loeser, 1974). In our study we selected six trace elements, copper, calcium, iron, magnesium, manganese, and zinc, to assess in neural tissues of the hen after treatment with two isomers of tritolyl phosphate, tri-ortho-tolyl phosphate or tri-meta-tolyl phosphate.

MATERIALS AND METHODS

Chemicals. Tri-*o*-tolyl phosphate and tri-*m*-tolyl phosphate (TMTP) were obtained from Pfaltz and Bauer, Inc. (Stamford, CT). Atropine sulfate was obtained from Sigma Chemical Co. (St. Louis, MO). Deionized water from Continental Water Systems (San Antonio, TX) was used for the homogenization step and for dilution of the tissue digests during metals analyses. Concentrated nitric acid from J. T. Baker Chemical Co. (Phillipsburg, NJ) was used for tissue digestion.

Treatment of animals. Adult White Leghorn hens, 18 months old and weighing approximately 1.4 kg, were used. Six TOTP-treated hens and six TMTP-treated hens were given 5 mg/kg atropine sulfate intraperitoneally 15 min prior to the administration of a single oral dose of the organophosphorus compound and every 6 hr for the next 18 hours to alleviate the cholinergic effects of the toxicants. Organophosphorus-treated hens were given a single oral dose of 200 mg/kg TOTP or TMTP mixed in corn oil by intubation. For a period of 48 hr postdosing, control and treated animals were monitored closely for signs of acute cholinergic poisoning. Following this period the animals were observed daily for mortality and the development of delayed neurotoxic symptoms. The hens were evaluated 27 to 43 days after treatment with TOTP or TMTP, the degrees of ataxia being scored at least twice each week. The degree of ataxia prior to complete paralysis was given one of four grades (T1 through T4), according to a standard scoring scheme (Abou-Donia and Graham, 1979). After the periods of treatment and observation, the hens were killed by decapitation. All of the TOTP-dosed birds were sacrificed at the time of maximal locomotor impairment, which occurred on Day 27 to 35 postdosing (one on Day 27, one on Day 29, three on Day 31, and one on Day 35). All of the TMTP-dosed hens and eight control hens were sacrificed between Days 27 to 43.

Preparation of tissues. Immediately following decapitation, the entire brain, spinal cord, and the sciatic nerves were removed by dissection and placed in ice-cold deionized water. Using a motor driven Teflon pestle and glass tube, the brain and spinal cord specimens were prepared as 10% w/v homogenates and sciatic nerves as 20% w/v homogenates. Aliquots of fresh homogenized tissues were transferred to vials, lyophilized, and stored at −20°C.

Trace element analysis. The homogenized tissues were thawed, weighed, and

placed into 1 ml nitric acid/0.1 g tissue for digestion overnight. The next day the digests were heated in a 60°C water bath for 1 hr to solubilize any remaining fat. To monitor percentage recovery of the metals, two aliquots of National Bureau of Standards (NBS) bovine liver standard reference material (SRM 1577a) were digested and analyzed for the same trace elements. The average percentage recovery for each trace element was 98% (Cu), 92% (Ca), 87% (Fe), 97% (Mg), 88% (Mn), and 93% (Zn). Using a Hitachi Model 180-70 polarized Zeeman atomic absorption spectrophotometer, the concentrations of the trace elements were determined by the Zeeman flame atomic absorption technique (Pleban *et al.*, 1981). The Mann–Whitney *U*-Wilcoxon rank sum *W* test was used to determine if differences in trace element concentrations between control and each treated group were statistically significant ($P < 0.05$).

RESULTS AND DISCUSSION

Atropine administration alleviated the cholinergic signs after oral dosing with TOTP and TMTP. All the TOTP- and TMTP-treated hens appeared normal up to 9 days postexposure, until diminished leg movement and reluctance to walk were noted in the hens dosed with TOTP. As detailed in our report (Luttrell *et al.*, 1988), all of the TOTP-dosed hens showed clinical signs of severe delayed neurotoxicity, consistent with an earlier report (Sprague *et al.*, 1980). The hens receiving TMTP and the controls remained normal during the entire experimental period, consistent with another report (Johnson, 1975).

As summarized in Table 1, homogenized tissue concentrations of copper, iron, magnesium, manganese, and zinc were not significantly different in the brain, spinal cord, and sciatic nerves of the treated animals compared to control values. Tissue calcium was also not significantly different in the brain and spinal cord of the treated animals compared to control values. However, relative to control hens, total calcium in homogenates of the sciatic nerves was decreased significantly in TOTP-treated hens.

Therefore, data in this study indicate decreased total calcium in the sciatic nerve homogenates from the TOTP-treated birds and the data from the TOTP-treated group represent levels of trace elements in a degenerated nerve. Additional work is required before definitive statements can be made to explain these observations. Future studies may include the injection of radioactive calcium into sciatic nerve cells to follow the dynamics of the ion movement during the development of organophosphorus ester-induced delayed neurotoxicity (OPIDN) and to detect the possible loss of normal intracellular and/or extracellular ionic gradients in axons.

CONCLUSIONS

Only the ortho isomer of tritolylphosphate elicited delayed neurotoxicity in the hen, and only treatment with the ortho isomer resulted in an effect on total calcium content in the sciatic nerve homogenate. Analysis of the neural tissues at the time of maximal locomotor impairment reflected secondary events in the degradative processes, since the initial assault of TOTP on NTE happens early after toxicant administration (Johnson, 1987). Therefore, at fully developed

TABLE 1
EFFECT OF TRI-*o*-TOLYL PHOSPHATE (TOTP) OR TRI-*m*-TOLYL PHOSPHATE (TMTP)[a] ON TRACE ELEMENT CONCENTRATIONS[b] IN HEN NEURAL TISSUES

Trace element	Control group	TOTP-treated group	TMTP-treated group
Brain			
Calcium	996.9 ± 124.8[c]	762.8 ± 97.1	844.2 ± 182.2
Copper	30.3 ± 3.4	33.6 ± 3.0	37.9 ± 1.7
Iron	69.1 ± 18.8	90.4 ± 21.1	85.1 ± 16.2
Magnesium	656.6 ± 16.8	637.7 ± 4.6	649.9 ± 30.8
Manganese	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.2
Zinc	49.4 ± 3.8	50.2 ± 4.5	45.7 ± 1.5
Spinal cord			
Calcium	473.7 ± 52.4	499.8 ± 94.0	615.7 ± 125.8
Copper	18.6 ± 2.4	21.9 ± 2.4	25.7 ± 3.1
Iron	129.1 ± 43.9	106.9 ± 13.1	74.0 ± 17.1
Magnesium	405.9 ± 7.2	394.4 ± 6.9	409.9 ± 3.2
Manganese	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Zinc	19.1 ± 4.4	17.4 ± 0.9	16.2 ± 3.7
Sciatic nerves			
Calcium	278.2 ± 61.6	112.0 ± 18.4*	206.8 ± 17.9
Copper	38.5 ± 3.3	31.0 ± 2.7	28.8 ± 6.0
Iron	262.9 ± 95.1	340.8 ± 101.0	142.6 ± 44.9
Magnesium	423.7 ± 17.2	381.9 ± 36.8	351.4 ± 31.0
Manganese	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Zinc	46.3 ± 12.3	36.7 ± 8.8	29.9 ± 4.7

[a] A single, oral dose of 200 mg/kg TOTP or TMTP was administered.
[b] μg/g dry tissue weight.
[c] Mean ± its standard error.
* When $P < 0.05$, the difference between the control and treated group is considered statistically significant.

OPIDN the alteration of the calcium balance is an indicator of axonopathy in a degenerated nerve following chemical injury. If altered calcium homeostasis is a factor in the mechanisms responsible for OPIDN, as suggested by a recent study with Verapamil, a calcium channel blocker (El-Fawal *et al.*, 1989), knowledge of the extracellular and intracellular calcium concentrations from the time of exposure to the time of maximal locomotor impairment must be determined in future studies.

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Polyneuropathy Due to Ethylene Oxide, Propylene Oxide, and Butylene Oxide[1]

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Axonal neuropathy occurs due to occupational ethylene oxide (EtO) exposure. The experimental model of human EtO neuropathy was established. In addition, the neurotoxic effects of propylene oxide (PpO) and butylene oxide (BtO) were demonstrated in rats. Although no human neuropathy due to PpO or BtO is reported, both chemicals must be considered to be neurotoxic, based on this study.

INTRODUCTION

Ethylene oxide (EtO) (Hess and Tilton, 1950; Glaser, 1979; Korpela *et al.*, 1983) is a major industrial chemical and is also used by medical industries and hospitals to sterilize a variety of medical devices and other products which might be damaged or destroyed by other sterilization methods. It has been used in the sterilizing units in many major health care facilities. Therefore, workers in these fields in various countries may be exposed to EtO. Recent studies have shown that it is an effective alkylating agent and mutagen in humans, animals, and plants. Clinical polyneuropathy after occupational exposure to EtO has been reported (Gross *et al.*, 1979; Finelli *et al.*, 1983; Kuzuhara *et al.*, 1983; Schröder *et al.*, 1985). These neurological effects may be assumed from animal experiments (Hollingworth *et al.*, 1956; Jacobsen *et al.*, 1956) reported 30 years ago. In this report we review cases of human EtO neuropathy and characterize the clinical features. In addition, we also review the animal model of neuropathy produced by chronic intoxication of EtO, propylene oxide (PpO), or butylene oxide (BtO).

HUMAN EtO NEUROPATHY

Human EtO neuropathy has been reported from the United States, Japan, Italy, and other countries. Twelve patients selected for the review showed the following clinical characteristics.

1. All were engaged in sterilizing work with EtO in the factory or hospital.
2. In 2 patients, sensorimotor neuropathy developed within 3 and 5 months of exposure, respectively. They had been repeatedly exposed to EtO up to several hundred ppm. Other patients with a more chronic sensorimotor neuropathy seem to have been exposed to EtO on the order of 10 ppm.
3. Complaints at the onset included muscle weakness, hypesthesia, and a tin-

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gling sensation in distal lower limbs, although distal upper limbs were also sometimes involved.

4. In neurological examinations, 10 of 12 patients showed muscle weakness and decreased sensation in distal lower limbs. Ankle jerk was absent in 9 and decreased in 1 of these 10 patients. Two others without muscle weakness and decreased sensation showed normal ankle jerk and were diagnosed as having EtO neuropathy based on abnormalities in conduction studies of the limb nerve and on their occupational history. A vibrating sensation was likely to be involved, most often among the sensory modalities.

5. Needle EMG revealed neurogenic changes in 8 of 11 patients (not studied in 1 of 12 patients selected for the analysis). Nerve conduction studies of limb nerves were abnormal in 8 of 10 (not studied in 2). A relatively mild decrease of motor and sensory conduction velocities with a decrease of the amplitude of nerve and muscle action potentials indicated axonal degeneration of both motor and sensory nerve fibers.

6. Histological studies of the sural nerve biopsied in 3 patients revealed mild abnormalities. They include decreased density of large myelinated fibers, reduction of cross-sectional area of axon, reduction of axonal circularity, and presence of myelin ovoids and Bünger's bands, and are compatible with a mild degree of axonal degeneration.

7. Cerebrospinal fluid studies showed elevated protein in 2 (150 and 53 mg/dl, respectively) of 6 patients. Both patients had a history of exposure of EtO of up to several hundred ppm.

8. When not exposed to EtO, improvement occurred in the majority of cases. In one study, the motor and sensory nerve conduction studies showed normal results with clinical improvement in the 3 symptomatic patients examined periodically over a 4-year period (Greenberg and Swift, 1982).

Thus, human sensorimotor neuropathy induced by occupational chronic exposure to EtO is characterized by relatively mild axonal degeneration, of large myelinated fiber in particular, and recovery is good.

EXPERIMENTAL EtO NEUROPATHY

Materials and Methods. In the first experiment, 5 Wistar rats were subjected to a single exposure to EtO for 6 hr at a concentration of 500 ppm, three times a week for 13 weeks, and served as test animals. Another 5 rats, exposed to filtered room air, were pair-fed and served as controls. In the second experiment, 21 rats were put into three groups: test, end control, and onset control. Seven test rats were subjected to 6-hr exposure to EtO at a concentration of 250 ppm five times a week for 9 months. Systematic clinical and histopathologic studies were performed.

Results. In the first experiment, in the 5th to 8th week of the exposure, all the test rats showed an awkward gait, and in the 9th to 10th week of the exposure, they showed slight to moderate ataxia and abnormal posture of the hindleg. The nature of the myelinated fiber degeneration was axonal. The mean frequency of myelinated fibers showing axonal degeneration was greater in the test than in the control in all the nerves. The difference was statistically significant in the distal sural and peroneal nerves. The distal sural nerve was more often affected than the

proximal sural nerve, but the difference was not significant. Under light microscopy of Epon-embedded preparations, myelin ovoids were seen in all the limb nerves examined in test rats. The frequency of myelinated fiber degeneration appeared to be more prominent in the peroneal, tibial, and distal sural nerves than in the proximal sural nerve. In the sixth lumbar ventral and dorsal roots and dorsal root ganglion, no definite abnormalities were found in the test rats compared with control rats. Under electron microscopy, myelinated fibers showing granular disintegration of axoplasmic organelles, with or without myelin breakdown, were frequently found in limb nerves. Numbers of both large and small myelinated fibers per nerve were similar between test and control in all the nerves. In the fasciculus gracilis, myelin ovoids were seen at both the third cervical and the fifth thoracic segment, except at the fifth thoracic segment in one rat. Only at the third cervical segment was the mean density of myelinated fibers significantly decreased in test compared with control.

In the second experiment, in which test rats were exposed to 250 ppm EtO, no rat showed abnormal gait throughout the 9-month experimental period. The nature of myelinated fiber degeneration was axonal. The mean frequency of myelinated fibers showing axonal degeneration was significantly greater in test than in end-control only in the peroneal nerve. Under light microscopy of Epon-embedded preparations, myelin ovoids were occasionally seen in distal sural and peroneal nerves of test rats. In the evaluation of the numbers and median diameters of myelinated fibers in the distal sural nerve, total myelinated fiber numbers per nerve were similar among test, onset-control, and end-control groups. The median diameter of myelinated fibers in test rats was significantly smaller than that in end-control rats, but significantly greater than that in onset-control rats. In the size distribution histograms of myelinated fibers of the three nerves studied, the large myelinated fiber number in test rats was significantly smaller than that in end-control rats, but significantly greater than that in onset-control rats. In the fasciculus gracilis of test rats, myelin ovoids were frequently observed at both the third cervical and the fifth thoracic segment. The mean density of myelinated fibers was about 15% less in test than in end-control rats at the third cervical segment, but the difference did not reach statistical significance.

In summary, the rats exposed to 500 ppm EtO developed neuropathy. Distal axonal degeneration was found in both peripheral and central myelinated axons of lumbar primary sensory neurons of rats exposed to EtO at concentrations of 500 and 250 ppm (Ohnishi *et al.*, 1985, 1986). In hindleg nerves, the rats exposed to 250 ppm EtO showed a retardation of growth and maturation of myelinated fibers in the presence of mild axonal degeneration.

EXPERIMENTAL PpO NEUROPATHY

Materials and methods. Based on the preliminary studies of the effects of various concentrations of PpO, test rats were subjected daily to a 6-hr exposure to PpO at a concentration of 1500 ppm, five times a week for 7 weeks. Systematic clinical and histopathologic studies were performed.

Results. Test rats developed an obvious ataxic gait at the end of 3 to 4 weeks exposure. In Epon-embedded sections of limb nerves, myelin ovoids were found,

especially in peroneal nerve. In the posterior column of the spinal cord, degeneration of myelinated fibers was found in the fasciculus gracilis at both the third cervical and the fifth thoracic segments. The myelinated fiber density was significantly less in test than in control at the third cervical segment, but it was similar between test and control at the fifth thoracic segment. No definite abnormalities were found in the first sacral spinal roots and the first sacral dorsal root ganglion.

Therefore, it was concluded that PpO induces distal axonal degeneration of the myelinated fibers in the hindleg nerve and in the fasciculus gracilis belonging to the lumbar primary sensory neuron (Ohnishi *et al.*, 1988).

EXPERIMENTAL BtO NEUROPATHY

Materials and methods. Based on preliminary studies, test rats were subjected daily to a 6-hr exposure to BtO at a concentration of 2000 ppm, four times a week for 5 months. Systematic clinical and histopathologic studies were performed.

