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FIELD WORKER EXPOSURE DURING PESTICIDE APPLICATION

Proceedings of the Fifth International Workshop of the Scientific Committee on Pesticides of the International Association on Occupational Health, The Hague, The Netherlands, October 9–11, 1979

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of the

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OPENING ADDRESS

L. ROSIVAL, chairman

Ladies and Gentlemen,

On behalf of the Scientific Committee on Pesticides of the International Association on Occupational Health I have the honour to welcome you in The Hague at the Vth International Workshop entitled Field Worker Exposure during Pesticide Application organised by the Scientific Committee on Pesticides.

It is a great pleasure for me to welcome among us Prof. Zielhuis, the first chairman of our Committee, whose broad knowledge in the field of occupational health problems and enthusiasm created a fruitful basis for the effective work of this Committee since the year 1971.

I would like to extend my thanks to Dr. Copplestone from WHO, for the acceptance of our invitation to take part in this workshop. We evaluate it as the expression of the great interest of WHO in the work of this Committee devoted to the topical problems which are an integral part of the international programme on chemical safety.

In its resolution WHA30.47 the 30th World Health Assembly requested the Director-General to study, in collaboration with appropriate national institutions and international organisations, the health problems related to the increasing use of chemicals and the long-term strategies needed in this field, and to examine the options for international collaboration aiming at:

i. accelerated and more effective evaluation of health risks from exposure to chemicals;
ii. promoting the use of experimental and epidemiological methods that will produce internationally comparable results;
iii. exchanging information on new chemical hazards to public health;
iv. providing rapid and effective response in emergencies and developing arrangements for mutual assistance among Member States and
v. developing manpower in this field.

In view of these objectives, it was proposed to establish an international programme on chemical safety based on and incorporating current WHO activities. The proposed programme would be concerned primarily with evaluations of health risks from exposure to chemicals. Other priority tasks of the programme would include the promotion of appropriate methods for laboratory testing, epidemiolo-

gical studies and risk and hazard assessments; promotion of effective international cooperation in emergencies and accidents involving chemicals; promotion of technical cooperation among Member States, and training of manpower. The programme would be implemented through a network of national and other participating institutions, coordinated by a WHO central unit, an advisory board and a technical committee.

The eight years of existence of our Committee gives us the opportunity to have a bird's eye view of our activities. These activities were carried out in a world where rapid identification of new or potential chemical hazards as well as appropriate forecasting mechanisms are obviously needed and in a period where the pesticides have been the object of considerable controversy in various countries.

Since 1971, the Scientific Committee on Pesticides has been concentrating upon the following activities:

In 1971, a workshop on Epidemiological toxicology of pesticides was held in Amsterdam with the topics: a) exposure to and body burden of chlorinated hydrocarbon pesticides, b) biological responses in subjects mainly exposed to anticholinesterase compounds, c) biological responses as specified health phenomena, and d) miscellaneous topics. The meeting, with participation of selected scientific workers from Europe, USA, South America, Africa and Australia, resulted in conclusions concerning criteria for the evaluation of professional exposure to chlorinated and organophosphate pesticides. The conclusions and recommendations have been published in the Archives of Environmental Health.

In 1974, a workshop was organized by the Committee in Sofia with the aim of obtaining basic information on toxicological data that are required for registration of pesticides. Experts from Europe, South America and Africa attended this meeting. Agreement was reached on recommendations concerning methodology of experimental toxicology in relation to occupational exposure to pesticides. The conclusions and recommendations have been published in the International Archives of Occupational Health.

In 1975, a workshop on cholinesterase enzymes took place in Cambridge. Selected scientific workers from Europe, USA and Japan discussed topical problems associated with the methodology of cholinesterase determination and with the evaluation of criteria for occupational exposure to organophosphate pesticides.

In 1977, a Committee workshop was organized in Bratislava dealing with the problems of toxicological classification of pesticides and evaluation of health hazards in aerial application of pesticides. Invited experts from Europe and South America and representatives of WHO and ILO attended this workshop. At the meeting, recommendations were approved resulting in suggestions for further activities of the Committee. The conclusions and recommendations were published in the International Archives of Occupational and Environmental Health.

The recommendations from our workshops were very positively accepted in the scientific circles all over the world.

For the XIX International Congress on Occupational Health in Dubrovnik in 1978 our Committee has prepared a section on Pesticides in close cooperation with the organizing committee; two half day sessions were devoted to the worker exposure to pesticides and to the general effects of pesticide poisoning; furthermore a poster session was held. We have had here many good discussions and the new information in this field had made it possible to orient our future work.

During the fourth workshop of the Committee, held in Bratislava in the year 1977, the subject of field worker exposure was chosen for the next workshop in order to discuss in more detail what tests and what supervision is required from an occupational health point of view before clearing the full scale use of pesticides.

Occupational exposure to pesticides, including exposure to a "cocktail" of chemicals, can occur in many different situations. It starts with formulation, continues with transport to the storage or application site and the transfer from containers to the application equipment. The next phases are application, field work involving contact with residues, drift of the pesticide out of the application area, disposal of the unused chemicals or containers, cleaning and repair of application equipment.

The greatest hazard is usually not from the application itself, although there may be applications with greater hazards, for instance aerial application of pesticides.

The greatest hazard exists during handling, mixing and filling of concentrated formulations into mixing tanks and spray equipment. A careful selection of formulation is essential in order to minimise the exposure and the potential hazards. These problems will be discussed in detail during this workshop.

The further critical points in the exposure are the methods of application; environmental aspects, especially meteorological conditions; the position of the worker and the physical effort during the work; length of the working time; personal protective measures and the attitude of the worker.

Concerning these problems and in particular on the medical surveillance of exposed workers directed to the specific hazards to which the individual in agriculture may be subjected, many interesting papers will be presented.

The situation in the developing countries creates a new dimension for our work. In these countries the working populations are affected by the general diseases prevailing in the community as well as by many uncontrolled hazardous agents at work. The successful adoption and application of preventive measures is limited by the vulnerability of the selected populations, the combined exposure to many biological and chemical agents, the unknown biological effects of certain agents

and the administrative constraints.

This situation was discussed also at the eleventh session of the Codex Committee on Pesticide Residues this year in The Hague. A special committee came to the following conclusion.

Many developing countries do not possess adequate facilities to undertake preregistration trials on pesticides and their formulations; toxicity tests; pesticide residue analysis in crops, stored food grains, animal products and processed food products; generation of appropriate data on intake of pesticide residues and impact on the environment. FAO/WHO should intensify its assistance in establishing suitable facilities for these activities either at national or at regional level.

FAO/WHO and other international bodies such as UNDP, UNEP, UNIDO, IAEA should intensify their assistance to developing countries in training personnel involved in these programmes, such as application of pesticides, techniques of sampling, methods of analysis and documentation.

FAO/WHO should prepare a document indicating the presently available facilities and expertise in this field in the developing countries, preferably on a regional basis.

As a collaborative effort among countries, Regional Committees on Pesticides should be established to discuss problems related to pesticides in the region. Seminars and conferences for exchange of technical information and experience gained in this field should be held frequently.

With respect to WHO's proposed new programme on the "evaluation of the effects of chemicals on health", the implications especially concerning developing countries should be examined.

FAO/WHO and other international bodies should prepare digests on toxicological data and efficacy of newer pesticides and formulations and supply these to the developing countries.

Guidelines for good practice in the use of pesticides, for evaluating toxicological hazards, for precautions to be taken and also for legislation and control should be prepared and supplied to developing countries.

In closing my opening address I would like to express the wish that our workshop will be a success and that you all will have a pleasant stay in the beautiful city of The Hague.

OCCUPATIONAL PESTICIDE HAZARDS IN THE ACTIVITIES OF WHO'S OFFICE OF OCCUPATIONAL HEALTH: HEMATOLOGICAL CRITERIA FOR HEALTH ASSESSMENT

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ABSTRACT

Pesticides are dealt with in the activities concerning health-based permissible levels in occupational exposure to harmful agents, early detection of health impairment in occupational exposure to health hazards, health of agricultural workers and occupational hygiene technology.

The authors analyze the two former activities. They emphasize the necessity to base the decisions on the evidence of adverse effects of long-term exposure, not only on acute toxicity. Blood dyscrasias attributed by some authors to the pesticides (especially gamma-hexachlorocyclohexane) are used to demonstrate gaps of knowledge and needs for further research.

Occupational pesticide hazards are dealt with in the following activities of WHO's Office of Occupational Health: health-based permissible levels in occupational exposure to harmful agents, early detection of health impairment in occupational exposure to health hazards, occupational health technology and occupational health-care in agriculture. In the two latter activities, methods for measuring blood acetylcholinesterase will be considered, and the agricultural workers' health risk of pesticide poisoning will be evaluated together with other agricultural health hazards. The former two activities will be discussed in detail in this paper.

Abbreviations used: (a) hematology: Band = band neutrophils, Eo = eosinophils, Gr = granulocytes, Hb = hemoglobin (concentration), Htc = hematocrit (VPRC, volume of packed red cells), Ly = lymphocytes, MCHC = mean corpuscular hemoglobin concentration, Mo = monocytes, Pl = platelets (thrombocytes), RBC = red blood cell (count), Rtc = reticulocytes, Seg = segmented neutrophils, WBC = white blood cell (count) (ref. 80).

(b) pesticides: DDT = dichlorodiphenyltrichloroethane. Metabolites of DDT: DDA = dichlorodiphenylacetic acid, DDE = dichlorodiphenyl dichloroethylene. HCH = hexachlorocyclohexane (benzene hexachloride; gamma-isomer = lindane). OP = organophosphorus compounds.

Based on the general conclusions of the Expert Committee on Methods used in Establishing Permissible Levels in Occupational Exposure to Harmful Agents (ref. 51), a project for recommendations of health-based permissible levels for chemical substances has been launched. Recognizing that thousands of toxic agents are found in the workplaces, priorities had to be set for the selection of substances to be included in this effort. Within each of the major groups of substances, at most only four or five agents could be handled initially. It was thought that the most appropriate criteria for selection of hazardous agents should involve among others:

1. Distribution and use of the substances, number of workers exposed throughout the world.

2. Potential for serious (disabling) morbidity and/or mortality to develop as a result of occupational exposure.

3. Availability of experimental and epidemiological information on which to base permissible levels.

The above criteria were then applied to a number of toxic metals, organic solvents and pesticides and the most appropriate substances were selected for further processing. In the group of pesticides, malathion, carbaryl, DDT, aldrin and dieldrin were chosen. The corresponding document for health-based permissible levels shall be prepared in the near future.

As the WHO recommended permissible levels are based predominantly on epidemiological or clinical evidence of non-adverse effect levels, a close link exists between this project and the early detection of health impairment due to occupational exposure to harmful agents. Both fields need to be backed by sufficient data obtained by epidemiological studies, and further research is to be encouraged whenever lack of knowledge is apparent. Some of the typical problems will be discussed on the three groups of above-mentioned pesticides, i.e. organophosphorus compounds (further OP), carbamates and chlorinated hydrocarbons in order to call attention to the collaborating investigators.

With respect to the high acute toxicity of some of the OP and carbamates, permissible levels of exposure established in different countries are based mainly to prevent acute poisoning. Activity of blood acetylcholinesterase is usually considered as a measure of health risk. It is also believed that if inhibition of acetylcholinesterase is prevented, other effects will be prevented too, including those which are claimed by some authors to be a result of long-term low-level exposure. However, some reports brought suggestive evidence that at least some adverse effects may occur independently of cholinesterase inhibition (ref. 10).

The acute toxicity of organochlorine insecticides, which is generally lower than that of organophosphates, is usually not decisive for establishing permissible levels Although specific phenomena of health impairment due to chronic exposure are not known, some reports again point to the possibility of influencing the morbidity of workers. Among the most heavily discussed questions is the possible hematotoxic effect of pesticides, because of the fatal course of some of the hematological diseases attributed to pesticide exposure. However, it has not been decided yet if blood dyscrasias should be included into considerations for early detection of health impairment and for health-based permissible levels.

Therefore, a survey of data from literature has been prepared. The three main sources of information on hematological changes in pesticide exposure - case reports, epidemiological studies, and experiments on volunteers - were analyzed in order to get scientifically sound conclusions on hematological hazards due to pesticides.

CASE REPORTS

The physicians observing single patients were the first ones who raised the question of the hematotoxicity of pesticides. Sixty-five case reports found in the literature are summarized in Table 1. The descriptions of cases are rather stereotype: exposure to pesticide(s) is reported in the history of the patient suffering from blood dyscrasia, usually from acute cytopenia. As no contact with other well known hematotoxic drugs or chemicals is found, the causal relationship between the pesticide exposure and the disease is supposed. Less than one half of the patients were exposed in their occupations, whereas nonoccupational exposure prevailed. Attempts of quantitative definitions of exposure levels were made only exceptionally, e.g. in terms of concentrations of pesticides found in the blood or in tissues post mortem. The duration of the exposure to pesticides lasted for some days (e.g. disinsection a flat) to several years (usually in agricultural workers). The onset of the blood dyscrasia started during the contact with the pesticide, or days, weeks and even several months after its termination. In about one half of the patients, the course of the disease was fatal. There was no consistency as to the types of nonoccupational use of pesticides, with the exemption of several patients who lived in rooms where vaporizers of HCH had been used (refs. 25, 79). In nonfatal cases, reexposure to pesticides was prevented as a rule, but there are a few observations of relapses of the diseases after reentry to the previous activity or after repeated contact with the pesticide formulation (refs. 3, 56, 63). Blood dyscrasia can be attributed to pesticide exposure with reasonable probability only in these patients as well as in a few patients who developed immunohematological alterations (refs. 56, 70). In the prevailing majority a simple coincidence of

TABLE 1

Case reports on blood dyscrasias attributed to pesticides.

Substance(s)	Blood dyscrasia	No. of patients	References
DDT	thrombocytopenia ("purpura")	7	38, 42, 65
	pancytopenia	2	59, 63
DDT + dichlorvos	thrombocytopenia	1	41
DDT + pyrethrum	agranulocytosis	1	82
HCH	pancytopenia	21	18, 25, 45, 46, 69, 70, 79, 81
	anemia	3	48, 75, 79
	thrombocytopenia	2	69, 70
HCH (+ benzene)	leukemia, acute	2	33
HCH + DDT	pancytopenia	9	1, 3, 16, 66, 70, 81
HCH + DDT + chlordane	pancytopenia	1	49
HCH + DDT + OP	pancytopenia	1	70
chlordane	pancytopenia	3	30, 60
	leukemia	2	30
	anemia	1	17
chlordane + OP	pancytopenia	1	30
chlordane + OP + carbamates	pancytopenia	1	30
chlordane + toxaphene + heptachlor + dieldrin	pancytopenia	1	56
chlordane + OP + other	leukemia	1	30
OP	pancytopenia	3	19, 57, 77
OP + carbamates	agranulocytosis	1	83
dichlorvos + DDT + HCH	leukemia	1	28

NOTE: the term pancytopenia has been used in the Table consistently instead of different terms used in original papers, like aplastic anemia, hypoplastic anemia, myelophthisis, etc. characterized by the reduction of all formed elements of the blood.

blood disease with pesticide exposure is at least as good an explanation as their causal relationship.

EPIDEMIOLOGICAL STUDIES

Mixtures of pesticides

Although a large number of epidemiological studies on the health effects of pesticides exists, only few of them have included hematological investigations. Most of the studies deal with agricultural application or industrial production and formulation of a large variety of pesticides and do not enable identification of any specific effect of a single pesticide. The main results of these epidemiological investigations are summarized in Table 2.

TABLE 2

Clinical hematology in workers occupationally exposed to combinations of pesticides in agriculture and/or industry.

Abbreviations used: E = exposed group, C = control group.

No. of persons	Hematology	Exposure level	Refs.
E 148	Low Hb levels in 23%, associated mainly with exposure over 5 years. The value for control group is not given.	Exposure mainly to HCH in antimalaria campaign. Level not estimated.	78
	$\bar{x}$ s.d.		
E 441	WBC 5798 ± 109	About 1% of the toxic dose	8, 34
Ep 170	5785 ± 208	absorbed at work (e.g. malathion	
C 162	6719 ± 187	5.03 mg/h/person, carbaryl 30.83	
	No blood dyscrasias. Incidence of leukopenias showed a positive correlation with the duration of exposure less than 5 and more than 10 years.	mg/h/person, parathion 5.85 mg/h/person).	
Note: Ep = population living in the area with extensive pesticide application.			
	Hb(%) WBC $P1.10^3$		
E 1637	75 ± 1.1 6100 ± 31 220 ± 2.1		
C ?	81 ± 3.1 6900 ± 80 317 ± 4.1	Exposure to different chlorinated compounds (like chlorinated methane, dichloroethane, epichlorhydrine, etc.), not only to pesticides. Number of subjects exposed to pesticides not defined.	40
	Pancytopenia in 3 workers exposed to DDT and polychloropinene		
E 120	No blood dyscrasias.	Exposure duration average 12 years.	64
C 50	WBC, RBC, Htc higher in some groups of the exposed workers (absolute values not quoted).	Elevated DDT level and diminished acetylcholinesterase activity in blood of some groups.	
E 70	No blood dyscrasias. Ly (%) 36.2 ± 8.0 Seg (%) 53.7 ± 8.5 Hb (g/100 ml) 15.7 ± 1.1	Serum concentrations (ppb): p,p'DDT 9.2 ± 11.7 p,p'DDE 29.3 ± 21.1 dieldrin 3.6 ± 6.3 Beta-HCH 2.3 ± 2.9	76
	Inconsistant differences between some of the occupational groups and controls in Hb, Htc, Band, Ly, Mo, Eo.		
	No correlation between serum DDE and hematology, with the exception of Band.		
C 30	Ly (%) 37.9 ± 8.3 Seg (%) 52.2 ± 8.2 Hb (g/100 ml) 15.7 ± 1.1	p,p'DDT 3.6 ± 2.7 p,p'DDE 19.6 ± 9.5 dieldrin 1.1 ± 1.6 Beta-HCH 1.3 ± 1.5	

	Hb(%) WBC		
E_1 586	80.4 ± 0.20	6900 ± 40	Slight acute poisoning in the history of 19% of the workers. 5
E_2 515	75.6 ± 0.42	6000 ± 85	
C ?	90.0 ± 0.56	7000 ± 60	
	Lower average values also for RBC, Eo, Seg, Band, Mo.		
Note: E_2 had higher general exposure to pesticides than E_1.			
E 149 C 86	No blood dyscrasias. Slightly lower % of Eo and Mo.	In heavy exposed group 50 to 100 times higher exposure than in general population.	11
E 58	No blood dyscrasias. Several times leukocytosis up to 17000, shift to the left, toxic granules.	Not described.	26
E 585 Ep 211 C 101	Neither blood dyscrasias nor significant blood count changes. Cytochemically diminished activity of peroxidase, cytochrome C, cytochromoxidase in leukocytes.	Duration of exposure 5 to 10 years, level not given.	39
E 2620 C 1049	No blood dyscrasias. Hb (g/100 ml) a) C 15.2 E_1 14.3 b) C 16.1 E_3 15.4 MCHC (%) a) C 33.7 E_1 33.0	Serum concentrations (median, ppb): DDT DDE C 4.5 32 E_1 5.2 34 E_2 5.8 33 E_3 6.5 41 Median exposure duration in E_3: 13 years.	53

Note: E_1, E_2, E_3 denote exposed groups of different occupations, a) and b) denote different localities where investigations were carried out.

In addition, one questionnaire survey of health of 1396 pesticide exposed agricultural workers (1105 responded) was carried out. Only three cases of blood dyscrasias were reported. Two of them had been known before the exposure started (lymphogranuloma and leukemia); in the third patient leukemia developed during the work with pesticides (ref. 68).

Single pesticides

In plants producing pesticides, the exposure level is often higher than in field application of pesticides, and it is usually considered as less mixed. However, the raw materials used for syntheses and the intermediate products may reach high concentrations in the air of workrooms and also exert adverse health effects. The results of main reported studies are presented in Table 3.
Two more studies on workers in chlordane production (refs. 2, 61) are quoted by Fishbein et al (ref. 15); no blood dyscrasias were found.

TABLE 3

Clinical hematology in workers occupationally exposed in pesticide production.

No. of persons	Hematology	Pesticide	Exposure level	Ref.
		DDT		
E 35	No blood dyscrasias. Ratio Ly:Gr greater than 1 in 5 subjects (14% = normal range).		Exposure duration 11 to 19 years. Average of DDT derived compounds in serum (total as p,p'-DDT) 0.59 ppm (general population 0.07 ppm).	43
E 40	No blood dyscrasias.		DDA in urine 0.12 to 7.56 ppm.	58
		HCH		
E 40 C 40	No blood dyscrasias. E C WBC 8630 7000 Seg 5379 3989 Rtc (%) 2 1.5		HCH in blood: E 11.9 ppb, C 0.1 ppb.	52
E 79	No blood dyscrasias. Elevated number of Mo.		Mean blood HCH levels in four different groups 2.2 to 30.6 ppb.	62
E 59	Hypochromic anemia 12 times, leukopenia 16, shift to the left 22, eosinophilia 26, thrombocytopenia 11 times. (No absolute values or limits for normal values are given).		HCH in the air of workplaces up to 34 mg/m^3.	6
		Chlordane		
E 15	No blood dyscrasias.		Exposure duration 1 to 15 years. Chlordane in the air of workplaces 0.012 to 0.017 mg/m^3.	15
		Dieldrin, aldrin, endrin, telodrin		
E 233	No blood dyscrasias. Positive correlation between DEq (raising from <0.01 to >0.40) and Hb level.		Exposure duration over 4 years. DEq <0.01 to >0.40	32
		OP		
E 130	Slight hypochromic anemia, leukopenia, thrombocytopenia.			73
E 112 C 92	Slight decrease of Hb.			14

Note: DEq = dieldrin equivalent (sum of the serum concentration of dieldrin and the ten-fold concentration of telodrin, both in µg/ml).

Other studies

Only few attempts were made for case-reference studies. The most extensive one is by Komarova (ref. 39) who investigated 1083 patients suffering from different hematological diseases (leukemias, hypoplastic anemias, agranulocytoses, hemorrhagic diseases) and 694 reference patients with other somatic diseases, mainly cardiovascular, gastrointestinal or respiratory. The history of exposure to pesticides in occupational or living environment was announced by 17.4% of the hematological patients and only by 6% of the other group. The methodology of history-taking and of forming the both groups is not described, so that it is difficult to assess the validity of the results.

Several authors tried to correlate the concentrations of chlorinated hydrocarbon pesticides (DDT, HCH and their metabolites, dieldrin) in blood and tissues (bone marrow, liver, fat, lymph nodes) to the pathological changes, but the discrepancies in their results are striking. Some investigators were unable to prove any difference between the patients suffering from blood diseases and from other diseases, or those persons who suddenly died of accidents (refs. 27, 54). On the contrary, other authors found higher concentrations of pesticides in patients who suffered from or died of hematological diseases (refs. 35, 36, 39, 72, 74).

EXPERIMENTS ON VOLUNTEERS

Hematological investigations were several times included into pharmacokinetic or clinical studies of chronic pesticide exposure on volunteers. Hayes et al (refs. 23, 24) and Morgan and Roon (ref. 55) did not observe any hematological changes in subjects to whom DDT was given in doses up to 35 mg/day for periods up to 21 months. Likewise, daily doses of dieldrin up to 210 µg for 18 months did not cause any hematological changes. (ref. 29).

DISCUSSION

Only those patients were summarized in Table 1, whose clinical picture had been described in the papers and the type of exposure given. In several hematological surveys of blood dyscrasias and their causes and in Registries of drug-induced blood dyscrasias (ref. 4) additional numbers of cases attributed to pesticide exposure are given but without any details. We have not included them into the Table 1 deliberately, as it was impossible to identify the subjects (they might have been the same as in case reports) and their exposure. Moreover, these surveys stand between case reports and epidemiology; their weakness is that they do not respect epidemiological principles of proving causal relationship, and do not bring enough details of single patients. Anyway, the percentage of supposed pesticide-caused blood dyscrasias is low. Among 865 cases of pancytopenia and erythroid hypoplasia, only about 20 were reported to have had exposure to pesticides, and in about 40 further patients the exposure to pesticides was

combined with the administration of other drugs, mostly with known hematotoxicity (ref. 80).

Among the cases in Table 1, all but five patients suffered from cytopenia. The whole number reported over a period of more than 30 years is surprisingly low. Pancytopenias are, of course, relatively rare diseases, and if the suspected chemical has not strong hematotoxic properties (like benzene), its contribution to the increase of the incidence of the disease can hardly be significant. It is estimated that in about 50% of the patients with cytopenias no cause of the disease is found or suspected. Of 1067 cases of aplastic anemia reported to the AMA Council of Drugs, 376 of the patients have received chloramphenicol therapy, whereas other drugs or chemicals appeared to be relatively infrequent offenders. A few drugs only have been reported as being associated with hypoplastic anemia in 20 or more persons, while a large number - including pesticides - have only rarely been reported as being associated with this dyscrasia (ref. 80). The risk of developing fatal aplastic anemia may be in the range of 1 in 60,000 or 1 in 20,000 chloramphenicol treated patients, which is about 13 times higher than the frequency of aplastic anemia in the population not so exposed (ref. 80). The number of cases attributed to pesticides is at least one order lower than those attributed to chloramphenicol. Therefore the difficulty of any statistical approach.

The low number of cases connected with the exposure to OP as well as DDT and chlordane (Table 1) seems not to favour the possibility of causal relationship. In fact, the broad use of pesticides makes inevitable the simple accidental coincidence of exposure and any disease. However, more than half of all cases were attributed to HCH alone or to its combination with other pesticides. This relation does not correspond to the equivalent of HCH used in comparison with other pesticides. The problem was brought to attention as early as 1953 (ref. 25), and still has not been decided firmly.

In almost all of the epidemiological studies minor, but statistically significant hematological differences between the exposed and control groups were proved. However, the average values and the standard deviations fall mostly into the normal range. Moreover, there is no consistency in the described changes, i.e. both increases or decreases of the numbers of the same cells were found. This fact could imply that not always all necessary precautions for exact epidemiological studies were maintained. Other factors than pesticide exposure could have been undiscovered. Another possible explanation could be the inhomogenity of exposure of different groups. In fact, there are no hematological observations of the effects of single substances, with the exception of a few studies on volunteers. Even the simplest formulation contains also other substances than the pesticide (and its inevitable technical impurities). The complexity of the exposure in

pesticide production and especially in field application has been stressed above, and is reflected in some analyses. The changes found in workers in OP production (refs. 14, 73) are considered not only due to a direct hematotoxic effect of the substances taking place in OP production, but mainly as secondary to functional changes of the liver and of gastric secretion, which are claimed to have been proved. As Heinz bodies were also found in erythrocytes, a hemolytic component due to aromatic amino- or nitrocompounds, used in the production, is to be assumed. No doubt this is the case in the observation of Levina and Kurando (ref. 44) from the production of carbamates; occurrence of methemoglobinemia (up to 28%), Heinz bodies and decrease of Hb can be attributed most likely to the exposure to aniline.

The most important fact remains that no serious blood dyscrasias were reported in epidemiological studies. It has been also proved that mortality on aplastic anemias did not increase since the modern pesticides had been introduced (ref. 21), and that mortality on aplastic anemias and leukemias did not differ in areas with high pesticide application in comparison to the general population (ref. 50). If hematological changes were registered in epidemiological studies, they can be explained as a part of general unspecific reactions of the organism to the work load (including the chemicals), like numerous subtle biochemical changes proved in these studies too.

Some observations do not exclude, however, the possibility of individual hypersensitivity or of an immunological alteration due to some pesticides. Antierythrocytic antibodies were found in 19 of 24 healthy workers exposed mainly to DDT (ref. 67). Recently, immune hemolytic anemia has been induced in dogs by pirimicarb (ref. 31). Migratory activity of leukocytes of patients with blood dyscrasias - but not of healthy subjects - could be depressed in vitro by adding DDT, DDE or HCH to the cultivating media (ref. 71). The quoted observations of positive Coomb's test or leuko- and thrombocytoagglutinins (refs. 25, 79) are also in favour of this possibility.

The suspected hematotoxicity of pesticides has been analyzed in several surveys. The authors of case reports are convinced of the causal relationship of blood dyscrasia to pesticide exposure, although reasons supporting their views are given only in the minority of observations. Sudden onset of the disease and again sudden improvement after cessation of the exposure are believed to give some support, as well as relapses after reexposure, or positivity of immunohematological tests (Coomb's test, agglutination of blood elements, etc). However, such evidence is found only exceptionally.

Other authors are more cautious in considering association versus causation (ref. 84). They do not accept easily that the coincidence of exposure followed by a disease is a satisfactory proof of causation (refs. 7, 20, 47). The lack of

evidence of blood dyscrasias in epidemiological studies is the main argument for not accepting the general hematotoxicity of pesticides (refs. 9, 13, 22, 37). The number of subjects included into epidemiological studies has increased so that this opinion seems to be justified. The members of an international workshop (ref. 12) did not even include hematological examinations among the recommended tests for the programme of investigations of long-term effects of pesticide exposure. However, the exceptional possibility of developing bone marrow failure on the basis of individual hypersensitivity cannot be rejected.

In conclusion, early diagnostics of occupational diseases rely on signs which appear early, are specific and develop gradually. Health-based permissible levels of exposure are derived from known dose-effect relationships. Hematological examinations in pesticide exposure cannot contribute to these objectives. There is no conclusive evidence that pesticides are the cause of blood dyscrasias. The only exceptions seem to be the extremely rare cases of adverse hematological reactions in subjects having possible individual hypersensitivity, mainly due to HCH. In those patients blood dyscrasias appeared suddenly, unexpectedly and even after minimal exposure. No preceding hematological examination could have prevented them, and there is no possibility to establish any permissible level of exposure based on dose-effect relationship. These diseases seem to be very rare and it is extremely difficult to prove the causal relationship. It is to be hoped that future development of knowledge and methods in hematology will provide the investigator with more efficient diagnostic tools.

The validity of these conclusions is limited to the groups of existing and broadly used pesticides and must not be enlarged to any new pesticide which will be synthetized in the future. Then full testing - including hematology - is to be performed in laboratory conditions as well as in clinical observations and epidemiological studies.

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METHODS FOR FIELD ASSESSMENT OF EXPOSURE TO PESTICIDES

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ABSTRACT

There is a need for much more information from field surveys on exposure of operators to pesticides during application. The available methodology of carrying this out is reviewed. The only advance in recent years has been the development of a spectrophotometric field kit for cholinesterase determination. There is a need for research into methodology as well as for actual surveys.

INTRODUCTION

Field assessment of exposure to pesticides may be for two main purposes: first, as a routine measure for the surveillance of personnel who are subject to a certain degree of hazard for prolonged periods of time; and second, in order to obtain information on the likely exposure of operators or the general public during specific pesticide applications. The second purpose may give an indication as to whether routine surveillance may be needed or it may be used to assess the level of protection and the type of preventive measures necessary to ensure minimum hazard in the application of the pesticide. The need for much more data of this type has been emphasized by two WHO Expert Committees on the Safe Use of Pesticides (refs. 1-2), and the work has been given impetus by the requirement of some registration authorities that human exposure data should be obtained under actual conditions of use wherever possible.

The methodology of carrying out such tests has not in general kept pace with the need, and there are no field methods which do not have some practical disadvantages or which do not require some degree of extrapolation. However, in the dynamic situation of field exposure, it is unwise to expect high degrees of correlation in the results. The factory situation tends to be more stable; the uncontrollable variables that may influence exposure in the field leads one to expect wide scatter in the results and to be wary of too close concordance (ref. 3). Rather, it is the hard task of the field investigator to examine this scatter, to try to decide how

much of this is due to ordinary (as compared to extraordinary) exposure, on this basis to calculate the highest probable exposure, and then to determine whether this exposure is acceptable or to define the additional preventive measures needed.

In the case of some pesticides particularly the organophosphates, it is possible to measure exposure in the field by the estimation of cholinesterase activity. At present, there are three main methods of doing this:

1. colorimetric changes in test papers; these usually refer to the plasma only and the separation of blood in the field is sometimes difficult.

2. the colorimetric Tintometric method, originally described by Edson (ref. 4); this measures whole blood activity which is more relevant than plasma measurements, but it relies on the cholinesterase level of the operator as the basis of comparison. An advantage of the commercial kit, which incorporates improvements, is its portability and cost.

3. a spectrophotometric method which has recently been developed by WHO (ref. 5) based on the Ellman (ref. 6) method: it requires some laboratory backup and expertise but is suitable for field use, particularly where a greater degree of accuracy is required.

Another method of assessing exposure is the measurement of metabolites excreted in the urine, and this has been used for the assessment of exposure to some pyrethroids, such as permethrin. This is not strictly a field method since the analysis itself is a demanding laboratory procedure, but the taking of samples has to be carried out in the field. It is very difficult to expect 24 hour specimens to be collected accurately and in many cases, only casual specimens may be available. The estimation can be standardized either on creatinine content or on specific gravity. Although the former is usually used, I wonder whether it is possible that the latter may be a more appropriate method if the subjects belong to races consuming a considerably different protein diet from that found in temperate zones and developed countries.

For some compounds (e.g. endosulfan , decamethrin), no method of estimating either effect or excretion is readily available. However, some general data on exposure can be obtained from the use of exposure pads using a method based on the work of Durham and Wolfe (ref. 7) and outlined in the WHO Standard Protocol for the Survey of Exposure to Organophosphorus Pesticides in Agriculture (ref. 8). This is a more general method and requires considerable extrapolation, but using a "worst probable usual case" approach, it is possible to arrive at general conclusions. This method has been used by WHO in the field with acceptable results (ref. 9) but, like the assessment of excretion products, requires sophisticated analysis to assay the very small deposits of the compound on the pads.

Sampling in the field, can give rise to a number of errors. The need to avoid

contamination of samples is obvious but this is not always easy, particularly if the fingers are used for capillary blood sampling. Venepuncture is more accurate, but often men object to it and the number of samples that can be taken daily or on successive days is necessarily limited, if only for humane reasons. As mentioned above, urine samples tend to be uncertain in supply unless taken under supervision. Contamination of urine is less important when the compound to be analyzed is a metabolite.

The conservation and transportation of specimens is apt to be complicated and to produce unexpected difficulties. It is essential that spiked specimens should be examined well before an actual survey if this is possible. For example, the transport of exposure pads in plastic bags - the simplest method - is usually not possible due to adsorption of the chemical from the pads by the plastic. For some compounds the pads can be rolled and put dry into dry glass test tubes but in one case, it was found necessary to transfer dimethoate contaminated pads in benzene as the compound broke down considerably during the transportation period. Glass jars often have plastic liners to caps that may cause analytical difficulties. Therefore it is essential that the field worker works closely with the chemist in the design stage of any survey, and that prepared samples should be subjected to the same treatment as it might be expected that the field samples will undergo.

The importance of field surveys and the assessment of exposure of operators to pesticides under actual application conditions is that it provides essential data and is the only alternative to extrapolation from animal exposures. This is true human toxicology. Not only is there a need for more surveys using the tools available, but there is a need for the refinement of methodology which, except for the new WHO kit, has developed little in the last 10 years. As scientists, we sometimes decry the extrapolations that have to be made in field work while ignoring the extrapolations, possibly of greater degree, that we use daily in working with animal data. When I was teaching public health to postgraduate students, I used to tell them that they couldn't learn that subject with clean shoes. The same applies to human pesticide toxicology.

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Note: Unpublished documents are available to interested scientists on request to Pesticide Development and Safe Use, WHO, 1211 Geneva 27, Switzerland.

FIELD STUDIES MONITORING EXPOSURE AND EFFECTS IN THE DEVELOPMENT OF PESTICIDES

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ABSTRACT

Safety recommendations for handling and application of new pesticides are based on the extrapolation of experimental toxicity data from animals to man. Although the soundness of this approach has proved satisfactory in practice, experimental toxicity tests and/or the use pattern may provide specific indications for the need to check this extrapolation by observation on man himself. Medical surveillance of exposed workers in the early phases of development of new pesticides, and of workers engaged in the development of novel formulations or new application techniques in the field will provide the necessary data for this check when the exposure and possible biomedical effects are monitored.

A well controlled dose-excretion study is essential for the identification in man of the most suitable metabolite(s) to monitor biologically and for an assessment of its excretion rate(s). Conditions which such studies should meet in order to be ethically and morally acceptable are discussed.

A short introduction is given to the design and procedure of a field study that was carried out to obtain information on the possible health effects of a pyrethroid applied by means of hand held ultra low volume apparatus.

INTRODUCTION

At the previous Workshop of the Scientific Committee on Pesticides of the International Association on Occupational Health held in Bratislava in 1977, it was decided to devote this Workshop to the subject of "Field worker exposure during pesticide application". The recommendations of the previous Workshop include the following items:

- further development of methods of measuring exposure;
- development of practical exposure tests for application on a wide scale;
- more attention should be paid to the use of information obtained from human studies on the determination of dose-response relationships;
- manufacturers are well placed to assist in collecting the data from coordinated field studies in order to give scientifically sound advice on health protection

of workers in the field.

As a medical toxicologist with a background in occupational health and working for a company which manufactures pesticides, I am very grateful for the invitation to give a short presentation on ways in which industry develops the knowledge and experience necessary to assure the safe use of pesticides in the field.

Nowadays, in the development of pesticides extensive animal testing, including short term and long term tests, is carried out to assess the toxicity of a candidate compound. Safety recommendations for handling and application are based on the extrapolation of these animal data to man. The long history of safe use when pesticides have been handled according to such recommendations, bears out the soundness of this approach. Sometimes, however, cases arise where it is useful to check the extrapolation from animals to man by medical observations on people who are occupationally exposed, paying special attention to effects that the animal data may point to. Such observations assist in:

- identifying and determining the degree of specific hazard of specific applications which may involve different characteristics of exposure;
- developing practicable parameters of exposure and of biomedical effects which can be used in occupational health surveillance programmes.

I intend to deal in this paper with procedures for the development of data on exposure and possible biomedical effects on field workers involved in the application of a new pesticide at an early stage in its development.
In some respects, the development of such data is analagous to the clinical observations made during the early stages of development of a new pharmaceutical product. In this case also, the data are developed to confirm the safe use of the product in practical conditions. In the case of pesticides, however, there are two important differences. Firstly pesticides are not administered to the applicator but to the crop, hence exposure by the applicator is of an incidental nature. Secondly the toxicity testing of pesticides is performed only on animals.