Results. Test rats developed mild ataxia in the hindleg in the second and third week of the fifth month of exposure. Test rats were sacrificed at the end of the fifth month of exposure along with the pair-fed control rats. In teased fiber preparations of peroneal, proximal, and distal sural nerves, nerve to soleus muscle, and proximal and distal dorsal caudal trunks, the difference in the frequency of the abnormality between test and control was not statistically significant. In Epon-embedded sections, no abnormality of myelinated fibers in test compared with control was found in the limb nerves and the dorsal caudal trunk. Similarly, no abnormality was found in the sixth lumbar and the third sacral dorsal roots and dorsal root ganglion. On the other hand, in fasciculus gracilis, degeneration of myelinated fibers was found in all test rats at the third cervical segment, but not at the fifth thoracic segment. Morphometric evaluation of peroneal nerve and proximal and distal dorsal caudal trunks showed that the transverse fascicular area, myelinated fiber density, myelinated fiber number per nerve, and median and mean diameters of myelinated fibers were all similar between test and control. The extent of the distribution of the distal degeneration of the myelinated axon in the fasciculus gracilis was analyzed. The centrally directed myelinated axon of the primary sensory neuron seemed to be dying or degenerating back to the fourth cervical segment and down to the third thoracic segment. The densities and the median diameters of myelinated fibers of the dorsomedian portion of the fasciculus gracilis at the third cervical and fifth thoracic segments were evaluated. Myelinated fiber density in test was significantly less than that in control only at the third cervical segment. Median diameter was similar between test and control in both segments.

In BtO intoxication, obvious axonal degeneration of myelinated fibers was preferentially found in the dorsal portion of the fasciculus gracilis (Ohnishi *et al.*, in preparation), where the centrally directed myelinated axon of the lumbosacrococcygeal primary sensory neuron is distributed.

DISCUSSION

The results of these experimental studies were summarized and compared (Table 1).

TABLE I
EXPERIMENTAL CONDITIONS TO PRODUCE NEUROPATHY AND ITS HISTOLOGIC RESULTS

Chemicals	Concentration (ppm)	Exposure (6 hr in single exposure)	Distribution of fiber degeneration[a]		
			Sural nerve	Dorsal root ganglion	Fasciculus gracilis
Ethylene oxide	500	3/W for 13 weeks	+	−	+
	250	5/W for 9 months	+	−	+
Propylene oxide	1500	5/W for 7 weeks	+	−	+
Butylene oxide	2000	4/W for 5 months	−	−	+

[a] +, Present; −, absent.

With regard to EtO neuropathy, histological findings in the limb nerve are very similar in humans and rats. In human EtO neuropathy, patients had been repeatedly exposed to up to several hundred ppm EtO. Therefore, the exposure of rats to 250 ppm EtO is a reasonable condition for the induction of experimental EtO neuropathy. Whether the degeneration of myelinated fibers in fasciculus gracilis, known to be poor in regeneration, is found in humans, as in rats, remains unknown.

In the comparison of the experimental conditions necessary to produce neuropathy, EtO showed the strongest and BtO the weakest neurotoxicity.

With regard to the distribution of axonal degeneration of myelinated fibers in BtO intoxication, preferential involvement of the centrally directed myelinated axon of the lumbosacrococcygeal primary sensory neuron is unique and further studies of the pathogenesis of such unique distribution are needed.

PpO and BtO are not known to produce neuropathy in humans; however, both produced ataxia of the hindleg and distal axonal degeneration of myelinated fibers of the lumbosacral primary sensory neuron in rats. Therefore, both must be considered to be neurotoxic. Although the concentration of PpO and BtO needed to produce neuropathy in rats is much greater than the exposure limits (100 ppm for PpO and not determined for BtO) recommended by the National Institute of Occupational Safety and Health, PpO and BtO may cause neuropathies in exposed workers. Recognition of the neurotoxicity of both chemicals seems to be very important for better understanding the relationship between the chemicals and the distribution of morphologic alterations of the lumbosacral primary sensory neuron, one of the most vulnerable targets of the neurotoxic substances (Thomas, 1980).

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Effects of Neurotoxins on Brain Creatine Kinase Activity[1]

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The effects of ethylene oxide (EO), acrylamide, *N,N'*-methylene-bis-acrylamide (bis-acrylamide), and methyl mercury chloride (MMC) on brain creatine kinase (CK) activity were examined *in vivo*. EO and acrylamide, both of which cause central–peripheral distal axonopathy, inhibited CK activity in the brain and spinal cord. On the other hand, neither bis-acrylamide, a nonneurotoxic analogue, nor MMC which causes neuronopathy affected brain CK activity significantly. The inhibition of CK may play a role in the pathogenesis of distal axonal degeneration in the central and peripheral nervous systems.

INTRODUCTION

In order to elucidate the mechanisms of neurotoxicity of chemicals, much attention has been paid to the effects on energy-producing systems such as glycolytic pathways and respiratory chains (Sabri and Spencer, 1980; Chang, 1980) because energy metabolism seems extremely important for normal function of the nervous system (Erecińska and Silver, 1989). While creatine kinase (CK, EC 2.7.3.2) is not directly involved in producing ATP, the enzyme appears important to maintain a normal level of ATP in tissues because it catalyzes the reaction: ATP + creatine ⇔ ADP + creatine phosphate (Bais and Edwards, 1982). Recently, we found that ethylene oxide (EO) and acrylamide, both of which cause central–peripheral distal axonopathy, can suppress CK activity in rat brain and spinal cord *in vivo* (Matsuoka *et al.*, 1990a,b).

In this communication, we examined whether another kind of potent neurotoxin, methyl mercury chloride (MMC) which causes neuronopathy (Spencer and Schaumburg, 1980), can also suppress brain CK activity *in vivo* to explore the relation between the suppression of CK activity and neurotoxicity, and the effects of EO, acrylamide, and its nonneurotoxic analogue, *N,N'*-methylene-bis-acrylamide (bis-acrylamide), on brain CK activity *in vivo* were also studied.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 150–180 g (for the exposure to EO, acrylamide, and bis-acrylamide) or 330–370 g (for the exposure to MMC) were used. Food and water were given *ad libitum* throughout the experimental period except for the

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case of EO exposure in which food intake was matched by pair-feeding between exposure and control groups.

EO Exposure

The air and EO gas (pure EO 20 wt%, CO_2 80 wt%) were constantly introduced into the chamber using a mass flow meter. The concentration of EO in the exposing chamber was kept at 500 ppm and monitored periodically by gas chromatographic analysis. We have found that this concentration of EO causes peripheral neuropathy in rats after 13 weeks of exposure (Ohnishi *et al.*, 1985). One exposure lasted for 6 hr and the exposure was repeated 3 times in a week for 4 or 12 weeks. Control animals were exposed to ambient air in the same manner. Forty hours after the final exposure, animals were killed by decapitation. The whole brain (cerebrum and cerebellum) and entire spinal cord were removed and kept at −40°C.

Acrylamide and Bis-acrylamide Exposure

Animals were divided into four groups and injected intraperitoneally for eight consecutive days. Acrylamide and bis-acrylamide were dissolved in isotonic saline and injected as follows: group I, 50 mg acrylamide/kg body wt/day (total dose; 400 mg/kg body wt); group II, 50 mg bis-acrylamide/kg body wt/day (total dose; 400 mg/kg body wt); group III, 100 mg bis-acrylamide/kg body wt/day (total dose; 800 mg/kg body wt). Control group (group IV) was administered an equal volume of isotonic saline (3.3 ml/kg body wt/day). Twenty-four hours after the last injection, rats were killed by decapitation. The cerebrum, cerebellum, and spinal cord were carefully dissected on ice and kept at −40°C.

MMC Exposure

The MMC-intoxicated animals were prepared according to the acute intoxicated rat model of methyl mercury hydroxide by Klein *et al.* (1972); MMC was dissolved in isotonic saline and injected at a dose of 10 mg/kg body wt/day for 7 consecutive days subcutaneously. Rats were killed by decapitation on the 15th day, 8 days after the last of 7 doses of MMC. Control group was administered an equal volume of isotonic saline (7.5 ml/kg body wt/day). After decapitation, the whole brain was quickly removed. The anterior cortex, midcortex, posterior cortex, striatum, and cerebellum were then carefully dissected on ice and kept at −40°C.

Preparation of Tissue

Frozen tissues were thawed at 37°C for 5 min. Subsequent procedures were all carried out at 4°C. Each tissue was homogenized with 9 vol of cold 20 m*M* $NaHCO_3$ using a Potter–Elvehjem homogenizer and whole homogenates were used for enzyme assays.

Enzyme Assays

Activities of CK, aspartate aminotransferase (ASAT) (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1), and lactate dehydrogenase (LDH) (L-

lactate: NAD oxidoreductase, EC 1.1.1.27) in tissue homogenate were determined using the method of Oliver (1955), Karmen *et al.* (1955), and Wróblewski and LaDue (1955), respectively. Statistical differences between means were determined by Student's *t* test.

RESULTS

In the case of EO exposure, body weight of the exposure group did not differ from that of the control group due to pair-feeding. The exposed rats began to show ataxic gait at the sixth week.

Rats given either acrylamide or bis-acrylamide (400 and 800 mg/kg body wt) showed significantly depressed gain of body weight when compared to control (88.3, 89.7, and 76.2% of control, respectively). However, only rats injected with acrylamide developed definite weakness of the hind limbs on the seventh or eighth day.

Rats given MMC began to show significant weight loss after the fourth day and the body weight on the 15th day was 54.1% of control. The MMC-intoxicated rats developed weakness of the hind limbs on the 10th or 11th day. On the 14th day, crossing phenomenon of the hind limbs was observed in all of the intoxicated rats.

Table 1 shows the effects of EO on enzyme activities. The CK activity was inhibited in the brain and spinal cord after 4 weeks exposure; however, no inhibition was found in ASAT and LDH activity from these tissues. After 12 weeks exposure, CK activity in the brain was more suppressed than after 4 weeks.

Table 2 shows the effects of acrylamide and bis-acrylamide on enzyme activi-

TABLE 1
EFFECTS OF EO ON CREATINE KINASE (CK), ASPARTATE AMINOTRANSFERASE (ASAT), AND LACTATE DEHYDROGENASE (LDH) ACTIVITIES

	CK[a]	ASAT[a]	LDH[a]
	4 weeks exposure		
Brain[b]			
Control (4)[c]	315.9 ± 10.8[d]	34.8 ± 0.8	65.8 ± 3.2
EO (6)	232.0 ± 4.5* (73.4)[e]	34.6 ± 1.2	65.8 ± 2.2
Spinal cord			
Control (4)	195.1 ± 9.5	19.7 ± 1.1	30.6 ± 0.7
EO (6)	150.8 ± 3.3* (77.3)	19.3 ± 0.7	30.7 ± 1.3
	12 weeks exposure		
Brain[b]			
Control (6)	298.1 ± 31.3	ND[f]	ND
EO (6)	203.2 ± 21.0* (68.2)	ND	ND

Note. Data are from Matsuoka *et al.*, 1990a.
[a] The activities are expressed in terms of μmole/min/g tissue.
[b] Cerebrum and cerebellum.
[c] Number of samples.
[d] Means ± SD.
[e] Percentage of control value of CK activity.
[f] ND, not done.
* Significantly different from the control ($P < 0.001$).

TABLE 2
EFFECTS OF ACRYLAMIDE AND BIS-ACRYLAMIDE ON CREATINE KINASE (CK), ASPARTATE AMINOTRANSFERASE (ASAT), AND LACTATE DEHYDROGENASE (LDH) ACTIVITIES

	CK[a]	ASAT[a]	LDH[a]
Cerebrum			
Control	272.1 ± 10.7[b]	35.9 ± 2.2	62.9 ± 1.2
Acrylamide (400 mg/kg body wt)	184.0 ± 11.6* (67.6)[c]	38.7 ± 2.4	62.3 ± 1.6
Bis-acrylamide (400 mg/kg body wt)	270.6 ± 11.7 (99.4)	38.3 ± 3.5	64.0 ± 0.9
Bis-acrylamide (800 mg/kg body wt)	268.5 ± 9.5 (98.7)	ND[d]	ND
Cerebellum			
Control	381.1 ± 13.1	36.8 ± 1.4	71.0 ± 2.9
Acrylamide (400 mg/kg body wt)	291.8 ± 8.6* (76.6)	36.5 ± 2.8	71.7 ± 3.9
Bis-acrylamide (400 mg/kg body wt)	386.7 ± 9.8 (101.5)	37.6 ± 2.3	73.8 ± 1.9
Spinal cord			
Control	191.3 ± 20.5	17.1 ± 0.7	36.3 ± 1.4
Acrylamide (400 mg/kg body wt)	128.4 ± 5.5* (67.1)	18.1 ± 0.9	36.0 ± 1.8
Bis-acrylamide (400 mg/kg body wt)	190.3 ± 12.9 (99.5)	17.9 ± 1.2	37.4 ± 1.6
Bis-acrylamide (800 mg/kg body wt)	185.6 ± 9.6 (97.0)	ND	ND

Note. Data are from Matsuoka *et al.*, 1990b.
[a] The activities are expressed in terms of μmole/min/g tissue.
[b] Means ± SD of six animals.
[c] Percentage of control value of CK activity.
[d] ND, not done.
* Significantly different from the control ($P < 0.001$).

ties. There was no difference in ASAT or LDH activity in tissues between the control and acrylamide or bis-acrylamide (400 and 800 mg/kg body wt) groups. Although the dose of bis-acrylamide was increased twofold (800 mg/kg body wt), no suppression of CK activity was found in any tissues from the bis-acrylamide group. However, CK activity was clearly inhibited in the cerebrum, cerebellum, and spinal cord from rats given acrylamide (400 mg/kg body wt).

Table 3 shows the effects of MMC on enzyme activities in the brain. CK and ASAT activities were mildly inhibited in the anterior cortex, midcortex, and posterior cortex. LDH activity was also inhibited mildly in the midcortex and posterior cortex. On the other hand, no definite inhibition of the enzyme activity was found in the striatum and cerebellum.

DISCUSSION

In this study, it was found that EO and acrylamide inhibited CK activity in the brain and spinal cord without any alterations of ASAT and LDH activities and induced neurological abnormalities. However, bis-acrylamide, a nonneurotoxic analogue of acrylamide, did not inhibit CK activity.

On the other hand, while MMC induced definite neurotoxic signs in rats, the degree of the suppression of brain CK activity was relatively mild (less than 12%). No inhibition of CK activity was found in the cerebellum, while MMC-induced pathological changes in the rat brain are usually most severe in the cerebellum and brain stem (Berlin, 1986). Moreover, other enzyme activities (ASAT and LDH) were also inhibited in some parts of the cerebral cortex to almost the same extent

TABLE 3
EFFECTS OF MMC ON CREATINE KINASE (CK), ASPARTATE AMINOTRANSFERASE (ASAT), AND LACTATE DEHYDROGENASE (LDH) ACTIVITIES

	CK[a]	ASAT[a]	LDH[a]
Anterior cortex			
Control	259.5 ± 12.0[b]	34.8 ± 1.5	69.9 ± 3.9
MMC	242.8 ± 16.1* (93.6)[c]	32.6 ± 1.7*	68.5 ± 3.2
Midcortex			
Control	254.3 ± 9.8	32.8 ± 1.6	65.8 ± 2.5
MMC	225.5 ± 23.8** (88.7)	30.1 ± 2.6*	61.9 ± 3.7*
Posterior cortex			
Control	227.8 ± 10.5	33.1 ± 1.1	69.2 ± 3.1
MMC	209.6 ± 17.4* (92.0)	30.3 ± 1.7***	63.3 ± 2.6***
Striatum			
Control	243.0 ± 38.2	33.7 ± 4.5	56.5 ± 7.8
MMC	230.6 ± 34.3 (94.9)	33.3 ± 4.3	58.9 ± 5.2
Cerebellum			
Control	337.5 ± 16.9	39.2 ± 2.3	64.7 ± 1.5
MMC	342.0 ± 10.5 (101.3)	40.3 ± 0.9	66.4 ± 2.8

[a] The activities are expressed in terms of μmole/min/g tissue.
[b] Means ± SD of eight animals.
[c] Percentage of control value of CK activity.
* Significantly different from the control ($P < 0.05$).
** Significantly different from the control ($P < 0.01$).
*** Significantly different from the control ($P < 0.005$).

as CK activity. Thus, unlike the cases of EO or acrylamide intoxication, it appears that the inhibition of CK activity is not concerned directly with the pathogenesis of MMC intoxication.