SOURCES OF INFORMATION ON POSSIBLE HUMAN HEALTH HAZARDS IN EARLY PHASES OF DEVELOPMENT.

Data on possible human health hazards can be obtained from:

- close occupational health surveillance of laboratory workers testing the compound for efficacy in the early phases of research and development; A drawback may be that these laboratory workers are exposed to so many different compounds that no compound specific data can be obtained.
- medically supervised field trials carried out by Research and Development (R and D) people or their extension field workers for the purpose of efficacy testing under field conditions;

- close occupational health surveillance of workers engaged in pilot plant manufacturing and formulation of pesticides;
- special studies on domestic insecticides. In certain specific circumstances it may be indicated to study exposure and biomedical effects in domestic applications under controlled conditions in the development phase, i.e. prior to marketing.

The R and D field studies seem to be the most suitable for our purpose to assess possible human health hazards and to develop methods for monitoring both exposure and health effects in practical application. This is a well controlled situation with selected and experienced sprayers who will understand the importance of intensive measurements and examinations from which they themselves will benefit in the first place.

Thus an R and D field study has in addition to the efficacy testing of the compound the following objectives:
- assessment of possible human health hazards under the condition of practical application by measuring exposure - if possible also absorption - and by measuring biomedical effects, if any, which may result in establishing dose-effect/response curves, although mostly only a limited section of these curves will be covered;
- assessment of health hazards arising from specific application techniques or from novel formulations which may result in modifications to safe use recommendations derived from animal data;
- provision of occupational health data which would be considered in the decision making process leading to large scale manufacturing and marketing;
- development of simple screening tests for both assessment of exposure and (prediction of) biomedical effects under conditions of practical application by both professional and non-professional sprayers.

EXPOSURE MONITORING GROUP

In order to be able to advise on and to carry out such studies a group of experts, called the Exposure Monitoring Group has been formed in my company. This group is a multifunctional working group covering the fields of toxicology, occupational health and industrial hygiene.
Drs. Prinsen and Van Sittert will report in this Workshop on an extensive field study which was undertaken to make sure that there were no unexpected health hazards associated with hand held ULV spraying of the pyrethroid selected for this purpose.
This field study consisted of the following elements:
- measurement of dermal exposure with aluminium foils and surgical gauze according to the recommendations of WHO (ref. 1);
- measurement of exposure via the respiratory route with personal air samplers;

- measurement of excretion of metabolites in the urine (biological monitoring);
- general medical and extensive neurological examination;
- extensive biochemical tests of the blood;
- measurement of selected peripheral neuro-muscular function by examination of conduction velocities and action potentials.

The study was designed in such a way that by measuring the nerve function several times before and after spraying, individual variability could be controlled and any change outside this variation could be detected. Changes in the neuro-physiological parameters in an individual can be caused by many factors. Changes which are consistent in a group of persons who have a specific factor in common which could cause the changes, may indicate that indeed this factor was active. Therefore in this study a group of 8 pyrethroid sprayers were examined before and after spraying. An additional group of 8 persons who sprayed a blank formulation was also examined.
In a follow-up study the 8 persons who sprayed initially the blank formulation sprayed cotton during one season with the selected pyrethroid. This field study provided valuable data on skin and inhalational exposure during spraying, on the excretion pattern of the metabolites in the urine and on the health of the sprayers.

DEVELOPMENT OF SIMPLE PARAMETERS FOR HEALTH SURVEILLANCE

An extensive field study, such as we have done on the hand held ULV application of a pyrethroid, is extremely labour-intensive and should therefore result in (relatively) simple techniques and simple parameters or indicators which can be used in routine health surveillance.
Indicators of the dose are frequently used in routine health surveillance. These estimates of the dose are then related to effects and responses which previously have been established in animal studies on dose-effect/response relationships.
As an indicator of the total absorbed dose we measured pyrethroid-metabolites in the urine of the sprayers and related this to the dermal and respiratory exposure.
Although this procedure gives valuable information, a well controlled dose-excretion study in man is the only way to assess the excretion rate of metabolites and subsequently to be able to identify the most suitable metabolite for monitoring and also to identify points of time for urine sampling. We performed such a controlled dose-excretion study by administering an oral dose of the pyrethroid and collecting subsequently the urine over a time period of 48 hours. Metabolites were determined in 24 hours urine portions. Such a study is the most reliable way to obtain information on the relationship between excretion and exposure.
Controlled dose-excretion studies are ethically and morally acceptable when they are in compliance with the Declaration of Helsinki (ref.2) prepared by the

World Medical Association and with the Nürenberg code (ref.3).
It is, in particular, necessary that:
- full scale animal toxicology has been carried out;
- metabolism studies in animals have been done;
- a dose can be calculated that confidently can be considered as safe;
- the persons who are examined cooperate of their own free will, are fully aware of and understand the objectives of the study and are well informed about and understand the toxicological background and the health aspects.

In practice this means that it is preferable to have the assistance of a few professionals in the field of medicine and toxicology, although this cooperation may ask for special measures as to the control of the protocol and the actual dose-excretion study.

As a result of the work done in our dose-excretion study and in our field study, we have identified the most suitable metabolite, we have information on the excretion rates and on how and when the urine should be collected. Most important was that we were able to relate the metabolite excreted in the urine of the exposed workers to an oral dose, and via an oral dose to the results of our biomedical examinations.

As I have said already, the dose effect/response relationship which can be assessed in field studies will only cover a limited section. For exposure is limited and biomedical effects should be absent or minimal if the prediction of human toxicity based on animal experiments was adequately precise. In our field study we found that in the observed range of metabolite excretion, which represents a certain range of exposure and absorption, no compound related effects occurred. This means that in future work urine sampling and determination of the selected metabolite is sufficient to perform a health hazard assessment, of course with the restriction that urinary excretion does not exceed significantly the range of excretion found in our study.

CONCLUSION

In the development of pharmaceutical drugs a "clinical trial" phase is used to evaluate efficacy and toxicity in humans after completion of a full scale animal experimental phase.

Although pesticides are not intended to be taken internally and exposure should be avoided, exposure may occur depending on the type of application and the skill and discipline of the applicator.

By analogy therefore with pharmaceutical drug development, it is recommended that the predictions based upon extrapolation of animal data to man should be checked out by carefully organised and conducted field observations on people involved in R and D field trials of the chemical under practical conditions.

Exposure measurements, preferably by biological monitoring, will give information on any special hazards associated with different types of application techniques. In addition, the data generated from such field studies will serve to confirm the adequacy or otherwise of safe use recommendations derived from animal data. In many occasions accurate information on absorption and excretion patterns can only be obtained when controlled dose-excretion studies in humans are done. The acceptability of candidate compounds from the view-point of potential health hazards can be assessed on the bases of the results from these field studies. Another objective is the development of simple parameters which may be used to monitor field workers. A simple parameter which gives information on exposure should also have predictive value as to the biomedical effects and to the health implications.

In this short presentation I have tried to convey to you our ideas on early human health hazard assessment in the development of new pesticides. I have tried to illustrate this by explaining the objectives and procedures of a recently carried out field study which was undertaken to evaluate possible health hazards of a specific type of application technique used with a relatively new pesticide. My colleagues Drs. Prinsen and Van Sittert will inform you in more detail on the procedure and the results of this field study.

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EFFECTS OF FORMULATION UPON THE SAFE USE OF PESTICIDES

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INTRODUCTION

Pesticides are rarely applied in their original form. The physical form of technical grades of pesticides varies from volatile liquids, through semi-liquid/solids to crystalline solids. For agricultural outlets, this material must be distributed uniformly, often at rates of less than 1 kg/ha, and it must therefore be transposed into a product that can be suitably applied by available equipment. Formulation is the process whereby pesticides are put into a form in which they can be suitably stored, transported and applied by practical methods to achieve effective, safe, convenient and economic use. In addition to agricultural outlets pesticides are used in public health, horticulture, animal health and in industrial and domestic situations. Accordingly, many types of formulations are available. Some of those most widely used in agricultural outlets are given in Table 1.

TABLE 1

Some of the formulations most widely used in agricultural outlets

Formulation Type	Physical State	How applied
Emulsifiable Concentrate (EC)	Liquid	Diluted with water
Water-miscible Concentrate (WMC)	"	"
Aqueous Solution (SL)	"	"
Suspension Concentrate (SC)	"	"
Mayonnaise Emulsion (EW)	"	"
Ultra Low Volume Concentrate (ULV)	"	Without further dilution
Spreading Oil (SO)	"	"
Fogging Concentrate (FC)	"	(Usually) diluted with solvent
Dust (FSD)	Solid	Without further dilution
Granule (GR)	"	"
Wettable Powder (WP)	"	Diluted with water

WPs and ECs are probably the most widely used formulation types. As shown in Table 1, formulations can be classified into different groups depending upon their physical state or how they are applied but such broad classifications give no real indication of the hazard potential. Virtually all pesticide formulations possess a toxicological hazard and some have an additional hazard - flammability. The toxicological hazard of a formulation is a combination of the inherent toxicity

of the pesticide and other ingredients together with exposure. The three main routes of exposure are inhalation, dermal and ingestion and the relative importance of these routes varies not only with formulation type but also with the activity being carried out by the user. The two most important characteristics of pesticide formulations that affect toxicological hazard are probably physical properties and composition.

CONTRIBUTION OF THE PHYSICAL PROPERTIES OF FORMULATIONS

Solid formulations vary from particulate dusts to coarse granules and liquid formulations vary from free flowing solutions to viscous mayonnaise emulsions often with poor flowability. The approximate volume median diameter (VMD) of some solid formulations is given below.

Formulation Type	Typical VMD (microns)	Pesticide content (g/kg)
WP	5	250-800
FSD (Field Strength Dust)	25	10-50
Microgranule	200	10-200
Granule	300	10-200

The actual particle size distribution of all these formulations can be quite large. For example, FSD formulations may contain a considerable proportion of particles above 40 microns and below 10 microns. These powder formulations are dry, free flowing, often of low bulk density and may give rise to dust clouds during measuring and other handling operations. Theoretically a 10 micron particle of a typical dust takes over 100 seconds and a one micron particle over three hours to settle three feet. Thus a major exposure from WP and dust formulations is through inhalation. To have sufficient amount of product for satisfactory distribution on crops, the pesticide concentration of FSDs (say 10-50 g/kg) is low. Since WPs are diluted with water for application purposes the pesticide concentration need not be so low and, in the interests of economy in inert ingredients, packaging and transport costs, typical concentrations are 250-800 g/kg. A dust cloud from a WP formulation is therefore likely to contain a relatively high pesticide content and remain suspended in the atmosphere for much longer than FSDs thus presenting a potential toxic hazard.

In granular formulations the pesticide concentration is generally low to medium (10-200 g/kg) and the particle size sufficiently large to greatly reduce inhalation hazard. Since both the inert granular carrier and, where necessary, the finished product can be screened, it is possible to obtain a narrow range of particle size and to remove dust at the time of manufacture.

The pesticide concentration of liquid formulations varies considerably from relatively low (100 g/l) to very high (>800 g/l). Since they exist as bulk liquids, prior to use the inhalation exposure is not as great as for WPs and FSDs. However, because of their low viscosity they may readily spread over the skin and give rise

to a dermal exposure. Gross spillage of liquid formulations can quickly lead to contamination of large floor areas, drains and other channels. Mayonnaise emulsions and certain SC formulations are much more viscous and do not spread so rapidly on surfaces thus presenting a lesser dermal exposure.

CONTRIBUTION OF THE COMPOSITION OF FORMULATIONS

The components of pesticide formulations consists of:

(i) the pesticide

(ii) the "carrier" - such as organic solvent for ECs, mineral clays for WPs etc.

(iii) surface-active agents (where required)

(iv) other ingredients - efficacy improvers, stabilisers, dyes etc.

Surface-active agents are present in significant quantities (e.g. often 30-100 g/l) in formulations that are diluted with water for application purposes. The amount of other ingredients vary considerably from only a few parts per million for dyes up to 500 g/l for agents controlling volatility of ULV formulations.

Although many different organic solvents are available, relatively few are used extensively in EC, WMC etc formulations. Certain solvents are not used because of their inherent toxicological properties (e.g. benzene, chloroform) but other factors such as cost, availability as well as the physico-chemical properties of the solvents also impose constraints. Pesticide solubility varies considerably. Xylene and closely related materials are good solvents for many organophosphate and organochlorine insecticides and, in view of their extensive use in other industries, are readily available in most parts of the world. Although acetone and other lower ketones and alcohols are good solvents for many pesticides they are extremely volatile and highly flammable. The use of volatile solvents can lead to rapid crystallisation of pesticide solutions when exposed to freely venting atmospheres. Blockage of screw threads with subsequent leakage through closures is a typical result from their use. The Threshold Limit Value (TLV) of solvents is generally at least one and often two or three orders of magnitude higher than that of most pesticides (ref.1). Examples are given in table 2. However, it should not be inferred that they are without hazard since the vapour pressure of many solvents is relatively high and open containers can quickly lead to the build-up of vapour in poorly vented areas such as warehouses and farm stores. Hydrocarbon solvents with low aromatic contents and higher TLVs are available and may be suitable for certain industrial outlets (ref.2) but in view of their relatively poor solvent power are unlikely to have wide application in pesticide usage at this time.

Solvents influence the percutaneous toxicity of forumulations by altering the dermal penetration. A comparison of the single dose acute percutaneous toxicity of dicrotophos showed that solutions in cotton seed oil and xylene (LD_{50} approximately 50 mg/kg to male rats) were more toxic than solutions in isopropanol or water (LD_{50} 190 and 140 mg/kg respectively). Dicrotophos in n-octanol (LD_{50} 25 mg/kg)

TABLE 2

Threshold limit values of some pesticides and solvents used in pesticide formulations

Substance	Time weighted average value (mg/m^3)
Parathion (skin)	0.10
Aldrin (skin)	0.25
Propoxur	0.50
DDT	1.00
Captan	5.00
Diuron	10.00
Hexylene glycol	125
Cyclohexanone	200
Xylene (skin)	435
Methylene chloride	720
Acetone	2400

TABLE 3

The acute percutaneous toxicities of some liquid and solid formulations of pesticides tested in CFE rats

Product Tested		Approximate LD_{50} mg/kg as active material
Active material	Formulation	
Chlorfenvinphos	50 g/kg dust	Males >800* Females 400
	240 g/l EC	27
Dieldrin	500 g/kg WP	Males 1350 Females 606
	200 g/l EC	182
Monocrotophos	50 g/kg granules	> 500
	240 g/l WMC	67.4

* Limit of solid material that could be applied.

was found to exert the maximum penetration rate of the solutions tested (ref. 3). Comparisons have also been reported (Table 3) between the percutaneous toxicity of some liquid and solid formulations of a chlorinated hydrocarbon insecticide (dieldrin) and that of two organophosphate insecticides (chlorfenvinphos and monocrotophos).(ref.4) In each case the liquid formulation was the more toxic and differences were particularly noticeable in results involving low content dust and granule formulations.

A great deal of attention has been given to skin penetration but much less to penetration of protective clothing. Some simple comparative tests on the permeability of candidate materials for protective clothing were carried out with technical mevinphos and a mevinphos 240 g/l EC.(ref.5) The results are given in Table 4 and show that

(i) permeability varies for different materials,

(ii) the EC gives rise to much more rapid penetration.

TABLE 4

The Permeability of Candidate Materials for Protective Clothing to mevinphos and a mevinphos EC

Test Material		Time to reach 100% larval mortality[1] (h)	
Type	Thickness (mm)	When material exposed to mevinphos	When material exposed to mevinphos 240 g/l EC[2]
Brailene nylon coated with polyurethane (siliconised)	0.38	0.75	0.50
Nylon-backed multithene MH3	0.36	0.80	0.50
Nitril rubber coated	0.27	3.0	0.75
'Green' PVC	0.25	3.7	0.75
Duranil hypalon coated	0.30	7.5	0.75
Neoprene	0.32	10.5	0.90
695 Chlorobutyl nylon	0.29	47.0	1.25
Butyl rubber coated	0.39	32.0	1.70

(1) Test materials were placed over the necks of 250 ml glass jars containing mevinphos or mevinphos EC and secured to prevent loss of liquid. The jars were inverted and immersed to a depth of 2 mm in a beaker of water containing 5-day old Aedes aegypti. The time taken for 100% mortality was noted.

(2) Mevinphos 240 g/l EC based on aromatic solvent plus anionic/nonionic surfactants (60 g/l).

The rate of penetration of pesticides will depend, inter alia, upon the pesticide, the composition of the formulation, the chemical composition and thickness of the material used and the time of contact between the formulation and the material. It should not be concluded therefore that similar results would be obtained with other liquid pesticide formulations. However, careful attention should be given to the selection of material chosen for protective clothing and procedures such as removing contaminants from protective clothing as soon as possible to minimise

absorption of pesticide and the frequent replacement of protective clothing worn by operators should be practised.

In a similar manner plastics used as container materials are, to a greater or lesser extent, permeable to organic solvents. The use of aromatic solvents in polyethylene and ketones in PVC containers cause softening of the container walls as well as loss of solvent by permeation. Packs become deformed and can ultimately collapse. Egress of pesticide to the outside surface of plastic containers can also occur. Although standard instructions are that gloves should be worn whenever handling pesticide containers e.g. whilst unloading lorries etc., users could be exposed to an unexpected hazard since it is quite possible that the containers would be handled without protective clothing when not actually sampling the contents. To avoid this it is essential that rigorous testing of formulations is carried out on all candidate container materials before final selection and recommendations are made.

Carriers used in solid formulations are often innocuous and are therefore less likely to constitute a hazard than are solvents used for ECs etc. Indeed, kaolinites and calcium carbonates which are widely used in the pesticide industry are also used in medical remedies. The use of synthetic silica, however, must be considered as less desirable due to its association with silicosis. Silica is incorporated into some pesticidal powder formulations. It is used to increase the sorptive capacity of the carrier system as a milling aid, to ensure that the product remains dry and free flowing for easy handling and application and to prevent "caking" in the container during prolonged ambient storage. Whilst the use of silica is limited because of cost, it is used, especially in WP formulations.

There is little published information on the composition of SC formulations and it is more difficult to judge the likely contribution the inert components could have upon the toxicological hazard. Perhaps two encouraging features are that the vast majority of SC formulations to date are aqueous based and that the concentration of other ingredients (e.g. surfactants) seems to be relatively low. SCs are often considered as wettable powders dispersed in a liquid to give a preformed, concentrated, liquid product. This is not usually the case. Generally, there is no significant amount of mineral carrier present and the dispersed particles consist only of pesticide. To minimise sedimentation of particles during prolonged storage in SC formulations, the size of the particles is relatively small. VMDs of about 2 micron are typical and the size distribution ranges from below 0.2 to above 10 micron. In view of this, SC deposits tend to adhere more strongly to plant surfaces than do WPs and this would equally be expected on other surfaces such as spray equipment, floors etc.

THE CONTRIBUTION OF FORMULATION DESIGN AND QUALITY CONTROL SPECIFICATIONS.

Appropriate design and stringent quality control are essential to obtain for-

mulations that are both safe and reliable. To design appropriate formulations requires knowledge of a vast amount of information not only about the pesticide, the pest and crop involved but also about the production facilities, the storage conditions, methods of transport, application equipment and application methods, know-how of users and many factors such as dilution rate, water hardness and temperatures which can affect the performance characteristics of the product. It is a complex task and requires close collaboration between personnel of different disciplines. It leads to the establishment of a design specification which it is believed will ensure acceptability of the product throughout its life. Similarly, the quality control specification must be able to distinguish between products that comply with the basic design requirements and those that do not.

Some of the problems experienced by users are given in Table 5 together with comments on the likely cause.

TABLE 5

Typical field problems and their relationship to formulation design and quality control

Problem	Possible Cause	Fault Origin
High dust content in granular products	a) Attrition during transport due to inappropriate selection of carrier	Form.Design
	b) Use of carrier with high dust content	Qual.Control
Rapid settling of WP in spray tank. Nozzle blockage	a) Product insufficiently milled	Qual.Control
	b) Water hardness different from that in which WP was tested during development stage	Form.Design
Crystallisation of ECs in spray tank leading to nozzle blockage	The use of excessive amounts of water-soluble solvent in EC	Form.Design
Unwanted drift from ULV application	Use of a volatile solvent which evaporates from spray particles before hitting target	Form.Design
Excessive free liquid and compacted sediment in SC container	Insufficient "stabilisers" in formulation	Form.Design
Excessive foam in spray tank	Too much surfactant in formulation	Form.Design
Blockage of dust applicator	Poor flowability of formulation due to a) wrong choice of carrier	Form.Design
	b) use of wet carrier	Qual.Control

The above problems have two things in common, firstly exposure is increased to those handling or using the products, and secondly they could all be avoided by better formulation design and/or quality control. International bodies such as

WHO and FAO have published specifications to define the basic quality standards of many products. Increasingly, countries are devising their own local specifications. This is an important development. It reflects a recognition that there is a great diversity of general conditions and use patterns in different countries and highlights the need to have specifications relevant to local as well as international needs to achieve safe and reliable formulations.

CHOICE OF FORMULATION AND FUTURE TRENDS

Based on the considerations mentioned above, an attempt has been made to assess the potential exposure of different types of formulations relative to each other for activities involved in handling etc up to the point of application. The exposure resulting from application of pesticide formulations is discussed in an associated paper being presented at this Workshop (ref.6).Clearly, any classification does not necessarily hold for all pesticides since factors, like extremely high volatility of the pesticide, could override many formulation differences. However, general guidelines can be distinguished. For example, a granule formulation is likely to constitute less hazard than any other formulation. Aqueous suspensions and emulsions where the pesticide is contained in the discontinuous phase - as normally found in mayonnaise emulsions - are probably the next most favourable. Organic solution types (e.g. ECs) are one of the most widely used but appear bottom of the league due to flammability and to relatively high percutaneous toxicity. Aqueous solution types may be considered as intermediate. Although the inhalation exposure is likely to be relatively low and products are non-flammable, their resemblance to soft drinks leads to a relatively high ingestion exposure potential. The mandatory incorporation of stench agents to aqueous solutions would probably change this to a relatively low category.

It seems, therefore, that the least hazardous type of formulations (GRs, SCs) are currently the least widely used and one must question whether or not this situation is likely to continue. Although granules are used for insecticide and herbicide soil applications they are generally unsuitable for foliage application. The recent commercial introduction of encapsulated pesticides may alter this situation in some cases, but it remains to be seen whether such a presentation is widely applicable for foliage use. Encapsulation certainly affords a very effective way of reducing the hazard from highly volatile pesticides. SC formulations are suitable for both soil and foliage application. With increasing developments in SC technology, the trend from WP to SC formulations that has taken place over the last 5-10 years is likely to continue and even accelerate as greater production capacity is installed. Owing to constraints of chemical stability and other physicochemical properties, not all pesticides are amenable to formulation as aqueous, or even non-aqueous based SCs. We must therefore expect that WPs and ECs will continue to be the most widely used type of formulations as long as overall

broadcasting of pesticides remains the major method of application. For WPs, the use of unit dose water-soluble sachets greatly reduces the risk of inhalation exposure during measuring and dosing operations and provides a valuable aid to achieving correct application rates. The tendency for these sachets to dissolve incompletely or too slowly has led to nozzle blockage etc and some improvements are required here. The presentation of WPs in a granular form, which reverts to a normal WP form when added to water in the spray tank, will also lead to a reduced inhalation exposure from WP formulations.

Looking further into the future, it is likely that the way in which formulation technology could most improve safe use of pesticides is through the introduction of products that do not require overall broadcasting. It is possible to envisage the more widespread use of insecticide bait formulations and the development of special seed treatment formulations that contain insecticides and fungicides to give protection of the growing plant as well as to the seed and young seedlings. In selective herbicide outlets, where complete and even distribution of the chemical is essential, such placement techniques are less likely to be viable.

CONCLUSION

It is concluded that formulation can have a considerable effect upon the safe use of pesticides and that careful selection of formulation for a particular pesticide is essential in order to minimise exposure and the consequent potential hazards. In compiling safe handling instructions for labels, training programmes etc due recognition must be given to the physical and chemical characteristics of formulations as well as to their inherent toxicity and the activities of users. Current trends in formulation use and developments in new formulation technology will lead to further improvements in safe handling but well established formulations will probably continue in widespread use for many years. In this context the use of reasoned and responsible formulation design principles and appropriate quality control procedures remain of great importance in the safe use of pesticide formulations.

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FIELD WORKER EXPOSURE DURING PESTICIDE APPLICATION

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The protection of agricultural crops and the control of disease vector by pesticides require the distribution of liquid drops or solid particles containing the active ingredient over crop surfaces or as a space spray. Man comes into contact with these chemicals during their manufacture, distribution and application. Contact can occur during:

(a) formulation
(b) transport to storage or application site
(c) transfer from container to application equipment
(d) application
(e) field work involving contact with residues
(f) drift of the pesticide out of the application area
(g) disposal of the unused chemical or containers
(h) cleaning and repair of application equipment.

Some idea of the toxicological hazards from pesticides at these points of contact can be gleaned from the data given by physicians to the California Health Department on illnesses due to pesticide exposure (Table 1). A high number of illnesses in any occupational group does not necessarily indicate a higher hazard as there is no indication of the total number of persons engaged in that occupation. However, the Table does differentiate between systemic, i.e. ingestion and inhalation, and dermal hazards. For instance a field worker exposed to residues on a crop runs a greater risk of dermal exposure than inhalation hazard whereas the pilot of an agricultural aircraft is protected from the direct spray but has a high risk of exposure to pesticide vapour and small inhalable drops. Thus the risk of illness attributed to pesticides will vary with the occupation. It will also vary with the application technique.

There are three methods used commercially for the atomisation of liquid agricultural chemicals. Hydraulic nozzles, twin fluid nozzles and rotary atomisers. Liquid is pumped to the hydraulic nozzle under pressure and the size and shape of the restriction and orifice determine the flow rate, spray pattern and drop spectrum. Hydraulic nozzles are the most widely used method of breaking up liquids

TABLE I

Illness due to exposure to pesticides. reported to the California Health Department. 1975

Occupation	Total No.	%	Classification (%)			
			Systemic	Skin	Eye	Eye/Skin
ground applicator (mixer. loader, applicator)	270	20.1	36	36	24	4
mixer, loader (aircraft)	131	9.8	57	15	26	2
field worker exposed to residues	165	12.3	17	69	12	2
gardener	107	8.0	22	26	38	13
nursery or greenhouse worker	100	7.4	25	54	19	2
formulation plant worker	56	4.2	73	9	14	4
warehouse worker, truck loader	45	3.4	42	33	22	2
structural pest control worker	35	2.6	20	40	40	0
fumigator of fields	22	1.6	41	23	27	9
cleaner or repairer of machinery	35	2.6	29	26	34	11
fireman exposed to pesticide fires	37	2.8	100	0	0	0
tractor driver or irrigator	23	1.7	39	44	13	4
worker exposed to drift	31	2.3	26	29	45	0
flagman for aircraft application	16	1.2	63	25	12	0
pilot of agricultural aircraft	8	0.6	75	0	25	0
indoor worker	79	5.9	63	16	19	1
other type of pesticide user	183	13.6	45	27	26	3

Denotes highest percentage

into spray drops. Twin fluid nozzles use an airstream to shear off drops from a liquid jet or to give further atomisation to drops issuing from a nozzle. Rotary atomisers produce spray drops by directing the liquid on to a rotating surface, the sheet of liquid so formed breaking off as drops from the surface periphery.

EQUIPMENT

Sprayers can be manually or power operated. Manually operated sprayers range from the simple piston operated intermittent sprayer to lever operated or compression type knapsack sprayers. These sprayers can only be used for application on small areas. Power operated sprayers include knapsack mist-blowers with 2-stroke internal combustion engines as the power source, tractor-mounted boom and nozzle equipment using the power take-off from the tractor or large orchard sprayers with their own power unit. Aircraft can be equipped with the boom and nozzle for conventional applications or rotary atomisers for ultra-low volume spraying (ULV). ULV is defined as the application of less than 5 litre/ha. Other rates of application can be classified as:

very low volume	5 - 20 l/ha
medium volume	20 - 150 l/ha
high volume	>150 l/ha.

Rotary atomisers have been in use for aerial application for over 20 years, particularly for ULV applications for the control of locusts and disease vectors of malaria and trypanosomiasis. More recently they have been used for agricultural applications not only for aerial spraying but as hand-held sprayers operated by dry cell batteries. The Micron ULVA and the Turbair X are being used extensively in many countries for pesticide application by peasant farmers on areas of less than one hectare, particularly for the control of pests on cotton in the developing countries. The pesticide formulated for ULV is contained in a plastic bottle of one litre capacity which screws on to the atomiser head. The head consists of a disc, 9 cm in diameter, with a serrated edge driven by a 12 volt electric motor at a speed of about 7,000 - 9,000 rev/min. In the ULVA sprayer the 8 x 1.5 v batteries are contained in the hollow handle of the sprayer. The Turbair X has a metal handle and the batteries are hung on a strap over the shoulder or fixed to a waist belt. When the bottle is inverted the liquid flows by gravity on to the revolving disc and is thrown off tangentially from the periphery of the disc as small drops.

The most widely used type of equipment for ULV aerial applications is the Micronair atomiser. It consists of a cylindrical gauze about 15 cm in diameter and 15 cm in length which rotates about a fixed spindle. Power is supplied from the aircraft slipstream by five blades fixed to the hub of the spindle; the speed of rotation of the gauze cylinder is determined by the pitch of the blades. The drop spectrum of the spray produced by the Micronair is determined by the blade angle, the flow

rate and the viscosity of the formulation.

DROP SPECTRA

Spray drop sizes are usually defined by median diameters. The median diameter divides the drop spectrum so that 50% of the number or the volume is above or below the stated diameter. The Number or Volume median diameters (NMD and VMD) alone give no indication of the range of drop sizes contained in the spray but the ratio (r) of VMD/NMD is related to the geometric standard deviation.

$$r = e^{3(\ln\sigma_g)^2}.$$

The width of the drop spectrum produced by hydraulic nozzles can be very wide with the ratio r as high as 10-15, whereas for rotary sprayers r is generally less than 1.5. However, sprays are often classified by their VMD viz.

Aerosols	<50 μm
Mists	50 - 100 μm
Fine sprays	100 - 400 μm
Coarse sprays	>400 μm

DRY FORMULATIONS

Dry formulations can be classified as:

Fine dusts with particle sizes less than 45 μm
Coarse dusts 45 - 100 μm
Micro-granules 100 - 300 μm
Coarse granules >300 μm.

Applicators can range from small hand-held dusters operated by bellows or fans to large vehicle-mounted power operated blowers. On aircraft the dust is fed from the hopper into a venturi spreader that utilises the aircraft slipstream for dispersal. Granules are often applied by feeding them through a metering device on to a ribbed rotating plate from which they are thrown off at the periphery.

HAZARDS

Dermal

For users of pesticides the main route of exposure to liquid pesticides is through the skin and not through the respiratory tract (ref.1) and the greatest hazard is generally not from the application itself but during the handling, mixing and filling of concentrate formulations into mixing tanks and spray equipment. A high proportion of all pesticide application use low to medium volumes as emulsion concentrates (EC) diluted in water. However, the EC themselves consist of high concentrations of the active ingredient in hydrocarbon solvents, frequently aromatic such as xylene, which penetrate the skin much more rapidly than does the dilute emulsion. ULV formulations in relatively non-volatile oils are applied without

dilution, thus eliminating mixing stage.

Inhalation and ingestion

Only spray drops less than 10 µm in diameter can reach the lungs, and the percentage by volume of drops below this diameter in sprays used for pesticide application is relatively small. However, drops between 50 and 100 µm can be inhaled and impact on the nasal lining. As the drop size gets smaller than 50 µm the further they can penetrate into the respiratory tract and drops between 5 and 10 µm can reach as far as the smallest bronchioles. Drops less than 1 µm are completely airborne and are exhaled. Generally the volume applied is directly related to the VMD of the spray and for conventional applications with volumes above 5 l/ha hydraulic nozzles are used, and these produce a very wide spectrum of drop sizes. ULV applications are usually made with rotary atomisers which, when operated correctly, produce a narrow range of sizes. For example, conventional aerial applications using hydraulic nozzles have a VMD of 200 - 400 µm and for ULV agricultural applications the VMD of the spray is between 80 - 150 µm. Table 2 gives a comparison of the volume and mass contained in drops less than 50 µm in diameter from typical aerial applications applied conventionally and by ULV. Under certain meteorological conditions a high proportion of drops less than 50 µm in diameter drift out of the application area. However, because the spectrum produced by the rotary atomiser is much narrower than that produced by a nozzle, the mass of active material liable to drift out of the area sprayed conventionally is nearly six times that from the ULV application despite the fact that the VMD of conventional spray is twice that of the ULV application. In practice the difference in drift between the two application techniques would be greater as the water in the conventional spray will evaporate, leaving drops containing only the pesticide and the relatively non-volatile emulsifiers, whereas a ULV spray is usually formulated to contain no more than 50% of volatile material. If there is a temperature inversion at the time of the application, the drift is concentrated in a layer of air below the level of emission and this concentration of respirable drops may extend several kilometers downwind from the application area.

TABLE 2

Comparison of the volume and mass contained in drops less than 50 µm in diameter from aerial application techniques

aerial application technique	pesticide concentration g/l	dose rate g/ha	volume rate l/ha	VMD µm	<50 µm		
					volume %	volume litre	mass of pesticide g
Conventional	25	500	20	200	4.0	0.8	20
ULV	250	500	2	100	0.7	0.014	3.5

SAFETY REGULATIONS

Many countries where pesticides have been used extensively have developed Codes of Practice or legislative requirements for handling and applying agricultural chemicals. Recommendations are also made for residues on edible crops, for the protection of livestock, fish and useful insects such as bees. The enforcement of such regulations varies considerably between countries and, in the USA, between States. Legislation on the use of pesticides in California is ahead of that in other States and the rest of the world. New regulations on pesticide worker safety came into operation there in 1974. They specify safe work practices for mixing, loading, applying and handling pesticides, including instruction, supervision, operation, use of protective clothing and modification of application equipment. A new and important innovation is the regulation requiring closed system mixing and handling of pesticides. 'Closed mixing system' means transferring a pesticide from its original container into a closed mixing tank without the exposure of any person to the pesticide. 'Closed loading system' means transferring a pesticide from a mixing tank into an application tank by a closed system of hoses, pipes and couplings that connect the two directly. These requirements also extend to the loading of agricultural aircraft. Obviously such stringent requirements could only be enforced where pesticide application is a highly sophisticated technique and where inspectorate personnel are available.

Problems occur in the developing countries especially where pesticides are being used for the first time. For instance, in Nigeria, the World Bank provided funds for Agricultural Development Projects. Included in these Projects was the provision of pesticides to small farmers through Farm Service Centres. The pesticides were used for the control of insect pests on cotton. The average size of holding was four hectares of which one ha was reserved for cotton. The pesticide application was made with the ULVA hand-held ULV sprayer. The spray with a VMD between 80-100 µm is dispersed by the wind and the operator walks in the crop at right angles to the wind direction. Operator contamination can be high if he is not instructed in the correct use of the equipment. Although protective clothing is recommended the operator is often clothed only in shorts and a short-sleeved shirt. In this instance the Chemical Company who sold the pesticide co-operated with the staff employed by the Nigerian Federal Department of Rural Development in organising Courses for Agricultural Assistants who in turn instructed the farmers. Filmstrips and booklets were also used to show the correct method of handling and applying the pesticide. Considerable contamination occurred during the filling of the one litre plastic container as the pesticide was supplied to the farmer in 25 litre drums. These containers were specified by the Department of Rural Development, contrary to the advice of the Chemical Company. Apart from the hazard of filling from these drums there was also the problem of storing the drum in an African house

and the probability that the empty drum would be used for the storage of water. These problems could have been solved by supplying the farmer with the pesticide in one litre plastic containers to screw directly to the ULVA sufficient for one application and requiring the farmer to return the bottles before giving him the pesticide for the second application. Over half a million of these sprayers are now being used in Africa alone.

In those countries where recommendations and legislation for the control of pesticide use are enacted and enforced the greatest hazards come from accidental spillage, misapplication and from drift of the pesticide out of the area of application. However, in some developing countries even if regulations on the safe use of pesticides exist they are not enforced and it is left to the Chemical Companies supplying the pesticides to provide educational courses to ensure that the pesticides are properly applied.

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SOLUBILITY FACTORS AFFECTING PESTICIDE PENETRATION THROUGH SKIN AND PROTECTIVE CLOTHING

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INTRODUCTION

The percutaneous penetration of pesticides is of interest for therapeutic use in veterinary medicine for the control of endo- and ectoparasites in farm animals as well as regarding possible human percutaneous poisoning following application of pesticide formulations. In the case of therapeutic use, a definite amount of insecticide has to be resorbed via the skin to give a certain level in the blood circulation and a satisfactory control of the parasites. On the other hand, for a safer use of pesticides in agriculture the hazards by dermal exposure and skin penetration should be reduced, as the main route of exposure during work with pesticide formulations is through the skin. Solubility factors influencing penetration of pesticides are of interest for all these aspects of pesticide use.