It has been reported that EO causes encephalopathy and central–peripheral distal axonopathy (Ohnishi *et al.*, 1985), and acrylamide also causes encephalopathy as well as distal axonopathy (Igisu *et al.*, 1975). Thus, two apparently unrelated chemicals can induce encephalopathy and the same type of neuropathy and inhibit brain CK activity. The functioning of the nervous system depends on the ATP level (Adams, 1982) and the ATP levels in tissues are partially conserved by CK (Erecińska and Silver, 1989). Moreover, CK is one of the components carried in the axon (Brady and Lasek, 1981). Therefore, the inhibition of CK activity may play a role in the genesis of toxicity of neurotoxins which cause encephalopathy and distal axonopathy.

Because CK exists both in neurons and astrocytes (Yoshimine *et al.*, 1983), and because the regional difference of CK activity in the central nervous system has been known (Maker *et al.*, 1981), the effects of neurotoxins on CK and related energy metabolites in each compartment remain to be examined. In our laboratory, a subsequent study using a large number of animals and other types of neurotoxins is now in progress.

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Effects of Ozone and Nitrogen Dioxide on Drinking and Eating Behaviors in Mice[1]

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Male ICR mice were exposed continuously to ozone (O_3) and nitrogen dioxide (NO_2) for 7 days to examine the effects on drinking and eating behaviors. Ozone at 0.1 ppm did not affect drinking and eating activities, whereas drinking activity decreased in a concentration-dependent manner to 47.7, 12.8, and 3.0% of the control value with 2-day exposures to 0.2, 0.4, and 0.8 ppm O_3, respectively, and eating activity decreased to 35.2 and 8.7% of the control value at 0.4 and 0.8 ppm O_3, respectively. Body weight also decreased markedly by 2.0, 4.6, and 7.5 g at 0.2, 0.4, and 0.8 ppm O_3, respectively. These decrements reached a maximum on the second day of exposure. However, alterations in drinking and eating activities and body weight were transient, leading to recovery during the continuous O_3 exposures. The recovery processes were dependent on the concentrations of O_3. Nitrogen dioxide at 4 ppm did not affect drinking and eating activities, whereas drinking activity decreased in a concentration-dependent manner to 56.8, 8.3, and 18.7% of the control value with 2-day exposures to 6, 8, and 12 ppm NO_2, respectively, and eating activity decreased markedly to 21.8 and 16.4% at 8 and 12 ppm NO_2, respectively. Body weight also decreased by 2.5, 5.5, and 6.1 g at 6, 8, and 12 ppm NO_2, respectively. These decrements reached a maximum on the second day of exposure. As in the O_3 exposures, the decrements in drinking and eating activities and body weight were transient and recovered during the continuous exposures to NO_2 depending on the concentrations of NO_2. Drinking and eating activities and body weights of mice that had been previously exposed to 12 ppm NO_2 for 7 days did not show changes when the mice were exposed to 0.4 ppm O_3 9 days after NO_2 exposure. The present study demonstrates that photochemical oxidants suppress drinking and eating behaviors in mice and that they recover thereafter under the continuous exposure conditions.

INTRODUCTION

Ozone (O_3) and nitrogen dioxide (NO_2) are principal oxidant pollutants of photochemical smogs. Although many studies on the effects of these gaseous pollutants on human and several animal species have been carried out, much of this work has been performed to reveal the effects on the respiratory systems because the first target organs of O_3 and NO_2 are lung and trachea. On the other hand, it has been suggested that the effects of O_3 and NO_2 on human health are not necessarily limited to respiratory diseases (Grisworld *et al.*, 1957; Posin *et al.*, 1978). However, the kinds of extrapulmonary functions in human and experimental animals affected by exposures to O_3 and NO_2 are not well known.

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In recent years, studies on the effects of toxic agents on physiological functions have been advanced by using behavioral indicators of animals in the field of behavioral toxicology (Burt, 1972). Animal behaviors are based on whole-body physiological functions; therefore, although real effects of toxic agents cannot be detected directly, some functional effects can be detected through behavioral changes. Observation of behaviors of intact animals helps researchers to accurately assess the effects of toxic agents on health, inasmuch as the physiological conditions of intact animals are not modified by anesthesia, surgery, and treatment with various drugs, which are often used in physiological studies. Furthermore, by using indicators of behavioral changes, long-term observations of the same animals are made possible. Hence, it is possible to examine dynamic processes from an outbreak of illness to recovery or death.

Few studies on the behavioral effects of NO_2 have been reported (Murphy *et al.*, 1964). On the other hand, several studies have found that exposures to O_3 result in changes of various kinds of behavioral functions, such as the wheel-running activity, the gross motor activity, and the operant behavior in rats (Konigsberg and Bachman, 1970; Murphy *et al.*, 1964; Tepper *et al.*, 1982, 1985; Tepper and Wood, 1984; Tepper and Weiss, 1986; Weiss *et al.*, 1981). However, not only the threshold at which O_3 and NO_2 affect behavioral functions but also the kinds of physiological functions affected by these gases remain to be clarified. Therefore, a systematic study of the behavioral changes is needed to reveal the health effects of O_3 and NO_2.

Among many kinds of behaviors displayed by experimental animals, drinking and eating behaviors are the most fundamental. To maintain homeostasis of the internal environments of the living body, these daily behaviors must be sustained constantly. Hence, it is possible to judge abnormal changes when drinking and eating rise above the normal level or decrease below it. Therefore, quantitative and qualitative analyses of drinking and eating behaviors are expected to provide useful information on the health effects of toxic agents. Previously, Umezu *et al.* (1987) have shown that the drinking activity of rats decreased by exposure to low concentrations of O_3 in a concentration-dependent manner. This result leads to the assumption that physiological conditions will be perturbed at an ambient O_3 level. It is therefore important to examine further the precise effects of O_3 and NO_2 on these behaviors. Knowledge of species differences in exposure to toxic agents is important in extrapolating the effects of toxic agents on animals to the effects on human health. However, species differences in the effects of O_3 and NO_2 on physiological functions are hardly known. It is therefore also important to examine the changes in drinking and eating behaviors caused by exposure to O_3 and NO_2 in different species of animals.

In the present study, we examined the effects of O_3 and NO_2 on drinking and eating behaviors in mice and compared the effect of O_3 with that of NO_2. We discuss the differences in the effect of O_3 in rats and mice.

MATERIALS AND METHODS

Animals

Forty-eight 8- to 26-week-old male ICR strain mice (Japan Clea, Tokyo) were

used in this study. These animals were divided into eight groups ($N = 6$, in each groups): (1) 0.1 ppm O_3 group; (2) 0.2 ppm O_3 group; (3) 0.4 ppm O_3 group; (4) 0.8 ppm O_3 group; (5) 4 ppm NO_2 group; (6) 6 ppm NO_2 group; (7) 8 ppm NO_2 group; (8) 12 ppm NO_2 group. Animals of each group were housed in stainless-steel mesh cages (one per a cage) which were placed in a gas-exposure chamber. The animals were allowed to take commercial food and tap water *ad libitum* throughout the experiments.

Measurement of Drinking and Eating Activities in Mice

The animals were taken out of the chamber daily at the fixed time, 6:00 PM, to measure body weight and the amount of food intake. Approximately 20 min were required for these manipulations. After these measurements, the animals were housed again in the exposure chamber. Because handling was considered to have an effect on drinking activity, the measurement of drinking activity between 6:00 and 7:00 PM was eliminated from the data analysis.

The drinking activity in the mouse was measured according to the method described by Kuribara *et al.* (1978) using commercial cylindrical cartridges (O'Hara & Co., Ltd., Tokyo). The cartridge is made of an acryl fiber case and a pair of stainless-steel tubes. Drops of water fall through the tubes while the mice drink water from a drinking spout. The mean volume of one drop of water is 0.05 ml, as described by Kuribara *et al.* (1978) and confirmed by the authors. In order to count the number of water drops, a device with an electronic circuit to drive an electromagnetic counter was fabricated, according to the modified method of Reidelberger and Heusner (1982). When the stainless-steel tubes are mediated by a water drop, the electronic circuit is closed and the counter drives. The number displayed by each counter for six mice is automatically recorded with a TV camera and a videotape recorder at 10-min intervals. Recordings are played back on the videotape recorder and the water drops counted.

Condition of the Gas-Exposure Chamber

A stainless-steel–glass chamber of 560-liter volume was used in the present study. The windows of the chamber were completely shielded with thick black paper to block out the light around the chamber. The chamber was illuminated by a fluorescent bulb (10 W), and the illuminance was 180 lux at the center of the chamber. The lighting schedule was controlled by a timer to be dark from 7:00 PM to 7:00 AM and light from 7:00 AM to 7:00 PM. Throughout the experiments, temperature and humidity did not alter markedly (24 ± 2°C, 50–75%). The chamber was ventilated continuously with filtered room air or with air containing O_3 or NO_2 during the exposure period.

Regulation of O_3 and NO_2 Concentrations

Ozone was generated by an ozone-gas generator (Japan Clea, Model CGNS-69) and was passed into the chamber through the electromagnetic valve. A discharge tube in the generator produced O_3 from oxygen supplied by a cylinder containing pure air. The concentration of O_3 in the chamber was monitored with an ozone analyzer (Monitor Labs, Model 8410E). The signals from this analyzer controlled

the electromagnetic valve so that the O_3 concentration was stabilized at the designated level.

Nitrogen dioxide was supplied by a cylinder containing NO_2 and N_2, and the gas was passed into the chamber through the electromagnetic valve. The concentration of NO_2 in the chamber was monitored with a nitrogen oxide analyzer (Monitor Labs, Model 8440H). The signals from this analyzer controlled the electromagnetic valve so that NO_2 concentration was stabilized at the designated level.

Exposure Procedure

After the mice were placed in the chamber, they were bred in the clean air for 4 days. Thereafter, they were continuously exposed to 0.1, 0.2, 0.4, and 0.8 ppm O_3 or 4, 6, 8, and 12 ppm NO_2. Exposures commenced 7:00 PM on the fifth day. The mice were exposed to O_3 for 7 days and to NO_2 for 5–7 days.

In some experiments, animals of the 12 ppm NO_2 group were exposed to clean air for 9 days after the termination of 7 days of NO_2 exposure. Thereafter, they were exposed to 0.4 ppm O_3 for 23 hr (on Day 21 in Fig. 9).

Data Analysis

The statistically significant difference (paired-sample t test) in the number of water drops, food intake, or body weight between the last day in the control period (Day 4) and each day during the exposure period was tested. The concentration–suppression relationships were examined by a Kruskal–Wallis analysis of variance of ranks or a Mann–Whitney U test. Five percent was adopted as the significant level.

RESULTS

Changes in Drinking Activity with Exposure to O_3

Drinking activities in mice were maintained at a relatively constant value between the second and fourth days, although they were often decreased considerably on the first day (Fig. 1). Mice showed 77–212 counts per day in drinking activity during the control period. The drinking activity in mice was not altered by exposure to 0.1 ppm O_3, whereas it decreased significantly at 0.2–0.8 ppm O_3 exposure compared to the drinking activity on Day 4. On the second day of O_3 exposure, drinking activity decreased to 47.7, 12.8, and 3.0% of the control value in the 0.2, 0.4, and 0.8 ppm O_3 groups, respectively. Kruskal–Wallis analysis of variance of ranks revealed that these differences in drinking activities on the second day of O_3 exposure were statistically significant ($H = 12.433$, $P < 0.05$), showing that the degree of decrease in the drinking activity of each group was significantly different. This means that the decrease is dependent on O_3 concentration. On the third day (Day 7) of O_3 exposure, the drinking activity in the 0.2–0.8 ppm O_3 groups tended to recover toward the control level (Fig. 1). However, the recovery process was not similar among the groups. To reach the control level, 3 days were required for the 0.4 ppm group, whereas the 0.2 ppm group recovered to the control level on the third day (Day 7). The 0.8 ppm O_3 group did not recover completely during the 7-day exposure period.

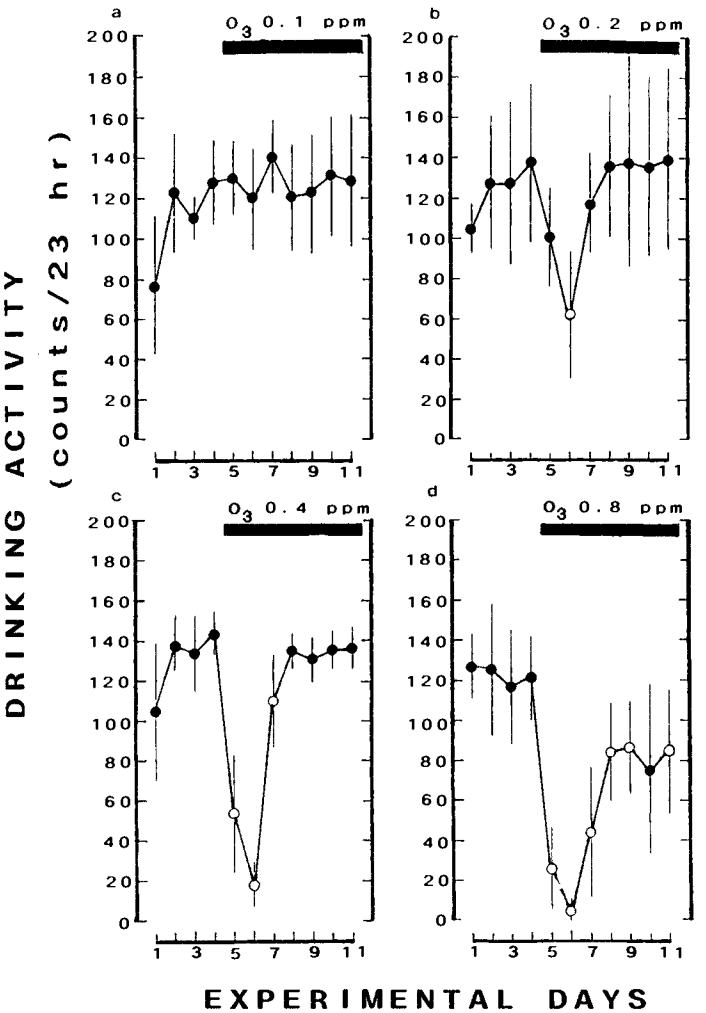

FIG. 1. Alterations in drinking activity of mice exposed to O_3: (a) 0.1 ppm O_3 group, $N = 6$; (b) 0.2 ppm O_3 group, $N = 6$; (c) 0.4 ppm O_3 group, $N = 6$; and (d) 0.8 ppm O_3 group, $N = 6$. Open and closed circles indicate mean values of drinking activity per day and vertical bars denote standard deviations. Open circles indicate that the drinking activity on the day is statistically significant compared to the value on Day 4 (the last day of the control period) in the same animals (paired-sample t test $P < 0.05$).

Figure 2 shows changes in the patterns of drinking activity of the 0.1, 0.2, 0.4 and 0.8 ppm O_3 groups, where the activity is represented as accumulated water drops at 3-hr intervals. Apparently, the drinking activity in all groups showed the nocturnal rythmicity. The circadian rythmicity was also observed in all groups during O_3 exposures.