SOLUBILITY

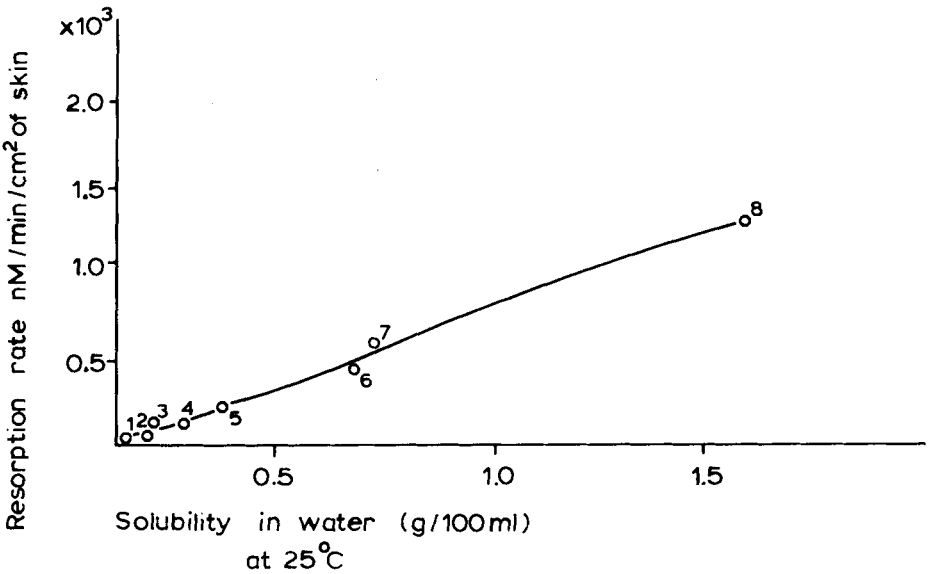
Solubility of pesticides

Penetration of pesticides without solvent is depending on the water solubility (w.s.) rather than on the oil solubility. Penetration (and therefore also distribution in the body) is increasing with a better solubility of the pesticide in water. For example parathion/paraoxon has a ratio of w.s. of 1:100. Paraoxon, a toxic metabolite of parathion with strong cholinesterase inhibition, is distributed in the body based on its good w.s. and penetration properties, whereas parathion is mainly remaining in the fat resp. hydrophobic parts of the body. This behaviour is important because of its toxicological consequences: cholinesterase, playing a central role in organophosphate and carbamate intoxication, is located in the hydrophilic parts of the body which are penetrated better by hydrophilic compounds, i.e. those with a better water solubility.
The solubilities in water of 738 pesticides are given in Residue Reviews Vol. 20 (ref. 1).

Solubility of solvents

For the in vivo percutaneous absorption in mice of 8 chlorinated hydrocarbon

solvents a linear relationship between the absorption rate of the skin and the water solubility was found by TSURUTA (ref. 2) (Fig. 1). This result gives rise to the supposition, that solvents with a good skin permeability, i.e. good water solubility, would generally promote the penetration of pesticides; however, compare with results in Table 1, which do not confirm the supposition.



1 Tetrachloroethylene
2 Tetrachloromethane
3 1,1,2-Trichloroethane
4 1,2 - Dichloroethane
5 1,1,1 - Trichloroethane
6 1,1,2,2-Tetrachloroethane
7 Trichloromethane
8 Dichloromethane

Fig.1. Relationship between the percutaneous absorption rate and the solubility in water for 8 chlorinated hydrocarbon solvents (quoted from TSURUTA, 1975)

SOLVENT-PESTICIDE INTERACTION

We found for the percutaneous absorption of cattle skin of organophosphates in definite solvents the following relations (refs. 3, 4, 5) (see table 1)

- Polar compounds are penetrating better in nonpolar solvents.
- Nonpolar compounds are penetrating better in polar solvents.

(For practical use, the term polar is associated with good w.s., and nonpolar with low w.s.). In pesticide formulations of the EC type mainly nonpolar solvents are used (aromatic hydrocarbons, cyclic ketones), and therefore the pesticides with high skin penetration rates and, consequently, high toxicological hazards are those

of more polar properties, i.e. better water solubility.
Nevertheless, organophosphates of low w.s. (about <0.5%) are showing generally smaller penetration rates, irrespective of the solvent used (compare phosmet and butonate with trichlorphon and dimethoate, Table 1).

TABLE 1

In vitro percutaneous absorption of organophosphates from solvents of different polarity

(Concentration 1%, cattle skin, amounts in $\mu g/cm^2$ after 22 h at 37.5°C; Σ dielectric constant, organophosphates labelled by ^{32}P, w.s. in g/litre).

Solvent	Σ	trichlorphon w.s. 154	dimethoate w.s. 39	butonate w.s. 3.5	phosmet w.s. 0.25
Glycerol	56.2	220 ± 53	227 ± 19	257 ± 14	-
DMSO	48.9	226 ± 21	32 ± 12	260 ± 41	256 ± 48
Ethyleneglycol	41.2	236 ± 15	229 ± 14	114 ± 10	-
Ethanol	27.8	272 ± 42	257 ± 17	68 ± 6	-
Acetone	21.5	393 ± 49	140 ± 25	186 ± 28	364 ± 39
Dichloromethane	-	620 ± 89	760 ± 84	56 ± 7	212 ± 32
Trichloromethane	5.2	1170 ± 195	1170 ± 127	15 ± 4	228 ± 23
Tetrachloromethane	2.2	809 ± 93	1640 ± 217	52 ± 4	-
Benzene	2.3	1003 ± 148	1325 ± 168	42 ± 8	56 ± 15
Toluene	2.3	963 ± 115	1046 ± 125	21 ± 5	138 ± 21
n-Heptane	1.9	-	-	216 ± 28	-

Regarding concentration as a factor affecting penetration, the solubility of the pesticide in the certain solvent is of importance. Maximum penetration rates are found in saturated solutions, irrespective of the absolute concentration. Concentration has to be considered in relation to the solubility in the solvent, according to Nernst's law of partition (ref. 3).

PROTECTIVE CLOTHING

In pure organic polymer layers the solubility of compounds and therefore the penetration rate should be proportional to the solubility in the specific organic material, and approximately inversely proportional to the w.s. of the compound. We found with some organophosphates an outstanding penetration for the very non-polar methylparathion (refs.6, 7). The materials testes with the best protecting properties were butyl rubber vulcanisates (Table 2).
The topics mentioned in this paper are discussed in detail in the papers given in the references.

TABLE 2

The permeability of polymer materials for protective clothing to organophosphates. (Solutions of 1 mg/50 µl of acetone, amounts in µg/cm^2 after 24 h at 25°C, w.s. in g/litre, d thickness in mm).

Test Material	d	methylparathion, w.s. 0.06	butonate w.s. 3.5	dichlorvos* w.s. 10	dimethoate w.s. 39
PVC	1.20	34 - 37	0.13-0.30	0.57-0.65	0.05
Buna NWL oilstable	0.50	8.8 - 9.0	0.55-0.58	4.0 -5.0	0.34-0.38
Buna SS	0.55	0.26- 0.60	0.05-0.1	<0.1	0.05-0.20
Chloroprene LW 5301	0.48	28 - 41	-	10.7 -16.4	0.30-0.34
Chloroprene + Butyl rubber-vulc. 602	0.34	0.05- 0.13	-	0.05	0.05-0.09
Butyl rubber + resin vulc.	0.48	<0.05	-	<0.05	<0.05
Butyl rubber + sulfur vulc.	0.40	<0.05	-	0.08-0.13	<0.05
Polyethylene, chlorsulfonat.	0.40	0.23- 0.30	-	0.05-0.09	0.05-0.20

*amounts of dichlorvos are likely influenced by the special high vapour pressure of dichlorvos

w.s. = water solubility

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SCOPE AND NEED OF TOXICOLOGICAL EVALUATION OF PESTICIDES UNDER FIELD CONDITIONS - MEDICAL SURVEILLANCE OF MALARIA SPRAYMEN EXPOSED TO HCH (HEXACHLOROCYCLOHEXANE) IN INDIA

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ABSTRACT

In developing countries like India, pesticides play a critical role in agriculture and public health programmes. An extensive use of these potentially toxic chemicals may involve risks of environmental contamination and health hazards to the community. The risk-benefit picture will differ in developing countries from developed countries. The hazards will vary due to differences in environmental conditions and host factors i.e. health status, nutritional status etc. Therefore, toxicological evaluation of pesticides under field conditions is highly desirable.

The observations on malaria spraymen exposed to HCH compared to unexposed controls are presented to assess the chemical, biochemical, haematological and serum residue changes. The results show significant changes in serum A-G ratio, glucose levels and HCH residues in spraymen when measured before and after exposure to HCH. Only limited differences in these parameters were noted when spraymen were compared with controls in a transversal study. This indicates the need for and importance of longitudinal studies for field evaluation of pesticides.

INTRODUCTION

India is a developing country having a population of over 647 millions; to provide the necessary food and basic public health and sanitation facilities is a tremendous task. The use of pesticides plays a critical role in agriculture in our country and is a key factor in the "green revolution" and self-sufficiency in food. The direct and indirect social and economic benefits from the use of pesticides are well recognised all over the world . It is reasonable to ask at this stage, whether the use of these chemicals has any disadvantage. Is there a price to be paid?

There is every reason to believe that these chemicals, purposely developed to be toxic to pests, might produce adverse effects on non-target organisms including man himself. This is the basic cause for public concern regarding risks involved

in large scale use of pesticides. What is required of us is to weigh all the risks against the benefits and to ensure the programme is proceeding with maximum possible care and a reasonable margin of safety. We all should realize that the total cost benefit picture from pesticide use will differ appreciably from a developed country to a developing country, this is primarily due to widely differing socio-economic situations. For developing countries, it is unthinkable to do without chemical pesticides, as no one would prefer famine, hunger and communicable diseases like malaria, at the cost of reasonable risks. It may be expedient to accept a reasonable degree of risk in these countries. In other words, the approach should be pragmatic. The introduction of pesticides should be based on scientific judgement and not on commercial exploitation.

SCOPE AND NEED FOR TOXICOLOGICAL EVALUTION OF PESTICIDES

For over a decade now, since the enactment of the Insecticides Act (1968) in our country, the registration of all these pest control chemicals is compulsory before clearance for import, production and use. We are one of the first countries to emphasize and enforce 'Toxicological evaluation of these chemicals under local conditions' as a pre-requisite for registration. However, this approach has been repeatedly challenged on grounds that:

1. pesticides are commercially marketed only after a series of toxicity tests in internationally reputable laboratories;
2. the cost involved in undertaking toxicity testing is exorbitant;
3. these chemicals are cleared or registered in the developed countries.

It is quite reasonable that if one is to keep in mind the welfare of the community at large, we need to be more concerned and give higher weighting to pesticide hazards rather than toxicity data based on experimental studies on animals. Because the risks involved from the use of pesticides depend not only on the toxicity of the chemical but also on environmental factors determining the fate and persistance of the chemical, hence the potential period of exposure and the host factors like genetic predisposition, health status, nutritional status, concomittant diseases and infections, etc. determining the nature of the response.

Out of these three groups of factors (toxicity, environmental and host factors) only the toxicity of a chemical is constant as it is an inherent property. Since toxicity is established after a series of conventional controlled laboratory experiments, the data should be acceptable to all. The mere repetition of these tests does not carry any basis in scientific logic and would certainly amount to a waste of money and effort.

However, this does not hold true for environmental and host factors which are equally important as the toxicity of the chemical in determining overall risks. These factors will vary from one geographical region to another. Therefore, the

data worked out in a developed country under entirely different conditions, on a population in relatively better health, will not be applicable in toto for a developing country. There may not be differences in the type or nature of the response in one population group as compared to other but in all probability the magnitude or severity of the response will differ and this will make the difference between risk and safety.

The question arises, how to assess the risks from the combined effect of so many variable environmental and host factors. Controlled exposure studies may provide some information but have their limitations. The only answer to this problem is to undertake "toxicological evaluation under field conditions" at least to begin with when a pest control chemical is introduced for the first time in the country, if one is genuinely concerned and interested to preserve the environment. It is heartening that now more and more countries are being converted to this approach, and international agencies too are correctly emphasizing the need of field studies. Therefore, all efforts now need to be directed to develop "proper methodology" for field evaluation of pesticides.

FIELD STUDY ON MALARIA SPRAYMEN EXPOSED TO HCH

In India, with the development of resistance against DDT by mosquitoes, the insecticide HCH (Hexachlorocyclohexane) is the main weapon in the National Malaria Eradication Programme (Table 1). Therefore, it was necessary to ascertain the overall risks involved. The insecticide was applied as a 1% solution of a 50% WDP (water dispersible powder) formulation using a Stirupp pump.

TABLE 1

Pesticide consumption and requirements for public health programme (in tonnes).

year	DDT (45% WDP*)	HCH (50% WDP*)	Malathion (25% WDP*)
1974-75	8933	5377	1062
1975-76	9567	13300	6000
1976-77	9567	14060	4000
1982-83**	9834	21548	13811 (Technical grade)

source:

1. Pesticide Association of India (1977)
2. Pesticide, Vol.xii (1978)

* WDP = water dispersible powder

**Anticipated Requirements

Objectives

The main objective of the study was to assess the adverse effects of HCH on malaria spraymen using clinical signs and symptoms, biochemical and haematological abnormalities and serum residue changes after short and repeated exposures to this insecticide.

Plan of study

Main study. In this survey, 464 spraymen and 201 comparable unexposed control subjects were examined during the spray season (= 16 weeks).
The spraymen were divided in four groups on the basis of the number of seasons previously exposed and the average duration of the exposure to HCH in weeks.

First follow-up examination. This was done on the lines similar to the main study in 310 exposed and 146 control (unexposed) subjects 8 months after the main study and just before the next spray operation.
The main objective of the study was to assess the reversibility of changes and to provide pre-exposure data for assessing the effects of one round of spray operation.

Second follow-up examination. This included the repeat examination of 218 malaria spraymen and 32 of the control subjects who also took part in the HCH spray operation. This examination was done after completion of the HCH spray operation (about 16 weeks after the first follow-up examination). The objective of this was to assess the changes after a one round of spray operation.

The distribution of spraymen examined during the main study and the two follow-up examinations is given in Table 2.

Methodology

Medical examination

Clinical findings, detailed medical and occupational history were recorded in a precoded proforma designed on identical lines to the one used in the WHO/ICMR project on spraymen exposed to DDT.

Biomedical investigations

- S.G.P.T. (Reitman and Frankel, 1957)
- Alkaline phosphatase (Kind and King,1954)
- Serum proteins and A.G. ratio (Reinhold,1953)
- Serum sugar (Folin and Wu method)

Haematological investigations

- Haemoglobin
- Total and differential W.B.C.'s count.

TABLE 2

Groupwise distribution of the subjects examined during the main study and two follow-up examinations

GROUP *	No. of Subjects		
	main study	first follow-up	second follow-up
Control	201	148	32**
I	96	66	27
II	168	116	82
III	120	82	68
IV	80	46	39

* previously exposed for:
I : one season
II : two seasons
III: three seasons
IV : > three seasons

** Control subjects engaged in HCH spray operation

Serum residue levels

Concentrations of HCH, individual isomers, were estimated by using gas liquid chromatography with electron capture detection (Stretz and Stahr, 1973).

RESULTS

Medical examination

In the spraymen, skin lesions and neurological abnormalities of coordination, balance and motor reflexes were more frequent than in the controls. The comparison of deviated neurological response in the main study and two follow up examinations (Table 3) indicate that the changes are not striking and cannot be attributed to HCH exposure.

Biochemical investigations

The results of the biochemical tests (Table 4) show that SGPT and alkaline phosphatase enzymes are well within the normal range in the controls and all groups of spraymen. There are no significant changes before and after spray operations. Significant changes are observed in serum proteins in the form of an increase in A.G. ratio and in glucose levels in spraymen before and after exposure to HCH (Table 5)

TABLE 3

Comparison of neurological response of the spraymen involved in all the three phases of the field study (= 216 subjects)

main study	first follow-up	second follow-up	No. of subjects
D →	D →	N	5 (2.3%)
D →	N →	N	2 (0.9%)
N →	N →	D	11 (5.1%)
N →	N →	N	198 (91.7%)
		Total... ...	216 (100%)

N = Neurologically normal

D = Deviated neurological response

TABLE 4

Changes in SGPT and alkaline phosphatase enzymes in controls and spraymen, examined during the main study, as well as in two follow up studies.

	GROUPS				
	0	I	II	III	IV
S.G.P.T. (Wroblewsky Units)					
M	6.3±3.0	9.0±5.1	7.7±4.3	7.9±3.8	7.3±3.6
F_1	9.2±4.9	9.6±5.2	9.5±3.8	10.3±3.9	11.1±3.4
F_2	8.7±4.4*	8.3±4.6	11.6±7.8	10.3±6.5	10.3±5.0
Alkaline Phosphatase (K.A. Units)					
M	6.8±3.7	6.9±4.6	7.4±2.9	6.5±2.8	7.4±4.1
F_1	8.7±2.9	7.9±3.0	9.0±2.5	9.1±2.3	8.7±2.4
F_2	7.9±3.0*	7.9±2.5	7.2±3.5	8.0±2.7	8.1±2.4

M = Main study; F_1 = First follow-up; F_2 = Second follow-up.

*Control subjects engaged in HCH spray operations

TABLE 5

Changes in A.G. ratio and glucose levels in controls and spraymen examined in all phases of study.

	GROUPS				
	0	I	II	III	IV
glucose (mg%)					
M	80.2±14.6	78.5±21.6	81.7±15.2	76.6±21.5	69.7±21.1
F_1	82.8±22.3	86.0±15.6	84.0±13.1	90.0±15.3	93.0±12.3
F_2	109.0±33.3*	111.0±35.6	112.0±32.3	111.0±25.5	113.0±34.9
A/G ratio					
M	1.37	1.45	1.36	1.39	1.51
F_1	1.64	1.58	1.37	1.66	1.26
F_2	1.94*	2.46	2.04	2.27	2.69

M = Main study; F_1 = First follow-up; F_2 = Second follow-up.

* Control subjects engaged in HCH spray operations

Haematological investigations

These do not reveal any specific changes in spraymen as compared to controls before or after exposure to HCH.

Serum residue levels

The results of serum HCH levels (Table 6) show significantly higher concentrations of total HCH residues in all the exposed groups of spraymen as compared to controls. The comparison of residues in successive exposure groups reveal an increasing trend upto group III only, and a significant decrease from group III to group IV of spraymen. This decrease could be a result of intercompartemental shift from blood to tissues or of enhanced metabolism due to enzyme induction.

TABLE 6

Comparison of serum HCH - residues (µg/l) in controls and malaria spraymen (main study).

HCH Isomers	Controls	Spraymen			
		I	II	III	IV
Alpha	53±71	79±56	90±58	121±116	41±35
Gamma	35±38	66±40	45±31	75±74	31±19
Beta	86±105	209±173	292±174	399±202	214±107
Total HCH	174±165	354±246	427±248	595±287	286±139
Significance Test		(P≤0.01)	(P<0.01)	(P<0.01)	(P<0.01)

The residue levels of total HCH show a significant increase for all groups of subjects (Table 7) after one season of exposure. The increase in residues is maximal (five times increase) in subjects engaged in spray operations for the first time as compared to those with previous exposure.

TABLE 7

Comparison of serum HCH concentrations (µg/l) before (first follow-up study) and after (second follow-up study) exposure in different groups of spraymen.

Subjects	Total HCH (µg/l)		Significance test
	Pre-exposure	Post-exposure	
Group 0	78±38	371±109*	P<0.01
I	116±84	435±125	P<0.01
II	261±125	356±135	P<0.01
III	312±151	481±172	P<0.01
IV	165±70	301±112	P<0.01

*Control subjects engaged in HCH spray operations

INFLUENCE OF SMOKING, DIETARY HABIT, ALCOHOL INTAKE AND PAST-EXPOSURE TO DDT

The comparison of clinical findings, biochemical and haematological parameters and residue levels in smokers and non-smokers, vegetarians and non-vegetarians,

alcoholics and non-alcoholics, with and without past-exposure to DDT do not reveal any specific, statistically significant consistent changes which can be attributed to these factors.

CONCLUSIONS

1. Clinically detectable neurological abnormalities observed in a small percentage of spraymen are not attributable to exposure to HCH.
2. Serum GPT and alkaline phosphatase enzyme changes are only minimal and do not indicate specific organ dysfunction.
3. Significant increase in blood sugar levels after exposure to HCH - suggest neoglucogenesis or insulin depression.
4. Increase in A.G. ratio without specific change in total proteins indicate suppression of globulin synthesis.
5. In spraymen, serum HCH residues show increase upto '3' seasons exposure and a decline thereafter.
6. Comparison of the results in the control (unexposed) subjects with those of the spraymen obtained in the main study gives no indication of the biochemical changes as observed in the follow up studies (before and after exposure) emphasising the importance of longitudinal studies.

THE EFFECTS OF CONTINUOUS EXPOSURE TO ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES ON CHOLINESTERASE (CHE) LEVELS IN HUMANS

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ABSTRACT

To assess the effects of continuous exposure to organophosphorus and carbamate insecticides on the cholinesterase levels in humans, the whole-blood cholinesterase from each clinically examined subject was determined using the tintometric method. The plasma cholinesterase level was spectrophotometrically determined in the laboratory. Our results show a reduction of the plasma cholinesterase enzyme, statistically significant at the 0.025 probability level, in subjects working in the Agricultural Entomology Sections at the Tropical Pesticides Research Institute (TPRI) Arusha, and Lyamungu Agricultural Research Institute, Moshi. No statistically significant reduction in the concentration of the whole-blood cholinesterase enzyme was observed in the subjects examined. These results show the degree of sensitivity of the second method rather than the actual inhibition of the cholinesterase enzyme.

INTRODUCTION

In the past 15 years the use of pesticides has increased tremendously in Kenya, Uganda and Tanzania. These chemicals are toxic to humans and animals and thus pose a potential hazard to those individuals who because of the nature of their work (farmers and spray operators) constantly run the risk of being exposed to these chemicals. Although some form of protective measure against such hazards is practised by those who use pesticides constantly, poisoning, be it accidental or intentional, cannot be completely ruled out.

The toxicity of organophosphorus and carbamate insecticides is mainly due to their ability to inhibit cholinesterase enzyme. Some of these compounds are readily absorbed into the human body through the skin, lungs and gastrointestinal tract.

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This experiment was designed specifically for screening purposes to reveal the presence of cholinesterase inhibitors in the whole-blood and plasma.

MATERIALS AND METHODS

Employees from four sections of the Tropical Pesticides Research Institute, Arusha, (Agricultural Entomology, Physics, Botany and Administration) and employees from two sections of Lyamungu Agricultural Research Institute, Moshi (Administration and Agricultural Entomology) volunteered to participate in this experiment. The Administrative personnel from the two Institutions served as the control group of their respective Institution while those who mix and spray pesticides in the two Institutions were in the experimental group.

At the time this experiment was carried out (March and April 1977), only the Agricultural Entomology Section of Lyamungu was actively engaged in spraying coffee plants using a mixture of fenthion, carbendazim and endosulfan. During the preceding two months, however, TPRI Sections had handled the compounds shown in Table I.

TABLE I

Chemicals handled at TPRI

section	chemicals
Agricultural Entomology	chlorpyrifos, dimethoate, fenthion, trichlorphon, methomyl, dichlorvos, carbofuran
Botany	DNOC, 2,4-D, atrazine, oxadiazon
Physics	dichlorvos

The whole-blood cholinesterase levels were determined using the tintometric method. This is a field method developed and described by Edson (ref. 2). The plasma cholinesterase levels were determined in the laboratory spectrophotometrically using the method described by Ellman et al. (ref. 3). One or two cholinesterase determinations were performed for each clinically examined person.

RESULTS

In the results shown in Table II, the whole-blood cholinesterase is expressed as a percentage of the activity of the normal pre-exposure value.

TABLE II

The whole-blood cholinesterase activity determined tintometrically

section	date	institution	mean %	±	S.D.	(n)
Administration	29 March &4 April	TPRI	95.8	±	9.4	(19)
Botany	18 March	"	93.5	±	8.5	(7)
Physics	28 March	"	93.1	±	6.6	(9)
Agricultural Entomology	25 March	"	94.0	±	9.9	(16)
Administration	19 April	Lyamungu	94.8	±	7.1	(12)
Agricultural Entomology	6 April	"	94.8	±	8.2	(27)
Agricultural Entomology	19 April	"	91.2	±	7.6	(29)

S.D. - Standard deviation
(n) - No of individuals

The plasma cholinesterase activity (Table III) is expressed in the kinetic units per litre (ku/l), using butyrylthiocholine at 25°C and at a pH of 7.7. The normal range is 3.0-8.0 ku/l.

TABLE III

The plasma cholinesterase activity determined by Ellman's spectrophotometric method

section	date	institution	mean ku/l	±	S.D.	(n)
Administration	29 March &4 April	TPRI	4.34	±	1.94	(17)
Botany	18 March	"	4.10	±	0.75	(7)
Physics	28 March	"	4.17	±	1.13	(9)
Agricultural Entomology	25 March	"	2.94*	±	0.98	(14)
Administration	19 April	Lyamungu	3.77	±	0.75	(10)
Agricultural Entomology	6 April	"	2.80*	±	1.14	(24)
Agricultural Entomology	19 April	"	3.08	±	1.02	(22)

* Statistically significant at the 0.025 probability level

DISCUSSION

Our results show a statistically significant reduction of the plasma cholinesterase enzyme at the 0.025 probability level (Table III) but no significant reduction in the concentration of the whole-blood cholinesterase in all persons examined (Table II). The inconsistency of the results shown by the two methods suggest that Ellman's spectrophotometric method enables the detection of a slight but significant reduction of the plasma enzyme whereas by using the tintometric method, plasma and erythrocyte cholinesterase activities are not

determined separately.

It should be mentioned that some of the mixers and spraymen we examined from the two Institutions have been applying these chemicals for many years without the use of any appropriate protective clothing and without any special cleansing routine after a day's work. The significance of this type of working habit to the depression of blood or plasma cholinesterase enzyme is uncertain.

Since there has been little information published on the effects of continuous exposure of organophosphorus and carbamate pesticides on the cholinesterase levels in humans in the tropics, we feel that more data will be needed before any conclusion is reached on this subject.

On the reactivation of the cholinesterase enzyme after inhibition by these pesticides, however, there have been reports that a complete reversal of the cholinesterase enzyme inhibition by organophosphorus or carbamate insecticides does occur by the hydrolysis of the phosphorylated or carbamylated enzyme at different rates depending upon the physiochemical properties of the inhibitor (refs. 1,4).

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STUDY OF PUBLIC HEALTH SECRETARIAT PESTICIDE APPLICATORS DURING THE ANTI-MALARIA CAMPAIGN 1978

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INTRODUCTION

In Argentina the Public Health Secretariat have been using different pesticides for many years to control the vectors of malaria and Chagas disease.

During the campaigns from 1959 to 1971, 5,415,000 kg of HCH and 517,391 kg of DDT were used.

The results of the campaign against Anopheles mosquitoes (combined with other sanitary measures) show the success of the programme. In two different groups of Provinces there was a steady reduction in the number of cases of malaria until 1964. There was an increase in cases during 1966/69, perhaps because of a lack of the proper use of the pesticides, or because of the banning of organochlorine pesticides, which was later reviewed for Public Health use.

In the case of triatomides (known as "vinchucas") the results showed an important decrease in morbidity, not only in the provincial capital cities but in all the small towns in the area. In the Province of Salta where the diagnosis was made by laboratory methods, the difference between the areas without application of pesticides and treated areas was very marked.

There is no discussion about the need for the use of residual pesticides against the vector of malaria and Chagas disease in spite of inevitable ecological contamination. The benefit-risk evaluation is here beyond any question.

EPIDEMIOLOGICAL ASPECTS

It was considered imperative to supervise and control all the applicators involved in the campaigns. Notwithstanding the preventive measures and the correct use of pesticides, there was a high level of contamination in these men compared with a control group of administrative staff working in the same campaigns. A complete examination was also undertaken periodically in order to ensure that no adverse effects occurred. Fortunately, there were no accidents and during a five year period the index of absenteeism was at an average level.

The comparison between the workers and the control group is shown in table 1.

TABLE 1

Epidemiological aspects of the application of chlorinated pesticides in Argentina.

Blood levels in applicators who worked for five years with HCH (n=50) compared with controls (n=50)

	mean µg/l of blood	
	exposed (n=50)	control (n=50)
HCH γ	70	0.39
HCH β	237	6.67
Dieldrin	1	0.80
DDE	15	6.80
DDT	3.86	-
op'DDT	-	0.62
pp'DDT	-	3.59

It will be noted that:

a) there is a high concentration of β-HCH since the product used in Argentina is not a pure gamma isomer (lindane);

b) none of the children under study showed general clinical or biological disturbances;

c) the workers who worked for 5 years with chlorinated pesticides for combating malaria and who show high indices of β-HCH showed no clinical symptoms of any kind. Owing to the impossibility of conducting specific tests (enzymograms, hepatic biopsy, etc.) we based our findings on the absenteeism index. During 5 years these men attended work with the same regularity and in a similar state as the administrative employees engaged in the anti-malaria campaign who were not in contact with chlorinated pesticides and who were used as controls for the purpose of our study.

I would like to give an account of the supervision of pesticide applicators during the last campaign against Chagas and malaria disease in Chaco Province (Argentina).

SURVEY IN 1978

Using some parts of the protocol VBC/75.9 of the Vector Biology and Control Division of WHO, we designed a survey to evaluate the hypothetic chronic effects of organochlorine pesticides in the last campaign against triatomides in 1978. We tried not only to study the degree of contamination between different groups of workers i.e. smokers vs. non-smokers, drinkers vs. non-drinkers, protected vs. unprotected, etc. during the application of pesticides, but also to evaluate

possible enzyme and metabolic effects with emphasis on the presence of alpha fetoprotein.

Material and methods

Exposed people: 48 male workers aged between 21 and 56 years (mean age 36.3 years) with 1 to 25 years in the field (mean 8.2 years). At the time of our study they were clinically healthy. Venous blood samples were taken and processed within 48 hours.

Control group: 5 males were selected with the background of not being in contact professionally with pesticides. They also were clinically healthy.

Studies performed:

a) all the applicators answered the WHO protocol questions;

b) the VDRL reaction and the Chagas-Mazza test (Yanowsky test), by direct agglutination with previous reduction by 2-mercaptoethanol, were performed in all people;

c) enzyme studies. Taking into account the possibility of hepatic enzyme induction by organochlorine pesticides, we performed the following analyses:

S.G.O.T. & S.G.T.P. (Reitman and Frankel, 1957; ref.1)
Alkaline Phosphatase (Schwartz, 1960; ref.2)
Lactic Dehydrogenase (LDH) (King 1969; ref.3)
Creatin-Phosphokinase (CPK) (Raikin & Zaccara 1974; ref. 4)
Cholinesterase (Cinetic method, Boehringer; ref.5)
Alpha-Fetoprotein (electrophoresis)
Immunotoxicological analysis (Schoidegger methods)

d) concentration of chlorinated hydrocarbons in the blood (Dale, 1966 and g.c.; ref.6)

RESULTS

The results are presented in tables 2A and 2B.

TABLE 2A

Results of serologic, enzyme, immunological and blood levels studies in exposed (n=48) and non-exposed (n=5) persons.

Serologic studies	Control group	Exposed
VDRL	0	4.1%
Machado Guerreiro	16%	12.5%

Enzyme studies	Control group	Exposed
S.G.O.T.	14.9± 2.9 MilliUnit/ml	13.0± 4.3 MilliUnit/ml
S.G.P.T.	10.8± 4.6 "	9.5± 3.5 "
Alkaline phosphatase	27.3± 8.1 "	28.0± 6.3 "
Cholinesterase	2325 ± .175 "	2,383 ± .418 "
LDH	98.7±14.1 "	102.3±19.4 "
CPK	16.5± 5.1 "	22.7± 8.6 "
Inmunological studies		
αFetoprotein	Negative	Negative
Inmunoelectrophoresis	No abnormalities	No abnormalities
Blood levels of chlorinated hydrocarbons		
αHCH	0.3 µg/l	2 µg/l
βHCH	32 "	104 "
γHCH	7 "	16 "
Dieldrin	1 "	13 "
pp'DDE	10 "	21 "
pp'DDT	16 "	31 "

TABLE 2B

Influence of protective clothing, smoking and drinking on the concentration of chlorinated hydrocarbons in the blood of exposed people

Influence of protective clothing	pinafore, shoes, hats (24 cases)	boots, mask, gloves (24 cases)
αHCH	4 µg/l	1 µg/l
βHCH	106 "	102 "
γHCH	17 "	16 "
Dieldrin	17 "	10 "
pp'DDE	24 "	18 "
pp'DDT	34 "	28 "
Influence of smoking habits	Smokers (23 cases)	Non-smokers (25 cases)
α HCH	3 µg/l	3 µg/l
β HCH	167 "	100 "
γ HCH	16 "	17 "

	Smokers (23 cases)	Non-smokers (25 cases)
Dieldrin	15 µg/l	12 µg/l
pp'DDE	22 "	19 "
pp'DDT	31 "	30 "

Influence of drinking during the application period

	Drinkers (27 cases)	Non-Drinkers (21 cases)
αHCH	3 µg/l	3 µg/l
βHCH	109 "	92 "
γHCH	17 "	15 "
Dieldrin	14 "	13 "
pp'DDE	21 "	21 "
pp'DDT	31 "	30 "

CONCLUSIONS

We studied the VDRL andChagas-Mazza tests although these are not included in the WHO protocol but from a medical point of view it was imperative to check-up on these two important disease in men in Argentina. Perhaps it was the only opportunity for these people to have such an analysis.

In the enzyme and immunological studies no abnormalities were observed. The main contaminant in the blood was found to be β-HCH. There was no important difference in the chlorinated hydrocarbon levels in blood of smokers and non-smokers neither was there a difference between the levels of people who had been drinking during the application period and those who did not.

In view of the importance of this kind of studies there is a great need for more laboratoria and more technical experts in the field.

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FIELD APPLICATION OF PHENOXY ACID HERBICIDES

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ABSTRACT

Occupational exposure in forestry to phenoxy acids (2,4-D and 2,4,5-T) as esters has been studied in four spraymen spraying a 2% emulsion in water from a tractor driven equipment. Air-borne concentrations measured with a stationary sampler and measured in the air from the individual breathing zone showed a mean 2,4-D and 2,4,5-T concentration of 0.1 - 0.2 mg/m^3. Plasma and urine levels were monitored during a week of exposure. The highest levels of phenoxy acids were found in urine with a mean of 8 (2,4-D) and 4.5 (2,4,5-T) µg/ml and ranging from 3 to 14 µg/ml for 2,4-D and from 1 to 11 µg/ml for 2,4,5-T in the afternoon after a day of exposure. The mean 24 hour excretion in urine was 9 mg of 2,4-D and about 1 mg of 2,4,5-T. The elimination in the urine was rapid. Uptake of the phenoxy acid esters seems to be caused both by inhalation and by dermal absorption. Improved hygienic conditions are suggested to decrease exposure. No symptoms indicating intoxication appeared. In control subjects with a low and indirect exposure no 2,4-D and 2,4,5-T could be detected in plasma or urine. Lowest detectable concentration in urine of 2,4-D and 2,4,5-T was 0.05 µg/ml.

Occupational exposure for agricultural purpose to phenoxy acids (MCPA and dichlorprop) as salts has been studied in 8 professional spraymen and 13 farmers. They were exposed to 1-2 per cent water soluble salts. Air-borne concentrations in the individual breathing zone showed a mean of 0.011 mg/m^3 for MCPA and 0.017 mg/m^3 for dichlorprop. Urine samples were taken during and after work. The levels of MCPA and dichlorprop ranged from non detectable (less than 0.05 µg/ml) to 12 µg/ml.

INTRODUCTION

In Sweden around 200 tons of phenoxy acids are used annually in forestry (2,4-D and MCPA-esters, and up to 1977 also 2,4,5-T-esters). For agricultural purposes salts of mainly MCPA, dichlorprop and mecoprop, around 2 000 tons, are used.

Lack of information concerning the exposure of the application of these

herbicides prompted this investigation.

The following questions were posed:

1) Can the blood or urine concentrations be indicators of exposure?
2) When should the samples be taken in relation to the working periods?
3) Is there any accumulation of phenoxy acids at the end of a week with exposure?
4) How rapidly would 2,4-D, 2,4,5-T, MCPA and dichlorprop levels decline after a week of exposure?

MATERIAL

Forestry

Four persons spraying a 2 per cent emulsion in water of phenoxy acid esters by tractor were monitored. They were all men, with a mean age of 39 years, all smokers. Years of previous exposure to phenoxy acids varied between 2 and 14 years. Two men of the staff, following the sprayers, served as controls. Butoxyethyl esters of 2,4-D and 2,4,5-T were used.

Agriculture

Eight professional spraymen and 13 farmers were monitored during the spring season. Years of previous exposure to phenoxy herbicides varied between 2 and 24 years. The workers mixed MCPA, dichlorprop, mecoprop, ioxynil, bromoxynil and sometimes 2,4-D individually in the tractor driven tank. The herbicides, mainly MCPA and dichlorprop, were used as 1-2 per cent water solutions of their salts. Until 1975 MCPA was used mainly as a powder formulation. Since then a liquid formulation was used.

METHODS

Sampling

Forestry Blood samples were drawn before the start of the work and immediately after spraying. This was done at the start of a work period and at the end of a week with exposure. Blood samples were also drawn at 12, 24 and 36 hours after the end of exposure.

Urine samples were delivered at the start and the end of a day with exposure and in a second investigation, in 12 hours sampling periods during and after exposure.

Agriculture. Urine samples were taken at the beginning and at the end of a day with exposure and at the end of a week with exposure. The elimination was studied during 1-2 days after the last exposure.

Forestry and agriculture. Air was sampled in impinger flasks placed in the breathing zones of the individuals for 60 minutes periods. Air flow was 1 liter/min. The absorption liquid was ethanol for the esters and water for the salts.

Analysis

Forestry. The air samples were analyzed by UV-spectrophotometry and thin-layer

chromatography (ref. 1). After hydrolysis the phenoxy acid levels in plasma and urine were determined by electron capture gas chromatography using an internal standard technique. The lowest detectable concentration of 2,4-D and 2,4,5-T was 0.02 µg/ml in plasma and 0.05 µg/ml in urine.

Agriculture. MCPA and other agricultural herbicides in air were analysed with a new HPLC method (ref. 2). Urine analyses were done with electron-capture gas chromatography using extractive derivatization. Lowest detectable concentration of MCPA and dichlorprop in air was 0.005 mg/m^3 (0.01 µg/ml and in urine 0.05 µg/ml.

RESULTS

Forestry

The air concentrations of 2,4-D and 2,4,5-T esters were mostly 0.1 - 0.2 mg/m^3 with occasional higher levels.

The plasma levels ranged from the detection limit up to 0.1 - 0.2 µg/ml. The levels varied due to intermittent exposure. No rising trend towards the end of the working week was noted. After exposure the levels declined overnight almost to the limit of detection.

The urine levels of phenoxy acids were much higher than those found in plasma. The concentrations of 2,4-D and 2,4,5-T were on an average 8 and 3.5 µg/ml respectively, and ranged from 3 to 14 µg/ml for 2,4-D and from 1 to 11 µg/ml for 2,4,5-T in the afternoon after a day of exposure. In a second investigation the mean 24 hour excretion in urine was 9 mg of 2,4-D and around 1 mg of 2,4,5-T. Thus a mean concentration of around 10 µg/ml 2,4-D in urine results from spraying for 2-4 hours/day (=effective spraying time).

In control subjects with a low and indirect exposure neither 2,4-D nor 2,4,5-T could be detected in urine with the above mentioned detection limits.

The elimination in urine was rapid (Fig. 1).

Agriculture

Air concentrations ranged from n.d. to 0.026 mg/m^3 with a mean of 0.011 mg/m^3 for MCPA and from n.d. to 0.036 mg/m^3 with a mean of 0.017 mg/m^3 for dichlorprop. In urine the concentration of MCPA ranged between 0.2 and 12 µg/ml for professionals and from n.d. to 1.8 µg/ml for farmers. The figures for dichlorprop were n.d. to 1.6 and n.d. to 0.9 µg/ml respectively. The elimination of MCPA seems to be longer than 12-24 hours, see Fig. 1. Dichlorprop follows the same pattern.

Symptoms

None of the subjects in our study, neither in forestry nor in agriculture, reported clinical symptoms except one man who mentioned slight irritation of the eyes after direct contact with the spray liquid. Symptoms of severe intoxication reported in literature are headache and nausea (ref.3), and rarely polyneuropathy (only reported in the 1950-ies). Lately sarcoma in workers exposed to various

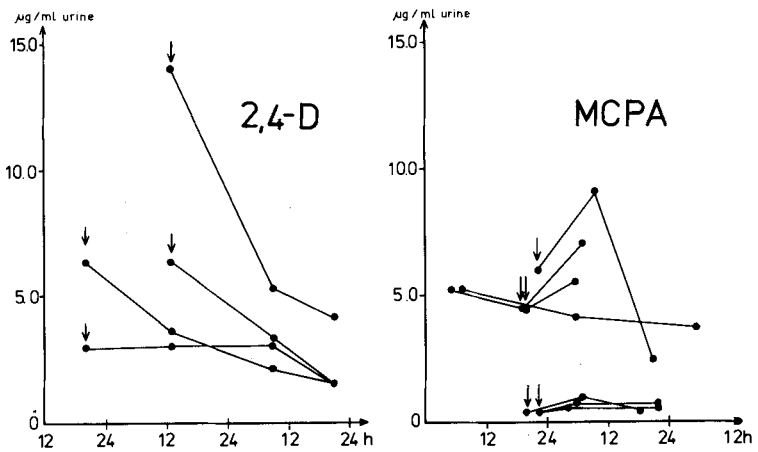

Fig. 1. Urinary elimination of herbicides from individuals spraying 2,4-D-ester or MCPA-salt. Arrows indicate end of work.

phenoxy acides in forestry and in agriculture has been discussed by the Swedes Hardell and coworkers (ref. 4 and 5).

CONCLUSION

Uptake of phenoxy acids seems to occur both by inhalation and by dermal absorption. The experimental conditions in this investigation did not allow an assessment of the relative importance of the routes of absorption. However, the urine concentrations after exposure to phenoxy acid salts are higher than might be expected according to the air concentrations. Improved hygienic conditions are suggested to decrease the exposure.

It is concluded that exposure to 2,4-D and 2,4,5-T esters could best be monitored by measurement of the phenoxy acid levels in the urine. The sampling should preferably be made in the afternoon of a working day or the following morning. For phenoxy acid salts urine sampling on the morning following the working day seems preferable.

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EXPOSURE OF FINNISH FORESTRY NURSERY WORKERS TO QUINTOZENE AND MANEB

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INTRODUCTION

In Finland exist about 40 forestry nurseries totaling almost 800 hectares. Most of the forestry nurseries are pine nurseries. The use of pesticides in pine nurseries is 10-20 tons annually, and fungicides constitute 70-80% of all pesticides used. By far the most common fungicides are quintozene (pentachloronitrobenzene) and maneb (manganese ethylenebisdithiocarbamate), 7-15 tons of which are used per year. Maneb is sprayed in pine nurseries twice a month during summertime and quintozene is used in the autumn just before winter. This work is done mechanically by 1-2 workers in the pine nursery and the number of persons dealing with pesticides in Finnish forestry nurseries is about 60.

The purpose of this survey was to study pine nursery workers' exposure to maneb and quintozene. We compared different application methods and observed possible mistakes in the use of pesticides. An additional aspect was to find out if it is possible to follow exposure by biological monitoring, i.e. analyzing metabolites of quintozene and maneb in urine. The survey was done in four forestry nurseries situated in central Finland.

METHODS OF SAMPLING

Samples were taken from the breathing zone of workers spraying pesticides in pine nurseries. For the exposure monitoring of the pesticide a personal sampling pump (MSA model S) was used to suck air through a membrane filter (Millipore AAW P 0037). Personal samples were collected for 3-4 hours a workday. During the sampling the pump flow rate was 2.8 l/min. While the workers were weighing the pesticide and diluting it in a mixing chamber samples of about 10 minutes were collected from their breathing zone. During this work the pesticide was collected with a more effective pump (20 l/min.).

Immediately after collection the samples were placed into an exsiccator and weighed after 24 hours. Urine samples were taken at the end of the workday and during the following night.

ANALYSES OF SAMPLES

Maneb is decomposed by boiling collected samples in diluted hydrochloric acid. Liberated carbon disulfide is trapped and determined with a sprectrophotometer at 435 nm (ref. 1). Quintozene was analyzed as pentachloronitrobenzene by a gaschromatograph using a 5% QF-1 column and FID-detector (ref.2). Ethylene diamine and pentachloroaniline were determined in urine samples being metabolites of maneb resp. quintozene.

In the hydrolyzed urine samples ethylene diamine was determined as pentafluorbenzoyl derivative with the gaschromatograph. The column was 1.5% carbowax 20 M-TPA and an EC-detector was used (ref.3).

Pentachloroaniline in the urine samples was determined after acid hydrolysis by a gaschromatograph equipped with a 2% SE-30 column and EC-detector (ref. 4).

The metabolite concentrations were calculated against the specific gravity of the urine.

RESULTS

Maneb

About 1.2 kg maneb/hectare was sprayed from a tractor pulled tank. Fungicide was mixed with water (30 g/10 l) in a tank either by using one kilogram bags or by weighing out maneb from the big 50 kg sacks. Exposure in both cases and the daily spraying exposure were measured. The time needed to apportion maneb into a tank was about 10 minutes. When this work was done by weighing out maneb from the sacks, the concentration of maneb in the breathing zone was found to average 6.7 mg/m^3. During weighing (2-3 min) concentrations of 12 mg maneb/m^3 were measured. When one kilogram bags were used samples taken from the workers' breathing zone averaged 1.3 mg maneb/m^3. If a tractor driver weighed out maneb from sacks his daily exposure to maneb averaged 0.2 mg/m^3.

In nurseries where weighing was not needed, a driver's daily exposure to maneb was below 0.1 mg/m^3.

In the breathing zone of assistant workers the maneb concentration was on the average 0.1 mg/m^3. This was so when maneb was apportioned in one kilogram bags. In a nursery where weighing was needed the daily exposure to maneb was on the average 0.2 mg/m^3. Results are presented in table 1. Ethylenediamine was not found in the urine of the workers.

TABLE 1

Exposure of workers to maneb

object	average daily exposure mg Mn-EBDC/m^3/8 h	mean values during sampling		number of samples
		Mn-EBDC mg/m^3	total dust mg/m^3	
exposure to maneb in different work periods:				
tractor driver				
- use of 1 kg bags	0.1	0.1	0.4	20
- use of 50 kg bags	0.2	0.3	0.5	10
assistant worker	0.1	0.1	0.4	10
exposure during dilution of maneb:				
- use of 1 kg bags		1.3	1.5	24
- use of 50 kg bags		6.7	6.8	16
exposure during weighing of maneb		11.9	12.4	2

Mn-EBDC = Manganese ethylenebisdithiocarbamate

Quintozene

Quintozene is applied at 10-50 kg/hectare. It can be mixed with water (200 g/10 l) or applied in powder form. Usually the fungicide is applied with a tractor, but sometimes especially in greenhouses where tractors cannot be used, quintozene is applied manually.

When quintozene was applied as a water mixture, the pentachloronitrobenzene concentration in a tractor driver's breathing zone averaged 0.4 mg/m^3. If the powder formulation was used this concentration rose to 1.3 mg/m^3. In cases where quintozene dust was applied manually, the worker was exposed to 3.5 mg/m^3 of fungicide. The more detailed results are presented in table 2.

TABLE 2

Exposure of workers to quintozene

object	mean values during sampling		number of samples	mean values of PCA µg/l urine
	PCNB mg/m^3	Total dust mg/m^3		
exposure to quintozene in different work periods :				
use of spray				
- tractor driver	0.4	0.8	8	1.4
use of powder				
- tractor driver	1.3	6.8	4	1.7
- assistent	0.5	0.9	4	1.4
- manual applicator	3.5	18.2	6	2.4
exposure during filling:				
use of spray				
- filling of tractor tank	2.6	2.8	5	
use of powder				
- filling of manual applicator	2.0	5.8	4	
- filling of tractor applicator	1.6	3.7	4	
exposure during application:				
- spray by tractor	0.2	0.5	2	
- dust by tractor	1.1	6.7	2	

PCNB = Pentachloronitrobenzene
PCA = Pentachloroaniline

The mean values of pentachloroaniline in the urine samples of different workers ranged from 1.4 µg/l to 2.4 µg/l. The highest pentachloroaniline concentration was in the urine samples of the person who manually applied quintozene. Results are presented in figure 1.

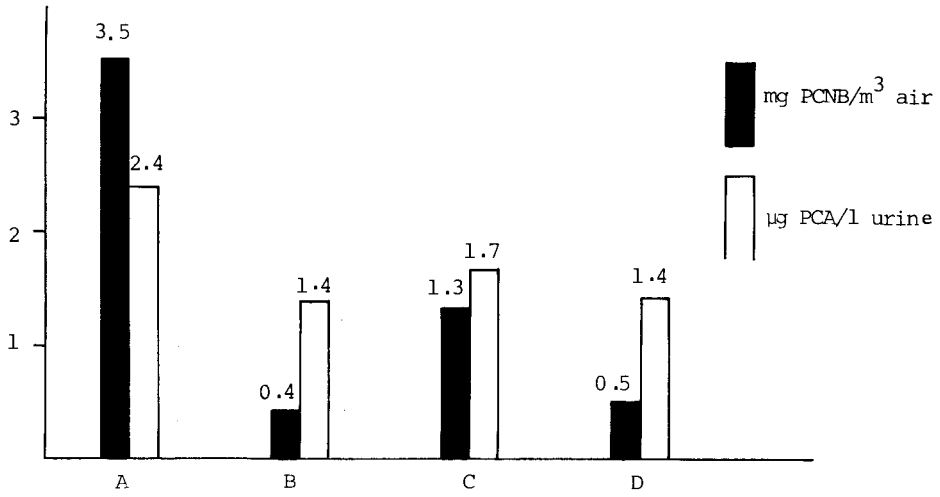

Fig. 1. Average concentrations of quintozene in the breathing zone and mean concentrations of the urinary metabolite pentachloroaniline in different work procedures:
A powder formulation applied manually
B application of water mixture with tractor
C application of powderformulation with tractor
D assistant worker in case C.

DISCUSSION

The acute toxicity of maneb and quintozene is relatively low (maneb oral LD_{50} rat ≈ 6750 mg/kg; quintozene oral LD_{50} rat over 12000 mg/kg) (ref.5). Both pesticides are excreted in a few days and they do not accumulate in mammals (refs. 6, 8, 10).

Maneb has been used continuously for a long time and is regarded as a relatively harmless chemical. In experimental animals treated with maneb, thyroid function has been increased. With the Ames Test maneb has been found to be a doubtful mutagen (ref. 7). In the human body maneb is metabolized into ethylenethiourea which is suspected to be a carcinogenic substance (ref. 8). It has been found in animal experiments that maneb has a negative effect on blood coagulation and hematopoietic organs (ref. 7). Changes in LDH isoenzyme fractions have been reported in the tests on rats exposed to 100 µg maneb/m^3 in respiratory air (ref. 7). Maneb has been reported as a strong skin sensitizer in experiments with guinea pigs (ref. 9). Some occupational dermatitis caused by maneb has been reported (ref. 10).

The chronic toxicity of quintozene is low. No evidence of adverse effects were detected when dogs were fed 30 ppm of pentachloronitrobenzene in a daily diet

during two years (ref. 11). In some cases quintozene has been reported as a skin irritant (ref. *11, 12*). *Quintozene* may contain as an impurity *hexachloro*-benzene, which is a far more toxic substance. It may seriously affect the liver and kidneys. Hexachlorobenzene has been found to have teratogenic effects (ref. 13).

The worker's exposure to quintozene and maneb is quite low during a workday when he is spraying a water mixture of these fungicides. If the work is done by using powder formulated quintozene the pesticide concentrations in his breathing zone will be higher. In these cases, especially when the pesticide is applied manually, personal protective equipment will be needed. During the weighing of fungicides into a dilution tank, high transitory concentrations of the fungicides were measured in the breathing zone of the worker. It would be advisable to use only small fungicide bags with a known weight in forestry nurseries. Because these fungicides are skin irritants and maneb is a skin sensitizer, the persons who are dealing with them should take good care of their personal hygiene. It seems that the pentachloroaniline concentration in urine follows the exposure to quintozene but its suitability for biological monitoring of field workers needs more detailed study. Because of the relatively minor exposure and limited period of use it is obvious that quintozene and maneb as applied in Finnish forestry nurseries do not present any significant health hazard.

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PESTICIDE EXPOSURE IN TROPICAL AND SUB-TROPICAL AUSTRALIA

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Queensland is a very large State with a total area of 1,728,000 sq. kilometres, representing 22½% of the area of Australia. The area within the tropics (North of the Tropic of Capricorn) is 934,000 sq. kilometres, representing 54% of the State. Its greatest length is 2,100 kilometres and the greatest breadth 1,450 kilometres. (7 x area of U.K.; 49 x area of Holland).

Within this vast area are maintained sheep and beef cattle, sheep on the open grass lands in the south and central west and cattle on the rougher and more wooded pastures of the east and north and the dry far west. There are vast cane-sugar farms stretching up the east coast, and becoming very extensive in the tropics around Mackay, Cairns and Innisfail. Wheat, cotton and tobacco are extensively cultivated, and obviously tropical fruits such as pineapples, peanuts and bananas are produced in large quantities. North Queensland supplies most of Australia's requirements for long grain rice, and tea has recently become available in the same area of the Burdekin River and Ingham areas. Afforestation and timber preservation is a large resource industry extending throughout the State.

The utilisation of pesticides in this vast area for plant and animal disease control is a production and economic necessity. This pattern is repeated in other States with large area and distance problems like New South Wales and Western Australia. The development of mechanical methods of application for large-scale applications, and the extensive use of aerial spraying techniques has been a direct result of these special attributes of the Queensland and Australian scene as regards pesticide usage. ULV dispersion over the crops of interest is the preferred method of application by air.

It will be appreciated that the capability and capacity for control, supervision and survey opportunities of pesticide operators and pesticide application

programmes is limited by these factors. There are unique and special opportunities such as locust eradication programmes which occur sporadically and may involve hundreds of men with little experience in spray techniques or the handling of toxic chemicals.

Workers will be exposed to economic pesticides for the best reasons as an assumption. These relate to pest control, crop adequacy and disease control, and economic and market basket benefit to the nation. Information of a basic type will inevitably be available at least to experts on the mammalian (human) toxicology of the product. Tests will be available or considered depending on the information.

For instance, in Australia there has been the most invidious pressure placed on available analytical methods by popular clamour, most especially illustrated by 2, 4, 5-T. Methods have been developed at the now recognised standard of detection of nanograms/ml , and occupational health units have in fact been forced to simply accept an arbitrary level for 2, 4, 5-T from expected minimal presence in exposed operators. The level chosen is 100 ng/ml (100 ppb) of 2, 4, 5-T and 2, 4-D in urine, but it is emphasised that this figure bears no relevance to poisoning nor is it any indication of hazard. It merely indicates a level beyond which one may assume some carelessness in application techniques, errors in equipment maintenance, neglect in wearing of protective clothing or indifferent personal hygiene. This comment of course also applies to "cut-off" limits for other pesticides or contaminants in the working environment.

Blood levels of 2, 4, 5-T do not appear to correlate in any way with urinary levels and a judgement of exposure only may be made. Levels of 200 to 300 ng/ml in the blood may be found with urinary levels of 18 to 22 ng/ml, and perhaps an opportunity for further study exists in this field.

Medical supervision can only relate to defined groups of operators under field conditions. Sporadic cases of spectacular type do not assist in defining the picture as pertains the experienced worker, neither for pesticide exposure nor indeed for other toxic substances encountered in industry.

As far as clearance for full-scale use is concerned, the release of a reliable chemical of proven characteristics, should not be inhibited at an impractical level of guaranteed safety requirements at national or international levels. In fact there have been several decades of use of materials with very low dose LD_{50} 's with the established expectation that field worker exposure at spray dilution percentages must be massive to induce symptomatic expression.

There is a very delicate balance between effectiveness of the material, toxicity to humans and economic persistence. A cheap, harmless insecticide requiring relatively frequent application will never serve vast tracts of Australian extent or similar needs in other countries. Efforts in Queensland to monitor the health of workers in the field have been directed to defined groups or areas in the positively identified occupational situations. The times of the year for spraying programmes are virtually confined to the October/April bracket. Visits are made to areas where large-scale spraying is being undertaken, either by aerial spraying methods or by power-spray systems. This may entail flying up to Emerald, 1000 km from Brisbane, or making a car trip out to Goondiwindi, 375 km from Brisbane, and covering other centres on the way there and back. Occasional surveys may be 1500-2000 km away (Charters Towers, Cairns, etc.). In addition surveys are arranged when indicated, with the help of local practitioners in these far-flung places, with all specimens being sent by air to the central laboratory for assessment.

During these visits the opportunity is taken of talking to the groups examined about safety, spraying techniques, the machines, mixing methods and symptoms of inappropriate exposure.

It should be realised that contract spraying is uncommon in Australia except by air. Virtually all other spraying, dipping or other methods applied for the control of plant or animal pests is undertaken by the farmer, his family and the few men employed on the larger properties. Their independence and ingenuity is their most remarkable characteristic, but their comprehension of toxicity is not always at the academic level, and is further bedevilled by the mass of propaganda perpetrated by the type of media presentation to which all countries have become accustomed.

Monitoring of the chlorinated hydrocarbon series of pesticides and cholinesterase levels for organo-phosphates and carbamates is constantly undertaken. In addition there are groups of men who undertake methyl bromide fumigation of wheat and other products, workers who are exposed to ethylene dibromide, phosphine fumigation of silos on farm properties and chlordimeform as an aerial spray for cotton pests. There has been significant arsenic exposure in weed control and tick control in some areas though this has been markedly diminished over the last 20-25 years with the availability of the newer pesticides and herbicides. Organic mercury compounds are used extensively on sugar-cane farms for pre-planting protection of the cane sets, and paraquat and other agents find their

place where indicated. In addition of course there is an extensive programme in forest control for underbrush and other weeds of the invasive type in urban or rural areas such as groundsel, where the usual herbicides are used such as 2,4,5-T, 2,4-D and Tordon. There should be no support from informed scientists of the emotional outbursts relating to these materials and the host of articles appearing on Agent Orange or the Alsea affair, whether by proclamation of the Environment Protection Agency or by representatives of movements such as CATS (Citizens Against Toxic Sprays).

An extremely useful chemical has been sacrificed on the altar of political and legalistic expediency. In Australia the National Health and Medical Research Council has officially stated its view, that it did not recommend any additional restrictions on the use of 2,4,5-T. The N.H. & M.R.C. concluded that its view of past evidence and its examination of new material did not provide any scientific evidence of a causal link between use of 2,4,5-T and an excessive occurrence of spontaneous abortion and human birth defects as had been suggested. (June 1979).

It will be appreciated that review of the data is very thorough, and there is no doubt that these matters are totally relevant to the topic of discussion. As in New Zealand, Australian health authorities have been forced into extensive enquiries into the alleged association between the use of 2,4,5-T and 2,4-D and birth defects in Queensland, and in the Yarram district of Victoria.

Somehow committees of expertise such as this Vth International Workshop must initiate attitudes of reasonableness and rationality into the edicts of respected bodies within the technologically advanced nations of the world. Legalistic interpretations and strenuous adherence to a mass of documentary protocol are creating chaos out of the ability of departments to make decisions relating to economic chemicals, including pesticides.

Reviews in Queensland and in Australia of pesticide exposure in the field relate to these problems. In the first place the Division of Industrial Medicine in Queensland monitors workers on the basis of occupational health supervision at the advisory level. It also perforce may be required to produce the data as a result of any local activity stimulated by media articles and reviews of a national or indeed international character of the type delineated. Data is available relating to surveys of all the described exposures named above though data is limited in those workers whose exposure is minimal - e.g. organic mercury is confined to a very few packers and to the few officers preparing solutions for cane dipping of sugar-cane sets.

Queensland surveys of organochlorine exposure show a preponderance of DDT, DDE and HCB presence. The spread for DDT and metabolites is from 2-53 nanogram/gram and of HCB from 1-4 ng/g. Occasional figures may of course exceed the higher of the figures quoted. Figures for dieldrin are very low, and often negative as use has declined markedly. The suggested upper levels of acceptance in blood for dieldrin, aldrin, heptachlor and chlordane are 50ng/ml (ppb), at which point cautionary advice is proffered.

Cholinesterase levels are most often quite normal but there have been occasional incidents of symptomatic expression in loaders of spray for aircraft use.

Should spectacular incidents occur emergency visits are arranged by air or motor vehicle, and the assistance of local officers requested when needed. It is nearly always possible to obtain specimens by air from virtually any region of Queensland.

A programme for the monitoring of the exposure of fumigators using methyl bromide has been developed as a result of the fairly extensive use of this fumigant in Queensland for the treatment of wheat, wood-borer and other pests.

A blood bromide level of 20 micrograms/ml is an indication for removal from exposure and has been adopted on the basis of experience which suggests that symptoms are possible at about 28 micrograms/ml (ref. 1), but any positive tendency for figures to rise from the non-exposed population base-line of 6-8 micrograms/ml indicates a need for tightening of procedures. This introduces by election a large safety factor for a hazardous occupation.

Verberk et al (ref.2) report that a worker with blood bromine greater than 12ppm has 3.5 times the probability of having a slightly disturbed EEG, but this figure bears no relationship to exposure or subjective symptoms.

A classic example of the efforts exerted relates to chlordimeform. There are no cases of human toxicity or other untoward incidents for which chlordimeform as such has been responsible in the field but the product was voluntarily withdrawn by the manufacturers as an alleged carcinogen in September, 1976. Its value on cotton seems to be very successful for Heliothis control, and extensive controlled trials were carried out in N.S.W. in 1978/79 in the field to determine adequate safety programmes based on urinary estimation of chlordimeform in urine.

Urine samples were collected:

1. prior to commencement of the programme (base sample)
2. 12 hours after application (morning after)

3. 24 hours later (36 hour sample)
4. 24 hours later (60 hour sample).

Of 464 samples, 57 (12.3%) were above the limit of detection of 0.03 ppm. This contrasts markedly with the only reported series of cases of symptomatic response due to gross contamination by the technical material (ref. 3). In these cases of industrial exposure on a packing line, 9 out of 22 workers developed a haemorrhagic cystitis, with urine levels of 2 to 19 ppm. All cases recovered completely.

The conclusions appear to indicate that the great majority of workers are not unduly exposed in these large scale aerial spraying programmes. These tests were part of an International Chlordimeform Monitoring programme carried out in Columbia, Guatemala, Nicaragua, U.S.A., Sudan, comprising over 16,000 samples.

It is considered that an adequate programme once proven should be available as a data base for decision. There is no doubt that values vary from country to country depending on sophistication of equipment and capacity for training of the worker groups. We do in fact know this to be the case, and should adjust our advice and approaches on this knowledge.

At the monitoring level factories where insecticides are manufactured and packed are quite obviously regarded as "field worker exposure" and in Queensland and in other States this philosophy directs our attention to these workers on the same pattern of supervision or investigation described.

In the past inhalation tests using pumps or personal samplers have been carried out to assess potential exposure in the worker environment in the field, and results are in conformity with those reported from other countries. The results, most often expressed as a percentage of the postulated LD_{50}, only indicate the degree of efficiency of method and protective equipment.

It is probably not justifiable to repeat tests of this type, or filter paper tests applied to forearms, back etc., except for special reasons, in order to estimate dermal dose in the light of the present availability of protective equipment and design of modern spraying gear. Pure research, standardisation of new equipment, or the introduction of new techniques or methods, could conjecturally provide the rationale for such experimental programmes.

The need for occasional surveys at the biological level remains as an impetus to lectures and exhortations to safety in the field for farmers and workers using

economic pesticides in all countries. Academic research must remain available as the refined tool for deeper understanding. For instance electromyography is a very sensitive test, but the cumbersome nature of the equipment and need for a power supply makes its use in the field rather impractical.* It is superb as a research tool or in the investigation of factory workers.

CONCLUSIONS

1. Efforts to establish the level of field worker exposure to pesticides are essential to safety programmes in all countries.
2. Repetitious surveys of established techniques do not add to our knowledge but simply fortify known and expected responses.
3. Such data should be available at an international level for utilisation and appraisal by expert bodies.
4. Decisions on usage, control and supervision should be based on mammalian toxicology and dose-rate expectation in the field, and not on the ephemeral imputation of carcinogenicity and genetic threat at levels of absorption quite unrelated to the practical situation.
5. Reassurance at a positive level must be given by responsible scientists on all available data.
6. Our presence as scientists, investigators and advisors should be as prominent as the emotional adversaries of that technology which characterises our modern states, and which makes the possibilities of adequate nutrition, clothing, housing and health a practical hope for all people, everywhere.

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* David Roberts (Liverpool) reports that easily portable systems have in fact been designed and hold promise for the future.

MONITORING PROGRAM FOR THE ASSESSMENT OF HUMAN SAFETY IN OPERATIONS INVOLVING ORGANOPHOSPHORUS INSECTICIDES

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ABSTRACT

Spray teams working in various field projects are examined periodically for blood cholinesterase (ChE) activities. Regular checking intervals are 3 to 4 weeks. Extra tests are taken after special duty and in case of accidental exposure.

Individual ChE activities remained remarkably constant. Whereas diminished plasma activity occurred sporadically, no case of diminished erythrocyte activity has yet been recorded in our spray teams. It is concluded that the favourable ChE activity records are due to adequate discipline in the teams. The monitoring scheme is considered essential to maintain awareness of exposed personnel and to detect effective exposure if it should occur. If suspicious activities are confirmed in a duplicate test, the suspect is subjected to a general medical checkup. The physician in charge decides on further precautionary steps. Spray team members view the scheme favourably and collaborate spontaneously.

INTRODUCTION

Organophosphorous insecticides (OP) are inhibitors of cholinesterase (ChE) activity. The effect of a single sublethal OP dose to a human subject is slowly reversible. Continued exposure to small doses will therefore gradually diminish the ChE activity, until symptoms appear which indicate a potentially dangerous level of poisoning.

Periodic determination of ChE activity in peripheral blood is a dependable way of recognizing effective exposure to an inhibitor substance in the preclinical stage. The procedure is therefore a must where insecticidal products are frequently handled as a process of professional routine.

I will give an outlay of the monitoring scheme which was developed by the medical service of Ciba-Geigy Switzerland to check employees who handle ChE inhibitors professionally, and I will present the results from monitoring spray teams who are engaged in experimental field application of various insecticides, mostly of the OP group and often at an early stage of development.

METHODS

I shall describe the organisation of monitoring personnel that are employed in Switzerland, and the methods which are used in the monitoring. In foreign operations, many details have to be adjusted to local conditions, therefore general statements would be virtually impossible and shall not be attempted.

Monitoring Scheme

Fig. 1 shows the organisation of the intramural monitoring scheme.

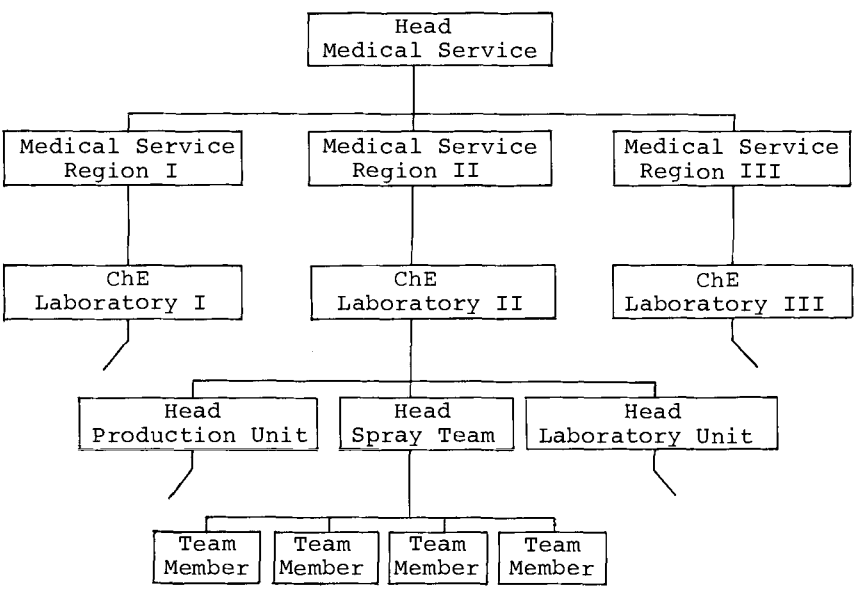

Fig.1. Organigram of the ChE Monitoring Scheme

The Company's medical service maintains a list of all employees who have professional contact with OP materials. They are members of production units, laboratory units and spray teams, respectively. Heads of the various units and teams are responsible for keeping the lists updated.

The ChE laboratory arranges for periodic blood tests in three to four week intervals. Testing days are posted in advance, so as to enable team members to call in time. Every individual is instructed to call for an additional test after heavier-than-usual duty, especially after incurring accidental contamination with OP material.

Three laboratories carry out determinations independently in three separate plants where the company conducts OP activities. All three laboratories report to their deputy physician, who in turn connects with the head of the service in the main plant, thus assuring coordinated procedures and integrated evaluation.

The result of every ChE determination is communicated by the laboratory to the individual employee. This immediate contact promotes the desirable awareness that the laboratory takes care and deserves confidence.

Cases of markedly lowered ChE activity are picked up by the physician in charge. He will give the suspect a thorough medical checkup to exclude other causes of altered enzyme activity. This provides a decision basis for further precautionary steps, which might culminate in suspension from work to provide for recovery of the enzyme activity, and investigation of the source of contamination in order to achieve improvement.

Assay method for ChE activity

The enzyme activity is determined in peripheral blood samples. The method is based on colorimetry of thiocholine with dithio-bis-nitrobenzoic acid (DTNB) after enzymatic hydrolysis of choline esters (ref.1). The method was modified for simultaneous determination of total activity and of plasma activity in microsamples of whole blood (ref.2).

Quality control is maintained by including VALIDATE® standard serum in each series. One determination per blood sample is considered sufficient. A duplicate sample is assayed the following day if a serious deviation from normal is determined in the primary sample.

RESULTS

The topic of the present Workshop is "Field Worker Exposure". Data from factory workers will therefore be excluded from consideration.

Cholinesterase activity records of 16 field workers were available for examination. Two record sheets date back to 1972, the majority began 1975/76. Depending on the frequency of assignments abroad, between 3 and 15 determinations per year were taken from individual employees in the Swiss ChE laboratories under the present monitoring scheme.

In agreement with common experience, there is considerable difference in enzyme activities between individuals. In a group of 8 employees of whom frequent data are available from the same laboratory, mean plasma ChE activities have a range from 90 to 127 Klett units (KU). Erythrocyte activities in the same sample range from 108 to 152 KU. On the other hand, the data of any one individual have only slight variability. In the same group of 8 employees, who were examined through 27 man years, all plasma ChE data of two individuals are within 10 % of the individual's mean. In 5 others, activity reductions between 11 and 20 % were recorded in 9 instances. 40 % from the individual's mean, representing the extreme deviant, was recorded once.

In all cases of decreased plasma cholinesterase activity, the activity in the erythrocytes remained normal, i.e. within 10 % of the mean. Reverting again to the sample of 8 employees, erythrocyte cholinesterase activity remained within 10 % of the mean in all determinations of three individuals. In the remaining 5, a reduction between 11 and 20 % was recorded in 7 instances.

The findings of diminished ChE activity were not connected with episodes of heavier-than-usual exposure to OP materials.

A representative section from one ChE activity record sheet is reproduced in table 1.

TABLE 1

Representative section from a field applicator's ChE activity record

Date	Ery (KU)	Plasma (KU)
16.12.76	108	102
26. 1.77	101	113
9. 2.77	(93)	119
9. 3.77	98	111
23. 3.77	99	105
26. 4.77	100	104
10. 5.77	107	99
1. 6.77	126	96
15. 6.77	109	106
29. 6.77	104	110
21. 9.77	106	114
30.11.77	106	100
14.12.77	112	(85)
28.12.77	119	97
11. 1.78	106	101

Three-year means (in Klett Units, KU):
erythrocytes 108
plasma 106

Circled:
Deviations exceeding 10 % from the mean

DISCUSSION

A certain variability of individual ChE activities is common. In clinical practice, activity decreases up to 20-30 % from pre-exposure level are to be considered normal (refs. 3-5). The independent occurrence of lowered activities in erythrocytes versus plasma in our samples confirms the clinical irrelevance of minor deviations in the 20 % range.

According to standard views, the one case of 40 % decrease of plasma ChE activity is reaching clinical relevance. Even this case had no obvious exposure in the anamnesis, and he recovered without specific measures being taken. The only case of presumably real ChE inhibition is in line with the rule that plasma ChE is less stable than erythrocyte ChE, and that a decrease of activity is not serious until it affects the erythrocyte ChE.

A recent report from Sweden, surveying eleven professional spraymen who worked with a variety of insecticides including OP, matches our data: no clinical symptoms of poisoning, one single case of decrease in plasma activity exceeding the normal intra-individual variation. Neurophysiological parameters in the study further corroborated the biochemical findings (ref. 6).

We conclude from our data that the established precautionary measures are adequate to protect professional spraymen from effective OP contamination. Overall, headgear, mask, boots and gloves are the regular personal equipment for applicators of experimental OP products. The implementation of appropriate measures is in the responsibility of team heads. The results of the personnel monitoring program provide feedback data to instruct and direct individual team members adequately.

ACKNOWLEDGEMENTS

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A LONGITUDINAL STUDY OF RED CELL AND PLASMA CHOLINESTERASE IN TWO GROUPS OF ORGANOPHOSPHORUS WORKERS

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INTRODUCTION

Although it is 28 years since Callaway, Davies and Rutland first measured blood cholinesterase (ChE) of organophosphorus pesticide workers (ref. 1) and while this parameter is in widespread use as an indicator of exposure to inhibitor compounds, there are few published accounts of the results and particularly of their effectiveness as a means of reducing exposure to whatever is considered to be an acceptable level. This paper presents the results of a longitudinal study of the red cell and plasma ChE in two groups of workers engaged in the manufacture and formulation of an organophosphorus pesticide.

FIRST STUDY OF PLASMA AND RBC CHOLINESTERASE LEVELS

The first study was made at the start of production and formulation of an OP pesticide in a plant with no previous work with OP compounds; it lasted five months, during which there were three production runs as indicated by the horizontal bars marked E in Fig. 1.

During this time, plasma ChE was about 50% of the pre-exposure mean value and showed no significant fluctuation correlating with the intermittent production runs. If plasma ChE indicated exposure to the OP compound then the exposure or its effects on the plasma enzyme persisted in spite of breaks in production lasting 10 and 30 days. The probability that exposure continued during these breaks was increased by observation of contamination of the work site with the finished product. In this example, depression of plasma ChE clearly indicated the need for improved house-keeping.

Over the same period, when plasma ChE was depressed, the laboratory responsible for ChE measurement continued to report no effect on RBC ChE. However, retrospective examination of the results showed that the RBC ChE increased above the pre-exposure value and that this increase was interrupted by depressions during the second and third production runs. A possible explanation for this pattern of response is that a low level of exposure to an inhibitor agent caused an increase

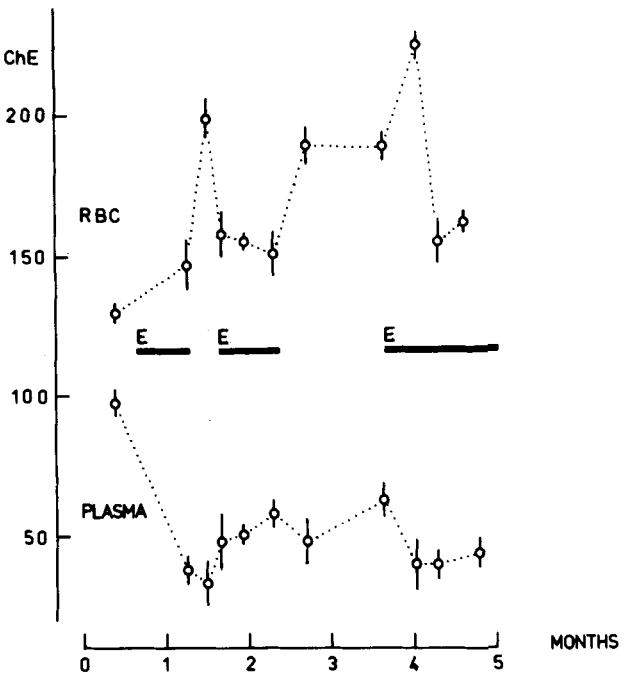

Fig.1 Mean ± 1 SEM values for RBC and plasma ChE found in 10 workers engaged in production and formulation of an OP pesticide. The horizontal bars marked E denote production runs with plant cleaning and maintenance taking place in the intervening periods.

in RBC ChE by enzyme induction. In such circumstances, inhibition of part of the increased total enzyme could occur even though normal or as in this case, above-normal RBC ChE values may be reported. In the absence of a method for measuring the total enzyme present, whether inhibited or not, evaluation of RBC ChE results is difficult.