Changes in Eating Activity with Exposure to O_3

Figure 3 shows alterations in eating activities of the 0.1, 0.2, 0.4, and 0.8 ppm O_3 groups. During the control period (Days 1–4), the mice showed an eating

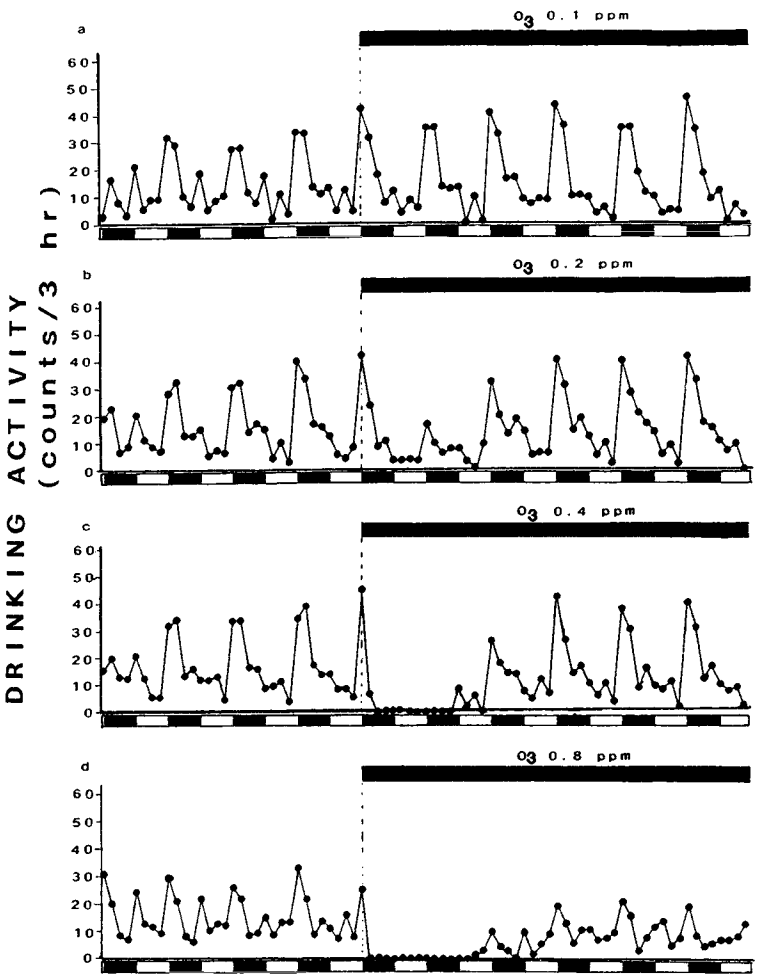

FIG. 2. Alternative changes in drinking activity of mice before and during O_3 exposure: (a) 0.1 ppm O_3 group, (b) 0.2 ppm O_3 group, (c) 0.4 ppm O_3 group, and (d) 0.8 ppm O_3 group. Closed circles indicate mean values of the drinking activity per 3 hr. The band at the bottom of each graph denotes light–dark alternations in the chamber. Shaded parts indicate dark period (7:00 PM–7:00 AM). O_3 exposures were started at 7:00 PM on the fifth day as shown.

activity of 2–10 g. During the 0.1–0.2 ppm O_3 exposures, the eating activity did not change, while it decreased markedly during the 0.4–0.8 ppm O_3 exposures. On the second day of exposure the eating activity was 35.2 and 8.7% of the control value in the 0.4 and 0.8 ppm O_3 groups, respectively. The value of the 0.4 ppm O_3 group was not significantly different from that of the 0.8 ppm O_3 group (Mann–Whitney U test, $U = 7.0$, $P > 0.05$). On the third day (Day 7) of O_3 exposure, the eating activity in the 0.4 and 0.8 ppm O_3 groups tended to increase toward the level during the control period. However, the recovery depended on the O_3 concentration (Fig. 3).

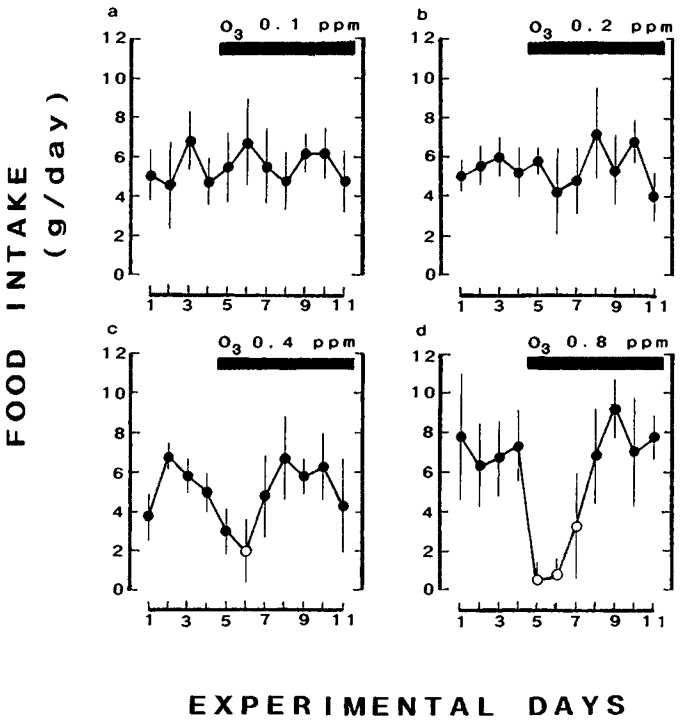

FIG. 3. Alterations in eating activity of mice exposed to O_3: (a) 0.1 ppm O_3 group, (b) 0.2 ppm O_3 group, (c) 0.4 ppm O_3 group, and (d) 0.8 ppm O_3 group. The data are shown in the same way as in Fig. 1.

Changes in Body Weight with Exposure to O_3

On Day 1, the body weights of the mice were 32.4–47.9 g and tended to increase during the control period. In the 0.1 ppm O_3 group, body weight continued to increase during O_3 exposure. On the other hand, body weight decreased significantly in the 0.2–0.8 ppm O_3 groups (Fig. 4). The decrease in body weight reached maximum on the second day of O_3 exposure by 2.0, 4.6, and 7.5 g in the 0.2, 0.4, and 0.8 ppm O_3 groups, respectively. These differences were significant (Kraskal–Wallis analysis of variance of ranks, $H = 14.0$, $P < 0.05$), showing that the decrease in body weight depended on the concentration of O_3. On the third day of O_3 exposure, the body weights of all groups showed recovery, and the recovery depended on the concentration of O_3 (Fig. 4).

Changes in Drinking Activity with Exposure to NO_2

The drinking activity in mice was altered little by 4 ppm NO_2 exposure, whereas it decreased markedly with 6–12 ppm NO_2 exposure (Fig. 5). On the second day of NO_2 exposure, the decrease in the drinking activity reached a maximum at 56.8, 8.3, and 18.7% of the control value in the 6, 8, and 12 ppm NO_2 groups, respectively. These values were significantly different (Kruskal–Wallis analysis of

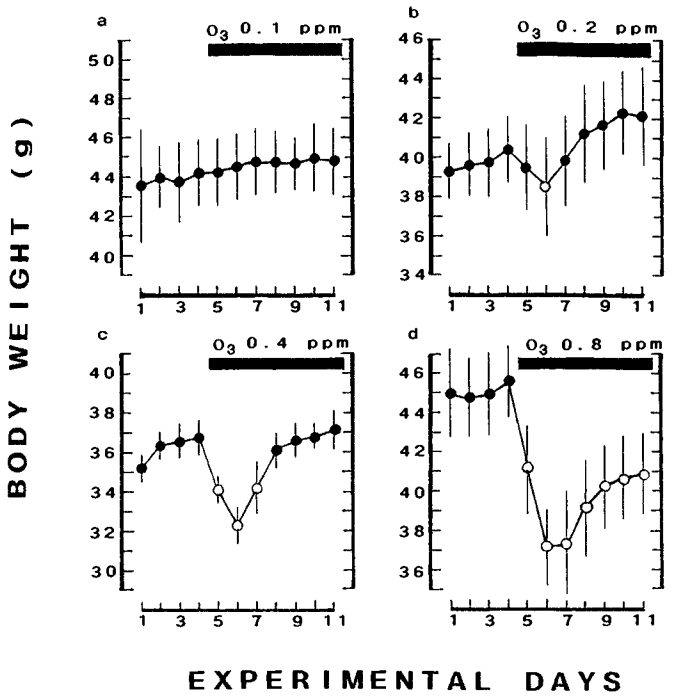

FIG. 4. Alterations in body weights of mice exposed to O_3: (a) 0.1 ppm O_3 group, (b) 0.2 ppm O_3 group, (c) 0.4 ppm O_3 group, and (d) 0.8 ppm O_3 group. The data are shown in the same way as in Fig. 1.

variance of ranks, $H = 6.89$, $P < 0.05$), suggesting that the decrement was dependent on the concentration of NO_2. On the third day (Day 7) of NO_2 exposure, the drinking activity tended to recover (Fig. 5), and the recovery depended on the NO_2 concentration. To reach the control level, 3 days were required for the 8 ppm NO_2 group, while the 6 ppm NO_2 group recovered to the control level on the third day (Day 7), and the 12 ppm NO_2 group did not recover during the 7-day exposure period.

Figure 6 shows the patterns in drinking activity of the 4, 6, 8, and 12 ppm NO_2 groups. Apparently, the drinking activity in all groups showed the nocturnal rhythms. The drinking activity also showed circadian rhythmicity during NO_2 exposure.

Changes in Eating Activity with Exposure to NO_2

The mice took 1–11 g food during the control period. During the period of 4–6 ppm NO_2 exposure, the eating activity did not change significantly (Fig. 7). However, when the mice were exposed to 8–12 ppm NO_2, the eating activity decreased markedly. The decrease reached a maximum on the second day when the eating activity was 21.8 and 16.4% of the control value in the 8 and 12 ppm NO_2 groups, respectively. These values were not significantly different (Mann–Whitney U test, $U = 14.5$, $P > 0.05$). On the third day (Day 7) of exposure, the eating activity in

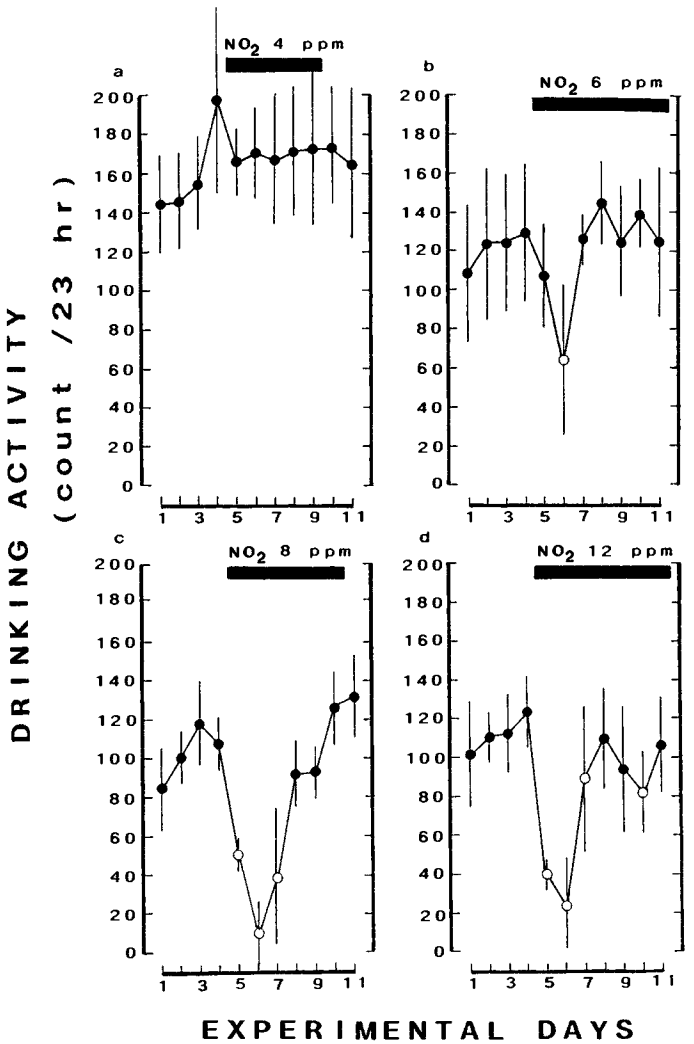

FIG. 5. Alterations in drinking activity of mice exposed to NO_2: (a) 4 ppm NO_2 group, $N = 6$; (b) 6 ppm NO_2 group, $N = 6$; (c) 8 ppm NO_2 group, $N = 6$; and (d) 12 ppm NO_2 group, $N = 6$. Open and closed circles indicate mean values of drinking activity per day and vertical bars denote standard deviations. Open circles indicate that the drinking activity on the day is statistically significant compared to the value on Day 4 (the last day of the control period) in the same animals (paired-sample t test, $P < 0.05$).

the 8 and 12 ppm NO_2 groups showed recovery, and the recovery depended on the concentration of NO_2.

Changes in Body Weight with Exposure to NO_2

On the first day of the control period, body weights of the mice were 39.6–58.0 g and tended to increase during the control period. In the 4 ppm NO_2 group, body weight continued to increase despite 4 ppm NO_2 exposure. However, body weight

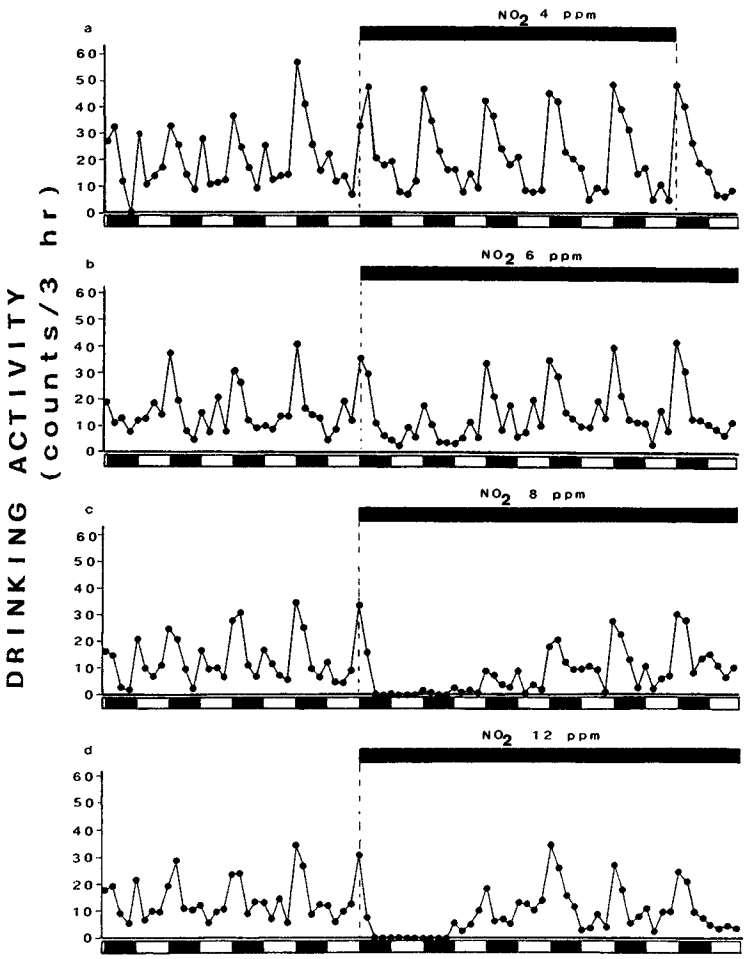

FIG. 6. Alternative changes in drinking activity of mice before and during NO_2 exposure: (a) 4 ppm NO_2 group, (b) 6 ppm NO_2 group, (c) 8 ppm NO_2 group, and (d) 12 ppm NO_2 group. Closed circles indicate mean values of the drinking activity per 3 hr. The band at the bottom of each graph denotes light–dark alternations in the chamber. Shaded parts indicate dark period (7:00 PM–7:00 AM). NO_2 exposures were started at 7:00 PM on the fifth day as shown in the figure.

showed a tendency to decrease in the 6 ppm NO_2 group and significantly decreased in the 8 and 12 ppm NO_2 groups (Fig. 8). On the second day of NO_2 exposure, body weight decreased by 2.5, 5.5, and 6.1 g in the 6, 8, and 12 ppm NO_2 groups, respectively. These values were significantly different (Kruskal–Wallis analysis of variance of ranks, $H = 6.43$, $P < 0.05$), showing that the decrease in body weight depended on concentration of NO_2. On the third day of NO_2 exposure, the body weights of all groups showed a tendency to recover, although the 12 ppm NO_2 group did not recover completely during the 7-day exposure period (Fig. 8).

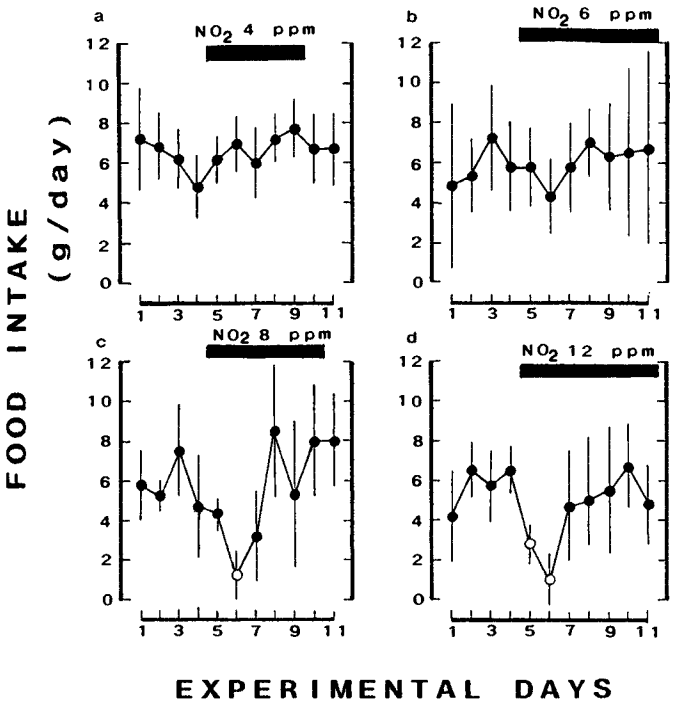

FIG. 7. Alterations in eating activity of mice exposed to NO_2: (a) 4 ppm NO_2 group, (b) 6 ppm NO_2 group, (c) 8 ppm NO_2 group, and (d) 12 ppm NO_2 group. The data are shown in the same way as in Fig. 5.