This study shows that, contrary to the initial laboratory report, there was an effect on both plasma and RBC ChE and that there is a need to evaluate blood ChE results over an adequate time span and at a frequency great enough to show up any long-term trends in enzyme activity levels.

THE SECOND STUDY

A second study was made during the commissioning of a new plant to manufacture and formulate the same OP pesticide. Over a period of 20 months covering the start of operations at a newly constructed OP plant, measurements of plasma and RBC ChE were made in the plant workers and in a control group not concerned with OP manufacture. Monthly average values for the two groups are expressed graphically

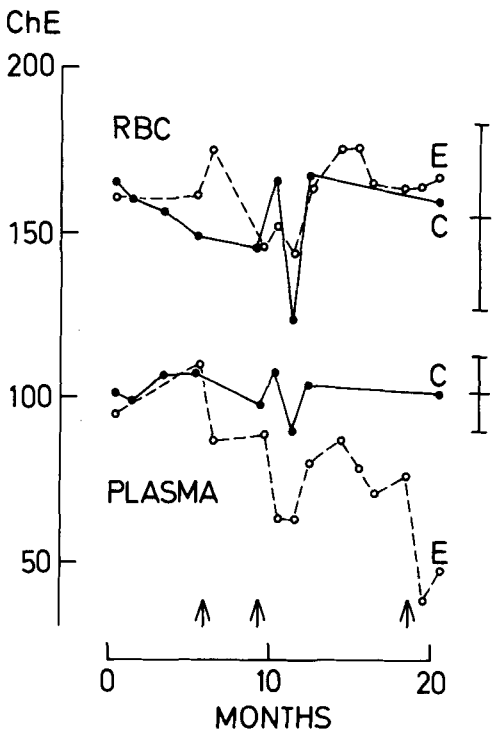

Fig. 2. Monthly average values for RBC and plasma ChE in 15 OP-pesticide (E) and 17 control (non-OP) workers (C). The mean and 95% confidence limits of monthly average values for control workers are indicated on the right of the figure. The significance of the vertical arrows is described in the text.

together with the overall mean and 95% confidence limits of monthly averages for the control group in Fig. 2. It may be noted that the wide range of monthly average values in the control group is due principally to the marked and unexplained fluctuation at 10 months.

From the 6th month onwards, the monthly averages for plasma ChE in the exposed group fall below the lower 95% confidence limit of the control group in a manner which appears to reflect three phases of the commissioning procedure, indicated by the three arrows in Fig. 2.

1) A small quantity of the pesticide was formulated.

2) Full-scale production commenced.

3) An automatic drum-filling system was introduced which, although theoretically cleaner than the manual system in use previously, in fact increased the environmental contamination and exposure of the workers. In the case of the RBC ChE, the exposed group monthly averages were always within the wide 95% confidence limits of the control group.

RETROSPECTIVE ANALYSIS WITH THE 'CUSUM' TECHNIQUE

In order to demonstrate more clearly any differences between control and exposed groups, the monthly average values were evaluated with the CUSUM technique. Exposed group monthly averages were subtracted from the overall mean of the control group over the 20 month period. The differences so obtained were then summed algebraically and the resultant cumulative differences were plotted against time in Fig. 3.

In retrospect, it can be seen that the plasma CUSUM differences deviated from zero following the first handling of the small quantity of pesticide (1st arrow) and that a more rapid deviation took place when production began (2nd arrow) and again when the new drum-filling device was installed (3rd arrow). These results indicate continuing exposure to an inhibitor compound and emphasize the need to take into account both duration and magnitude of enzyme inhibition when assessing the possible biological consequences. They were also of practical value to the plant manager as evidence of inadequate plant and personal hygiene throughout the operation.

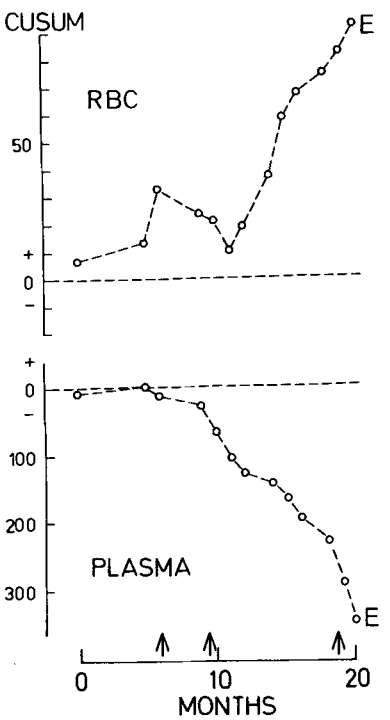

Fig. 3. CUSUM plot of monthly average values for RBC and plasma ChE in 15 OP pesticide workers. The zero points represent the average values for 17 control workers over the 20 month period. Significance of vertical arrows as in Fig. 2.

A similar retrospective examination of the RBC ChE values shows a continuing increase in the exposed group compared with the controls. This positive deviation began with the first formulation, it was reduced when production started - perhaps due to inhibition of the enzyme - and then continued to increase during the rest of the period under observation. Whatever the mechanism for this increase in enzyme activity, it indicates the need for caution in the interpretation of RBC ChE values.

CORRECTIVE ACTION BASED ON BLOOD CHOLINESTERASE LEVELS

In the light of these results on blood ChE, improvements were made in plant and personal hygiene, following which there was an increase in plasma ChE to 75% of the control value over a period of six months. However, RBC ChE still remained at 10% above the control value. These further results, which are part of a continuing study, are encouraging but they also emphasize the need for even more successful measures to limit exposure.

CONCLUSION

These two examples of longitudinal studies of blood ChE activity in workers occupationally exposed to an organophosphorus pesticide have been chosen in order to illustrate some problems of interpretation which can arise. They also demonstrate the value of retrospective analysis of group results as opposed to the short-term use of individual ChE values as indicators of exposure. Finally, although these studies were made at the site of production of the pesticide, there is no reason to suppose that they are not valid for field worker exposure during pesticide application.

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EXPOSURE AND MEDICAL MONITORING STUDY OF A NEW SYNTHETIC PYRETHROID AFTER ONE SEASON OF SPRAYING ON COTTON IN IVORY COAST

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ABSTRACT

In view of the neuropathies found in experimental animals given almost lethal oral doses and the subjective symptoms of skin sensations in workers occupationally exposed to a recently developed synthetic pyrethroid insecticide, a study was carried out in Ivory Coast to monitor exposure and possible adverse health effects in man after hand-held ULV application to cotton during one season of spraying. The operators sprayed a pyrethroid formulation at the rate of 50 grams a.i. per hectare in six spray sessions at fortnightly intervals. An extra application was made one month after the sixth spray session.

Exposure to the pyrethroid was monitored by the determination of the major metabolite derived from the acid fragment of the pyrethroid in urine collected during 24 hours after application. In many samples metabolite excretion was below the limit of detection indicating a low level of absorption. The absorption was calculated to be substantially less than the equivalent of an oral dose of 1 mg pyrethroid per spray session. This calculation is based on a controlled dose-urinary excretion study. Skin and inhalational exposures did not correlate well with the urinary excretion. Inhalational exposure was about 1% of skin exposure. General medical and extensive clinical and neurological examinations, blood biochemistry and peripheral nerve function tests (including the trigeminal nerve) did not show abnormalities either before or after the series of six spray sessions nor after the seventh spray session. In some electroneurophysiological tests (motor conduction velocity, slow fibre conduction velocity and cornea reflex) a statistically significant change within the normal range appeared to exist for the group of sprayers between pre- and post exposure measurements. There is no evidence that these changes are compound related; they probably reflect seasonal variations. The general conclusion is that under the conditions of this study the application of this pyrethroid caused no detectable adverse health effects.

INTRODUCTION

In view of the neuropathies found in experimental animals given almost lethal oral doses and the subjective symptoms of skin sensations in workers occupationally exposed, an exposure and medical monitoring study of a recently developed synthetic pyrethroid has been carried out in Ivory Coast to assess safety in use of this pyrethroid by hand-held ULV after repeated application. In this study seven indigenous Africans sprayed the pyrethroid every fortnight during 3 months (June-July-August 1978). The study was designed and guided by the Exposure Monitoring Group, a multi-functional group of experts in the field of toxicology and occupational health. The results of this study in terms of exposure measurements and medical examinations are described in this report.

METHODS

Application and application personnel

The applicators were seven indigenous Africans, working at the Institut de Recherches du Coton et des Textiles Exotiques (I.R.C.T.), Bouake, Ivory Coast. The sprayed pyrethroid formulation (25 g. a.i.* per litre) at the practical rate of 2 litres per hectare using Micron ULVA equipment in six spray sessions at fortnightly intervals. This part of the study was supervised by I.R.C.T. staff. An extra seventh application was supervised by members of the Exposure Monitoring Group. During application the operators wore long trousers and open-necked short sleeved shirts with no other protective equipment. Spraying conditions of the extra seventh application are given in Table 1.

Exposure measurements

Exposure was measured:

- by determination of the major metabolite derived from the acid moiety of the pyrethroid in the total amount of urine passed in the first 24 hours after each spray session (ref. 1);
- by determination of pyrethroid skin deposit, which was done by sampling the pyrethroid on aluminium foil attached to various parts of the body (only during the extra spray session)(ref. 1);
- by determination of the respiratory exposure by personal air sampling (only during the extra spray session). This was done both with a direct method in which the amount of pyrethroid sampled on nitrocellulose filters was determined and an indirect method in which the concentration of the solvent vehicle on charcoal tubes was determined (ref. 1).

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* a.i. is the abbreviation of active ingredient.

TABLE 1

Application details of part of the study supervised by members of the Exposure Monitoring Group in which 2 litres of pyrethroid formulation per ha (= 50 g a.i./ha) were sprayed.

Subject	Overall time in the field*	Angle between wind direction and cotton rows	Height of the crop	Windspeed (km/hour)	Temp. (°C)	Relative humidity (%)	Impact on sensitive paper
A2	60 min.	0 - 360°		0 - 12	29°C	46%	++
A4	90 min.	0 - 90°		0 - 8	25°C	72%	+
B2	60 min.	0 - 90°		0 - 8	25°C	72%	+
B4	60 min.	45°	70 cm-1 m	3 - 10	26°C	75%	+
C2	50 min.	0 - 45°		0 - 7	25°C	75%	++
C4	60 min.	45 - 90°		0 - 10	27°C	81%	+
D2	55 min.	45 - 90°		0 - 10	25°C	84%	+

* The time reported in this column includes the time necessary to refill the bottles with 1 l. of formulation. Actual spraying lasted about 45 minutes. Driving time from the field to the I.R.C.T. was about 1 hour. In this period the operators did not change clothes or washed their hands and faces.

An estimate of skin exposure during spraying was made by using special impact-sensitive chart recorder paper which was attached to the chest and back of the operator.

Medical examinations

Medical examinations were carried out before and after the series of six spray sessions and after the extra seventh spray session. The examinations consisted of:

a. Medical history including occupational, smoking, drinking and medication histories.

b. Extensive physical examination.

c. Extensive neurological examination with special emphasis on the cranial nerves.

d. Examination of 14 blood biochemical parameters.

e. Electrophysiological examinations of selected peripheral nerves including the trigeminal nerve.

Post spraying examinations further included a specific medical history relating to any complaints or symptoms which could be caused by exposure.

Electrophysiological techniques

A summary of the performed nerve function tests and the time of measurement is given in Table 2.

Apart from the sensory action potentials of the median- and sural nerve (SAPM, SAPS) and the sensory conduction velocity of the sural nerve (SCVS), base-line values of each parameter on each operator were measured in December 1977/ January 1978.

Base line values of the SAPM, SAPS, SCVS and additional base-line values in each operator of the SCVM were measured in May 1978 with recently acquired equipment from Medelec, a modular electromyograph MS6 (Medelec Limited, Woking, Surrey, England).

With the same equipment measurements were carried out of each parameter on each operator at the end of the sixth and the extra (seventh) spray session. For each occasion at least four separate measurements of each parameter on each operator were carried out on different days.

The tests took place in an air conditioned room at a temperature of 20-22^{o}C. In peripheral nerve function tests skin temperature measurements were carried out with a digital analogue temperature meter over the nerve tract at the elbow, wrist and middle-finger. If lower than 30^{o}C the arm was warmed in a water bath.

TABLE 2

Details of electrophysiological measurements

Nerve function parameter	Time of measurement
PAP1 Muscle action potential; adductor pollicis (before voluntary contraction)	Dec.'77-Sept.'78 * Sept.'78 - Sept. '78 **
PAP2 Muscle action potential; adductor pollicis (after voluntary contraction)	Dec.'77-Sept.'78 Sept.'78 - Sept.'78
MCV Maximum conduction velocity; ulnar nerve	Dec.'77-Sept.'78 Sept.'78 - Sept.'78
SFCV Slow fibre conduction velocity; ulnar nerve	Dec.'77-Sept.'78 Sept.'78 - Sept.'78
SCVM Sensory conduction velocity; median nerve	Dec.'77-Sept.'78 *** May'78-Sept.'78 Dec.'77-May'78 **** Sept.'78- Sept.'78
SCVS Sensory conduction velocity; sural nerve	May'78-Sept.'78 Sept.'78-Sept.'78
SAPM Sensory action potential; medican nerve	May'78-Sept.'78 Sept.'78 - Sept.'78
SAPS Sensory action potential; sural nerve	May'78-Sept.'78 Sept.'78 - Sept.'78
OORF (Orbicularis-oculi reflex)	Dec.'77-Sept.'78 Sept.'78-Sept.'78
CORN (Cornea reflex)	Dec.'77-Sept.'78 Sept.'78-Sept.'78
MAS (Masseter reflex)	Dec.'77-Sept.'78 Sept.'78-Sept.'78
MIH (Masseter inhibition)	Dec.'77-Sept.'78 Sept.'78 - Sept.'78

* results obtained in December 1977/January 1978 and at the end of a season of repeated spraying (September 1978) (refs. 1,2).

** results obtained in September 1978 at the end of a season of repeated spraying and in September 1978 after an additional spray session supervised by Exposure Monitoring Group (ref. 1).

*** results obtained in May 1978 before spraying and at the end of a season of repeated spraying (September 1978) (ref. 1).

**** results obtained in December 1977/January 1978 and May 1978 (both baseline values) (refs. 1,2).

RESULTS

Exposure Monitoring

Pyrethroid metabolites in urine*. Applicator's urine metabolite levels (the trans metabolite was a more reliable index of pyrethroid exposure than the cis-isomer, see Interpretation of exposure monitoring tests) were low, both in the six spray sessions supervised by I.R.C.T. staff and in the extra seventh session under supervision of members of the Exposure Monitoring Group and were in many cases below the limit of detection, which varied from 0.01-0.05 mg/l. Overall detectable 24 hours urinary trans metabolite levels were in the range of 0.008-0.13 mg (n = 9; mean value 0.032 mg) (ref.1).

In an attempt to simulate the worst conditions likely to be encountered in practice, two members of the Exposure Monitoring Group carried out a spray application with 2 litres of the pyrethroid formulation ensuring gross exposure. Also in these sprayers 24 hours urinary levels were low: 0.077 and 0.065 mg respectively.

Pyrethroid skin deposit on aluminium foil**. Calculation of dermal exposure was done by relating the quantity of the pyrethroid on the aluminium foil with the areas of unclothed body parts as given by WHO (ref. 3). Total pyrethroid dermal exposure of the operators during actual spraying time was in the range of 2.8-42.2 mg/hour (mean value: 14.8 mg/hour). Most exposure was on the hands and the arms. Dermal exposure levels of the two members of the Exposure Monitoring Group were 36.0 and 65.4 mg/hour respectively. There was a good relationship between the pyrethroid skin exposure and the patterns on impact sensitive paper (ref.1).

Pyrethroid levels in the operator's breathing zone. Apart from two measurements with the filter sampling method (direct method, see Exposure measurements) inhalable exposures of operators during actual spraying time were all below 0.10 mg/m^3 of pyrethroid; in two operators inhalable pyrethroid concentrations of 0.17 and 0.20 mg/m^3 were obtained. Inhalable exposure levels of the two members of the Exposure Monitoring Group were 0.48 mg/m^3 and 0.02 mg/m^3 respectively as determined with the filter sampling method (ref. 1).

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* Analyses of samples were carried out in Shell Toxicology Laboratory, Sittingbourne, U.K.

** Analyses of samples were carried out in Shell Biosciences Laboratory (Woodstock), Sittingbourne, U.K.

Medical examinations

Medical examinations carried out before the series of six spray sessions demonstrated that all application personnel were in good health (ref.2). Blood biochemical tests showed that one operator had a slightly increased level of SGOT, but this abnormality was not considered to be of medical significance.

Physical, neurological and blood biochemical examinations carried out at the end of the six spray sessions did not show abnormalities or changes in any operator, apart from the above mentioned operator, compared with examinations made before the spray sessions. In this operator there was a change in liver function test values: SGOT, SGPT and gamma GT were abnormal, while other liver function tests i.e. total bilirubin and alkalinephosphatase were borderline (ref.1).

Electrophysiological examinations

The individual results of the electrophysiological tests in each operator obtained in the periods specified in Table 2 are shown in reference 1.

Comparing the electrophysiological values obtained at the end of a season of repeated spraying with the base-line values of December 1977/January 1978 it was concluded that, as a group, there was a statistically significant decrease in both peripheral motor- and sensory conduction velocities MCV, SFCV and SCVM ($P < 0.05$) within the normal range. With respect to the trigeminal nerve function it can be concluded that there was a statistically significant decrease in latency time of the cornea reflex ($P < 0.05$) within the normal range. There were no statistically significant group changes in the other parameters (see figures 1a, 1b, 1c), although statistically significant individual changes were found in all parameters within the normal range.

Comparing the values at the end of a season of repeated spraying with the additional base-line values obtained in the sensory nerves in May 1978 it can be concluded that, as a group, no statistically significant differences were found in the parameters SCVM, SCVS and SAPM. A statistically significant increase was found in the SAPS but this was thought to be due to technical difficulties in the measurement of this particular parameter. Again some statistically significant individual changes were observed within the normal range in all parameters.

Comparing the electrophysiological values obtained after the extra (seventh) spray session with the values after the end of a season of repeated spraying it can be concluded that as a group there were no statistically significant differences in any parameter (see figures 1a, 1b, 1c). Occasionally individual changes were observed in some parameters within the normal range.

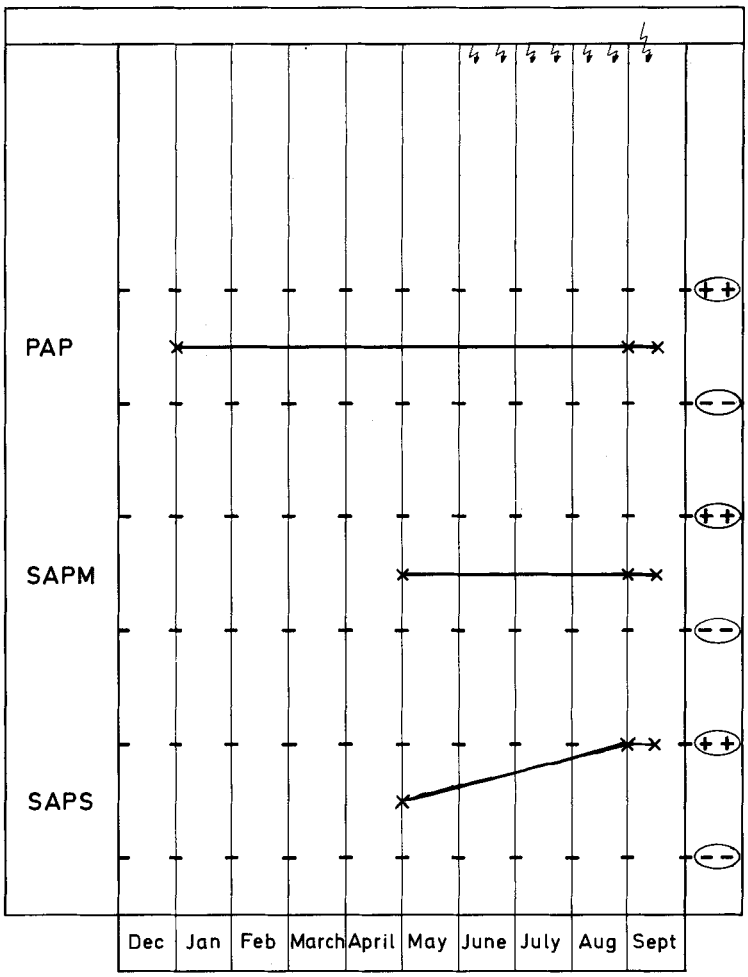

FIGURE 1a

Group changes in nerve function parameters
PAP (action potential of the adductor pollices muscle), SAPM (sensory action potential of the median nerve) and SAPS (sensory action potential of the sural nerve) of application personnel after a serie of six applications of a pyrethroid formulation and after an extra application of the formulation.
Values in September 1978 were compared with the base-line values measured in December/January 1977 and in May 1978

spray sessions supervised by personnel of I.R.C.T.

spray session supervised by members of the Exposure Monitoring Group

(++) significantly increased as a Group

(--) significantly decreased as a Group

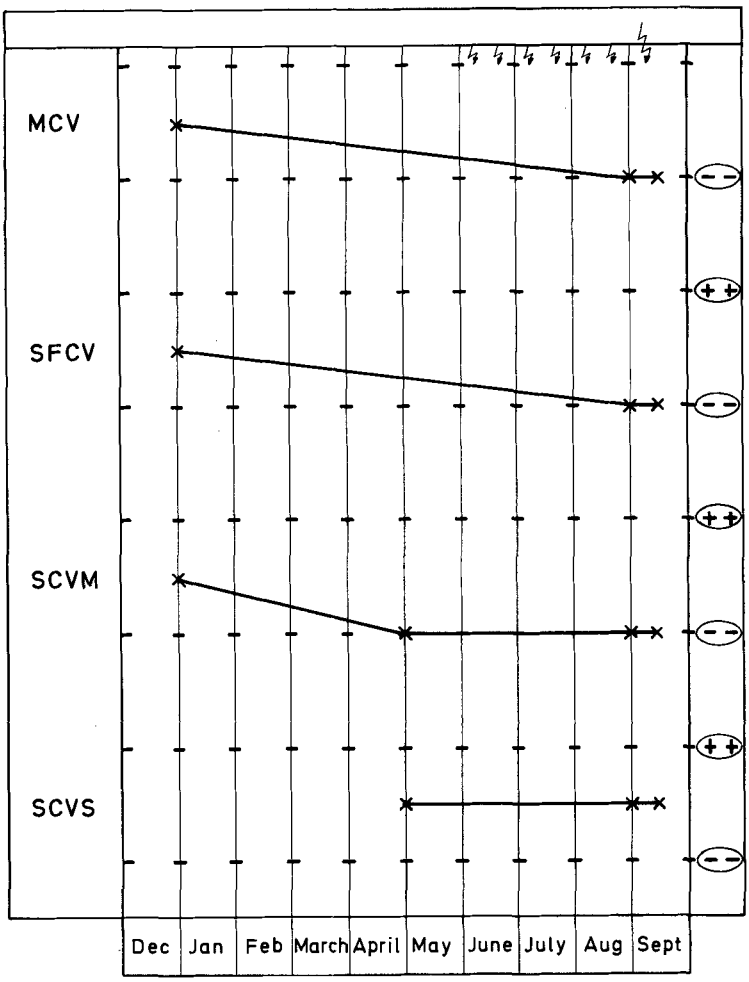

FIGURE 1b

Group changes in nerve function parameters
MCV (maximum conduction velocity of the ulnar nerve), SFCV (slow fibre conduction velocity of the ulnar nerve), SCVM (sensory conduction velocity of the median nerve) and SCVS (sensory conduction velocity of the sural nerve) of application personnel after a serie of six applications of a pyrethroid formulation and after an extra application of the formulation.
Values in September 1978 were compared with the base-line values measured in December/January 1977 and in May 1978

spray sessions supervised by personnel of I.R.C.T.

spray sessions supervised by members of the Exposure Monitoring Group

(++) significantly increased as a Group

(−−) significantly decreased as a Group

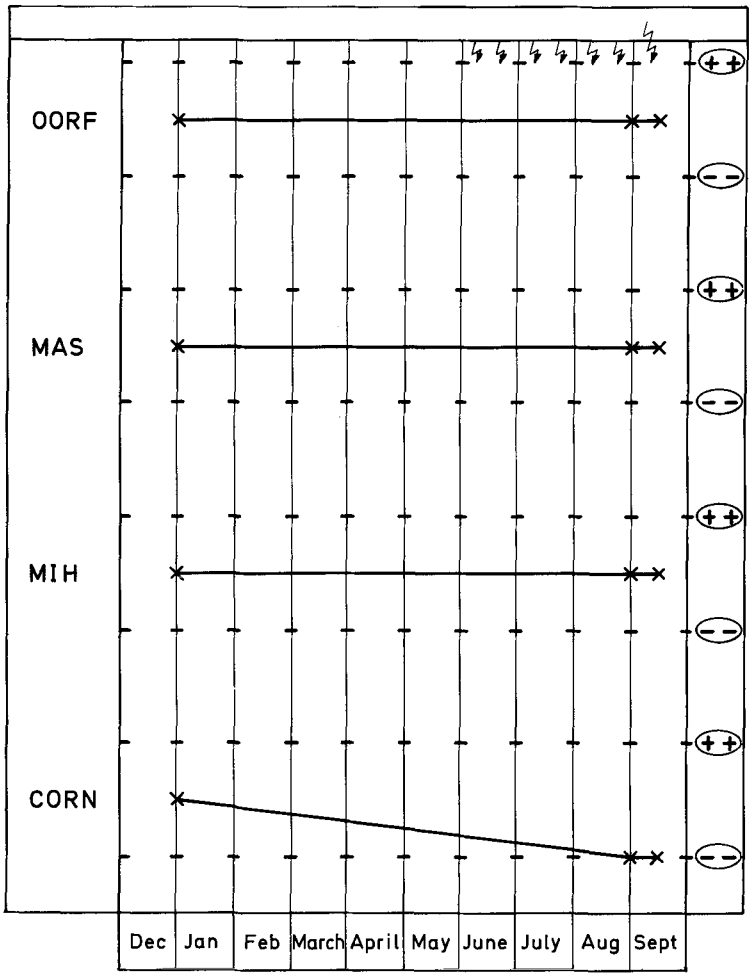

FIGURE 1c

Group changes in nerve function parameters
OORF (o-o reflex), MAS (masseter reflex), MIH (masseter inhibition) and CORN (cornea reflex) of application personnel after a serie of six applications of a pyrethroid formulation and after an extra application of the formulation.
Values in September 1978 were compared with the base-line values measured in December/January 1977 and in May 1978.

spray sessions supervised by personnel of I.R.C.T.

spray sessions supervised by members of the Exposure Monitoring Group

(++) significantly increased as a Group

(--) significantly decreased as a Group

DISCUSSION AND CONCLUSIONS

This repeated application study with a recently developed pyrethroid consisted of two parts:

(i) The major part of the study was supervised by personnel of I.R.C.T. during which six applications were done at fortnightly intervals. In this period the total amount of urine passed in the first 24 hours after each spraying session was collected with the aim to determine pyrethroid metabolite levels which are an indirect index of pyrethroid exposure. Before and after the six spraying sessions extensive medical and neurological examinations were carried out to establish any biological effects after a season of repeated spraying.

(ii) A seventh spray session was done under supervision of members of the Exposure Monitoring Group about 1 month after the final spray session as described in (i). This was done for the following reasons:

a. to study any possible medical effects, including blood biochemistry and peripheral nerve function tests, immediately after spraying in operators who had previously repeatedly applied pyrethroid ;

b. to study whether exposure to the pyrethroid determined by measuring the major metabolite derived from the acid fragment of the pyrethroid in urine was consistent with the results obtained in the IRCT personnel supervised part of the study ;

c. to study the relation between the concentration of urinary metabolite, pyrethroid skin exposure and pyrethroid respiratory exposure.

Medical and extensive neurological examination

No indications were found in these examinations of a compound related effect of exposure to the pyrethroid after a series of seven spraying sessions of the pyrethroid with hand-held ULV. Blood biochemistry tests showed a slightly abnormal liver function tests in one operator. However, this operator had already a slightly increased SGOT level at the beginning of the study. Apart from this operator there were no abnormalities in the other operators.

Electroneurophysiological measurements. The study design gave the opportunity to detect not only abnormal functioning of peripheral nerves but also to identify minute changes in nerve function parameters within the normal range by comparing pre- and post exposure values. Several measurements per operator were done to cope with intra individual variability. When changes within the normal range are found it might be difficult in individual cases to identify the cause. When a group of persons is examined it may be easier to find the cause of a change when the individuals in the group react in a similar pattern and when a common factor is present which could be responsible for the changes.

In this study no indications of abnormal functioning of peripheral nerves have been found. A few statistically significant changes within the normal range have been found in nerve function parameters of individuals when the measurements made before and after the extra seventh spray session were compared but no statistically significant changes were found when group results were compared (see figures 1a, 1b, 1c). The conclusion is that no compound related group changes have occurred in this period.

Comparing the results of the September 1978 measurements with those made in December 1977/January 1978 it appears that there are statistically significant changes within the normal range in all parameters in individuals.

For certain parameters (MCV, SCVM, SFCV, Cornea reflex) there were statistically significant changes within the normal range on a group basis (see figures 1a, 1b, 1c). The conclusion could be that these group changes are compound related, but when the measurements performed in May 1978 on the sensory conduction velocity (SCVM) are taken into account, the possibility for a compound related action on the SCVM disappears showing no group change between May and September and suggesting a seasonal change between Jan'78 and May'78 (see figures 1a, 1b, 1c).

There was also no group change in the period between May'78 and September '78 in the sensory action potential of the median nerve (SAPM) and the sensory conduction velocity of the sural nerve (SCVS). The change in sensory action potential of the sural nerve (SAPS) could be due to technical difficulties in the measurement of this particular parameter in May which may have lead to inaccuracy of base-line values.

As there are no measurements performed on motor conduction velocity (MCV) and slow fibre conduction velocity (SFCV) in May, the theory of a seasonal influenced change in all individuals taken place between January and May cannot be verified but seems altogether an appropriate explanation. The group changes in the cornea reflex are difficult to interprete as the other parameters of the function of the trigeminal nerve do not indicate any change on a group basis.

From the information obtained from these studies, the conclusion is that there is no evidence that the group changes that were found, are compound related.

Interpretation of exposure monitoring tests

As far as exposure is concerned urinary trans metabolite excretion levels in many cases were below limit of detection (0.01-0.05 mg/l) in both the part of the study supervised by I.R.C.T. personnel and by the Exposure Monitoring Group, which may indicate a low amount of pyrethroid absorption. Measurable urinary concentrations of trans metabolite were in the same order in both parts of the study indicating consistent exposure patterns.

An interpretation of urinary trans metabolite excretion levels in terms of absorption of the pyrethroid in the body can be made from data obtained from a previously carried out dose-excretion study in one person to whom an oral dose of 0.50 mg of the pyrethroid (0.25 mg trans and 0.25 mg cis isomer) was administered.

The results of this study showed that 78% of the maximum amount trans metabolite and 32% of the maximum amount of cis metabolite* that could be excreted was found in the urine passed in the first 24 hours after the dose, which is equivalent to 0.19 mg of trans pyrethroid and 0.08 mg cis pyrethroid respectively. From the above results the total oral intake of the pyrethroid can be calculated with the formula:

oral intake pyrethroid = $\frac{100}{39}$ x mg trans metabolite excreted in the first 24 hours urine x (1 + ratio $\frac{\text{cis}}{\text{trans}}$ in the pyrethroid) =

$\frac{256}{\%\text{ trans in the pyrethroid}}$ x mg trans metabolite in first 24 hours urine,

assuming that there is a lineair relationship between pyrethroid intake and urinary excretion of trans metabolite (ref.1).

It has been demonstrated in two persons that after spraying with the pyrethroid about 70% of the total excretion of trans metabolite was found in the first 24 hours urine, which is in the same order as in the human dose-excretion study, where 78% was found. Thus the elimination rates of the trans metabolite after spraying and after oral administration of the pyrethroid are in the same order. The above formula can be used for the calculation of the absorbed amount of pyrethroid in the body after spraying (expressed as equivalents of an oral dose) assuming that there are no major differences after oral administration and spraying of the pyrethroid with respect to e.g.:

a. absorption ratio of trans and cis pyrethroid in the body
b. metabolite of the pyrethroid
c. route of excretion of the trans metabolite.

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*equivalent to 39% of the trans isomer and 16% of the cis isomer in the pyrethroid

If the amount of pyrethroid absorption in the body of the operators is calculated with the above formula it may be concluded that the total absorption of the material per operator per spray session was always much lower than the equivalent of an oral dose of 1 mg, and that it even may be assumed that the total amount of six spray sessions did not exceed the equivalent of an oral dose of 1 mg.

The amounts of absorbed pyrethroid in operators in the part of the study supervised by the Exposure Monitoring Group are shown in Table 3.

TABLE 3

Survev of first 24 hours urinary trans metabolite levels, calculated pyrethroid absorption and pyrethroid skin and respiratory exposure in operators after application of 2 litres of pyrethroid formulation per hectare (=50 g a.i./ha) on cotton (seventh spray session)

Operator	First 24 hours urinary trans metabolite levels (mg)	Calculated pyrethroid absorption (mg) (oral equivalent)	Calculated pyrethroid skin exposure (mg/hr) during actual spraying	Calculated pyrethroid respiratory exposure (mg/hr) during actual spraying	
				Direct method	Indirect method
1	< 0.018	< 0.08	42.2	0.25	0.03
2	< 0.050	< 0.21	2.8	0.003	0.006
3	0.040	0.17	3.1	0.03	0.03
4	< 0.050	< 0.21	11.5	0.33	0.02
5	< 0.040	< 0.17	14.6	0.03	0.09
6	0.008	0.08	12.0	0.09	0.08
7*	0.13	0.55	17.4	0.08	0.13
8*	0.077	0.33	65.4	1.03	0.24
9*	0.065	0.28	36.0	0.03	-

* members of the Exposure Monitoring Group

In Table 3 pyrethroid skin and respiratory exposure results are also included. There was only a poor relationship between measurements of pyrethroid absorption in the body and pyrethroid skin or respiratory exposure during application of the pyrethroid. With respect to skin and respiratory exposure it can be concluded that also between these parameters there was a poor relationship. However, the mean percentage of respiratory exposure of the pyrethroid measured with the filter sampling (direct) method was 0.9% (range 0.1-2.9%) and with the vapour absorption (indirect) method 0.5% (range 0.1-1.0%) of pyrethroid skin exposure, which is in good agreement with data from other studies (refs. 4,5).

An interpretation of skin and respiratory exposure can be made by using the WHO formula for percentage toxic dose per hour:

$$\% \text{ toxic dose per hour} = \frac{\text{dermal exposure (mg/hr)} + [\text{respiratory exposure (mg/hr)} \times 10]}{\text{dermal } LD_{50} \text{ (mg/kg, rat)} \times 70} \times 100$$

For the operator with the highest dermal exposure the toxic dose per hour is then < 1.2% which has been calculated as follows:

$$\% \text{ toxic dose/hour} < \frac{42.2 + 2.5^{*}}{50^{**} \times 70} \times 100$$

It is not known what the actual value of the toxic dose per hour would be, because during dermal toxicity testing the highest dose that could be applied was 2000 mg/kg of formulation and there were no death amongst the animals tested at this dose level.

To summarise, although exposure has certainly occurred during the six fortnightly spraying sessions supervised by IRCT personnel, in many cases the excretion levels of urinary trans metabolite levels were below limit of detection. The urinary excretion levels measured after the seventh spray session supervised by the Exposure Monitoring Group were of the same order indicating that absorption is low. From previous limited studies on the relationship between oral uptake of the pyrethroid and excretion levels of metabolite in man and on excretion patterns after exposure in the field, the absorption can be calculated as previously explained.

It appears that per spray session much less than the equivalent of an oral dose of 1 mg pyrethroid is absorbed and that it is probable that the total amount in six spray sessions may not exceed the equivalent of an oral dose of 1 mg. The relation between calculated pyrethroid absorption and skin or respiratory exposure is poor. The highest dermal exposure was calculated to be less than 1.2% of the toxic dose/hour.

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* determined with the filter sampling method

** the dermal LD_{50} (rat) of the pyrethroid formulation was greater than 2000 mg/kg or greater than 50 mg a.i./kg (determined in Shell Toxicology Laboratory)

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EARLY STUDIES WITH AZINPHOS METHYL TO DETERMINE RE-ENTRY TIMES FOR CITRUS PICKERS

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INTRODUCTION

In the spring of 1970, azinphos methyl was implicated in an alleged poisoning incident with citrus pickers in California. Officials of the California Public Health Department investigated the history of the people who had been reported ill and determined that these workers could have been previously exposed to a wide variety of compounds, including dioxathion, dimethoate, parathion, ethion and azinphos methyl. However, they concluded that the principal problem had occurred in areas where both azinphos methyl and ethion residues existed in citrus groves. As a result of this conclusion, it was proposed to extend the re-entry interval from 7 to 30 days for citrus treated with azinphos methyl and ethion. It was decided to conduct monitoring tests in an effort to determine a realistic re-entry interval for azinphos methyl.

The test protocols provided for cholinesterase tests for workers and collection of residue data* for fruit, foliage, gloves, skin patches and air during a normal field operation. Three tests were conducted using different formulations and/or rates.

METHODS

The 3 separate monitoring studies were conducted in the State of California and in general are outlined in Table I. In all cases, pre-exposure erythrocyte and plasma cholinesterase values were determined for each worker. The analytical method was the pH stat assay for human blood cholinesterase, recommended by the United States Public Health Service. A local laboratory conducted all cholinesterase analyses immediately following each sampling interval. All workers were 21 years of age or older with no recent exposure to cholinesterase-inhibiting pesticides. Only workers whose cholinesterase levels were within the normal range participated in the test. A minimum of 15 workers was included in each test.