Changes in Drinking and Eating Activities and Body Weight with Exposure to NO_2 Followed by O_3

Figure 9 shows alterations in drinking activity (upper panel), food intake (middle panel), and body weight (lower panel) in the mice of the 12 ppm NO_2 group before, during, and after the NO_2 exposure. They were further exposed to 0.4 ppm O_3 for 23 hr on Day 21. Although the mice were exposed to 0.4 ppm O_3, a concentration at which the drinking and eating activities and body weight apparently decreased in naive mice (Figs. 1, 3, and 4), the drinking and eating activities and body weights of the mice in the 12 ppm NO_2 group did not change significantly upon subsequent O_3 exposure.

DISCUSSION

The present study shows that the drinking and eating activities of mice did not alter when they were exposed to 0.1 ppm O_3 for 7 days. On the other hand, the drinking activity decreased in a concentration-dependent manner when mice were exposed to 0.2–0.8 ppm O_3 (Fig. 1). The amount of drinking activity is parallel to that of water intake (Kuribara *et al.*, 1978). Therefore, the amount of water intake in mice decreased in a concentration-dependent manner on exposure to more than 0.2 ppm O_3. In addition, the eating activity decreased when mice were exposed to

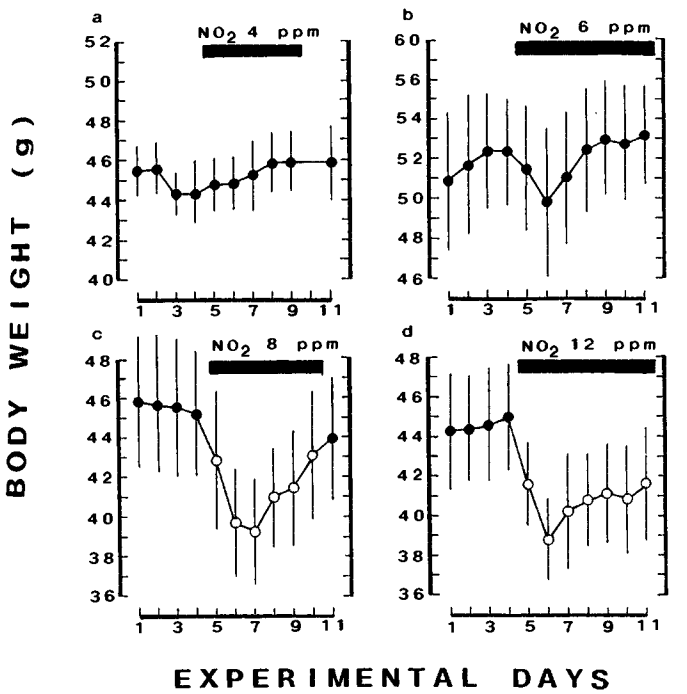

FIG. 8. Alterations in body weights of mice exposed to NO_2: (a) 4 ppm NO_2 group, (b) 6 ppm NO_2 group, (c) 8 ppm NO_2 group, and (d) 12 ppm NO_2 group. The data are shown in the same way as in Fig. 5.

more than 0.4 ppm O_3 (Fig. 3). The body weight also decreased upon exposure to more than 0.2 ppm O_3 (Fig. 4). The decreases in the amount of food and water intake should result in a decrease in body weight. Based on these results, it can be concluded that O_3 at more than 0.2 ppm suppresses drinking and eating behaviors in mice.

The previous study (Umezu *et al.*, 1987) has shown that drinking activity in rats exposed to more than 0.2 ppm O_3 decreased in a concentration-dependent manner. The authors also confirmed that not only drinking activity but also eating activity and body weight in rats decreased with 0.4 ppm O_3 exposure (data not shown). The minimum effective concentration of O_3 that suppresses drinking behavior seems to be almost the same in rats and mice, although the degree of decrease in drinking activity with 0.4 ppm O_3 exposure seems to be larger in mice than in rats. These results suggest that O_3 at more than 0.2 ppm suppresses drinking and eating behaviors in rodents.

There have been several reports of the effects of O_3 on behaviors in experimental animals (mainly rats). Konigsberg and Bachman (1970) reported that the gross motor activity in rats decreased with 0.05–1.0 ppm O_3 exposure. Several reports (Murphy *et al.*, 1964; Tepper *et al.*, 1982, 1985; Tepper and Weiss, 1986; Higuchi *et al.*, 1985) have shown that the wheel-running activity in rats also decreased in a concentration-dependent manner with more than 0.12 ppm O_3.

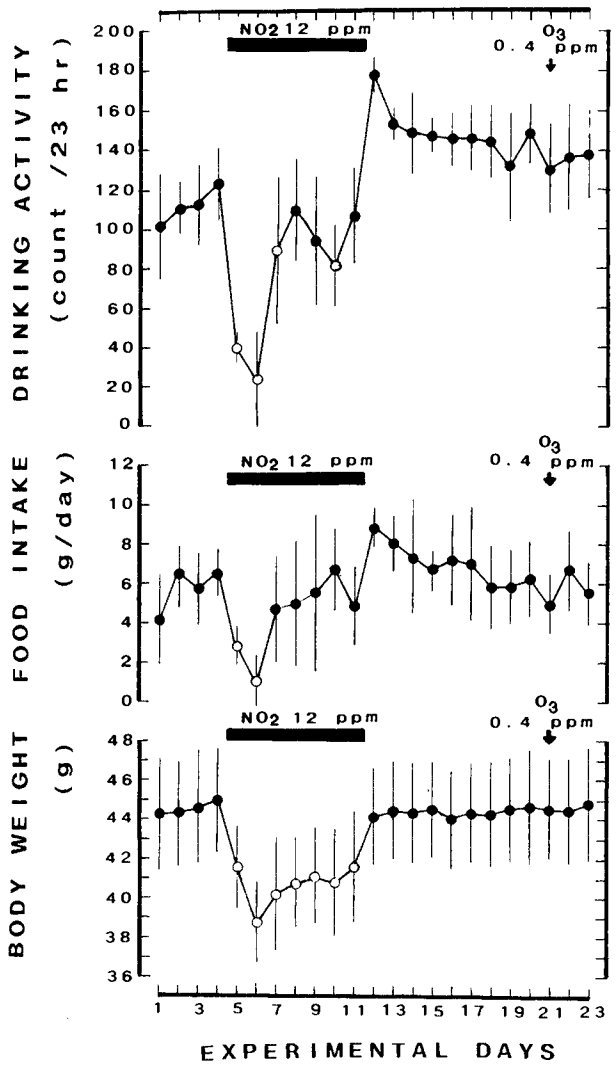

FIG. 9. Alterations in drinking activity (upper), eating activity (middle), and body weights (lower) of mice exposed to 12 ppm NO_2 for 7 days followed by exposure to O_3 for 23 hr on Day 21. The data are shown in the same way as in Fig. 5. The mice are the same as those in Figs. 5d, 7d, and 8d.

These studies suggest that spontaneous motor activities of experimental animals are suppressed at relatively low concentrations of O_3. It was also reported that operant behavior reinforced by food decreased with 0.12–2.0 ppm O_3 exposure in rats (Weiss *et al.*, 1981). In addition to these results, the present and previous studies confirmed that drinking and eating behaviors in rats and mice were suppressed by O_3 exposure. Therefore, effects of O_3 on behaviors in experimental animals may not be specific; that is, O_3 should nonspecifically suppress various physiological functions of the whole body in rodents.

The decrements in drinking and eating activities in mice were maximum on the

second day of O_3 exposure (Figs. 1 and 3). Thereafter these activities recovered to the control level depending on the concentration of O_3. Similar phenomena were observed in rats (Umezu *et al.*, 1987). This attenuation in the sensitivity to O_3 suggests the presence of physiological processes which change the sensitivity to O_3 effects and provide mice and rats with physiological defense mechanisms against O_3.

In the fields of pharmacology and toxicology, the phenomenon, in which effects of agents on physiological functions are attenuated gradually when the agents are administered repeatedly to human or animals, is termed "tolerance" (Goodman and Gillman, 1970). Therefore, the attenuation of O_3 effects on drinking and eating behaviors is considered as tolerance to O_3. On the other hand, the concept of tolerance on the health effects of O_3 has already been established (Stokinger *et al.*, 1956; Matzen, 1957; Fairchild, 1967; Nambu and Yokoyama, 1982; Evans *et al.*, 1985). This tolerance means that animals which have been briefly exposed to sublethal doses of O_3 acquire resistance to the effect of a later exposure to otherwise lethal or edematous doses. The present study suggests that functional tolerance to O_3 is induced by relatively low concentrations of O_3. Concerning the concept of tolerance, we should not confuse the former tolerance with the functional tolerance because the latter was developed under continuous exposure conditions to relatively lower concentrations of O_3. Murphy *et al.* (1964) showed that the wheel-running activity in mice recovered under continuous exposure to O_3. On the other hand, Tepper *et al.* (1982) reported that the degree of decrease in the wheel-running activity in rats did not change when the rats were exposed repeatedly to O_3. Hence, it cannot be concluded at present whether the degree of change in spontaneous motor activity of animals is affected when animals are exposed continuously or repeatedly to O_3.

In humans, continuous long-term exposure to O_3 is impossible. Thus, it is not known how physiological functions in humans are altered during long-term continuous O_3 exposure. The present study may predict that functional changes caused by O_3 exposure in humans are transient and recover thereafter under the O_3 exposure. On the other hand, there are reports (Farrel *et al.*, 1979; Folinsbee *et al.*, 1980; Hackney *et al.*, 1977) in which humans recovered when they were repeatedly exposed to O_3 for a few hours a day. They exhibited symptoms such as substernal soreness, tracheal irritation, cough, and headache on the first 2 days but they did not exhibit such symptoms thereafter; that is, susceptibility in humans to O_3 was attenuated by repeated O_3 exposure. This phenomenon is termed "adaptation." Therefore, the similarity in the time course between symptoms in humans and the changes in drinking and eating behaviors in rodents can be pointed out.

To our knowledge, there is only one report on the effects of NO_2 on animal behaviors. Murphy *et al.* (1964) reported that wheel-running activity in mice decreased with NO_2 exposure. The present experiments showed that drinking and eating activities in mice did not alter with 4 ppm NO_2 exposure. On the other hand, drinking activity decreased when mice were exposed to 6–12 ppm NO_2 (Fig. 5), and eating activity also decreased when they were exposed to 8–12 ppm NO_2 (Fig. 7). In addition, body weight decreased apparently by exposure to more than

8 ppm NO_2 (Fig. 8). Therefore, it is concluded that behaviors of mice change when they are exposed to more than 6 ppm NO_2. These results suggest that NO_2 at more than 6 ppm affects whole-body physiological functions in mice.

Ozone at 0.1 ppm did not affect drinking and eating behaviors, and at 0.2 ppm O_3 suppressed them (Figs. 1 and 3). NO_2 at 4 ppm did not affect behaviors of mice and at 6 ppm NO_2 did affect them (Figs. 5 and 7). NO_2 must be given at higher concentrations than O_3 to suppress drinking and eating behaviors. Many reports also show that O_3 affects these behaviors at lower concentrations than NO_2. It has been shown that O_3 is 40 times as potent as NO_2 (Chang *et al.*, 1988). The present study also confirmed that the effect of O_3 is stronger than that of NO_2 by means of behavioral indicators.

Both O_3 and NO_2 are major components of photochemical smog, and both are considered to affect human health. Usually, O_3 and NO_2 coexist in photochemical smog. Hence, health effects of photochemical smog will come from synergistic effects of O_3 and NO_2 at least in some cases. However, knowledge about the effects of combined exposures to O_3 and NO_2 on the physiological functions of the whole body is not enough. Especially, the combined effects of O_3 and NO_2 on animal behavior are not known at all. Therefore, there is the need to examine systematically not only the effects of a single exposure of O_3 or NO_2 but also the combined effects of O_3 and NO_2 on behaviors in animals. The results of those examinations may provide very useful information for studies about health effects of photochemical smog.

When mice were exposed to NO_2, drinking and eating behaviors decreased. However, the changes were transient and they recovered under continuous exposure to NO_2, as in the case of O_3 exposures. These results suggest that NO_2 affects physiological functions of the whole body in mice, but mice recover to normal conditions thereafter. These phenomena suggest that mice have physiological defense mechanisms against NO_2, too. Are these two defense mechanisms against O_3 and NO_2 independent of each other? The present experiments revealed that drinking and eating activities were scarcely altered by 0.4 ppm O_3 exposure in mice that had been previously exposed to 12 ppm NO_2 for 7 days. Naive mice showed decreases in drinking and eating activities when they were exposed to 0.4 ppm O_3 for the first time. Therefore, it is concluded that the susceptibility to O_3 is attenuated by NO_2 exposure. This fact suggests that physiological defense mechanism against NO_2 is effective against O_3. It is probable that the defense mechanism against NO_2 and O_3 is identical.

Both NO_2 and O_3 have strong oxidative characteristics. Each of them apparently affects the respiratory organs. Morphological studies on animals show that injury to trachea, bronchi, and alveoli occurs after inhalation of O_3 or NO_2. The small conducting airway and adjacent alveoli are the most sensitive. Stephens *et al.* (1973) reported that type I alveolar lining cells were very sensitive to O_3 and were severely damaged by 0.35 ppm O_3 exposure involving their removal from significant areas proximal alveoli. Furthermore, they showed that the lesional area was repaired through proliferation of type II cells and the repair was accomplished within approximately 48 hr. The same phenomena were observed in the case of NO_2 exposure (Rombaout *et al.*, 1982). Evans *et al.* (1976) reported that

the repaired lung is tolerant to the injurious action of O_3. These results suggest the possibility in which the injury of lung results in depression of drinking and eating behaviors and thereafter animals develop tolerance in behaviors accompanying the repair of the lung. The lung is innervated by vagus nerves, and there are various types of afferent neurons such as irritant receptors in the vagus nerves (Tsubone, 1986). Such receptors are involved in various physiological reflexes. Therefore, it is important to examine the relationships between injury in the lung and suppression of behaviors produced by O_3 and NO_2, and between repair of the lung and the appearance of tolerance in behaviors.

Finally, the present study revealed that photochemical oxidants suppress drinking and eating behaviors in rodents and they recover thereafter under continuous exposure to photochemical oxidants.

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ENVIRONMENTAL RESEARCH 60, 84–86 (1993)

WHO NCTB and Other Neurobehavioral Test Batteries

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This workshop considered the presentations made at the symposium and the reports of past workshops in this series of symposia. The workshop members came to the following conclusions: (1) We are at the very early stages of learning about neurotoxicology, and we understand the mechanisms of action of very few neurotoxic chemicals. It is thus important that we not limit the development and use of tests or test batteries and that we tailor them to the known effects of the chemicals under study. In short, we must remain flexible in the tests we employ. However, there was strong support for retaining the concept of using a core set of tests such as the WHO-recommended Neurobehavioral Core Test Battery (NCTB) in most if not all studies in order to continue building a database on the effects of diverse chemicals on common tests. No objections were raised to the set of tests in the NCTB as that core, and no alternatives were put forward. Rather, the concept of focusing on tests found consistently sensitive to the effects of industrial chemicals was suggested as a way to ensure an orderly incorporation of improved tests into widely-used test batteries.

The distinction between tests implemented on a computer and those administered by a human testor is a trivial distinction. One should always use the test best suited to evaluate the chemical under study, and the instrument chosen should be the instrument of greatest precision. Computer-implemented tests are thus often the best choice. It was noted that the ability to observe a subject's performance face to face often provided valuable information of subtle or even more obvious problems that had important implications for the interpretation of results or for follow-up testing. In computer-implemented tests, a more extended face-to-face interview with the subject may be preferable. It was noted that computer-implemented tests had limitations for assessing motor and sensory performance, but that they were particularly efficient and precise for cognitive behavior. However, their utility for any type of testing remained doubtful in developing countries where costs, availability of service, and familiarity with keyboards were limiting factors. Here especially, human-administered tests such as the NCTB were equivalent alternatives that could provide a good assessment of key neurotoxic effects in humans. They allow developing countries to participate as equal partners in the international effort to assess the neurotoxic effects of chemicals in their populations.