*Acknowledgement is given to Dr. C. A. Anderson and his Chemical Research Group, Mobay Chemical Corporation, for contributions to protocol design and residue analyses.

TABLE I

Outline of azinphos methyl citrus tests

day	exp. 1	exp. 2		exp. 3	
	block 1	block 2	block 3	block 4	block 5
0	Spray W.P. 6 oz per 100 gal, 900 gal per acre	Spray S.C. 4 oz per 100 gal, 900 gal per acre		Spray S.C. 6.7 oz per 100 gal, 900 gal per acre	
1					
2					
3					
4					
5					
6					
7	H.	H.		H.	Spray S.C. 6.7 oz per 100 gal, 900 gal per acre
8	H. Bl.			H. Bl.	
9	H.			H.	
10	H. Bl.	H.	Spray S.C. 4 oz per 100 gal, 900 gal per acre	H.	
11	H.	H. Bl.		H. Bl.	
12		H.		(Day 18 Blk.3)	
13		H.			
14		H. Bl.			H. Bl.
15					H.
16					H. Bl.
17			H.		
18			H. Bl.		
19			H.		
20			H.		
21			H. Bl.		
22					

H. - Harvest - days of work for citrus pickers
Bl. - Blood - sample intervals for citrus pickers
W.P. - Wettable Powder
S.C. - Spray Concentrate

In all cases, the spray applications to the citrus groves were made with commercial equipment. Before the tests were initiated, protocols were written and sent to the State of California for approval and suggestions. Representatives of the state visited and inspected the ongoing tests.

In the first test, the spray was prepared from a wettable powder formulation. The application was made at the rate of 6 oz. a.i. per 100 gallons, 900 gallons per acre being applied. Workers entered the field 7 days after the azinphos methyl spray. Blood samples were taken at the end of the 8th day. Workers continued to pick citrus through the 9th and 10th days. Additional blood samples were taken at the end of the 10th day. Examination of the blood samples taken at the end of the 10th day indicated significant plasma cholinesterase depression and, therefore, the test was aborted.

A second test was conducted with the azinphos methyl spray prepared from spray concentrate in which 4 oz. a.i. per 100 gallons of spray, 900 gallons per acre, were used. In this case, the spraying was done on Friday, the workers picked the following Friday, were off for 2 days over the weekend (days 8 and 9), then picked throughout the following week in that block of citrus, blood samples being taken on the 11th and 14th days. On the 17th day, they entered another block of citrus which had been sprayed 7 days before and picked for another working week in that block, blood samples being taken on the 18th and 21st days.

A third test was conducted with azinphos methyl spray concentrate - the application was made at the rate of 6.7 oz. a.i. per 100 gallons at 900 gallons per acre. In this case, the workers entered the fields on the 7th day after spraying and blood samples were taken on the 8th day. They continued to work through the 10th day. On the 11th day they picked in Block 3 from the previous experiment. Workers only picked 3 days from Block 5 the following week (i.e., 7, 8, and 9 days after spraying) or 14, 15 and 16 days from the beginning of Experiment No. 3. Unfortumately, as the third experiment was conducted late in the year, the amount of citrus available was limited. Therefore, the workers only worked half-days. In addition, the weather was cooler than normal citrus picking conditions.

RESULTS

In Table II, the cholinesterase values for the citrus pickers participating in the different experiments are summarized. There were 15 workers in Experiment No. 1, 15 workers in Experiment No. 2, and 19 workers in Experiment No. 3. Cholinesterase values are given as percentage of pre-exposure values. The values shown in the table are the means for all workers on the day indicated. These means demonstrate the trends which occurred. It must be emphasized that the variations for individuals were greater than is shown by these mean values.

TABLE II

Cholinesterase values* of pickers**
Azinphos methyl citrus tests

	exp. 1 (W.P. 6 oz per 100 gal)		exp. 2 (S.C. 4 oz per 100 gal)		exp.3 (S.C. 6.7 oz per 100 gal)	
day	plasma	eryth.***	plasma	eryth.	plasma	eryth.
8	72	86			104	
10	60	88				
11			119	100	101	104
14			98	78	110	96
16					110	104
18			105	72		
21			87	60		

* mean % of mean pre-exposure values

** number of workers: Exp. 1 - 15 persons, Exp. 2 - 15 persons, Exp. 3 - 19 persons

*** erythrocyte

These data show that in Experiment No. 1 at 10 days there was a significant depression of plasma cholinesterase. No significant depression of plasma cholinesterase occurred in Experiment No. 2 but there was a significant depression of erythrocyte cholinesterase. Erythrocyte cholinesterase showed a gradual decline throughout the study period. There was no depression either of plasma or erythrocyte cholinesterase in Experiment No. 3; but, as mentioned previously, working conditions for this experiment were not completely normal. No adequate explanation can be offered for the depression of the plasma cholinesterase in Experiment No. 1 and the erythrocyte cholinesterase depression in Experiment No. 2. On the basis of previous information, erythrocyte and not plasma cholinesterase would have been expected to be depressed as a result of exposure to azinphos methyl. It is possible that if Experiment No. 1 had run for a longer period, erythrocyte depression would have occurred. The data show that exposure was greater in Experiment No. 1 than in Experiment No. 2.

Surface residues were calculated for leaves by shaking the leaves for 2 minutes in water and analyzing the amount of residual material which was removed. Approximately 75% of the residue could be removed by this method. In Figures 1 and 2, the levels of surface residues on citrus leaves are shown for Experiments 1 and 2. From these data you can see there is great variability in the results and little or no indication of decline of residues up to approximately 30 days after spraying.

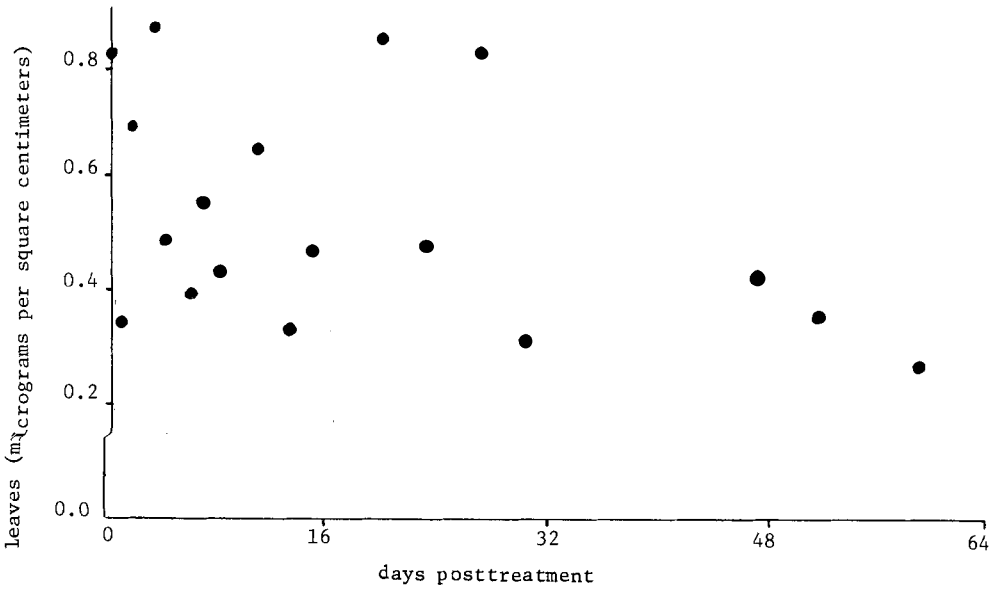

Fig. 1. Surface residues for leaves sprayed with azinphos methyl-wettable powder formulation (6 oz a.i. per 100 gallons, 900 gallons per acre)-Experiment No. 1.

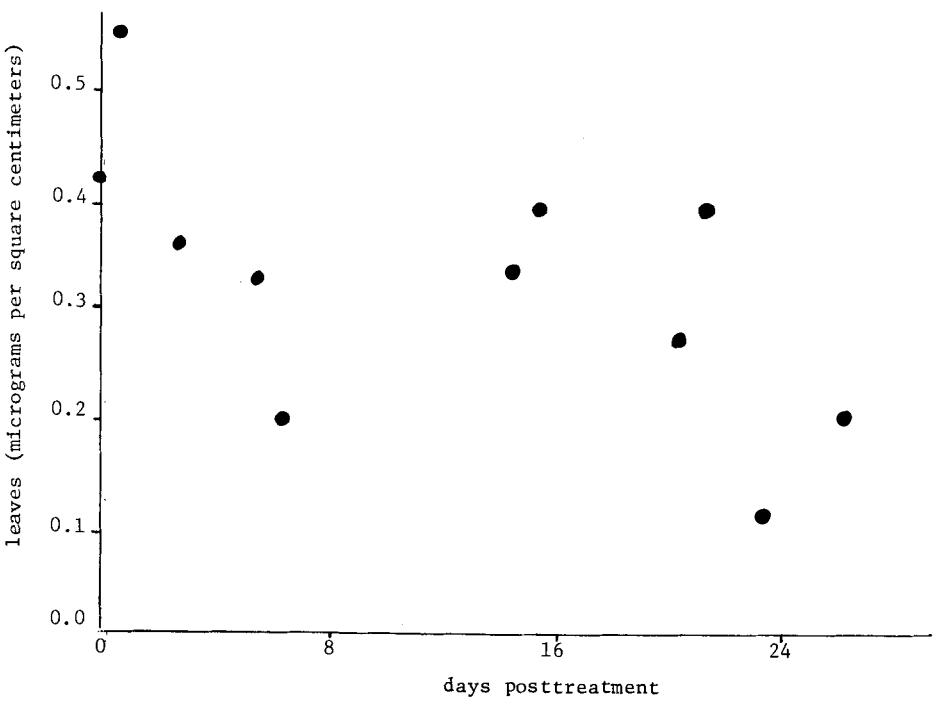

Fig.2. Surface residues for leaves sprayed with azinphos methyl-spray concentrate formulation (4 oz a.i. per 100 gallons, 900 gallons per acre)-Experiment No.2.

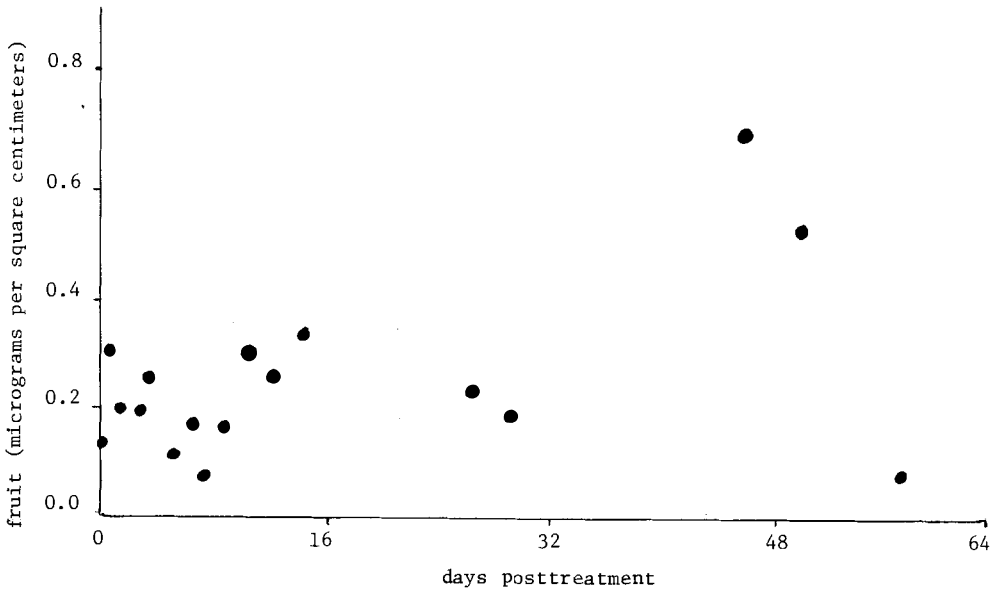

Fig. 3. Surface residues of fruit sprayed with azinphos methyl-wettable powder formulation (6 oz a.i. per 100 gallons, 900 gallons per acre)-Experiment No. 1

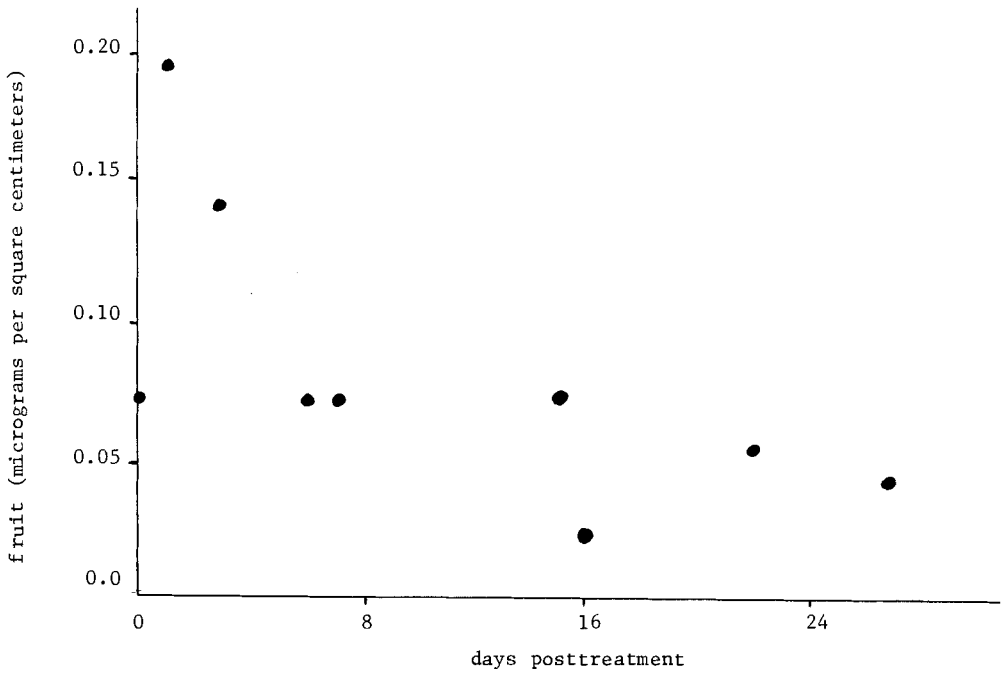

Fig.4. Surface residues for fruit sprayed with azinphos methyl-spray concentrate formulation (4 oz a.i. per 100 gallons, 900 gallons per acre)-Experiment No.2.

In figures 3 and 4, similar data for surface resiudes on fruit are shown. Again, in Experiment No. 1 there is no indication of a decline up to approximately 60 days after treatment. There may be some decline in Experiment No. 2; however, residues remain high throughout the treatment period.

In connection with these tests, a number of exposure measurements were made. Patches were placed on the arms and heads of workers for 60-minute periods. Aspirators were used to determine the amount of exposure which may have occurred as a result of inhalation. The results of these exposure tests, all expressed in micrograms per square centimeters, are shown in Table III for the 3 experiments. Approximate surface residue of leaves and fruit are also given. It can be seen from these results that the exposure in Experiment No. 2 was considerably less than that for Experiment No. 1. The exposure for the gloves in Experiment No. 3 is greater than that for Experiment No. 2, as would be expected from the higher rate of application. Very low concentrations of azinphos methyl were found in the air surrounding the pickers.

TABLE III

Surface residues (micrograms per square centimeters) for leaves, fruit and samples collected during azinphos methyl citrus tests

	exp. 1	exp. 2	exp. 3
Leaves	0.73	0.35	0.84
Fruit	0.15	0.07	0.24
Gloves*	52.6	20.4	46.1
Arm patches*	1.5	0.32	0.25
Head patches*	0.44	0.12	0.07
Concentrate* in air	0.14 µg/1	0.08 µg/1	0.05 µg/1

* 60 minute samples

CONCLUSIONS

1. Dermal exposure appeared to be much more serious than inhalation exposure.
2. The amount of exposure was related to the rate of application.
3. Decline of residues on citrus under California conditions was not rapid during the first 30 days following application.
4. Minimum protection from dermal exposure, such as gloves, daily change of work clothing and bathing, would considerably reduce the exposure hazard.
5. Future study designs of this nature should have the flexibility to accomodate local activities of land owners, unions, workers, normal operational procedures and scientific and public information services.

HEAT STRESS IN AERIAL SPRAY PILOTS

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ABSTRACT

Heat stress (WBGT index) in the cockpit of agricultural spray pilots, and its physiological and subjective effects, were measured for 9 pilots flying in hot weather for a large Israeli aerial spray company. There was concern that heat exposure may be one of the factors involved in a recent increase in fatal and non-fatal crash rate among Israeli spray pilots.

WBGT index calculations were based on sequential cockpit wet bulb, dry bulb and globe temperatures read when the aircraft landed. The WBGT was always above 25°C and exceeded 26.7°C in 70% of the observations.

In pilots, a daily loss of 0.6-1.2% of total body weight was usual, as were rectal temparature increases of 0.5°C. Daily water intakes ranged from less than 100 ml to 2000 ml per workshift. Questionnaires indicated that 42 of 45 pilots said they would drink more fluids if they were provided between flights by ground crews.

An unresolved problem was whether conventional threshold standards for heat exposure were set at levels that may produce insidious impairments in pilot psychomotor performance, especially when parathion, g-forces and dehydration were also present.

Cockpit air cooling is suggested as a measure for preventing heat stress in hot climates. However, appropriate filter technologies would be needed to prevent pesticide exposure during flight. Engineering and other measures to prevent heat stress and dehydration should rank high as part of a comprehensive program to protect health and performance levels of agricultural spray pilots.

INTRODUCTION

A recent increase in both fatal and non-fatal crashes among spray pilots in Israel has prompted an investigation into the possibility that heat stress is a contributing factor. Table 1 demonstrates the rise in the rate of accidents among Israeli, as compared to American spray pilots in recent years.

TABLE 1

Accident* Rates in Aerial Spraying: USA vs. Israel

		1974	1975	1976	1977	1978
HOURS FLOWN	USA	2,085,400	2,172,900	2,498,600	--	--
	ISRAEL	17,616	18,000	20,000	22,000	24,000
ACCIDENTS	USA	467	429	433	--	--
	ISRAEL	4	6	11	6	17
TOTAL ACCIDENT RATE (per 100,000)	USA	22.4	19.7	17.3	--	--
	ISRAEL	22.7	33.3	55.0	27.3	70.8

* Definition: U.S. National Transportation Safety Board.

There was a 150% increase in the accident rate among Israeli agricultural pilots from 1977 to 1978 (ref. 1). Post-crash investigations implied that some 60% of Israeli crashes resulted from pilot error. These data prompted our investigation.

The pilots' daily workload is demanding (early work hours, 4-5 hours flight time, 10-15 take-offs and landings, several hundred 180° turns, frequent passes under telephone and power lines) and includes exposures to noise, vibrations, gravitational forces and various pesticides, in addition to heat. Several studies show that heat alone can cause deterioration in performance (refs.2,3,4).

Israel offers a unique opportunity to investigate the working conditions of agricultural spray pilots, because all spraying is centrally controlled and administered by two companies. The study was carried out in the larger of the two, which employs 60 pilots, 50 loaders and 100 mechanics. This study was conducted at a field in northern Israel. We measured thermal conditions in the cockpit and estimated the resulting heat strain and dehydration in the pilots.

Earlier studies had already shown that when parathion was sprayed, cockpit exposure levels of this pesticide at times exceeded the Threshold Limit Value (TLV) of 50 $\mu g/m^3$ and Short-Term Exposure Limit of 300 $\mu g/m^3$ (ref. 5).

METHODS

Measurements were taken over an 8 day period in September 1978. The aircraft flown were the SNOW and PONY airplanes and the BELL 47 and BELL 206 helicopters.

Thermal Stress Measurements

Instruments which measured thermal conditions were placed in the cockpit before take-off and were read immediately after the aircraft landed. The following variables were recorded:

i. dry bulb temperature (T_{db})

ii. ventilated wet bulb temperature (T_{wb}) (measure of humidity)

iii. 150-mm black globe temperature (T_g) (measure of radiant heat).

The equipment was obtained from the Israeli meteorological service. All thermometers had a range of -20 to +110°C, with graduations of 1°C and an accuracy of ±1°C. In the evaluation of overall heat stress, the wet bulb globe temperature index (WBGT) was chosen, calculated according to the equation $WBGT = .7T_{wb} + .2T_g + .1T_{db}$. The three thermal sensors were positioned inside the cockpits of two planes (or plane and helicopter) as close to the pilots as possible, an area with artificial ventilation and exposed to ambient radiant heat. Readings were taken every time the plane/helicopter landed for refilling of pesticide (which varied from once every 10 minutes for the helicopters to once every ½ hour for the planes). The WBGT heat stress values that we recorded tend to be underestimates, because the pilots, despite instructions otherwise, often threw open their cockpit windows upon landings before the WB thermometer could be read, thus lowering the WB values and subsequent WBGT calculations. The outdoors ambient temperature, humidity and radiant heat values for date and hour were obtained from the local meteorological service station.

Physical measurements

The following physical measurements were made on the pilots:

i. weight in underwear and shoes before and after work

ii. rectal temperatures before and after work.

The hospital scale used had graduations of .05 kgm. with an accuracy of ±.05 kgm. The rectal thermometers were standard clinical thermometers with a range of 35° to 42°C, graduations of .1°C.

The pilots were instructed to maintain their usual eating and drinking habits during the work day. The weights and rectal temperatures were done before work (approximately 3:30 AM) and immediately upon final landing (approximately 10:00 AM). It was not possible to obtain control rectal temperatures for the pilots on similar flights in cooler weather.

Questionnaires

A questionnaire was completed daily by the pilots, covering several aspects of their work habits, including eating and drinking, and subjective experience of change in their performance during the workday. A separate questionnaire was also distributed to all the pilots of the company on the subject of drinking habits and preferences, and possible techniques for preventing dehydration.

Disclosure of findings

All data were reported to the pilots, together with an explanation of the findings.

RESULTS

Heat Stress

The rapid and marked rise in wet bulb, dry bulb and globe thermometer readings, and WBGT heat stress index calculations within one cockpit during the morning workshift, is shown in Figure 1. In addition, ambient WB and DB readings can be seen in the figure. Similar trends were demonstrated in other aircraft.

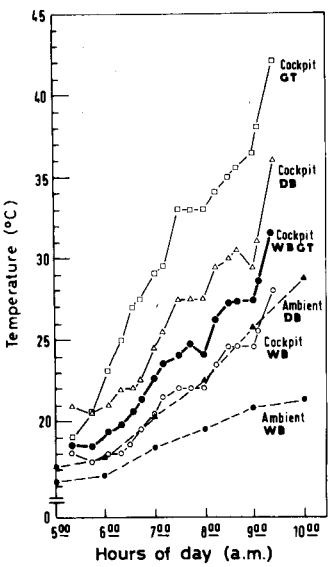

Fig. 1. Cockpit wet bulb (WB), dry bulb (DB), globe thermometer (GT) readings and WBGT heat stress index during workshift, BELL 47 helicopter in comparison with ambient data.

Physical measurements

Table 2 shows the rectal temperature rises during the workshift, and at the end of the workshift. Rectal temperatures increased by at least 0.5°C for 5 of 7 pilots ($t = 9.25$, $p<.001$, paired t-test). This rise is not greater than the expected normal diurnal variation (ref. 6). However, the average rectal temperatures at the end of the workshift exceeded 37.5°C for 4 of 7 pilots.

TABLE 2

Rectal temperature (T_{re}) changes in agricultural spray pilots

		T_{re} (°C) at end of workshift			T_{re} (°C) rise above baseline value	
pilot	no. days	average T_{re}	range	days exceeded 37.5°C	mean rise (°C)	range
A	5	37.6	37.3-37.8	3	.88	.6-1.2
B	3	37.7	37.7-37.7	3	.82	.6-1.0
C	3	37.5	37.5-37.6	1	.25	0-0.5
D	5	37.3	37.2-37.4	0	.60	.25-0.9
E	1	38.4	---	1	1.30	---
F	3	37.4	37.2-37.6	1	.37	0-0.6
G	3	37.5	37.3-37.6	1	1.00	.9-1.3

Average rectal temperature rises during the workshift for 7 of the 9 pilots, ($t = 9.25$, $p < .001$, paired t-test), September 1978, Beit Shean Valley, Israel.

Table 3 demonstrates that average daily weight losses during the workshifts of 0.6-1.2% of total body weight were seen in 6 of 7 pilots (who were eating and drinking during work 'as usual') ($t = 6.57$, $p < .001$, paired t-test). The average fluid intake as reported by the pilots, ranged from 100 ml or less per workshift (3 pilots), to 200-600 ml per workshift (5 pilots) to 2000 ml per workshift (1 pilot). These average daily fluid intakes for each pilot were fairly consistent with their recorded range of daily fluid consumptions, with the exception of 1 pilot who drank 1200 ml one day and nothing the following day.

TABLE 3

Weight changes (weight range of pilots: 72.2-84.3 kg.)

pilot	no. days	mean decrease in kg.	mean decrease in body weight %	range: % drop in body weight	average workshift fluid intake ml/day
A	4	.20	.28	0-.68	400 (range 0-800)
B	3	.25	.74	.4-1.4	2000 (range 1600-2400)
C	3	1.03	1.22	.66-1.9	60 (range 0-200)
D	5	.48	.61	0-1.0	340 (range 300-600)
E	3	.48	.74	.14-1.8	260 (range 200-400)
F	3	.60	.82	.69-1.1	340 (range 0.600)
G	2	.48	.62	.6-.65	0

Average workshift weight losses for 7 of the 9 pilots ($t = 6.57$, $p < .001$, paired t-test), September 1978, Beit Shean Valley, Israel.

Questionnaire

The daily questionnaires answered by the participating pilots indicated a subjective sense of impairment in alertness and in exactness of performance after 3 hours of work. Pilots' responses showed little day-to-day variation. Six of the nine pilots felt thirsty during the work day.

Fluid replacement was evaluated in a questionnaire distributed to the 60 company pilots of whom 45 responded. Nearly all (91%) felt that a supervised drinking program would prevent the symptoms of dehydration, and agreed that they would drink more during the workday, if the ground crew offered them drinks between flights. Beverage preferences were as follows: 29 chose juice, 14 water, 6 milk and 3 soda (some pilots listed more than one beverage). A number of pilots felt that chilling the fluids would increase their intake, and several suggested to carry a flask of fluid in the cockpit.

DISCUSSION

The data suggest that environmental heat stress is a hazard for Israeli spray pilots. The weather during the study was considerably cooler than the hottest part of the spraying season, suggesting that higher levels of cockpit heat stress are produced at the peak season.

The data collected on weight changes, while influenced by workshift food intake, suggests that for at least some of the pilots fluid replacement was insufficient. The reports of thirst during the workshift support this conclusion. Thirst itself generally occurs only after the occurrence of significant fluid depletion, as noted in studies of 'voluntary dehydration' (ref. 7).

In summary, both excessive heat stress, and in certain cases, the early signs of heat strain (weight loss, rectal temperatures above 37.5°C, and thirst) have been documented for the Israeli agricultural pilots.

Conventional threshold standards for heat exposure (ref. 8), designed for ground workers, are inappropriate in aviation medicine in general and for spray pilots in particular. The heat stress measured in the cockpits of the Israeli sprayers exceeded the recommended heat exposure threshold limit value (TLV) for continuous heavy work (25°C) during every observation, and was sustained for periods of up to 3½ hours. The TLV for continuous moderate work (26.7°C) was exceeded in 70% of observations. These standards are designed for purely physical work, according to worker energy expenditures, and are based on the prevention of gross heat strain, while we are more worried about subtle psychomotor changes in pilot performance. Nunneley has shown that with exposure to a WBGT of approximately 30°C for periods of 2 hours, vision and perceptual speed are adversely affected (ref. 2). There is a clear need for simple screening tests of psychomotor and neurobehavioral function in field settings. Because of the multiple hazards

and exposures of aerial spraying, there may be a need for threshold standards of heat exposure specifically for spray pilots. Figure 2 presents the suggested physiological interactions between 4 of these hazards (parathion, heat stress, dehydration and g-forces).

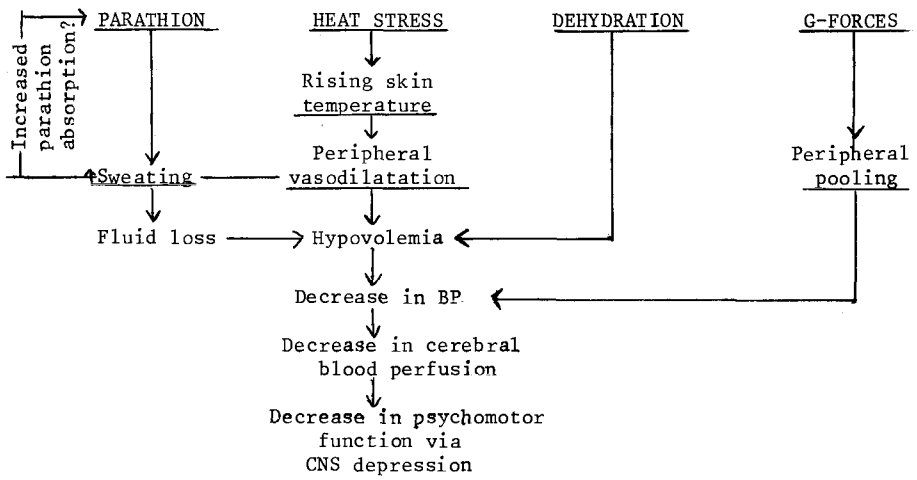

Fig. 2. Possible interactions between heat stress, dehydration, parathion and g-forces in aerial spray pilots.

The Fighter Index of Thermal Stress (FITS) is a set of guidelines which uses WBGT values but considers psychomotor and physiological changes of importance to pilot performance (ref. 9). The FITS estimates cockpit WBGT's, based on ambient wet and dry bulb temperatures, to designate zones of caution and danger in which specific precautionary measures and even flight cancellations are recommended. Research is needed to ascertain whether the FITS formula, or a variation thereof, is applicable to the specific conditions of aerial spraying.

RECOMMENDATIONS

Recommendations for the prevention of excessive heat stress include air-conditioning of the cockpits (with appropriate filter technology), or alternative methods of body cooling, and a supervised program of fluid intake by the pilots. These recommendations should be viewed as part of a comprehensive program designed to improve the working conditions of the agricultural spray pilot and to reduce the risk of injury and deaths from crashes.

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PRE-CROP SPRAYING OF VINEYARDS WITH MEVINPHOS

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ABSTRACT

The insecticide mevinphos was suggested as a very convenient preharvest spray on grapes for a variety of reasons. As no information was available on absorption by spray operators under local circumstances cholinesterase inhibition during actual spraying was assessed in 5 operators in one experiment and in 10 operators and 2 controls in a further study. The results indicated that under spraying conditions which could be presumed to represent normal practice in the Cape vineyard area mevinphos spraying does not significantly affect blood cholinesterase concentrations in farm labourers engaged in spraying operations.

INTRODUCTION

Certain insects present a grave problem in the period immediately preceding the harvesting of export grapes in the South Western Cape Province of the Republic of South Africa. The most important of these are mealy bugs (Pseudococcus spp) and fruit fly (Pterandrus rosa and Ceratitis capitata). The insecticide mevinphos was suggested as an ideal pre-harvest spray for the following reasons:

1 It is very effective against mealy bug and fruit fly.
2 It disappears in a matter of hours and leaves no residue.
3 It does not have an adverse effect on the taste or appearance of grapes.
4 It is a direct cholinesterase inhibitor and acts very rapidly. Consequently although it is extremely toxic - oral LD_{50} rat of 5-10 mg/kg - operators who absorb too much are immediately affected. They become aware of premonitory muscarine effects on the eyes and the lungs and withdraw from further exposure. This offers a most decided practical advantage over parathion which has a delayed effect of up to 12 hours and illness arising after exposure is therefore often not recognized as such with serious consequences.

METHODOLOGY

As no information was available on absorption by spray operators under local circumstances an experimental model was set up on a representative farm in the vineyard area and five random employees who normally do the spraying agreed to participate in the trial. Spraying was done by two units namely

1 a tractor drawing a tank with a spray pump, spraying being carried out by two men following on foot and handling spray guns on trailing hoses (operators 1, 2 and 3) and

2 a spray pump on a trailer at the side of the vineyard while two operators (nos 4 and 5) pulled out extension hoses and sprayed as they walked down the rows of vines.

We wished to carry out the experiment under the worst possible conditions. We were informed that ordinarily sprayers find it too hot and refuse to wear plastic overalls, gloves and respirators. As medical help would be available on the spot all day we gave no special instructions except to tell the workers that the insecticide was a dangerous one and had to be treated with the usual care. The purpose of the trial was explained to them. No protective clothing was worn. All men were dressed in long trousers, shirts, shoes and stockings. A concentrated emulsion of mevinphos was diluted to 0.15% (0.036% active ingredient) with water. Spraying continued all day. Venous blood was taken before spraying started and periodically thereafter during the day. Heparin was used as anticoagulant and the blood was kept cold until it could be analysed in a laboratory. The analysis was done according to the electrometric method of Michel. Results are shown in Table I.

Subsequently the work was criticised because samples had only been taken on the day of spraying and other possible later effects had not been examined. A further similar trial was thereafter set up in which random workers from three farms were asked to carry out their usual duties using two types of spray equipment. These were:

a tractor drawn spray units as in the first experiment each requiring a driver and two spraymen;

b one tractor drawn unit fitted with 2 oscillating spray booms at the rear end of the unit. Only the driver of the unit was involved.

To obtain a representative impression of the exposure hazard, "open" trellised Almeria vines were sprayed during the first half of the day and "closed over"

T A B L E I

Whole blood and red cell cholinesterase levels in five workers exposed to mevinphos. The measurements were made on one day.

OPERATOR		NO 1 TRACTOR DRIVER		NO 2 TRACTOR SPRAYER		NO 3 TRACTOR SPRAYER		NO 4 PUMP SPRAYER		NO 5 PUMP SPRAYER	
DETAILS OF SAMPLES		Whole Blood CHE	Red Cell CHE	Whole Blood CHE	Red Cell CHE	Whole Blood CHE	Red Cell CHE	Whole Blood CHE	Red Cell CHE	Whole Blood CHE	Red Cell CHE
09h30	Pre-Spray level	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
11h20	After 1st 1000 litres on open vines	105%	102%	98%	96%	100%	98%	98%	106%	111%	100%
12h30	After 2nd 1000 litres on open vines	100%	102%	98%	91%	98%	100%	95%	106%	109%	99%
13h30	After 3rd 1000 litres on open vines	103%	102%	93%	91%	100%	96%	93%	111%	109%	97%
15h15	After 1st 1000 litres on closed vines	103%	98%	90%	90%	100%	98%	95%	106%	107%	95%
16h30	After 2nd 1000 litres on closed vines	97%	98%	90%	87%	98%	100%	95%	106%	111%	95%

TABLE II

Serum cholinesterase levels of ten workers exposed to mevinphos and of three controls. The measurements were done before (-) and after (+) the spray operations.

		SERUM CHOLINESTERASE				
FARM	OPERATOR	- 7 Days	- 30 min.	+ 30 min.	+ 15 hours	+ 8 days
I	Tractor driver	100%	105%	108%	113%	95%
	Sprayer	100%	100%	90%	90%	93%
	Sprayer	100%	117%	102%	117%	100%
II	Tractor driver	100%	100%	88%	94%	88%
	Sprayer	100%	84%	71%	82%	76%
	Sprayer	100%	100%	82%	88%	94%
	Control	100%	91%	93%	107%	93%
	Control	100%	105%	115%	123%	105%
	Tractor driver	100%	113%	108%	121%	133%
	Sprayer	100%	100%	71%	94%	not available
	Sprayer	100%	153%	93%	100%	167%
	Control	100%	89%	83%	86%	89%
III	Tractor driver (Boom spray)	100%	93%	93%	107%	98%

Barlinka vines during the second half of the day. Blood was drawn as follows:

Sampling Occasion	Time elapsed before (-) or after (+) application
1	- 7 days
2	- 30 minutes
3	+ 30 minutes
4	+ 15 hours
5	+ 8 days

Three farm labourers who were not involved in spray operations acted as controls. Serum cholinesterase was determined at the nearby laboratories of the Stellenbosch Medical School. Results are shown in Table II.

DISCUSSION AND CONCLUSION

These results indicate that under spraying conditions which can be presumed to represent normal practice in the Cape vineyard area mevinphos spraying does not significantly affect blood cholinesterase concentrations in farm labourers engaged in spraying operations. In this particular area parathion had been totally withdrawn for two reasons. Firstly there existed the possibility that high residues might slip through the control net and harm the reputation of South African export grapes. Secondly many cases of parathion poisoning occurred amongst children who illicitly entered the vineyards and stole recently sprayed grapes.

Mevinphos completely eliminated the first objection to the use of parathion and greatly reduced the toxic hazard in the second instance. Consequently it was considered that mevinphos offered an excellent and comparitively safe pre-harvest spray provided it was handled with reasonable care.

MONITORING OF PUBLIC HEALTH WORKERS EXPOSED TO FENTHION

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ABSTRACT

This study set out to examine 125 public health workers exposed to fenthion; the results were compared with a control group not exposed to fenthion. It was observed that symptoms likely to result from exposure were commoner in the exposed group, though an associated depression of blood cholinesterase values was not observed. It is suggested that health education of the workers would minimise any problems.

INTRODUCTION

Few dispute the fact that the control of pests will benefit human health and welfare (ref. 1). In some quarters this contention is being questioned, but not supported by adequate scientific evidence. It is the responsibility of scientists to examine the problem objectively while it becomes the responsibility of decision makers to utilise this information appropriately. It is in this context that studies to monitor workers exposed to pesticides becomes relevant as such workers, particularly those engaged in public health programmes, are continuously exposed to pesticides in relatively small doses over a long period. The benefits of such studies are mainly (i) to provide early detection of toxic effects among the workers and thus enable remedial action to be taken; (ii) to provide baseline information on possible health hazards. The latter is particularly relevant socially as it would be a guide to the possible health hazards, if any that may be encountered in the community.

Though previously studies have been undertaken on agricultural and public health workers exposed to a variety of pesticides, the WHO Expert Committee on Vector Biology and Control (ref. 2), has indicated the need for the continuance of studies on this category of workers. It is in pursuance of this need that the present study was undertaken to examine public health workers exposed to fenthion.

METHODS

In Sri Lanka, fenthion is used as a larvicide against the Culex fatigans mosquito, the vector of filariasis in man. Fenthion is used for this purpose by the Anti-filariasis Campaign of the Department of Health as well as by the Colombo Municipal Council which is responsible for the public health activities of the city of Colombo. Fenthion has been used by the Colombo Municipal Council since 1970, whereas the Anti-filariasis Campaign of the Department of Health has used it since 1967. The chemicals used by the workers of these programmes are shown in Tables 1 and 2 respectively.

TABLE 1

Pesticides used by the spraymen of the Colombo Municipal Council

year	pesticide
1954-	DDT in diesel oil
1958-	HCH in diesel oil
1964-	malathion 95% in diesel oil
23 June 1970-	fenthion 50% in water

TABLE 2

Pesticides used by the spraymen of the Anti-filariasis Campaign, Department of Health

year	pesticide
1947-	DDT and thereafter dieldrin and malathion
1967-	fenthion in water

In the first stage of the present study, workers and controls in two selected health unit divisions of both the Anti-filariasis Campaign and the Colombo Municipal Council were examined and studied for the duration of the working week. The data were recorded according to the WHO protocol of the Division of Vector Biology and Control (VBC/75.9), in addition to personal data and examination protocol which was developed for the purpose of the study. The medical history and physical examination of the workers and the controls were done during the working week according to the project protocol.

The blood cholinesterase levels of the workers and controls were estimated using the field kit, tintometric method, in the morning before commencement of work, and in the evening at the end of the days work. This schedule was carried out for the 5 day working week. During this stage of the study, a sample of fenthion in the spray cans of the workers while in the field was taken and the concentration of fenthion was estimated using thin layer chromatography. The spraymen used a knapsack type of sprayer of 3½ gallon capacity; the nozzle was a Duro whirling pin type, which was discarded by workers of the Colombo Municipal Council. The environmental conditions during the morning and afternoon were measured, the mean relative humidity for this period was estimated in the

mornings and the afternoons during the period of study.

Analysis of the data at this stage of the study did not indicate a marked difference in cholinesterase levels between workers exposed to fenthion and the control group. In view of this, it was decided to undertake the next stage of the study among the rest of the spraymen of the Colombo Municipal Council, without estimating the cholinesterase levels. Accordingly the second stage of the study was continued with this one difference. For the purpose of the final analysis, the data of the spraymen and the controls of both stages of the study were combined.

RESULTS

The mean relative humidity for the period of study was estimated to be 80% while the mean wind velocity which was of variable direction was recorded at 0.2 m/sec. Further, it was observed that the variation during the day as well as for the period of study was minimal. Hence, no relationship was demonstrable between the presence of symptoms and environmental conditions.

The estimated concentration of fenthion in the spray cans of the workers is shown in Table 3.

TABLE 3

Concentration of fenthion in spray cans of workers and recommended (and therefore expected) concentration

Sample no.	Observed concentration g/100 ml	Expected concentration g/100 ml
1	0.09	1.04
2	0.03	1.04
3	0.60	1.04
4	0.78	1.04
5	0.07	1.04
6	0.05	1.04

The observed concentrations were found to be much lower than the expected value for solutions made up according to instructions using 1 oz. of 50% concentrate of fenthion mixed in 2.5 gallons of water.

Analysis of the blood cholinesterase levels of the workers exposed to fenthion showed no depression during the working day or over the period of study. This is indicated in Table 4, which shows the mean blood cholinesterase levels of the workers and controls.

TABLE 4

Comparison of blood cholinesterase levels before and after work among workers exposed to fenthion and among controls

Category of personnel	Mean blood cholinesterase value (%) for working week		
	before work	after work	difference (%)
Exposed group n = 28	94.0	89.0	- 5.0
Controls n = 10	98.8	97.9	- 0.9

Though there was no demonstrable depression of blood cholinesterase levels, it was observed that the spraymen had a higher incidence of symptoms than that of the controls (Table 5) an observation which is attributable to their exposure to fenthion.

TABLE 5

Analysis of symptoms of spraymen (125) exposed to fenthion and control group (51)

Symptom	Spraymen with symptom		Controls with symptom	
	Number	%	Number	%
Headache	38	30.4	4	7.8
Sweating	37	29.6	11	21.6
Pain and/or watering of the eyes	35	28.0	7	13.7
Impaired vision	29	23.2	4	7.8
Muscle cramps	26	20.8	2	3.9
Tightness of chest	23	18.4	6	11.8
Dermatitis	20	16.0	nil	0.0
Excessive Salivation	15	12.0	4	7.8
Excessive Secretions	6	4.8	2	3.9

Further, it was also observed that the blood cholinesterase levels were not associated with the presence of symptoms (Table 6).

TABLE 6

Relationship between symptoms related to fenthion exposure and blood cholinesterase levels of workers exposed to fenthion during the working week

Category of personnel	Mean blood cholinesterase value (%) for working week		
	before work	after work	difference (%)
Workers with symptoms (n = 18)	93.4	92.7	- 0.7
Workers without symptoms (n = 10)	95.0	92.0	- 3.0

DISCUSSION

The study demonstrated a higher incidence of symptoms attributable to fenthion among spraymen than in the control group. The commonest symptoms observed were headache, sweating, pain and/or watering of the eyes, impaired vision, muscle cramps, tightness of chest, dermatitis, excessive salivation and nasal secretions. But as shown in the results, the study did not demonstrate an associated reduction of blood cholinesterase levels. This discrepancy between the occurrence of symptoms without depression of blood cholinesterase levels was unusual. Similarly, Copplestone et al (ref. 3) in their study among agricultural workers did not demonstrate a lowering of cholinesterase level, although the occurrence of symptoms is not mentioned in this study.

It is possible that all of the symptoms described among the spraymen exposed to fenthion could result from local exposure of specific target sites. Systemic absorption of fenthion resulting in the depression of blood cholinesterase levels may not be essential to cause these symptoms (ref. 4). The headache, pain in the eyes and dimness of vision could be explained by local exposure of the ciliary body. Similarly, local exposure of the bronchial tree could result in tightness of chest; exposure of the nasal mucous membrane could result in rhinorrhoea, and even the sweating could be due to a local effect.

In this context, one of the other possible reasons for the normal blood cholinesterase levels observed may also be due to the possibility that the spraymen may have been particularly conscious of the presence of the research personnel and hence took more than the usual care about personel hygiene. A similar possibility was suggested by Baker et al (ref. 5) in a study of Malaria workers in Pakistan who developed symptoms as a result of exposure to malathion. In general, it was observed that the personal hygiene of spraymen was inadequate.

Dermatitis was another condition that was frequently observed among workers exposed to fenthion. Matushita et al (ref. 6) reported skin conditions resulting from exposure to pesticides to be common. In the present study in most instances, the dermatitis was considered to result from the aggravation of a previously existing dermatological problem. In one instance, the dermatitis was considered to be a photosensitive type of reaction. Similar findings have been reported by Japanese workers (ref. 7).

In the final analysis, it is evident that spraymen could develop mild symptoms of poisoning without a concomitant depression of blood cholinesterase levels. Further it was noted that none of the workmen used any form of protective clothing, a situation made worse by poor personal hygiene during spraying. This is a state of affairs which could be improved by education. It is felt that, particularly in the developing countries, this educational activity should be considered important and urgent.

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RISKS FROM COMBINED EXPOSURES TO DIFFERENT CHEMICALS

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INTRODUCTION

The problem of mixtures of different chemicals in the environment is particularly important in surface water as the aquatic organisms are exposed to the joint action of the mixture. In our laboratory Mr. Könemann studied this problem in a series of experiments with fish. Since his work may also be of interest to occupational health, I would like to report some of his results.

A helpful classification in experimental studies with mixed exposures, based on quantal response, is given by Plackett and Hewlett (ref 1) (Scheme 1).

	similar joint action	dissimilar joint action
interaction absent	simple similar action	independent action
interaction present	complex similar action	dependent action

Scheme 1. Classification of quantal response to mixed exposures.

In the case of complex mixtures as in polluted surface water the occasional occurrence of pairs of compounds with interaction (resulting in potentiation or antagonism) is not very important. What remains in the first place is simple similar action for compounds with the same site of action, as when more than one organophosphate is present. In the second place there is the independent action for mixtures of differently acting compounds, as for example with a mixture of dieldrin, parathion and fluoroacetic acid.

SIMPLE SIMILAR ACTION

In the case of simple similar action it is most convenient to refer to a fixed standard response level for both the single compounds and the mixture. In our case it was the LC50 for fish. At the standard response level each compound can be replaced by an equitoxic concentration of another.

Every compound contributes to the 50% lethal response by its number of toxic units, defined as the concentration of the compound divided by its LC50. At 50% response the sum of the toxic units is one. Without knowledge of the slope of the log concentration-response relationship it is not possible to predict other levels of response than the 50% level. This case is also called "concentration addition".

INDEPENDENT ACTION

In the case of independent action of compounds in a mixture, two extreme possibilities may be distinguished. In the first possibility the same fishes which are more sensitive to one of the compounds are also more sensitive to all the other compounds. The sensitivity is completely correlated (r=1) and the response to the mixture will be 50% if one of the components is present at its LC50. The other components present at the same or lower concentration do not contribute further to the lethal response, because the 50% more sensitive fishes are already dead. This case we called "no addition".

If the sensitivity for the different compounds is random among the fishes, or rather if there is no correlation (r=0), than each component will contribute to the total toxicity of the mixture in accordance with its own probability of killing at its given concentration and toxicity. The probability of surviving the mixture can be calculated as the product of the probabilities of survival for each component. If e.g. we have three compounds in a mixture, each at its LC50, or 50% survival level, the joint probability of survival is $0.5 \times 0.5 \times 0.5 = 0.125$, corresponding with a mortality level of 87.5%. This case is called "response addition".

In a real situation of independent action the degree of correlation will be somewhere between one and zero.

The importance of the distinction between these three extreme possibilities is demonstrated in Scheme 2 for a mixture of 10 compounds.

Scheme 2

Joint toxicity of an isotoxic mixture of 10 chemicals

A. Simple similar action
   (concentration addition)
   0.1 LC 50 of each compound
   → 50% mortality

B. Independent action (r=1)
   (no addition)
   1 LC 50 of one of the chemicals
   → 50% mortality

C. Independent action (r=0)
(response addition)
one LC 6.7 of each
$\longrightarrow$50% mortality
($\sim$ 0.75 LC 50 of each chemical, on the basis of slope 1.2 in log conc.-probit plot)

EXPERIMENTAL RESULTS

For 50 representatives of chloroaromatics, chloro alkanes, glycolderivatives and some related compounds the LC50 was determined for guppies (Poecilia reticulata, a small tropical fish species). None of these compounds was known to have a specific toxic action, they might all belong to the more or less lipophilic agents with a non-specific "physical" action, comparable to that of the volatile anesthetics. The homogeneous character of this group was indeed confirmed by its quantitative structure activity relationship (QSAR) (ref. 2).

$$\log 1/LC50 = 0.87 \log P_{oct.} - 4.87$$
$$(n= 50;\ r = 0.988;\ s = 0.24)$$

$P_{oct.}$ = partition coefficient of the compound between n. octanol and water.

It is expected that many other non-specific acting compounds will fall within the same QSAR, which means that their toxicity can be predicted from the partition coefficient. With these 50 compounds mixtures were made on the basis of an equi-toxic concentration of each component and for these mixtures the concentration level of 50% mortality was determined. It appeared that for the 50% response level of the mixture the sum of the toxic units (concentration divided by LC50) was 0.9. When the sum of the toxic units was calculated from the toxicity derived from the QSAR, instead of from the individual result, the sum of the toxic units was 1.0.

It is concluded that in this group of non-specific acting organic chemicals the joint toxicity is completely additive and that its action may be classified as "simple similar".

As a contrast another mixture was prepared of 10 compounds, mainly pesticides, with each a known different specific action. In that case the sum of the toxic units at the 50% response level was 2.5. Here, the joint action is much less than additive; each component being present at $\frac{1}{4}$th of its LC50.

It is of particular interest to note that in the case of the 50 compounds each one acted at a concentration which was as low as 2% of the chosen response level. This was in our case the level of 50% mortality, but the same may hold true if the response level was e.g. 1% mortality. There is no particular reason not to expect that also for the highest no toxic effect level the concentration addition would be applicable. In such a case concentrations which are much less than the no toxic effect level may add up when more lipophilic organic compounds are present.

The non-specific action of such compounds is related, as mentioned before, to their hydrophobicity. This characteristic may be explained by a filling of lipoid containing sites, probably in the first place in the biomembranes. Over a certain degree of filling, functional defects will follow. Probably the first defect to appear is loss of efficiency in the transmission of impulses in the central nervous system.

Not only the non-specific acting compounds will accumulate at these sites but also lipophilic organic compounds with specific actions, such as the organophosphates and carbamate insecticides. When present as a single compound the accumulation at the lipophilic sites is not of great interest, because the specific action, the inhibition of cholinesterase, will overrule any non-specific action. But at non-toxic dose levels it may be expected that these compounds will also contribute, according to their hydrophobicity, to the filling of the lipoid sites and when the filling from different sources added together is high enough the characteristic effects of central nervous system depression will follow, whatever the kind of the fillers.

In this hypothesis for every lipophilic organic compound there are at least two modes of action of interest: the specific one and the non-specific one. The specific action may not necessarily be present, as with ether, and a non-specific action may in some compounds only appear in a mixture with other compounds when their concentrations are below the threshold for the specific action. Further work to confirm or refute this hypothesis is in progress, but for the time being it is considered as a warning to be very careful with mixed exposures.

Of course, there are factors which may modify the actual joint response as compared to the calculated response on the basis of the hydrophobicity contribution. To mention only one: speed of elimination. When a compound is very quickly eliminated by detoxication its body burden may be much less than expected from the external exposure concentration. It is worth mentioning here that the exposure of fish to lipophilic compounds dissolved in water is very similar to the exposure of warm blooded animals or man to volatile lipophilic chemicals in air. In both cases the membranes of the gills and lung alveoli come into intimate contact with blood that will not first flow through the liver as is the case with the blood from the gut.

The response from the total of lipophilic compounds calculated by concentration addition is considered to be the maximum possible response for mixtures of large numbers of compounds, where the possibilities of potentiation is remote.

FROM FISH TO MAN

The problem of mixed exposures in industry is already for a long time a matter of interest in occupational toxicology. In the Appendix of the yearly issued ACGIH

booklet on threshold limit values the system of concentration addition is proposed to calculate the T.L.V. for mixtures from the individual TLV's. The examples given refer to organic solvents. On the contrary, the German brochure on MAK-values issued early by the Deutsche Forschungsgemeinschaft rejects the idea of such calculations and calls for more research in this field. In this respect it is important to remember that TLV or MAC-values are not based on toxicity alone.

The main purpose of this presentation is to call your attention to our results with fish where it is possible to get results with a much higher degree of accuracy than with warm blooded animals or man. We have to bear in mind when extrapolating from fish that the process of biotransformation is at a much higher rate in man. A more valid comparison should be made on the basis of data on body burden, e.g. concentrations in blood, instead of external concentrations in water or in air. In particular I would like to bring into the discussion our findings of the contribution of extremely low concentrations in mixtures of non-specific acting compounds and our expectation that other lipophilic compounds with specific actions may come into the picture of the non-specific action. Mixed exposures will be mainly a problem in industrial situations, but I think that this is also worth considering in the case of field workers exposed to the more lipophilic kind of herbicides, fungicides and their formulation additives. With insecticides in the field, I expect that the specific toxicity will limit the uptake sufficiently to prevent the occurrence of non-specific toxic actions. The symptoms will be as vague as can be expected from a non-specific action, such as fatigue, headache and perhaps in more extreme cases a (reversible) decrease in nerve conduction.

It is, of course, not new but it should be recalled that the drinking of alcohol at home and the use of tranquillizers or other drugs with action on the central nervous system may also contribute to the total of the non-specific action.

This is not the only area of simple similar action calling for concentration addition. The same may be said of mixtures of specific acting compounds with the same site of action, such as mixtures of cholinesterase inhibitors. I will not further deal with these cases, because the problems of additivity and potentiation have had sufficient attention already (see e.g. ref. 3). The same applies to the case of additivity in carcinogenic action (ref. 4).

I would only like to mention one particular case that may be overlooked. It is the case of sensory irritation by mixtures of chemical irritants, as was demonstrated in mice for formaldehyde and acroléin (ref. 5) and for man with mixtures of formaldehyde, NO_2 and hexane (ref. 6). Subthreshold concentrations of each component in a mixture may add to get a noticeable effect. Here again, modifying factors are present, e.g. the sensory nerve cells responsible for the effects as well as their anatomical sites are not always the same for each compound.

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THE PORPHYRINOGENIC POTENTIAL OF PESTICIDES WITH SPECIAL EMPHASIS ON ORGANOPHOSPHOROUS COMPOUNDS

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ABSTRACT

A number of pesticides were examined for their possible porphyrinogenic properties by means of an assay procedure based on the use of primary liver cell cultures of chicken embryos. Three out of twelve compounds tested appeared to be able to induce porphyria without previous treatment of the cells with a drug enzyme inducing compound (viz. HCB, β-HCH and chlorfenvinphos). Eight out of the twelve compounds responded in the induced system (viz. HCB, β-HCH, chlorfenvinphos, temephos, azinphosmethyl, benzoyl prop-ethyl, pentachlorophenol and photomirex). No effect was found with 2,4,5-T, carbaryl, captan and trichlorfon. Enzyme induction markedly enhanced the porphyrinogenic potential of HCB, β-HCH and chlorfenvinphos. It is suggested that the measurement of the urinary porphyrin pattern may be a valuable parameter for the purpose of human biological monitoring in relation to occupational exposure to pesticides. Especially when there is some likelihood that the workers are also affected by the drug enzyme inducing properties of pesticides and/or other chemicals like drugs.

INTRODUCTION

Hepatic porphyria is a disorder of porphyrin metabolism which can either be inherited as a congenital anomaly or which can be induced by certain chemical compounds.

In clinical cases in man the condition of hepatic porphyria is usually associated with a considerable increase in the total amounts of porphyrins, especially uroporphyrin, excreted in the urine. However, qualitative changes in the pattern of porphyrins in the urine provide a far more sensitive indication of these disturbances in the pathway of heme synthesis than the quantitative changes. The latter are characterized by an increase of the proportion of porphyrins, which contain eight and seven carboxylic groups, relative to those containing six, five and four of these groups (ref. 8).

The molecular mode of action of porphyrinogenic chemicals has not yet been elucidated. However, there is evidence that inhibition of the enzyme uro-

porhyrinogen decarboxylase fulfils an essential role in the onset of the disease (ref. 1).

An important finding is, that in most cases the ultimate porphyrinogenic action is not exerted by the parent compounds but by one or more activated metabolites (Debets and Strik, in prep.). Induction of the drug enzyme system markedly enhances the development of hepatic porphyria after exposure to porphyrinogenic chemicals.

Most porphyrinogenic chemicals known so far are halogenated hydrocarbons, including such commonly known compounds as the polychlorinated and polybrominated biphenyls (PCB's and PBB's respectively), vinylchloride (VC) and tetrachlorodibenzodioxin (TCDD) (ref. 9). A pesticide which is well known for its porphyrinogenic potential is the fungicide hexachlorobenzene (HCB) (ref. 5). Evidence so far indicates that among the pesticides HCB forms a rare exception, since most pesticides tested do not seem to have porphyrinogenic properties. However, as will be demonstrated in the present paper, the porphyrinogenic potential may have been overlooked under test conditions where insufficient attention was paid to the possible contribution of an induced drug enzyme system.

MATERIAL AND METHODS

Until recently the porphyrinogenic potential of chemical compounds was only discovered in short term (ca 1-2 months) oral feeding studies with warmblooded laboratory animals such as rats and Japanese quail (e.g. ref. 7). The development of a test model based on the use of primary liver cell cultures of chicken embryos by Granick (ref. 4), markedly improved the ability to identify the porphyrinogenic properties of chemicals. This method generally is much more sensitive than the experimental models used previously and moreover responds within a time span of only one or two days after the start of the experiment.

In the present study the porphyrinogenic potential of a number of pesticides was investigated by means of primary liver cell cultures of chicken embryos by using a modified procedure in order to study the effect of pre-exposure induction of the drug enzyme system of the hepatocytes used.

Chicken embryo liver cell cultures were prepared as follows. Fertilized eggs of White chicken (strain Hubbard) were obtained from a commercial supplier. After an incubation period of 15 - 18 days the eggs were opened and the embryos perfused through the heart with a sterile (Ca^{++}-free) Hanks solution, containing 20 mM Hepes and 0.04 per cent EDTA. Four livers prepared this way were cut into small pieces (ca. 1 mm diameter) with a pair of scissors. The fragments thus obtained were put into an erlenmeyer flask and treated with a 30 ml dispase solution under slow stirring (grade II solution 1 U/ml, Boehringer, Mannheim)

in (Ca^{++}-free) Hanks medium (buffered with 20 mM Hepes) at 37°C for 20 min. Thereafter the cell suspension was sedimented by centrifugation at 200xg for 3 min. Remaining red blood cells were removed by washing the pellet with buffered ammonium chloride according to Sassa and Kappas (ref. 6). The isolated hepatocytes were then resuspended (4 livers/100 ml) in Williams E medium supplemented with 10 per cent fetal calf serum and without the supply of any antibiotics.

The incubation procedure was carried out as follows. Cell suspensions of 2 ml containing circa $1.5-2.10^6$ cells were transferred into 3 cm ϕ plastic petri dishes (Costar). The cells were incubated at 37°C in a humidified incubator in an atmosphere of 5 per cent CO_2 in air. The liver cells attached to the bottom of the culture dish within 6 hours after plating and at that time appeared as monolayer colonies. Some of the plates were preincubated with β-naphthoflavone (3 µg/ml medium) between the 6th and 24th hour of culture in order to induce the drug enzyme system. After 24 hours of culture, the medium was replaced by 2 ml fresh medium. Thereafter the pesticides were administered in 1 µl DMSO at a concentration of 10 µg/ml medium. The cells were then incubated for another period of 24 hours.

The development of symptoms of porphyria was measured by the examination of the porphyrin fluorescence using a fluorescence microscope.

For the purpose of this study a number of pesticides was selected, which so far had not been investigated in any detail with respect to this property. Benzoyl-prop-ethyl was selected because recent circumstantial evidence suggested that this herbicide might be porphyrinogenic (ref. 3). Some organophosphorous compounds, one carbamate (carbaryl), 2,4,5-T and photomirex were studied in order to examine whether metabolic activation might turn these compounds into porphyrinogenic metabolites.

RESULTS

The results of the chicken embryo liver cell assay are illustrated in table 1. The table also summarizes some data from the literature for comparison. Three out of twelve compounds appear to be able to induce porphyria in the cell culture system without previous treatment of the cells with a drug enzyme inducer, whilst eight out of these twelve compounds respond in the induced system. Of the compounds considered only HCB, β-HCH and chlorfenvinphos are porphyrinogenic without induction. However, induction markedly enhances the porphyrinogenic potential of these compounds.

TABLE 1

Effects of some pesticides on porphyrin formation in chicken embryo liver cells

compound [a]	microscopic fluorescence of cell cultures		reference
	without induction	with induction [b]	
2,4,5-T	-	-	this study
benzoylprop-ethyl	-	+	" "
carbaryl	-	-	" "
captan	-	-	" "
trichlorfon	-	-	" "
temephos	-	+	" "
azinphosmethyl	-	+	" "
chlorfenvinphos	+	++	" "
photomirex	-	+/-	" "
hexachlorobenzene (HCB)	+	++	Debets et al. (in preparation)
β-hexachlorocyclohexane (β-HCH)	+	++	Debets and Strik (in preparation)
pentachlorophenol	-	+/-	Debets et al. (in preparation)

a) technically pure compounds were used. All compounds were added after 24 h of culture in a concentration of 10 µg/ml.

b) liver cells were pretreated with β-naphtoflavone (3 µg/ml) between the 6th and 24th h of culture.

++ indicates clear overall fluorescence, + clear fluorescence of clusters of cells in the culture, +/- weak fluorescence of cells, - no fluorescence.

DISCUSSION

The results of this study strongly support the hypothesis that the metabolic formation of reactive intermediates represents an essential element in the process of porphyria induction. For a review of the possible molecular mode of action of porphyrinogenic compounds see Debets and Strik (ref. 1). The only organophosphorous compound which proved to be porphyrinogenic in tissue culture without pre-induction was chlorfenvinphos. In this connection it is of interest to point to the presence of an allyl group in the structure of this insecticide. Allyl-containing compounds, e.g. 2-allyl-2-isopropylacetamide (AIA), secobarbital and other allyl-containing barbiturates are well known for their porphyrinogenic properties. There is strong evidence that these allyl-containing compounds are metabolized via reactive epoxide intermediates (ref. 2). Fig. 1 shows the hypothetical intermediate which might be involved in the porphyrinogenic activity of chlorfenvinphos.

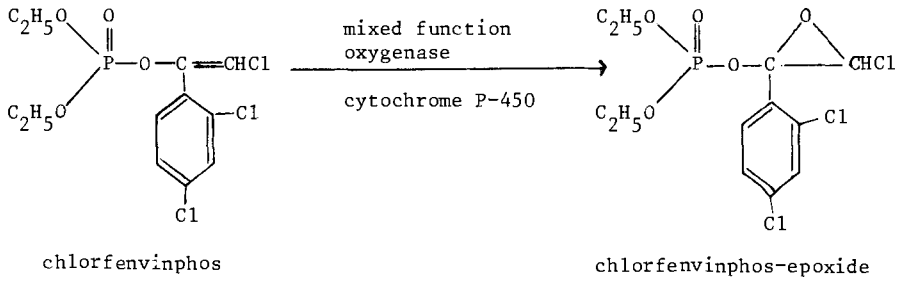

Fig. 1. Possible activation mechanism of chlorfenvinphos.

A possible mechanism could be that these epoxides are involved in the destruction of cytochrome P-450 and in the conversion of heme to socalled "green pigment". This causes a heme shortage and hence stimulation of porphyrin synthesis. However, no direct evidence for this possible role of epoxides has yet been obtained.

The outcome of the chicken embryo liver cell assay correlates strongly with the results of tests in which warmblooded experimental animals are used. This conclusion is based on experience with a fair number of compounds including drugs, industrial chemicals and a few pesticides (ref. 9). The chicken embryo liver cell technique may therefore be considered as a test model with respect to man. It is also important to mention that the induction of hepatic porphyria very probably preceeds all other known effects which may be induced by these chemicals after subacute or short term exposure. Therefore the measurement of the urinary porphyrin pattern may be a valuable parameter for the purpose of human biological monitoring in relation to occupational exposure to pesticides. Especially when there is some likelihood that the workers are also affected by the drug enzyme inducing properties of pesticides and/or other chemicals like drugs.

A final remark should be made about the herbicide benzoylprop-ethyl. Edling (ref. 3) reported a case of polyneuropathic porphyria (congenital porphyria) in a man who became ill after working in a field picking wild oats which had been sprayed with this compound. Examination revealed a palpable liver and laboratory tests showed increased transaminase. Coproporphyrin in the blood was at the limit value and urophorphyrin in the urine and protoporphyrin in the blood were slightly increased. No assessment could be made of the degree of exposure to the herbicide. Considering the porphyrinogenic properties of benzoylprop-ethyl as found in the present study it cannot be excluded that the compound contributed to the ill condition of this man.

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CONSEQUENCES FOR FIELD EXPOSURE OF IMPURITIES IN PESTICIDE FORMULATIONS

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INTRODUCTION

During recent years, the attention of toxicologists and chemists has been drawn to impurities in technical pesticide preparations. In this connection it has been shown that in the toxicological classification of pesticides this problem assumes enormous importance.

From the toxicological point of view this problem is not new. Diggle and Gage (ref. 3) pointed out, that marked differences in the toxicity of the insecticide parathion could be traced to contamination with small quantities of the oxygen analogue, paraoxon and the phosphorothiolate isomer which are more acutely toxic than the parent insecticide. Independantly Rosival and Bátora (refs. 2,5) have detected the presence of the oxygen analogue of parathion, paraoxon, as a metabolite in the liver of rats, after administration of parathion standard.

In assessing data one frequently encounters problems stemming from inadequate information on the composition of certain technical grade pesticides. Also of concern is the fact that some pesticides are produced by several manufacturers; thus the composition of technical products may vary, particularly with respect to contaminants. Certain pesticides are of unknown or variable composition, e.g. toxaphene and other chlorinated terpenes and technical grades of HCH (hexachlorocyclohexane). Consequently, it is impossible to relate the existing toxicological data on these compounds to the compounds actual used in agriculture.

This situation is likely to occur more frequently in the future as patent protection of various compounds expires, permitting them to be produced by a number of different manufacturers. If toxicological evaluations are to be applicable to products whatever their source, it is essential for such products to meet specifications in order to ensure a reasonable similarity of composition (ref. 1).

Potential hazards from exposure to impurities in pesticide preparations apply during their production and practical use as well as for pesticide residues, as based on their different metabolic pathways and their degradation in the environment and biological systems. In addition, their interactions with other chemical compounds cannot be underestimated. Therefore, it seems unavoidable to follow systematically all exposed workers in pesticide production and use for reactions that could signalize possible toxic action of impurities. Animals and plants can also be good indicators of such action, thus important information can be obtained from an ecotoxicological viewpoint as well.

The importance of these problems is also connected with safety measures not only during production but also during practical plant protection. On the other hand this can lead to repercussions on the philosophy of the evaluation of the maximal limits and acceptable daily intake for the residues of pesticides in the biotic and abiotic environment.

The traditional attitude to toxicological evaluation was based on the evaluation of technical preparations that resulted from an established technology, whereby the presence of impurities was included in the toxicological data. This stemmed mainly from the problems of synthetizing pure compounds, but also from those of the identification and determination of chemical impurities, and with the economics of pesticide manufacture. Although such evaluation was close to practical conditions, it made a comparison of toxicities of various technical preparations very complicated and difficult.

Another way of expressing toxicity is its calculation on the basis of pure (100%) active substance: however, this has not found a wider use in practice.

If the impurities represent a significant portion of the product or if their chemical properties or their chemical analogy to other known substances suggest they may have serious toxic properties, the impurities must be evaluated separately (ref. 4).

SOURCES OF IMPURITIES

There are two main sources of impurities in technical pesticide products:
a. the starting materials for pesticide production may contain impurities e.g. amines used in manufacturing alkyl amine salts of phenoxyalkane carboxylic acids may contain corresponding N-nitroso alkylamines; the production of camphechlor (toxaphene) by chlorinating camphene of technical quality only;
b. manufacturing technology will include chemical reactions which inevitably result in reaction products containing by-products e.g. the formation of S-alkyl isomers in organophosphorus thioates and dithioates; ETU formation in ethylene-bis-dithio-carbamates (EBDTC) production; formation of N-nitroso-alkylamines during the production of dinitroaniline herbicides (trifluralin,

benfluralin, isopropalin); formation of polychlorinated dioxines, polychlorinated furans during the production of PCP, hexachlorophene, 2,4,5-T.

Secondary sources include:

a. unsuitable formulation of technical grade active ingredient e.g. the isomerization of organophosphorus thioates and dithioates; formation of hydrophilic degradation products leading to alteration of toxicological and pesticidal properties;
b. unsuitable tank-mix preparation before practical application e.g. ETU formation from EBDTC fungicides;
c. unsuitable storage of formulations e.g. chemical/physical transformation of active ingredient.

TOXICOLOGICAL IMPORTANCE OF IMPURITIES

The importance of impurities in pesticides arises from the fact that they may have a decisive effect on the character of toxic manifestations. Therefore a specification at international level will be needed, including impurities in technical grade products. WHO and FAO should present criteria so as to require from manufacturers products of maximum possible purity, whereby health criteria and economic possibilities can be taken together. On the other hand, it would be useful from the side of the WHO to promote research projects, directed to identification and determination of impurities and side reaction products, and their complete biological activities. A successful solution of these problems can be illustrated by the manufacturing of 2,4-D acid where it has been shown that the technology used does not allow tetra-, hexa-, and octachlorodibenzo-p-dioxins to be formed. However, it does not exclude the presence of other impurities.

Organophosphorus compounds

Among the problems mentioned here, the organophosphorus pesticides have become of special importance where at the stage of production there are no means of avoiding the presence of interfering products of side - reactions.

In this sense we are considering the presence of S-alkyl isomers of thio- and dithiophosphoric acid triesters. Examples of this are the compounds of the parathion group, namely parathion - methyl, parathion, and fenitrothion.

From our work we would like to discuss the problem of fenitrothion S-methyl isomer (SMF). This isomer occurs as an interfering impurity in the technical product used for the production of the commercially available insecticide preparations. The concentration of SMF ranges between 0.5 - 1.5%. It can also be formed during production as a result of thermic isomerisation, or due to UV irradiation during storage of the product over long periods under inappro-

priate conditions. No other similar study has yet been undertaken. Such a study requires hardly available pure substances, fenitrothion and its stereoisomer SMF, stability of which under normal conditions is limited.

In our experiments the pI_{50} values (the negative logarithm to base 10 of the I 50, which is the molar concentration of inhibitor that gives 50% enzyme inhibition) for fenitrothion, SMF and oxidized fenitrothion were established in lyophilized horse serum, human blood serum and in homogenates of heads of the domestic fly.

The following results were obtained: fenitrothion: both human and horse serum 5, fly heads 6; oxidized fenitrothion: horse serum 7, human serum 8; SMF: horse serum 7, human serum 8, fly heads 9.

As it can be seen, SMF is in the range of 2-3 orders of magnitude more effective in inhibiting cholinesterase activity than fenitrothion. In the next step, the influence of SMF in mixtures with fenitrothion (1 and 5% of SMF) using cholinesterase from horse serum was studied.

As little as 1% SMF caused a decrease in the original I 50 of fenitrothion by 1 order of magnitude, while 5% SMF caused an even more marked decrease.

In the case of 1% SMF added to standard fenitrothion the inhibition of cholinesterase is largely due to SMF (35% against 12), while when a 5% SMF concentration was used, fenitrothion participated in the total inhibition to the extent of one tenth only. It is, therefore, obvious that the enzymatic method of organophosphorus pesticide determination requires standards free of impurities able to inhibit cholinesterase.

A further example of potential risk from impurities in technical pesticide products presents the case of isomalathion. This impurity can be formed by concurrent reaction mechanisms during malathion manufacturing and during unsuitable storage of formulated malathion (effect of temperature, humidity, packing material). As was shown in Pakistan, during the application of impure malathion formulation, a number of acute intoxications of applicators have been diagnosed. According to the chemical analysis, the malathion formulation used in Pakistan contained a high level of isomalathion. For this reason, it was recommended, that the level of isomalathion calculated on actual malathion content should not exceed 1.8% w/w.

2,4,5-T

Another group of pesticides which have caused considerable public concern in recent years are phenoxy acids, applied as herbicides in agriculture and forestry. Occasionally these compounds are contaminated with highly toxic chlorinated dioxins. Of these compounds the most hazardous is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It is formed as an unwanted contaminant in the

manufacture of 2,4,5-trichlorophenol precursor. The manufacture of 2,4,5-trichlorophenoxyacetic acid can be in such a way that the pesticide 2,4,5-T is contaminated with TCDD.

This compound is extremely toxic. For guineapigs, the ratio of the LD_{50} for 2,4,5-T to the LD_{50} of the dioxin is about 630000. In female rats, the acute oral LD_{50} for dioxin is about 1/10000 of the oral LD_{50} for 2,4,5-T. The major concern for the toxicity of 2,4,5-T should be therefore directed towards the contaminant rather than towards the herbicide itself (ref. 4).

By means of advanced technology it is possible to produce the pesticide 2,4,5-T with a dioxin content below the level of 0.1 mg/kg. This is accepted in many countries as the highest concentration of dioxin in a 2,4,5-T product.

Pentachlorophenol

In the case of pentachlorophenol (PCP) the chlorinated dioxin and dibenzofuran impurities are of particular concern. For instance, the hexa- and heptachloro-compounds can be present at a total of over 30 mg/kg and are much more toxic than the octachlorodibenzodioxin (OCDD). It must be stressed also, that the above mentioned TCDD can be present in some PCP at as much as 0.25 mg/kg. Although PCP has been found in a variety of environmental situations, data on PCP impurities and conversion products are almost nonexistent.

CONCLUDING REMARKS

The purity of the majority of active substances lies in the range of 90-100% therefore the toxicological properties of the remaining compounds up to 100% can be exceedingly important for human health.

In a current analysis of these problems we have established that only a fraction of the large quantity of active substances has a fully determined identity. Only a relatively small number of compounds from the group of organophosphates, carbamates, phenoxyalkanic herbicides, and a few chlorinated cyclodienic insecticides have been completely characterised.

The importance of these problems is emphasized by the fact that the FAO and the WHO make their international consent dependent on the data regarding the quantity and nature of byproducts. It must be stressed, however, that concern is only for the listing of impurities. Their toxicological characteristics have not so far gained proper attention.

Evaluation of biological activities of interfering substances present in pesticide preparations is of great value from the toxicologic point of view. It represents a new attitude contributing to the elucidation of the mechanism of action of such substances and enabling creation of objective criteria for their toxicological classification.

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METHODS FOR DETERMINATION OF EXPOSURE OF AGRICULTURAL WORKERS TO ORGANOPHOSPHORUS PESTICIDES

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INTRODUCTION

The problems concerning the safe application of pesticides for plant protection and the safe handling of plants treated with these chemicals are solved through the competence:

a) of those who apply the pesticides (which includes their choice of application method and formulation - liquid, solid, aerosol);
b) of the physician (who can detect early the toxic effect of the chemical on man);
c) of the chemist (who selects correctly methods of sampling and analysis of the plant and other biological material).

Thus, it is a complex solution. No matter how slight the toxicity of a chemical, irresponsible and uncontrolled application could lead to serious consequences.