(2) Deep concern was expressed regarding study design. It was recognized that longitudinal research in which the subject served as his own control was the ideal,

but was rarely practical. Rather, concern over health typically occurs when adverse health effects are suspected or identified. We recommend the use of control or reference subjects in all research, even though it is acknowledged that the "perfect control group" does not exist. Norms might be useable to replace reference subjects in restricted cases (e.g., when appropriate controls from the same geographical area had been recently tested), but the results would be more tenuous or questionable.

(3) There is a need to focus on improved exposure characterization (development of dose–response information) in all research. Nonetheless, it was recognized that most measures of dose only reflect current or recent exposure—nervous system effects reflect cumulative exposure and may actually be a better indicator of dose than current industrial hygiene measures provide. Strategies for improving relevance of industrial hygiene measures include simulations of past conditions. Measures of internal dose are an important supplement to measurement of the ambient environment. Industrial hygiene measurements are always needed for study credibility.

(4) Regarding the use of neurobehavioral test batteries, there are some clouds on the horizon, particularly regarding their utility in those developing countries or populations in any country with very limited education, based on findings presented at this symposium. These concerns form the basis for this workshop's recommendations for future research.

Research presented at this and other symposia consistently identifies high correlations between performance on behavioral tests and confounding variables such as age, education, sex, alcohol and cigarette consumption, and job characteristics (e.g., piece work). These confounding factors produce significant uncertainties when relating effect to chemical exposure. We believe that the role of these recognized confounding variables needs to be clarified through international research that includes substantial representation of developing as well as industrialized nations. The need to involve developing countries derives from concern that a different range of variables may be most relevant in developing countries. It was noted that the lower limit of the range of education was 8 years of schooling in many industrialized countries while the range began at 0 years in at least two presentations from developing countries at this symposium. Even in industrialized countries, there may be important differences not previously recognized in European research on such variables. For example, it was noted that the availability of education and many occupations may be very restricted for females in male-dominant cultures, thus adding to the impact of the effects of the variable of occupation.

Strategies should also be employed to reduce the impact of these variables and to better characterize them. For example, it was noted that more extended practice on some tests (which is rarely employed in field research but often utilized in laboratory research) might reduce the effects of the education variable and that the use of years in school to characterize education may be inadequate such as in some industrialized countries (e.g., the United States), where some persons identified as having high school diplomas (12 years of school) were unable to read or write. Lest these examples correlated to education appear to focus too much on

education, there was strong sentiment for including several major confounding factors in future research. An example from job characteristics or motivation was particularly noted where these tests may be used to qualify people for some jobs. An example of the impact of this variable is employees working on piece work who were extremely competitive and intense in completing many tests. They thus needed, at a minimum, equal representation in exposed and reference groups, although their presence would increase variability substantially in both groups.

(5) Some workshop members expressed concern that this focus on confounding variables could be seen as trivial against the background of urgent needs in developing countries. It was suggested that we might instead employ these precious resources in developing countries not for research but to reduce exposures to chemicals known to be toxic. This suggestion was countered by several responses: (a) many chemicals have no Maximum Allowable Concentration (MAC) or Permissible Exposure Limit (PEL) or there are insufficient health effects data on which to base standards (e.g., Landrigan, 1992 [this volume]); (b) standards set in industrialized countries may be too high for developing countries where nutritional or disease problems may greatly enhance the toxicity of chemicals. Finally, it was concluded that evidence of health effects was often critical in stimulating companies to reduce exposures or to stimulate local health departments to force companies to reduce exposures in the absence of national or international standards.

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ENVIRONMENTAL RESEARCH 60, 82–83 (1993)

Computerized Test Batteries

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Approximately 20 people met in a workshop to discuss computerized neurobehavioral test systems. A summary of this discussion and our recommendations follow.

SUMMARY OF WORKSHOP DISCUSSION

The discussions at the workshop were initiated by two short reviews, the first of which was a brief presentation of the major computerized tests systems that were developed primarily for use within the field of behavioral toxicology (Letz, 1991). These systems have been applied in a number of investigations utilizing a variety of study designs. Some of these test systems have been employed more successfully than others. In the long run the utility of a specific system will be measured by the number and the quality of the reports that eventually appear in the peer-reviewed scientific journals.

The second review comprised a short discussion of what may eventually be included in an "ideal" computerized test battery and the alternative solutions that should be considered before making various decisions regarding the design of any test system. These design considerations include aspects such as which hardware to use, which kinds of tasks to implement, how to manage the instructions and training, which performance measures to use, what measurement properties to attend to, and how to handle the data output from the systems.

A concern was expressed that too many studies of the behavioral effects from environmental exposures have been performed without the assistance of professional psychologists with proper research experience. This has undoubtedly had a deleterious effect on the general scientific level of some investigations. Also, lack of participation of appropriately trained personnel in development of some computerized test systems has undoubtedly limited their utility.

Another concern raised in the discussion was the need to establish explicit criteria for the evaluation of tests with respect to reliability, validity, sensitivity, ease of administration, etc. Such criteria should be established and published. In relation to this topic a brief report on an effort to make an evaluation of some 25 tests, paper-and-pencil as well as computerized ones, was given by one of the participants. This evaluation will be performed in a project financed by one of the medical research programs within the European Community.

Some participants felt strongly that all test batteries, computerized as well as traditional ones, should be developed on the basis of an explicit conceptual model from cognitive psychology or neuropsychology. This view, however, was not shared by all the participants. Proponents of this approach argued that results

from such a battery would allow discrimination of deficits in particular cognitive functions. Others countered that the proper evaluation of differential deficits will always encounter some extremely difficult psychometric problems.

Part of the lack of agreement on this issue and others during the discussions was due to the fact that different people want the tests to fulfill different purposes. One major difference in the purpose for testing is between the use of performance tests for diagnostic evaluation of individuals and their use in large groups of people with the sole aim of assessing the impact of the environment on performance.

During the discussions at the workshop, as well as at other times during the symposium, it was discussed whether the time might be ripe for strict standardization of at least a small set of computerized tests. There appears to be urgent need for such standardization, and we believe that an effort to this end should now be made. We have, in fact, recently made a proposal in this direction (Iregren and Letz, 1991) by proposing the MCCCB, i.e., the "Minimal Common Core Computerized Battery." This battery would comprise highly standardized versions of tests of simple reaction time, finger tapping, and symbol–digit substitution. The MCCCB is proposed to be included as part of the test battery in all studies using computerized tests to evaluate effects on performance of long-term environmental exposures.

It is our present belief that this previously proposed battery should be somewhat extended to be suitable for the screening for effects from environmental exposures. For example, in the original proposal for the MCCCB, there is a lack of tests measuring memory functions and eye–hand coordination.

RECOMMENDATIONS

To help realize the goal of standardizing computerized performance tests and their application, we conclude by making the following recommendations:

A meeting should be held in the near future among psychologists active in computerized test development to agree on a computerized test battery suitable for the screening of large groups of subjects and to establish a mechanism for extending the computerized screening battery.

Efforts should be strongly encouraged toward the standardization of hardware, software, and data formats used in computerized tests to simplify the exchange of information between users of different computerized test systems.

Development or application of behavioral research methods without the assistance of properly trained research psychologists should be discouraged.

A mechanism should be established for the training of investigators on a continuing basis in the use of computerized performance tests.

A mechanism should be established for providing technical support to investigators from countries using nonalphabetic character sets to assist in the translation and presentation of test instructions in computerized testing systems.

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Evoked Potentials

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Participants raised a variety of methodological questions concerning chemosensory-evoked potentials and the use of event-related potentials in occupational and environmental health research. Limitations in the use of pattern-reversal visual-, brainstem auditory-, and somatosensory-evoked potentials were also discussed.

I. CHEMOSENSORY-EVOKED POTENTIAL METHODOLOGY (CSEP)

(*A*) *Hardware and Software Requirements*

A brief description of the olfactometer used by Kobal and Hummel at the Institute of Pharmacology and Toxicology, University of Erlangen-Nurnberg, Germany, was provided. The basic requirement is a device that can deliver chemical stimuli in the form of square waves with steep onsets and offsets without altering the mechanical or thermal conditions of the nasal mucosa. The system designed by Kobal and Plattig (1978) mixes pulses of stimulants in a constantly flowing air stream with temperature maintained at 36.5°C and 80% relative humidity. A critical component of the system is a vacuum-operated switch which can deliver precise concentrations of odorant or clean air. The rise time of the odorant pulse cannot exceed 20 msec, while noise is used to mask auditory cues from the switch. The flow rate is variable, but 140 ml/sec is commonly used.

(*B*) *Velopharyngeal Closure*

Controlled breathing is necessary to avoid confounding the delivery of the odorant stimulus to the olfactory mucosa. A method of velopharyngeal closure (Kobal, 1981) is used in which respiratory air flow in the nose is avoided. In this procedure, subjects breathe through the mouth.

(*C*) *Sampling Rates*

Questions were also raised about the sampling rate for CSEPs and the number of trials needed to form a signal average. Kobal and Hummel recommend a minimum ISI of 30 sec and a minimum of 16 trials, per average.

(*D*) *Distinguishing Olfactory vs Trigeminal Responses*

Responses mediated by the olfactory and trigeminal nerves can be distinguished in two ways. (1) Subjects can easily localize which nostril has been stimulated by an irritant such as CO_2, whereas they are unable to localize the nostril stimulated by a pure odorant such as vanillin. (2) The topographical distribution of olfactory-evoked potentials (OEPs) and chemosomatosensory-evoked potentials (CSSEPs) differ. CSSEPs are maximal at the vertex, while OEPs are maximal postcentrally.

VI. MAGNETIC STIMULATION METHOD OF MOTOR CORTEX

Magnetic stimulation of the human brain and the recording of the resultant muscle action potentials has recently been reported (Barker *et al.*, 1987; Mills *et al.*, 1987). This is a painless method of stimulating the motor cortex and deep peripheral nerves in humans by magnetic field, and can be used to estimate central motor conduction time. However, magnetic stimulation has several disadvantages; i.e., the equipment required is relatively bulky and the site of stimulation is not well defined. Further research is needed to determine whether this method can be applied to the study of the occupational and environmental medicine.

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Neuroimaging Methods

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During the discussion it became clear that there has been an exponential increase in the number of publications relating to modern brain-imaging techniques in studying central nervous system (CNS) disorders. Recently published reviews cite more than 200 original articles regarding brain- and neuroimaging methods such as X-ray computed tomography (CAT), magnetic resonance imaging (MRI) or spectroscopy (MRS), positron emission tomography (PET), and single photon emission tomography (SPECT) (Alavi and Hirsch 1991, Lang *et al.*, 1990). However, for the field of neurotoxicology in humans the number of studies is limited. Furthermore, the participants agreed that the results are somewhat different depending on the researchers and the methods applied.

Summarizing current experience, well-known factors for brain atrophy are age, chronic alcoholism, and solvent abuse. Suspected causes are long-term and intensive exposures to solvents like toluene, carbon disulfide, and carbon monoxide. At present there is no hint of abnormal brain atrophy in workers exposed to solvent concentrations below TLV values.

Computed tomography (CAT) of the brain is the result of digital reproduction of computer-analyzed X-ray absorption by biological materials, such as bone, brain tissue, and liquid fluid.

Nuclear magnetic resonance (NMR, MRI) is based on the absorption of radio frequency energy by the magnetic moments of atomic nuclei in samples placed in a strong magnetic field (Moonen *et al.*, 1990). So-called "T_2-weighted" spin echoes provide information on the gray matter of the brain superior to that provided by a CAT scan. There was some discussion during the workshop about the reliability and reproducibility of images obtained with MRI. MRI constructs an image based on the behavior of nuclear spins and not simply based on transmission or emission of electromagnetic waves. The image obtained is thus dependent on multiple parameters such as spin density, longitudinal relaxation time (T_1), transverse relaxation time (T_2), and movement of spins. This complexity could cause some confusion for the analysis of the image. The best way to avoid this kind of confusion is to understand the principle behind the image and not to use MRI as a black box. We should say that a modern MR imager gives us a highly reproducible result when it is operated well within its limitation. Since neither MRI nor MRS has yet been used widely in the field of environmental health, we have to accumulate basic research and compile the results in order to make accurate diagnoses.

Positron emission tomography (*PET*) provides a method of measuring the metabolism, perfusion, and pharmacology of human organs *in vivo* (Brooks, 1991). A tracer tagged with a short-lived positron-emitting isotope is generated by a cyclotron and administered to the subject intravenously or by inhalation. Quantitative tomographic images of regional cerebral function can be generated using established mathematical models from the scans of regional cerebral uptake with the knowledge of the arterial plasma tracer activity.

Single photon emission computed tomography (*SPECT*) utilizes radionuclides that are capable of measuring cerebral function and are commercially available (Jagust *et al.*, 1987). Common tracers are *N*-isopropyl-*p*-iodoamphetamine (IMP), or hexamethylpropyleneaminoxin (HMPAO) marked with technetium-99m labeled with iodine-123.

In summary, CT and MRI give us anatomical and pathological images. PET, SPECT, and MRS supply functional or biochemical information which cannot be obtained with other methods in a noninvasive manner. The imaging of the brain can be a dynamic reflection of brain chemistry. The inaccessibility of brain tissue will necessitate various indirect methods of studying biochemical processes, such as oxidation, of normal and abnormal states. [^{31}P]MRS is useful for monitoring energy metabolism in the brain. Applications of [^{1}H] and [^{13}C]MRS are being exploited. However, the basic techniques of MRS, particularly for localized spectroscopy or for metabolite mapping have to be greatly improved.

Despite some advantages, such as costs and feasability, SPECT has an important limitation in that the measured activities are relative and not absolute values (English and Brown, 1986; Rootwelt *et al.*, 1986). The overall results therefore depend on the blood flow in the region of interest (ROI). They indicate perfusion and not necessarily disturbed brain metabolism (Deisenhammer *et al.*, 1989; Heiss *et al.*, 1988).

The group was aware of the problems created by difficult techniques (PET needs a cyclotron to get short-lived radionuclides), costs, and the availability of the methods. This is especially true for developing countries. On the other hand, an important aspect is the (relative) safety for the health of the patient. This makes the method appropriate for repeated use (especially MRI).

SPECIFIC RECOMMENDATIONS

Concerning the limited knowledge, more research is needed. This should cover the following areas:

— basic metabolic and biochemical research, e.g., investigation of possible associations between morphological, biochemical, and functional pathology in laboratory animals;

— standardization of the methods (application, evaluation);

— assessment of reliability criteria of the methods;

— imaging studies to rule out underlying pathology other than that potentially caused by toxins;

— studying the correlations between neuroimaging findings and clinical neurophysiological, as well as neurobehavioral parameters in exposed workers and in patients; and

— follow-up patients with clinical toxic encephalopathy and abnormal neuroimaging to get more information on the prognosis of the disease.

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Biochemical Methods in Neurobehavioral Toxicology

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The Workshop Group used the schematic concept of the National Academy of Sciences (U.S.) to discuss biochemical markers of exposure and response to neurotoxicants.

The importance of developing and validating biological markers of exposure was stressed, with discussion of work on hippuric acid (for toluene) and 2,5-hexanedione (for *n*-hexane) as examples. Exposure markers may or may not give direct information on target organ dose. However, markers of exposure should give information relevant to the types of toxicity endpoints being studied. For instance, for studies of *chronic* lead neurotoxicity it is important to evaluate exposure by means of markers that reflect *chronic* exposure. Research is needed to develop exposure markers for many important chemicals in the workplace and the general environment. It was proposed that in some cases researchers should obtain biological samples (such as blood, urine, hair, nails, breast milk, and sperm) in connection with large studies and that these samples should be banked so that in the future new developments in markers could be applied. It was also proposed that more studies using human volunteers should be undertaken to determine biochemical responses under controlled conditions. Biochemical markers of response were recognized to be difficult to develop because these markers are not likely to be useful for screening purposes, since they reflect specific types of cell response and require considerable knowledge of mechanism of neurotoxicity.

The practical needs and limits of clinical and epidemiological research will restrict the range of potential markers. Neuron- and glia-specific markers may be potentially useful, but at present may not be applicable, since these special response markers are often difficult to interpret in terms of relevance to eventual disease or dysfunction. Research in this area is further limited by a lack of funding for the "interface" of basic neurosciences and practical epidemiology. It should be remembered that DNA adducts, biological markers of exposure to chemical carcinogens, were developed out of 30 years' worth of basic research in molecular biology of carinogenesis. Areas for potential research including neuroendocrine and neuroimmunological pathways, because signals from the nervous system may be detected by altered states of target cells in the pituitary, gonad, and lymphocyte. Additionally, technological developments in detecting *in vivo* chemi-

cal signals in human brain using new neuroimaging methods may be useful, although limited by expense. However, many neurotoxins may not produce detectable changes in morphology, even when behavior and function are significantly affected.

The workshop participants recommend seeking opportunities for collaborative research, involving basic researchers in neuroscience so that information on neurobehavioral status can be integrated with measurements of biochemical markers of exposure and response.

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Neurotoxic Diseases

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Approximately 25 persons took part in the discussion in this workshop. The expertise of the participants covered occupational health, occupational medicine, legal medicine, neurology, neurophysiology, experimental and clinical toxicology, and *in vitro* toxicology. The workshop started with a general discussion about most important neurotoxic problems. Each participant reviewed his/her main interests in the research of neurotoxic diseases. A spectrum of important neurotoxic problems was shortly discussed, after which the group selected solvents as a point of discussion.

SUMMARY OF THE DISCUSSION

Mixture/Single Solvent

It was generally agreed that poor specification of solvent mixtures causes great problems in dealing with neurotoxic syndromes associated with solvents. Exposure to solvent mixtures is undoubtedly the most common form of exposure in industrial settings. Terms such as lacquer thinner and white spirit mineral turpentine tell little about the chemical constituents of the mixture. They may contain toluene, methyl-isobutyl ketone, isobutanol, acetone, butyl acetate, isopropanol, etc., in many combinations. The possibility of metabolic interactions in a number of ways in cases of exposure to mixtures of solvents renders it almost impossible to judge the relevance of exposure. However, it was emphasized that quantitative and qualitative assessment of exposure is a cornerstone in etiological diagnosis of neurotoxic diseases. Exposure indices should be used whenever it is possible. At the same time it was noticed that we actually know very little about solvent-specific neurotoxic syndromes.

The group discussed *n*-hexane and toluene, which were the targets for several ongoing clinical and epidemiological studies. Several case reports were reviewed by the participants. Severe cases of *n*-hexane intoxications occurred in Taiwan some years ago. These cases were discussed with regard to their prognosis. Recovery in the cases was rather good during the follow-up despite the severity of the clinical picture. It was emphasized that we know very little about the prognosis of solvent-induced neurotoxic syndromes, particularly of those due to long-term exposure to a mixture of solvents.

Individual Susceptibility

The issue of individual susceptibility was discussed in somewhat more detail. In neurobehavioral research, everything would be much easier without individual constitutional factors. These are defined as attributes of organism variation, which are associated with variation in the risk of developing toxicity. Individual susceptibility is one of the most important factors in determining the clinical

picture of a neurotoxic syndrome. In individual cases of neurotoxicity hygienic standards are of limited value because of this individual susceptibility.

Our current knowledge about factors behind individual susceptibility is limited. Theoretically, fast and slow metabolizers may have different susceptibilities to toxic agents due to different rates of metabolism. For example, poor metabolizer phenotype is associated with increased oral bioavailability, accumulation of toxin, decreased metabolic activation, and enhancement of usually minor pathway. The reverse would be the case when the activation step is involved as in, e.g., *n*-hexane poisoning. Hypersusceptible individuals may have different physiological or psychological constitutions for a variety of reasons. Molecular genetics is probably the field in which solution to this difficult problem will be found eventually. Co-twin control study design and longitudinal studies were considered in this context.

Neurobehavioral Tests in Neurotoxic Diseases

Neurobehavioral tests were discussed in relation to the diagnostic procedure of the neurotoxic disease due to solvent exposure. It was stressed that individual test results are always subject to a number of false interpretations. This is due to great interindividual and intraindividual variation in behavioral tests, quantitative and qualitative differences in exposure, and the possibility of systematic bias, either positive or negative. In this connection, the issue of the specificity and sensitivity of a test was considered. The problems in current neurobehavioral research seem to be the selection of relevant tests from a large group of available tests and achieving general consensus between different countries on using these tests.

Children as a Specific Target for Neurotoxins

Neurotoxicity in general should be considered separately in developing, degenerating, and aging nervous system. In this respect, children form a particularly susceptible population which is worth special attention. The developing nervous system seems to be particularly susceptible to a number of neurotoxins, including solvents, lead, and alcohol. The plasticity of the nervous tissue in children may partly compensate for the effects of neurotoxins but our current knowledge on this issue is scanty. The participants were particularly concerned about the influences of childhood exposure on brain function in adult life and aging. The difficulties in scientific research on this matter were noticed.

SPECIFIC RECOMMENDATIONS

- Qualitative and quantitative estimation of exposure is most important in dealing with neurotoxic diseases. This, together with the formation of exposure indices, should be emphasized.
- The problem of solvent mixtures and possible interactions should be studied more vigorously in future.
- Individual susceptibility and the factors underlying it are the most important targets for future scientific research.
- Children form a particular group in neurotoxicological research worthy of special attention.

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Neurological Diseases

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About 30 participants discussed various problems in this workshop. Expertise of the participants covered neurology, neurophysiology, toxicology, epidemiology, behavioral sciences, etc. The discussions were rather diverse with no focus on particular points, and there were no special conclusions or recommendations.

Concerns about the increasing burdens of neurological diseases due to aging were expressed. Not only in developed countries, but in relatively "young" countries, control of the environmental causes of death combined with declining birth rates inevitably increases the elderly population and, therefore, the incidence of age-related neurological diseases. This is predictable because neurons are postmitotic cells that age with the host individuals' age.

Cerebrovascular diseases (CVD) still predominate among the neurological diseases throughout the elderly ages although their mortality rates apparently are declining owing to effective preventions and changing lifestyles in various countries. Decline in CVD is not random, however, being more obvious in males, in the younger elderly, in hemorrhages than in infarctions, and in accidents involving larger arteries than those involving arterioles. As a consequence, we now see more cases of multiple recurrent thromboembolic processes in the late elderly ages, which underlie dementia, than focal neurological deficits.

Degenerative diseases are now increasingly important among the morbidities and mortalities in the elderly ages. In Japan, for example, they represented 18.9% of non-CVD neurological deaths in 1987. Although causes of neurodegenerations remain obscure, circumstantial evidence suggests that the cumulative effects of lifelong exposures to neurotoxic elements in daily and/or occupational life interplay with inherited predispositions, superimposing with "physiologic" aging of the neurons in the brain. Major disease entities include Parkinson's disease, Alzheimer's disease, motor neuron disease (MND), and spinocerebellar degenerations.

Guamanian Chamorros are noted for extremely high incidences of MND and Parkinsonism dementia complex (PDC). Currently, MND is nearly extinct, and PDC has largely declined, although it is showing changing clinical and pathological patterns. Extrapyramidal symptoms are now less obvious and autopsies have disclosed more senile plaques, indicating PDC to be closer to Alzheimer's disease observed elsewhere. Guam is an isolated island, which gives unique opportunities for finding cause(s) or risk factors of MND and PDC. Renewed studies should be carried out there focusing on the primary degenerative dementias.

Occupational neurological afflictions still occur, although they are subsiding in many countries owing to improving standards for controlling such hazards. Various specific problems were presented and discussed in this workshop.

Infectious diseases may not be serious in developed countries in the temperate zones, but they still take heavy tolls in the tropical and subtropical areas where research and therapeutic and surveillance systems are yet to be implemented adequately.

The participants recognized the importance of neurological disorders in the fields of occupational and environmental sciences. The nervous system, once destroyed, is extremely difficult to restore and more efforts for primary and secondary preventions are highly desired for each specific problem.

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Psychosocial Factors

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The workshop was based on presentations by two of the moderators, namely professors Milan Horváth and Lennart Levi. In his presentation, "Mental Work Stress and Health Promotions: A Fifteen-Year Follow Up," Professor Horváth identified the two main goals of his study as (1) selection of persons at risk from cardiovascular diseases along with catamnestic validation of prognostic cues and (2) proposals and evaluation of various interventions.

The population of this study was engaged in almost purely mental work (with low levels of physical activity) and had strong internal and external drives, high psychological demands combined with high levels of decision latitude, and high and persistent activation levels, which interfered with natural rhythms of activity and rest. Due to the absence of explicitly negative work factors, psychological and physiological reactivity stemming both from family and from personal history comes to the forefront. Stable characteristics of circulatory reactivity to standard challenges appeared to be specifically related to anamnestic and catamnestic data on the one hand and to humoral or cellular mechanisms on the other.

The following examination, performed 15 years after the initial study (retaining 70% of the original sample of 675 healthy, normotonic middle-age males), indicates resting systolic blood pressure, diastolic blood pressure reaction to emotional challenge, and Type A behavior pattern as first-order predictors of cardiovascular morbidity, with smoking, family history of hypertension, high blood cholesterol, low physical activity, psychological tension, and "hard driving" behavior as second order predictors.

In his presentation, "Work Stress and Health," professor Levi drew attention to the basic assumption for research on psychosocial factors in occupational health that they can precipitate, or counteract, ill health, influence wellbeing, and modify the outcome of health measures. Based on this assumption, he presented a heuristic model that involved (a) social structures and processes at and outside the place of work, viewed objectively and as perceived and appraised by the employee; (b) vulnerabilities and other characteristics of the employee; (c) pathogenic cognitive, emotional, behavioral, and physiological mechanisms in the employee's reactions; (d) outcomes in terms of occupational health and wellbeing; and (e) interacting and modifying influences, such as coping repertoire and social support.

To identify critically important components in this system for subsequent modification, we need a strategy comprising three consecutive steps: (1) epidemiological determination of environmental and health problems, using survey techniques and morbidity data; (2) intensive longitudinal, multidisciplinary, controlled

field studies of high-risk situations and high-risk groups and ways in which they intersect; and (3) evaluation of therapeutic and/or preventive interventions in laboratory and/or real-life settings.

Interventions for which supportive evidence suggests the value of application and evaluation are

- Increasing a worker's control of work arrangements and organization;
- Avoiding monotonous, machine-paced, and short but frequent work actions;
- Optimizing automation;
- Helping workers see their specific task in relation to the total product;
- Avoiding quantitative work overload or underload; and
- Facilitating communication and support systems among work mates and others.

In the subsequent discussion, it was pointed out that *Japan* has experienced an economic success almost without precedent. One reason for its success seems to be the greater amount of annual work time per worker than any major industrialized nation—on the average, 500 more hours on the job for Japanese than in West German and French workers. The average work time is 261 workdays per year as compared to that of Swedish industrial workers, approximately 188 days.

In addition to working long hours, Japanese workers are usually reported to exhibit high work intensity and to spend a long time commuting to and from work.

In spite of this seemingly high work load, employees by and large are said to use only half of their paid vacation time, generally 15 days a year.

In similarly industrialized Western countries, such indefatigable work habits are usually found to lead to stress and fatigue and subsequently to cardiovascular and mental ill health. There might be some incipient evidence of such phenomena also from Japanese working life. The increase of sudden death during long working hours even has a name: karoshi. However, so far there have not been any nationwide studies in Japan and very few in other countries on (a) actual exposures to occupational and other stressors, and (b) subjective and objective occupational stress reactions, as related to (c) subsequent, work-related morbidity and mortality.

The crucial questions concern (a) whether conditions of work in Japan are, indeed, rich in quantitative and/or qualitative stressors, (b) whether sizable groups of employees—with or without preexisting vulnerabilities—react with emotional, cognitive, behavioral, and/or physiological stress mechanisms to such exposures, and (c) whether these exposures are reflected in subsequent work-related morbidity and mortality, and—if so—when, in whom, and why, And—if not—why not.

Briefly, it would be highly interesting to know whether there is a prize in human terms to be paid for the Japanese "worker bee" ethic that seems to be the backbone of Japan's economic success. The answer may well be that this is not so, or that the prize is low. Total and cardiovascular mortality is low in Japan and life expectancy has correspondingly increased and is among the highest in the world.

The generally low morbidity and mortality rates may be due to genetic and dietary factors but conceivably also to cultural ones. The latter may include concepts such as mutual dependence (amae), propensity to pull one's weight (ki ga sumanai), and conflict avoidance (warawareru).

However, what if prevailing cultural norms become eroded in the process of the rapid changes the Japanese society and working life are undergoing?

Japanese demographic development points to a rapid increase in the proportion of elderly people in the population. Will this mean that the young and middle-aged will have to work even harder to maintain the present level of productivity and remuneration? Will this be compensated by an increasing proportion of mothers working outside home? If so, will this affect the ability and willingness of the latter to care for their children, parents, and in-laws?

In another highly industrialized and economically successful society, namely Sweden, a governmental commission on work environment has reviewed the evidence regarding relationships between exposure to various components of work and work environment and subsequent changes in worker's health and wellbeing. Based on such evidence, the Commission presented a series of amendments to the Swedish Work Environment Act, stipulating more clearly than existing provisions that

- working conditions are to be adapted to people's physical and psychological conditions;
- employees are to be given opportunities of participating in the arrangements of their own work situations and its transformation and development;
- technology, the organization of work and job contents are to be designed so that the employee is not exposed to physical or mental loads that may lead to ill health or accidents;
- forms of remuneration and work schedule that involve an appreciable risk of ill health or accidents are not to be used;
- strictly controlled or tied work is to be avoided or restricted;
- work should afford opportunities for variety, social contacts, cooperation, and a connection between individual tasks; and
- working conditions should provide opportunities for personal and occupational development, as well as for self-determination and professional responsibility.

These amendments were introduced in a bill by the Swedish Government to the Swedish Parliament, which passed the bill in late May 1991.

To promote practical work along these lines, the Swedish Working Life Fund was set up by decree of the Swedish Parliament. It is distributing 15 billion Swedish kronor (nearly 3 billion U.S. dollars) over a 6-year period, aiming at a radical renewal of Swedish working life. The money has been collected from Swedish employers through a special charge. Through financial grants to the employers the fund will promote a healthy work environment and work organization as well as active rehabilitation programs in the workplaces.

Briefly, then, this illustrates how research results can be, and actually are, translated into legislation and practical health action. Participants of the workshop expressed much interest in these experiences and developments and proposed that they should be evaluated.

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Prevention Strategies for Neurotoxicology

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Prevention of neurotoxic disease has been a consistent theme throughout this Symposium.

The workshop participants noted the centrality of prevention in the discipline of neurotoxicology. This central position reflects the fact that the central nervous system, once damaged, has little capacity for repair.

Many speakers at this international symposium have considered various aspects of prevention of neurotoxic illness. Their remarks stimulated discussion in the workshop. Among these persons were

- Director-General Nakajima of WHO, who discussed the contribution of neurotoxins to the causation of preventable neurotoxic diseases around the world;
- Professor Araki, who considered the central importance of prevention in his own research and in creating the structure of this symposium;
- Professor Gilioli, who considered the contribution of chronic exposure to neurotoxicants in the etiology of preventable neurotoxic illness;
- Dr. Kogi of ILO, who considered strategies for prevention of neurotoxicity in the workplace;
- Professor Jeyaratnam, who led the discussion on prevention of neurotoxic illness in developing countries;
- Dr. Barry Johnson, who discussed the coordinated approaches being developed in the United States for identification and prevention of neurotoxic illness;
- Dr. George Becking, who discussed the multidisciplinary work of the International Programme on Chemical Safety in the identification and prevention of exposure to environmental neurotoxins.

These and many other speakers at this international symposium formed the basis for the discussion in this workshop.

The workshop concluded that prevention of neurotoxic disease *must* be a multifaceted enterprise. It includes the following elements:

- *Toxicologic research* to identify neurotoxic substances *before* they are released into the human environment. This constitutes primary prevention.
- *Epidemiologic surveillance* of high-risk populations using sensitive indicators of early neurotoxic injury. This constitutes secondary prevention.
- *Communication of data* on neurotoxic risk to workers, citizens, manufactures, government officials, and all others who will need to know. Participants in the workshop stressed the importance of the transmission of knowledge or a tool for prevention. Information is a very powerful tool. Two specific applications are

(a) Transmission of information on toxic hazards can be very effective in

preventing export of those materials from developed to developing countries.

(b) Transmission of data on toxics from scientists to politicians and other policy makers is an underutilized tool of prevention, one which should be used with more frequency.

- *Education of workers,* a specific form of information transfer.
- *Legislation and vigorous enforcement of regulations.* Prevention of neurotoxic exposure is unfortunately not possible without regulation. Regulation is therefore an essential tool of public health.
- *Vigorous application of preventive strategies* in the workplace and in the environment. Emphasis should be placed on primary prevention of exposures:
  - Substitution of safer materials,
  - Prevention of toxic releases,
  - "Cradle-to-grave" management of hazardous substances,
  - Engineering controls.
- *Formation of research institutes and regulatory bodies.* These legally mandated institutes provide an essential substructure for prevention research and preventive intervention. The workshop participants considered their creation essential.

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Developing Countries

JERRY JEYARATNAM AND FENGSHENG HE

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Papers and posters were presented by the participants from 12 developing countries (Chile, China, Brazil, Egypt, India, Indonesia, Korea, Philippines, Malaysia, South Africa, Viet Nam, Singapore).

Several papers addressed the neurotoxic effects of a variety of chemicals such as solvents, carbon disulfide, carbon monoxide, lead, mercury, and organophosphate pesticides. The exposures were usually heavy, resulting from poor working conditions, particularly in the small-scale industries, inadequate information to workers, lack of legislation, the transfer of hazardous manufacturing processes from developed countries to developing countries, and air and waste pollution.

The neurotoxic effects of these chemicals were assessed by neurobehavioral methods, neurophysiological approaches such as electroneuromyography, evoked potentials, and analysis of symptoms. Environmental monitoring of exposures was also conducted in some studies. The results obtained contribute to the understanding and prevention of neurotoxic hazards.

There are some special concerns related to the developing countries:

1. It is necessary to systematically validate neurobehavioral methods in developing countries, since most of them cannot be applied in any developing countries because of differences in language, education, and cultural background. At present the NES might not be feasible in some of the developing countries. The WHO/NCTB needs to be assessed in developing countries.

2. In the interpretation of the results of neurobehavioral testings, it is generally accepted that age, sex, education, smoking and drinking habits are confounding factors that one must consider. Besides these, there are other variables to be considered in some developing countries, such as malnutrition, endemic hepatitis B, and hypoxia due to high altitude.

3. Some recommendations are made:
   (1) Provide adequate information about prevention of toxic effects of chemicals to the exposed workers.
   (2) Encourage the establishment and enforcement of legislation on chemical safety, occupational health, and environmental health in developing countries.
   (3) Prioritize occupational health research on the basis of limited resources.
   (4) Identify occupational and environmental health needs in developing countries as an important priority for WHO and ILO.

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Pesticides

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Although the subject of "pesticides" was ranked at the head of the program at this symposium, only 5 of the 170 papers presented concerned themselves with pesticides. Also, participation in the pesticides workshop was virtually limited to the reporters of the 5 papers stated above.

SUMMARY OF DISCUSSION

What Is "an Intermediate Syndrome"?

At first Dr. Kinebuchi, one of the comoderators, showed a documentary videotape of an experiment using hens which had been administered leptophos, an organophosphorus pesticide. Organophosphate-induced delayed neuropathy (OPIDN) was clearly presented. Discussion began concerning the neurotoxicity in organophosphorus pesticides. It has been established that some organophosphorus pesticides produce not only the well-known acute poisoning caused by the inhibition of cholinesterase but also characteristic OPIDN in exposed animals. However, Dr. Spencer, another comoderator, posed a third neurotoxicity caused by organophosphates. The new kind of neurotoxicity, an intermediate syndrome, was first reported from Sri Lanka some years ago in *New England Journal of Medicine* (Senanayake and Karalliedde, 1987). The syndrome developed after the acute cholinergic crisis and before the expected onset of OPIDN. Patients had paralysis of proximal limb muscles, neck flexors, motor cranial nerves, and respiratory muscles 24 to 96 hr after a well-defined cholinergic phase. The paralytic symptoms lasted up to 18 days. The symptoms are quite different from OPIDN, because the paralysis in OPIDN is usually limited to the distal muscles of limbs. The electromyographic findings in the intermediate syndrome were different from those seen in other neuromuscular disorders. It is possible that the neuromuscular junctional dysfunction in the intermediate syndrome is postsynaptic and that the dysfunction is the predominant factor in the pathogenesis of the intermediate syndrome. Discussion stressed that the entity of the intermediate syndrome has not been widely accepted. Discussion also stressed that the organophosphorus pesticides which caused the intermediate syndrome might or might not possess the capacity to produce OPIDN.

What Is "Neuropathy Target Esterase"?

OPIDN is one of the most mysterious diseases. It is said that the primary change causing OPIDN is phosphorylation in the active site of the specific enzyme "neuropathy target esterase" (NTE). Johnson (1970) who posed NTE hypothesis had first named it "neurotoxic esterase." Johnson's theory was discussed. It was

pointed out that the brain was used as an experimental material to predict the changes in the spinal cord and peripheral nerves. It was also suggested that the relationship between NTE and OPIDN was not very clear.

It is widely known that young animals have less sensitivity than older ones to the delayed neurotoxicity. The reason for OPIDN increase with advanced age remained unknown after the discussion. It is also a well-known fact that the sensitivity to the delayed neurotoxicity is different among animal species. For example, neither rats nor mice present OPIDN, whereas hens are most sensitive. The reason for this remained unknown as well.

The treatment of organophosphate intoxication was discussed. The first measure for treating acute poisoning caused by organophosphates is atropinization. The second specific measure is the administration of pralidoxime. However, there is no therapy for preventing OPIDN. The question was posed: What would be an effective treatment for OPIDN?

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SYMPOSIUM SYNTHESIS

Application of Neurobehavioral Methods in Environmental and Occupational Health[1]

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Based on more than 150 presentations at an international symposium, a critical review is undertaken to assess the current status of research using neurobehavioral methods in environmental and occupational health. Although empirical in nature, this research approach has provided forceful evidence of different manifestations of neurotoxicity and their etiologies. Neurotoxicity is a major adverse effect of chemical exposure and is particularly serious in developing countries. Further development of neurobehavioral methods is likely to benefit from developments in basic neurosciences, and powerful epidemiological designs will be needed to assess their validity when applied to environmental and occupational health. Having documented that neurobehavioral methods can be successfully developed and applied in this field, this research area is likely to show considerable advancement in the future.

NEED FOR A CRITICAL APPRAISAL

After 2 decades of extensive use of neurobehavioral tests in environmental and occupational health, and after four international symposia on this subject, it would appear justifiable to examine critically the achievements, as reflected by the 150 presentations at the 1991 symposium in Tokyo. This assessment would appear particularly necessary for two reasons.

First of all, environmental and occupational health constitute a scientific area that attracts much public attention and that involves considerable expenditures due to the health costs caused by adverse effects and to the economic expenses of prevention. Scientific progress is therefore necessary to expand the documentation as a basis for improved decision-making. Neurobehavioral signs and symptoms are among those most commonly elicited by chemical exposure simply because the nervous system is particularly sensitive both to relevant external stimuli and to adverse effects caused by hazardous exposures. Neurobehavioral dysfunction is likely to be the most prevalent type of adverse effect induced by chemical exposure. This perspective is of serious concern, as a well-functioning nervous system is considered a key to secure an optimal quality of life and high economic activity in society.

The second reason for critically examining this research area relates to its place within the biomedical fields of science. Our achievements also need to be scru-

[1] Presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8–11, 1991, Tokyo, Japan.

tinized from a philosophical point of view. By adopting neurobehavioral methods, we approach problems of mental health strictly from the empirical side. In particular, we tend to prefer limited psychometric and neurophysiological tests and to restrict ourselves to observational studies. We seldom use more comprehensive impressionistic methods, generally because those available are regarded less quantitative and therefore less useful. Thus, we tend to scratch the surface rather than penetrate into the heart of the matter. More importantly, only rarely do we manage to relate our findings to theories on mechanisms, as is done in biological psychiatry. Also, by concentrating on environmental and occupational exposures, our research strategy becomes even more atomistic; too often do we search for correlations between insensitive and nonspecific measures of both causes and effects.

I wanted to emphasize these two viewpoints because they have important bearings on the evaluation that follows. However, before going into more specific detail, one may ask the question, "Why we are actually doing this type of research despite the difficulties?" As I see it, there are several reasons for using neurobehavioral methods. First, medicine as a science is predominantly empirical and we generally require reproducible, standardized observations as a main component of a "proof." Thus, with neurobehavioral methods, we hope to have at our disposal a variety of tests that will produce objective and reliable information on processes in the nervous system. Methods fulfilling these requirements may allow comparisons across languages and cultures, and even, in some instance, across species, thus allowing a better understanding of the processes involved. Further, with valid neurobehavioral methods, we hope to learn more about the natural history of environmental and occupational diseases of the nervous system, and also to identify specific hazards. This research could then lead to intervention. Such objectives are, at least from an empirical point of view, relevant, though not necessarily realistic.

Given this, a critical assessment should then also assess the achievements of neurobehavioral research with regard to its success in allowing conclusions relevant for prevention. Thus, has the use of these methods added significantly to our understanding of the contribution of environmental and occupational exposures to nervous system disorders? More specifically, is this information useful in relation to, e.g., individual exposure limits or wider scale efforts, such as WHO's program for Health for All by the Year 2000? Although neurobehavioral research has certainly spurred some preventive actions, the symposium has mainly emphasized methodological aspects. I will therefore focus my remarks on scientific merits and try to address my concerns under four headings: test procedures, etiologies, time span, and subject materials.

CURRENT STATUS

This symposium has amply illustrated that the study of environmental and occupational health using neurobehavioral methods is thriving. We are seeing continued development and improvement of perceptual, performance, and physiological tests, and advances in computerization and in imaging techniques are rapidly being employed in our field. The span of methods goes from in-depth

examination and interview of single patients in illustrative case reports to more extensive surveys of neurobehavioral symptoms based on structured questionnaires. Some of the traditional tests that have been used for decades are still in use and provide a necessary basis for comparison, while new methods are examined on a pilot project scale. Also, better animal models are being developed, e.g., using neurophysiological methods and learning behavior, so that their relevance to human exposure situations is becoming more evident. Under the auspices of IPCS, a validation study is now being carried out to examine specificity and interlaboratory variability of a screening protocol.

Most of the studies presented have dealt with industrial chemicals of known or suspected neurotoxic potential. Within the chemical sphere, especially metals, solvents, and pesticides are continuously being examined for neurotoxic potentials. The general tendency has been to confirm and extend the dose–effect or dose–response relationships for these chemicals. In addition, a few authors at this symposium have sought to address the crucial issue of structure–activity relationships. Other hazardous exposures include naturally occurring chemicals, such as plant toxins, nutritional factors (including vitamin deficiencies), and life-style (in particular, exercise and alcohol habits).

For the first time in the series of symposia, special emphasis has been put on psychosocial factors, including stress at work, even among ministers of religion. Occupational stress and other psychosocial risks are of critical importance because they have direct implications for neurobehavioral functions, but they may also affect nervous system function indirectly, i.e., through associated alcohol or drug usage, and they could also result in differential attrition in epidemiological studies and thereby influence the outcome of a study.

The time span for most studies remains short. Even experimental studies are generally carried out within one generation of rodents, and often exposures and effects are condensed to within several weeks. The important studies of human volunteers also deal with acute effects only. Most epidemiological studies are cross-sectional, thus offering little insight into the natural history of the neurobehavioral dysfunctions identified and adding only limited information as to the exact etiology. However, pleasant surprises at the symposium were the long-term study of the chronic effects of acute carbon monoxide poisoning and the attempts to examine the effects of solvent exposure in prospective designs. Information of this type is of key relevance in evaluating the long-term health significance of the changes identified. Too little information is available on whether particular neurobehavioral effects are reversible or at least self-limiting. We must also consider the possible significance of environmental and occupational exposures with regard to neurodegenerative diseases. The possibility exists that apparently innocuous exposures with the passage of time could spur the development of such serious diseases as parkinsonism or Alzheimer's disease.

The subject materials have spanned from in-depth studies of single cases, e.g., of 2,6-dimethyl-4-heptanone as a new cause of neurotoxicity, and dramatic case series, e.g., of acute acrylamide poisoning, to larger scale studies of population groups. The epidemiological designs have followed traditional lines of thinking, with only few reports dealing with large population groups. More specifically, an

embarrassingly small number of speakers have dealt with exposures and events that occur either early or late in life. Mostly, we deal with economically active working populations of one sex only and have forgotten the frail subgroups. However, a sign of movement in the right direction is that new emphasis seems to be put on community studies and environmental health. Also, several authors have presented impressive evidence from developing countries. Although some of these presentations were of outstanding quality, they represent only a fledgling effort.

THE WAY AHEAD

Much remains to be understood in relation to nervous system disorders, but recent research has amply documented the useful role of neurobehavioral methods and the significance of environmental and occupational exposures. Let me emphasize a few key areas where neurobehavioral methods will play a major role and where intensified efforts will be needed.

The test batteries adopted by different groups of researchers have shown their usefulness. However, these standardized and, to some extent, computerized batteries have not solved all problems. I noted in particular, that one test battery is now recommended only for subjects with at least high-school education. That will leave out the main part of the world population. However, these tests should not be abandoned because of their limitations, because they will continue to provide an important basis for comparison. Rather, they should be part of a "tiered" approach, with other methods being used for further penetration. As the nervous system cannot be expected to react in a uniform way to different types of exposures, the variety of tests available would suggest that we are, in principle, well equipped to deal with both existing and new chemicals. The direction of further penetration is at this point hard to anticipate and it will, to a large extent, depend on suggestions from basic research, including the development of so-called biological markers.

Due to the complexities involved, studies of interactions in mixed exposure situations have been few. However, this area is becoming more important as the lack of relevant data is becoming more apparent. For example, can we expect that findings from an industrialized country can be directly applied in the developing world? What is the significance of chronic infections, malnutrition, and climate for the effects of neurotoxic exposures? Also, we need to explore the significance of other interactions, not only between individual chemicals, but also in relation to, e.g., alcohol use and psychosocial factors.

These concerns have important implications on the necessary time span of future studies and the choice of subject populations. Too often neurobehavioral studies have been of limited external validity, or the evidence has been regarded nonpositive due to methodological shortcomings. We have used imprecise or semiquantitative measures of exposure, and we have paid too little attention to sources of potential bias, e.g., in relation to confounding. We will need more powerful and, preferably, prospective studies that take into regard the existence of more than one of the risk factors that humans are likely to face. This approach requires multidisciplinary cooperation, and it demands patience and resources.

Our choice of epidemiological study objectives should be guided by results from experimental studies and the development of tests with better predictive validity. With improved test methods used also in experimental settings, we hope that extrapolation will become less troublesome, thus perhaps decreasing the pressure to conduct epidemiological studies for confirmation purposes.

Perhaps the most important suggestion for future studies is that cooperation must be sought between industrialized and developing countries. Evidence presented at this symposium has illustrated the tremendous prevalence of neurotoxicity in the latter countries and the need to assess etiologies and determine dose–effect relationships.

CONCLUSIONS

With the nervous system being particularly sensitive to chemical exposure, modern neurobehavioral methods constitute an important resource for research in environmental and occupational health. Neurotoxic impairment is likely to represent one of the most important preventable groups of disease. Although the use of neurobehavioral methods may be criticized as a reductionist and empiricist approach, this type of study is by no means unique within the biomedical sciences. As few alternatives are available, the approach should be judged with regard to its achievements. Thus, although not a specific topic of the symposium, we have seen several practical instances in which preventive efforts have been initiated or expanded due to new evidence in this area. More importantly, a range of neurobehavioral methods have already been successfully applied in environmental and occupational health, and this approach is certainly sufficiently valid to be considered viable and necessary for continued efforts. However, our epidemiological study designs have sometimes faltered.

The fact that research using neurobehavioral methods has failed in part on some of these accounts should not be taken to mean that this type of activity is wasted. We are only at the beginning of a new era. I consider this area of research one of the most intriguing that I know of, and I find the colleagues that I have met at these symposia to be a very enthusiastic and inspiring group. I have confidence that neurobehavioral research will continue to thrive and to unfold.

In the sense of science philosopher Thomas S. Kuhn, one might well conclude that we are currently working within a paradigmatic instability. Thus, on one hand, we are uncertain to which degree our neurobehavioral methods can contribute comprehensively to the understanding of nervous system disease. On the other hand, our empirical achievements suggest that we are at least moving in the right direction. I am therefore confident that our efforts will continue to contribute scientific rewards, and perhaps we will see a new paradigm emerge, one I hope will also help us to make this a better world to live in.

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