What should be the methods for studying residual quantities of pesticides and for analysis of biological material to establish the effects of these chemicals on man? They must be rapid, of high sensitivity and yet simple so that these measurements can be carried out in any chemical laboratory, without special, expensive equipment. Only then can measurements be carried out systematically anywhere in the world, a matter of special importance for the developing countries.

At present we are preparing a map of Bulgaria divided into regions where the following data will be illustrated: number and type of the pesticides used, method of application, number of warehouse premises, number of teams applying pesticides, type of crop, number and causation of intoxications during the last 5 years. This information is necessary for us to be able to work out minimum periods for safe reentry for work (m.p.s.w.) for the most frequently used pesticides in Bulgaria.

METHODS FOR ANALYSIS OF BIOLOGICAL MEDIA

Method for determination of ChE activity

For ChE activity we had prepared a rapid paper test (refs. 1 and 2) which has been applied for more than 10 years in Bulgaria.

Method for demonstrating ChE inhibitors in blood

In order to demonstrate the presence of ChE inhibitors in blood separately for serum and the erythrocytes, a rapid thin-layer chromatographic enzymatic method (ref.3) has been developed.Blood was taken for analysis from patients with severe organophosphate intoxication and with zero values for ChE activity. It was established that after extraction of 10 ml blood from the above mentioned patients followed by chromatography with enzymatic development, a chromatographic spot, or number of spots from the ChE inhibiting metabolites of the pesticide, had an area of at least 100 mm^2. This quantity of inhibitor (pesticide or ChE inhibiting metabolites) we accept as the quantity producing 100% inhibition of blood ChE. If there is no indication of inhibitors in the blood then the low values of ChE are assumed to be due to an injury to liver function preventing synthesis of the enzyme. In these cases, no drugs which can have additional toxic effects on the liver (for example the antibiotics chlornitromycin, climycin, etc., which have a clearly inhibitary effect on ChE activity, as we recently have observed (ref.3)) should be used.

LABORATORY STUDIES AND FIELD OBSERVATIONS

Our recent results (ref.6) on the effect of some pesticides on ChE activity have established a decrease in acetyl ChE and ChE activity clearly related to the appearance of clinical symptoms after oral administration of sun-flower seed oil solutions of the pesticides methyl-parathion, trichlorphon (organophosphates) and carbaryl (carbamate). It was established that the AcChE recovers more slowly than ChE. The clinical picture corresponds to the ChE inhibition only for the first 20 hours, while subsequently symptoms disappear in the presence of inhibited ChE.

Similar results are found in blood analysis of man with severe intoxication of organophosphates and carbamate compounds (the cases did not result from occupational poisonings, but were most frequently caused by attempts for suicide - samples were obtained from the Institute for Emergency Medical Care). It was established (refs. 4 and 7) that during acute poisonings with organophosphates (trichlorphon, methyl-parathion, dichlorvos) and carbamates (carbofuran and dimetilan) ChE activity decreases to 0.5 - 0.0 IU/ml.
While with the former the cholinesterase activity recovers slowly (sometimes over a period of one month or more), with the latter the reactivation of the enzyme occurs very quickly (over a period of several hours). Thus less than 12 hrs after intake of carbofuran the ChE level was near to the normal one - 2 IU/ml.

After poisoning with trichlorfon, the ChE inhibitors persist up to the 24th hour after oral intake, and after dermal treatment up to the 5th day.

The experiments carried out with both test animals and man show low values of ChE activity after elimination of the poison and metabolites from the body.

When blood analysis of a patient poisoned with methyl-parathion was carried out very interesting results were obtained. Zero values were established up to the 9th day and the presence of ChE inhibitor (not identified) in plasma and erythrocytes up to the 30th day of the intoxication while a maximum elimination was observed on the 23-24th day.*

The application of chemically treated papers for rapid ChE activity determination gives us the possibility to cover a large number of people and in cases of group poisoning (though not frequent, these are still observed in Bulgaria) to perform a quick and precise analysis. In an average severe intoxication with menazon (headache giddiness, weakness, nausea and vomiting) the ChE level decreases to 1.6 (1.8-1.6) IU/ml, with propoxur to 1.0 (1.6-1.0) IU/ml and with mevinphos to 0.0 (1.0-0.0) IU/ml. These results are important both from a hygienic point of view and for forensic purposes in determining the agent of poisoning and separating the organophosphate poisoning from other cases with similar symptoms.

On the basis of extensive observations on agricultural and glass-house workers, a method for determining minimum periods for safe re-entry for work with organophosphate pesticides has been established (ref. 5). This method has been used for determining minimum periods for safe re-entry for work for seven pesticides. We are in the process of determining safe periods for other frequently applied pesticides in Bulgaria.

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BLOOD CHOLINESTERASE MONITORING OF WORKERS EXPOSED TO ORGANOPHOSPHORUS PESTICIDES: THEORY AND PRACTICE

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INTRODUCTION

The Health and Safety at Work Act, 1974, refers to the duty of employers to ensure as far as reasonably practical that a substance "is safe and without risks to health when properly handled". In practice, it is the method of handling which should be subject to constant scrutiny in order to ensure a high level of safety and low health risk. While much can be done by good design of safety measures and equipment, and by effective supervision of the work, there is always the need to confirm the success of such procedures. In part, this is a function of regular medical examinations which may provide subclinical evidence of exposure. Alternatively, evidence of exposure may be sought by measuring blood and/or urine levels of the relevant chemicals or their metabolites. In the case of anticholinesterase compounds such as the organophosphorus and carbamate pesticides, depression of plasma and/or red cell cholinesterase activity has been used as an indirect indicator of exposure to and absorption of these chemicals. It is the purpose of this paper to examine the theoretical and practical aspects of this use of blood cholinesterase measurements as a guide to the safe handling of toxic chemicals.

THE BIOLOGICAL BASIS OF BLOOD ChE MONITORING

The use of blood ChE level as an indicator of exposure to an inhibitor agent is based on experimental observations, in vitro and in vivo of an inverse relationship between the concentration of inhibitor present and the level of enzyme activity. The relationship is not linear but sigmoid, and so enzyme activity is insensitive to changes in inhibitor concentration at the two extremes. In vitro, depression of enzyme activity by an inhibitor is measured when full equilibrium has been established between the two reactants. In vivo however, this ideal condition is unlikely to occur and the dose/response relation is modified by the factors of absorption, distribution, metabolism and excretion which are all dose and time dependent and subject to normal and pathological variation. It follows that a single ChE value can only reflect the state of the blood at the time of sampling and gives no information about the rate or direction of changes in enzyme

activity following exposure. The same value of ChE depression can have different meanings dependent on the time of sampling relative to exposure. For example, a 50% depression shortly after exposure while enzyme levels are falling is more evidence of a threat to health than the same value obtained some time later when exposure has ceased and enzyme levels are returning to normal values. Without exact information about the time of exposure it is impossible to resolve this difference. It must also be recognised that in practice, the delay between taking a blood sample and receiving the result may vary from a few hours to several weeks depending on the effectiveness of the laboratory service. Thus all blood ChE values are to a greater or lesser extent 'out-of-date' so that the action taken as a result of a particular value may be inappropriate at the time it is taken and sometimes too late to prevent further exposure.

RECOGNITION OF DEPRESSED BLOOD ChE

It may not always be fully appreciated that while it is the amount of inhibited enzyme which is dose-related to the concentration of inhibitor present in the blood, the parameter actually measured is the remaining active enzyme. Calculation of the amount of inhibited enzyme to provide a measure of exposure requires a prior knowledge of the total enzyme present, and this poses certain problems.

The use of a worker as his own control with pre-exposure enzyme level as a point of reference is subject to the limitations imposed by spontaneous fluctuation in enzyme activity. If only one pre-exposure value is obtained - a common procedure - subsequent values must differ from it by more than 20% (plasma) and 15% (red cell) to be considered significant at the $p = 5\%$ level. Even when three pre-exposure measurements are carried out the corresponding differences are still 16% and 13% (ref. 1).

Alternatively, statistical evidence of depressed enzyme activity in individual workers may be sought by comparison with the mean and standard deviation values for a non-exposed population. However, as Callaway et al.(ref.1) have pointed out, significant depression of an individual's enzyme level can occur without exceeding the limits of normality of a non-exposed population.

For these statistical reasons small degrees of inhibition are likely to escape detection and when the underlying exposure is allowed to persist, cumulative biological changes can occur. Of particular importance in this context are the development of pharmacological tolerance, with suppression of muscarinic warning signs and symptoms, and delayed neurotoxicity.

NON-INHIBITORY CHANGES IN BLOOD ChE ACTIVITY

In addition to physiological variations, changes in enzyme activity level can occur for reasons not associated with inhibition. Acute or chronic depression

of liver function results in a lower plasma ChE level (ref. 2) and the red cell enzyme reflects the level of haemopoietic activity (ref. 3). To complicate matters further, both enzyme production systems are subject to inductive effects. As a result, a low blood ChE value does not necessarily indicate exposure to an inhibitor substance and a value within normal limits may conceal a degree of inhibition when enzyme induction has raised the total concentration of enzyme above the pre-exposure value. These factors should be kept in mind particularly when workers are exposed, not only to inhibitor compounds, but also to solvents with toxic effects on liver and bone marrow enzyme systems.

PREDICTIVE SIGNIFICANCE OF LOW ChE VALUES

While depression of blood ChE enzymes is of no known biological significance it has been used as a measure of biological effects likely to arise from the inhibition of similar enzymes elsewhere in the body, notably in the nervous system. Particular use is made of the experimental observation that plasma ChE can, with controlled exposure, be depressed to very low values before signs and symptoms of intoxication are apparent. However, occupational exposure is a different matter and a brief but heavy exposure can result in the inhibitor substance moving from the plasma into other tissues in amounts which are not related to the inhibition of plasma ChE when an equilibrium has been established. Account has also to be taken of the different affinities of anti-ChE compounds for the enzyme systems in plasma, red cell, and nervous tissues which can result in unequal degrees of inhibition even in the unlikely event of equality of distribution of inhibitor throughout the body. For these reasons, the degree of inhibition of blood enzymes is of doubtful predictive value for inhibition of ChE in the nervous system, either in magnitude or in time course, nor can it give any indication of the extent or severity of functional changes due to inhibition of ChE in the nervous and other systems of the body.

DELAYED NEUROTOXICITY

The acute signs and symptoms of exposure to anti-ChE compounds are adequately explained by the inhibition of this enzyme system but it is clear since the work of Johnson (ref. 4) that the delayed neurotoxic action of some organophosphorus compounds is related to inhibition of a different enzyme system present within nerve tissue. It follows that blood ChE measurements can not be of any predictive value for the changes in structure and function found in the delayed neurotoxic state. From the practical point of view, it is important to note that delayed neurotoxic effects have occurred as a result of exposure to organophosphorus compounds in spite of negative blood ChE results (ref. 5).

CONCLUSIONS

In view of these theoretical and practical inadequacies of blood ChE as a monitoring system and because many field workers do not have ready access to medical and toxicological advisory services, there is need for a guiding statement from this workshop. This should deal with such points as :

1) preferred method of measurement of ChE activity, including appropriate quality control

2) number of pre-exposure values required to establish an adequate baseline

3) frequency of measurement to provide a realistic basis for control of exposure

4) statistical significance of 'below control values' as indices of exposure

5) biological significance of 'below control values' with respect to (a) acute and (b) chronic exposure

6) the appropriate use of blood ChE values to improve health and safety measures and to remove from exposure those workers who appear to be at risk if exposure is continued.

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SOME OCCUPATIONAL MEDICAL ASPECTS OF PESTICIDES HANDLING; INDUSTRY VIEWPOINT

DR. H.G.S. VAN RAALTE
GIFAP Toxicology Committee, The Hague (The Netherlands)

As chairman of GIFAP's Toxicology Committee, I have been invited to talk to this Workshop.

GIFAP stands for Groupement International des Associations nationales de Fabricants de Pesticides. It is the international trade-association of the manufacturers of pesticides. The federation, original European, was founded in 1960. GIFAP now represents more than 650 Companies in eighteen countries who produce ninety per cent of the pesticides used throughout the world. The secretariat and permanent staff is located in Brussels.

GIFAP's aims include:

1 the promotion of safe and sensible use of pesticides;
2 the promotion of the harmonisation of national and international legislation and regulations on pesticides;
3 to provide a forum for discussion of and expert advice and information on international scientific and technical problems such as toxicology, residues, environmental issues, regulatory affairs, analytical matters, economic affairs etc.;
4 to act as liaison agency and to place industry's views and expertise before among others international organisations such as the Food and Agricultural Organisation (FAO), the World Health Organization (WHO), the United Nations Environmental Programme (UNEP), the European Community (EC), the Council of Europe, the Organisation of Economic Co-operation and Development (OECD) and others.

It will be clear that I cannot ensure giving the views or opinions of all affiliated national associations and much less of those of all and every associated member company. There would not have been time to check or even to consult in advance with all company representatives. I can only give you what I believe is the industry's position on some aspects relevant to the theme of your Workshop.

This workshop has been concerned exclusively with protection of the health of pesticide applicators and other field workers and perhaps, I wonder, but I have not yet heard anything about it, of what we call "bystanders" i.e. all those people who happen to be, at some time or other, in the immediate vicinity of the process of

application itself or of its short-term results. GIFAP's Toxicology Committee is, of course, also concerned mainly with health effects. It is a Committee dealing exclusively with matters that relate to the science of toxicology. Its activities do not relate to commercial- or socio-economic or political aspects. I am neither qualified nor prepared to discuss such aspects.

As I said in the beginning, GIFAP's aims include promotion of the safe and sensible use of pesticides. A prerequisite thereto is toxicological research. Toxicological research is necessary to establish:-

1 precautions for application
2 diagnosis and treatment of poisoning
3 an ADI
4 a preharvest interval
5 precautions to prevent injury to animals.

The risk, and thus the relative safety, of a pesticide depends on the interaction of:

1 the people
2 the pesticide
3 the conditions of application.

The conditions of application vary widely from small farmer handspraying to aerial canopy spraying with all kind of equipment and formulations.

It is a fact which is not always realized that seventy-five per cent or more of all toxicological research on pesticides has been and is being carried out or sponsored by Industry. Therefore, a considerable amount of scientific and technical expertise and knowledge is present in Industry. GIFAP is prepared to share this expertise and make it available to Governments and International organisations. Just two examples:

1 our co-operation with Dr. Copplestone's group in WHO in a field study in Africa with the objective of assessing possible hazards of pesticide application in a tropical area without elaborate protective gear ;
2 our active participation in the FAO-Government Consultation on Harmonisation of registration requirements.

Co-operation with regard to hazards of specific pesticides is a matter for specific manufacturing companies. Co-operation on general, non-specific problems of toxic hazards is a matter for the entire Industry and sometimes for GIFAP, therefore.

As regards this co-operation there are two issues I would like to discuss briefly in the very short time available.

(a) It has been stated - and it was, of course, eagerly taken up by the media - that worldwide half a million casualties, one-tenth of them fatal, have resulted from the use of pesticides. GIFAP is seriously trying to find the real figures by looking into existing registrations of national Health, Labour and Agricultural ministries and Poison Control Centers. Up till now we have failed to find confirmation or substantion of this serious accusation. GIFAP would be grateful for co-operation and any help in getting hard data and true facts on both number of people occupationally exposed and real - confirmed and not alleged - casualties.

(b) Then there is the problem of international harmonisation of registration requirements. In this area GIFAP is actively participating in discussions in the FAO-Government Consultation Programme, the Council of Europe Brochure on Pesticides and wherever possible in the EC.

As has been said, at least seventy-five per cent of all toxicological and field studies on pesticides are being carried out by Industry. We know what can and cannot be done. There is great concern that the capacity for toxicological and ecotoxicological testing and for the evaluation of data have been outstripped by technical and chemical developments and by the ambitious requirements for data. It is clear that the scarce talent and limited resources available worldwide must be used wisely and managed with great care. This means we must be very selective in our requirements. It is certainly no longer just a matter of requiring data which <u>can</u> be generated but of requiring data that are really essential.

Then we must avoid unnecessary duplication.

Assessments of hazards from chemicals depends on evaluation of toxicity- and ecotoxicological data.

Data are an expression of properties.

Properties do not change or vary upon crossing of national boundaries.

It is true that human populations vary between regions but these variations are not large and they have already been taken into account by the use of an appropriate safety factor. Time does, unfortunately, not permit to discuss and elaborate upon this very interesting topic. For today, it would suffice to say - in the context of avoiding unnecessary duplication - that there is no conceivable ground for requiring local replication of studies designed to determine a pesticide's inherent mammalian toxicity in order to assess a possible hazard to human health.

REPORT OF RAPPORTEUR

W.F. TORDOIR

PREFACE

The Vth International Workshop of the Scientific Committee on Pesticides of the International Association on Occupational Health was held in The Hague, The Netherlands on October 9, 10 and 11, 1979.

The subject "Field worker exposure during pesticide application" was chosen during the previous Workshop held in Bratislava in 1977 which recommended:

- further development of methods for assessment of exposure
- development of practical exposure tests for application on a wide scale
- improvement of the use of information obtained from human studies on the determination of dose-response relationships
- assistance from manufacturers of pesticides in data collection from field studies for advice on health protection of field workers.

Thirty-eight invited experts from all continents and working in international organisations, governmental agencies, universities or industries discussed twenty-five papers. Each paper contributed in its own way to the evaluation of the progress that has been made in the implementation of the recommendations of the previous Workshops.

GENERAL INTRODUCTION

The chairman, Professor Rosival, opened the Workshop with a survey of the activities of the Scientific Committee on Pesticides since its inception in 1971. He stressed the task of the Committee in identifying and evaluating the specific hazards of the handling and application of pesticides, and the need to collaborate with WHO and other organisations on the promotion of health protection of exposed workers. The work of this Committee is an integral part of the international program on chemical safety based on and incorporating current WHO activities.

In the papers of this session, attention was drawn to the importance of assessment of exposure in the field and of medical examinations for compound related effects. This should be done in order to provide data for the formulation of guidelines on occupational health surveillance of workers and to evaluate

possible environmental health hazards.

Depending on the results of toxicity testing, it may be necessary to check the extrapolation from experimental animals to man by means of surveillance of exposed workers in the early phases of the development of new pesticides, novel formulations or new application techniques.

Adequate epidemiological studies are necessary to detect specific toxic effects, in particular when these effects only occur in the long term. These studies are difficult as exposures to several pesticides frequently occur simultaneously or sequentially.

In the discussion it was noted that, unfortunately, medical surveillance to protect the health of exposed people in which information is also obtained on human toxicology is sometimes inaccurately described as "human experimentation".

TECHNICAL PAPERS

Three papers were presented on technical aspects of pesticide formulation, application and personal protection. It was very clear that a good understanding of these technical aspects is essential for the control of hazards for occupationally and environmentally exposed people. Significant reduction of exposure and risk appears to result from the use of certain described methods in formulation and application.

National experts, in collaboration with government officials who know local conditions and cultural habits as well as other requirements, should collaborate closely with manufacturers to design appropriate formulations, packaging, labelling, and application methods, and to supervise instruction of personnel.

In the discussion it was suggested that too much should not be expected from protective clothing. Even when used, it may give false reassurance and, when not appropriate, may increase exposure and even lead to other conditions such as heat stress. Further work is needed to design effective and practicable protective clothing.

It was emphasised that personal hygiene is extremely important, although there are still gaps in our knowledge on the best means of removing chemical contamination of the skin. The use of alcohol-impregnated cleansing tissues may enhance absorption.

REPORTS ON FIELD STUDIES

Five reports on field studies were presented and discussed on the first day. These dealt with exposure to organochlorine compounds, organophosphorus compounds, carbamates and chlorophenoxy acetic acids.

From the pictures shown by some speakers it was evident that inappropriate handling of pesticides is a problem in both developed and developing countries.

Various techniques for the measurement of exposure were described. Biological monitoring of blood and/or urine was sometimes combined with, and related to, personal air sampling. In these cases an assessment of dermal absorption can be made which may stimulate a critical inspection of handling procedures and (re-) instruction of the workers.

In the discussion the following important observations were made.

- Experimental animal toxicology should be carried out in experienced and recognised laboratories: the results wherever obtained should then be valid everywhere. Field evaluations, on the other hand, should be made under local conditions or under similar conditions where the specific human and environmental factors are taken into account.
- There is an urgent need for practical, reliable and inexpensive methods and equipment for field work, in particular in the developing countries, to effect quick measurements giving rapid results.
- In some countries it may be impossible to find an adequate control population as the whole population may be exposed to some extent to the pesticide under investigation.
- It is essential in field studies, whenever possible, to obtain a medical history and to perform a physical examination of the subject. Sometimes this may be the only opportunity for a person to have a medical examination and be referred for treatment.

  If indicated and possible, sensitive techniques should be applied which measure functioning of relevant organs and/or physiologic systems.

Eight papers were presented on the second day dealing with the measurement of exposure of workers engaged in the handling of organophosphates, a pyrethroid and methylbromide. In some studies the biomedical effects were also measured. One paper reported on physical factors possibly influencing the performance of aerial sprayers (high temperatures, vibration and noise). The effect of these factors may be additive with and perhaps potentiate the effects of exposure to pesticides.

In the discussion several important observations were made and subjects were suggested for further research.

- There was no consensus on which types of cholinesterase should be measured. It appeared that there is still not enough knowledge about the biological significance of plasma ChE, whole blood ChE and erythrocyt ChE. This problem is particularly relevant in field work as one prefers to test only one type of ChE under field conditions.
- The possibility of allergic reactions in relation to pyrethroids apparently needs further evaluation as cases of possible allergic type reactions (skin and respiratory) were reported by some of the participants. However, other participants only had knowledge of the occurrence of the well known skin

sensations - in particular in the face - which are described as a local paraesthesia. It is not clear if the described allergic reactions were due to the pyrethroid itself or to the carrier. It appeared that in moderate or cold climates more subjective symptoms occur after exposure than in tropical areas. The reason for this is also an item for further investigation.

- Much attention was given to the need to inform workers and supervisors in understandable words on the objectives and procedures of field investigations. They should also be informed of the results in understandable terms. Relations with the publicity media need careful consideration when a field study is planned. Early involvement of the press and explanation about objectives and procedures may be a valuable, time-saving activity.
- It was recommended that an international code of practice for hazard elimination and health protection during aerial pesticide spraying should be devised.

MISCELLANEOUS CONTRIBUTIONS

The Workshop also considered six papers dealing with other scientific aspects relevant to field workers exposure.

- In the investigation of effects of mixtures of simple similar acting compounds it was found that equitoxic doses are usually additive to give a toxic effect, even if the individual compounds are present only in very low concentrations which would in themselves not give rise to such an effect.
- The porphyrinogenic potential of a number of pesticides was screened in chicken embryo cultures with and without enzyme induction. Some organophosphorus compounds as well as other pesticides had a porphyrinogenic action in the test system. Studies in exposed people are planned.
- The importance of impurities in technical products was discussed, mentioning examples illustrating possible health effects. Identification and evaluation of impurities was advocated.
- Two papers dealt with the basic principle and with techniques of the measurement of cholinesterase inhibition. Investigations were discussed which can be used for OP-compounds to calculate re-entry times into sprayed fields.
- The role and aims of GIFAP (Groupement International des Associations Nationals de Fabricants de Pesticides) were explained and it was stated that GIFAP will consider cooperating in field studies when general - rather than specific - product problems are involved.

RECOMMENDATIONS

1. The Workshop considers field studies essential to gather information on the exposure patterns, on possible biomedical effects and on development of methods for occupational surveillance of field workers. This is to enable hazard to be assessed and to define more precisely what routine surveillance may be needed for occupational groups.

2. Field studies should be carried out under representative conditions where human and environmental factors are taken into account.

3. The design and the protocol of a field study should take into account:
   - the specific properties of the pesticide
   - the symptomatology and the mode of action if known of the pesticide as derived from experimental toxicology
   - method of application
   - the local and ambient conditions
   - the cultural characteristics of the workers.

   Such designs should be as flexible as possible to anticipate unexpected events, but should always meet the scientific objectives.

4. Field studies should be carried out wherever possible with the approval of governmental authorities and if possible also in consultation with international organisations, such as WHO, ILO, FAO, etc. The importance of good public relations is stressed.

5. In our present stage of knowledge, determination of cholinesterase activity is the best practicable method of measuring exposure to organophosphorus pesticides. Field methods exist but could be refined; quality control and comparative studies should be encouraged. Many years of experience with cholinesterase determination in a wide variety of circumstances of organophosphorus exposure has indicated that, for the avoidance of acute effects, a person should cease to be exposed to these compounds when the whole blood, or red cell, cholinesterase activity falls more than 30% below a well established (mean of 3 tests) pre-exposure value, until the value rises to 80% of the pre-exposure value. Significant depressions of plasma cholinesterase on their own indicate exposure but should not lead to suspensory action. They nevertheless indicate a need for reviewing of safety precautions.

This recommendation is in line with the recommendation of previous Workshops of the Committee.

6. There is a need for a careful evaluation of possible local or allergic reactions caused by pyrethroids.

7. Agricultural pilots may be exposed to special hazards.
Codes of practice should be developed to minimise these.

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Vth International Workshop, "Field worker exposure during pesticide application"
Scientific Committee on Pesticides
International Association on Occupational Health
The Hague, 9-11 October 1979

CLOSING REMARKS

L. ROSIVAL, chairman

Ladies and Gentlemen,

We are coming to the final part of our workshop. My task is not very complicated because our rapporteur, Dr. Tordoir, did his best and I would like to extend my thanks for his excellent work. Our report reflects the results of our three days of presentations and discussions in regard to field worker exposure during pesticide application.

The purpose of our workshop was to report the present state of knowledge in the above mentioned field and to describe the efforts and approaches underway in solving and understanding these problems.

In this respect we have had for the first time in the history of our workshops the possibility to confront the situation in the six continents of the world from the tropical Australia to the regions of Sweden and Finland. We did assess the risk based on existing information and data. The results contributed to the designation of methods for laboratory testing and epidemiological methods that are suitable for risk assessment purposes. We could also clarify some problems concerning the appropriate methods for risk-benefit and exposure assessment. The presence of colleagues from developing countries made it possible to relate the situation in these countries to those in other parts of the world. In this sense our workshop helped to promote international cooperation with regard to the assessment of health hazards that may arise from the use of pesticides with particular reference to developing countries.

As I said in my introductory remarks, pesticides have in the past been the object of considerable controversy in various countries. It will be the same in the future. It is sometimes said that we have only two options, that is to die without pesticides as a result of starvation or to die as a consequence of intoxication by pesticides. I think a third possibility is to live with pesticides in co-existence because we know that the biological form of pest control is still in an embryonic stage of development. The use of pesticides sometimes raises emotions via the media. We as scientists and people of the practice have a great responsibility not only to present facts but also to <u>educate</u> the public. Sometimes we have the tendency to remain in an ivory tower of splendid isolation and then we are sometimes shocked with the events which occur.

The recommendations for the solution of the topical problems in field worker exposure are the most important outcome of our workshop.

As for the future, the organization of workshops in approximately two-year intervals with participation of selected scientific workers qualified in the respective fields will be of the greatest importance. Every workshop will elaborate recommendations that will be published in important international periodicals. Workshop materials will be submitted to publication in extenso; also, publishing of individual reports will be stimulated. The workshops will be organized in close cooperation with the WHO, ILO, COMECON and IUPAC organizations in order to grant transfer of scientific knowledge into the activity of these organisations, and to assure application in practice of this knowledge. Intensification of scientific contacts in this field and mutual information of the situation in various regions of the world concerning knowledge of experimental toxicology of pesticides and other chemicals is a closely associated aspect. It is proposed that the next workshop will be held in South America and should discuss Education and Safe Handling in Pesticide Application.

I express my thanks to the secretariat and the committee that organized the workshop on behalf of the Scientific Committee on Pesticides.

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PREVIOUS WORKSHOPS

Previous workshops organised by the Scientific Committee on Pesticides of the International Association on Occupational Health were held in Amsterdam, Sofia, Cambridge and Bratislava.
For easy reference the titles of these Workshops and the publications of reports and recommendations are listed below.

EPIDEMIOLOGICAL TOXICOLOGY OF PESTICIDE EXPOSURE
Amsterdam, The Netherlands, 8-10 September 1971
Chairman: Prof. Dr. R.L. Zielhuis
Report and recommendations were published in Archives of Environmental Health 25 (1972) 399-405.

OCCUPATIONAL HEALTH CRITERIA FOR THE EVALUATION OF PESTICIDES
Sofia, Bulgaria, 9-11 April 1974
Chairman: Prof. Dr. F. Kaloyanova
Conclusions and recommendations were published in International Archives of Occupational Health 33 (1974) 335-341.

BIOLOGICAL MONITORING IN EXPOSURE TO CHOLINESTERASE INHIBITORS
Cambridge, U.K., 8-10 September 1975
Chairman : Dr. M.R. Zavon
Report and recommendations were published in the International Archives of Occupational and Environmental Health 37 (1976) 65-71.

EVALUATION OF VARIOUS EXISTING CLASSIFICATIONS OF TOXICITY AND HAZARD, AS FAR AS RELEVANT FOR OCCUPATIONAL EXPOSURE TO PESTICIDES
Bratislava, Czechoslovakia, 23-25 August 1977
Chairman: Prof. L. Rosival
Report and recommendations were published in the International Archives of Occupational and Environmental Health 41 (1978) 287-290.

APPENDIX

WORLD HEALTH ORGANIZATION
Organisation mondiale de la santé
Division of Vector biology and control
WHO headquarters, Geneva

VBC/75.9
English only
(reprint with permission)

SURVEY OF EXPOSURE TO ORGANOPHOSPHORUS PESTICIDES[1] IN AGRICULTURE - STANDARD PROTOCOL

1. GENERAL AIM

To examine the exposure of men spraying toxic pesticides in tropical areas, in order to be able to deduce from the information obtained (i) the standards of protection required in tropical areas for similar operations using a pesticide of similar toxicity and mode of action, (ii) standards of protection including the clothing which needs to be used and/or (iii) the maximum time of allowable exposure per day.

2. SPECIFIC AIMS

2.1 By:

(a) defining accurately the environmental conditions under which the pesticide is applied, and the method and rate of application;
(b) collecting data on the potential exposure of spraymen, including (i) times of exposure, (ii) the volume of pesticide applied by each man, and (iii) the amount of pesticide coming into contact with the exterior of clothing and exposed skin;
(c) collecting data on the actual exposure of spraymen including (i) details of protection used, (ii) the amount of pesticide (if any) coming into contact with the skin below clothing and with exposed skin and (iii) biochemical tests of absorption.

To determine:

(a) whether the actual exposure fell above or below the "no effect" level of exposure;
(b) the parts of the body most exposed to contamination by the pesticide under the particular environmental conditions of the survey.

---

[1] The same protocol can be used for pesticides of other chemical groups if a means of measuring absorption by the man is available.

2.2

If the actual exposure exceeds the "no effect" level, (i) to determine the possible factors involved and their relative importance, i.e. type of protection provided, personal hygiene, quantity of pesticide applied, and hours of work, and (ii) to study the relationship of degree or absence of effect in individual men with the concept of the percentage of toxic dose per hour (see section 7) to which the man was potentially exposed.

2.3

If the actual exposure is below the "no effect" level in all men, to calculate the percentage of toxic dose per hour to which the man was potentially exposed, and to estimate the efficacy of the clothing worn.

3. CHOICE OF SURVEY SITE AND GROUP

3.1

The spraying operation should be under single administrative control and likely to last over two weeks.

3.2

The pesticide should be applied in uniform concentration by uniform means throughout the operation.

3.3

The group studied should be volunteers and should consist of whole time mixers and up to 10 spraymen.[1] Ideally, the group should not have been exposed to the pesticide used during the survey or to any other pesticide with a similar mode of action for one month preceding the commencement of the operation.

If it is necessary to select from volunteer spraymen, the basis of selection should be on the basis of typical working clothing worn in the area in order that this might be standardized as far as possible. Otherwise, the supervisors of the operation should be asked to request the men to wear the typical clothing. If possible, photographs of the men in their working clothing, should be taken.

4. SURVEY STAFF

4.1

Staff for the adequate supervision of the agricultural aspects of the operation, including proper application of the pesticide, have not been included as it is assumed that these would be provided for in a planned operation.

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[1] This is the optimum number that can be dealt with by the staff shown in Section 4. If more spraymen are included the staff will need to be increased accordingly. The minimum number for an adequate study according to this protocol is six spraymen.

4.2

The survey staff consists of a physician/toxicologist and one or two local support officers of technician status.

4.3

The toxicologist is responsible for the general organization of the survey, the keeping of records, and oversight of the health of spraymen during the operation, including the taking of blood tests where necessary, and for the training of local staff in the use of equipment for cholinesterase determination. He also supervises the processing of blood specimens, the application, removal, labelling and packing of exposure pads.

5. METHOD (ORGANOPHOSPHORUS PESTICIDES)

5.1 Cholinesterase determination

The main method of control is by estimation of whole blood cholinesterase by the tintometric method (Edson, E.P. (1958) World Crops, 10, 49, as modified by Tintometer Sales Limited, Salisbury, UK). Tests should be carried out on all mixers and on each sprayman included in the survey group:

(a) twice on days immediately preceding the start of application (pre-exposure value);

(b) on working days, daily at end of work.

The end-of-work test should be taken after the mixer or sprayman has washed or bathed and removed any protective clothing. If a gap of more than one day occurs during the operation, a pre-work test should also be performed on the day that work is resumed. If any mixer or sprayman in the survey group or any other sprayman complains of any symptoms of sickness which in the opinion of the toxicologist may be attributable to the pesticide, a cholinesterase test should be carried out immediately with the man's consent.

5.2 Exposure pads

5.2.1 Pads are prepared of α-cellulose, 10 cm^2, backed with glassine paper, previously extracted if necessary (see Section 7). Pads should be fixed with masking tape covering the edges of the pad only, and on clothing with safety pins through the edge of the pad. On each day of application, four spraymen from the survey group should be chosen serially to wear exposure pads throughout the day. Pads should be applied to mixers, on three days during the operation.

5.2.2 Pads should be applied as shown below. This is set out for right-handed man; if man is left-handed, opposite sides apply. Applied on clothing (if worn) or on skin:

Arm: Upper surface of left forearm held with elbow bent at right-angle

across body, midway between elbow and wrist.

Leg: (1) front of left leg, above ankle
(2) front of left leg, mid thigh

Trunk: (1) over sternum
(2) on back between shoulder blades

Head: If head not covered, forehead as high as possible to give good adhesion. If head covered, on hat as close as practicable to top of forehead.

5.2.3 In addition, one pad is applied to the skin under clothing to the upper abdomen at approximately the same height as the arm pad and below the sternal pad.

5.2.4 On removal at the end of the day, a 5 cm square should be cut from the pad. Care should be taken that the scissors or other cutting instruments are not contaminated.

Decontamination can be carried out with cotton wool swabs soaked in surgical spirit. The central portion of the pad should be placed either in a plastic envelope or in a bottle containing solvent (see Section 7). If a bottle is used, the cap of the bottle should have an aluminium liner inserted, the bottle closed and shaken gently to ensure that the whole pad is wetted with the solvent. The exterior of the envelope or bottle should be labelled clearly with:

(a) the man's number;

(b) the number of the day, preceded by the letter D;

(c) the site of the pad, and whether it was attached to clothing "C" or skin "S" (e.g. "6 D3 Thigh C").

5.2.5 In addition, on two days during the trial period, a control to determine laboratory recovering rate of the pesticide should be carried out. To 5 x 5 cm squares cut from exposure pads, add carefully measured[1] 0.5 and 1.0 ml samples of the pesticide concentrate. These pads are then treated in the same way as those taken from the spraymen.

A further control test can be carried out with the dilute solution as sprayed. In the same way as above 1.0 ml samples are added to five cut pads. The first of these is put immediately into the plastic envelope or into a bottle with solvent. The others are similarly treated after having been left in the sun for a half, one, two and three hours respectively.

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[1] These samples need to be measured by pipette, using a suction bulb to draw up the liquid.

5.3 Respirators

The wearing of respirators is optional: if not worn, respiratory exposure can be accounted for as shown in Section 8.

To estimate respiratory exposure, two of the men in each group of four wearing pads for the day should be asked to wear half-face cartridge-type respirators for the whole of the working day. Respirator pads should be fitted each morning on the external side of chemical filter cartridges; these pads are removed at the end of the day and placed entirely in a plastic envelope or bottle, clearly marked as in 5.2.4 (a) and (b) above. The man should be carefully instructed on the care of the respirator in order to avoid any contamination at times when it is removed. All respirators should be carefully washed at the end of each working day, after the chemical filter cartridges have been removed. If the men normally wear a surgical or gauge mask, respiratory exposure can be measured by fixing a pad to the underside of the mask in front of the nose. This is not as accurate as the wearing of respirators as set out above.

6. RECORDS

6.1 Record of operation details

On the day spraying commences, Form 1 (Annex 1) should be completed. On each day subsequently, the data on the form should be reviewed, and any changes recorded by completing a new form with details of the changes only.

6.2 Daily record and diary

Each day, from the day before spraying commences onwards, the daily record Form 2 (Annex 2) should be completed. The diary section of Form 2 should be used for recording any events which are relevant to the survey. Sickness in any man, whether or not he is included in the survey group, should be recorded, together with an account of action taken and result.

6.3 Daily personal record

Form 3 (Annex 3) should be started for each man in the survey group and should be entered daily.

7. LABORATORY PROCESSING OF EXPOSURE AND RESPIRATOR PADS

7.1 Before the survey

7.1.1 It is necessary to determine the recovery rate for the pesticide by the method used for its determination. In addition, it is necessary to estimate degradation of the pesticide between the time the exposure pad is removed and the time of processing, and whether this can be reduced by transportation of the pad in bottles containing solvent. This is done by preparing pads with known quantities of the pesticide and processing them immediately and after

one, two and three weeks. If after three weeks, recovery exceeds 75%, the pads can be transported in plastic envelopes. Otherwise a suitable solvent[1] must be tested similarly. The bottles for transportation which should be small but wide-necked, will probably need 10 ml solvent per bottle.

7.1.2 A blank estimation using the pad only should be carried out to determine whether the pads need prior extraction. This is not usually necessary.

7.2 After the survey

Pads should be processed as quickly as possible. The maximum number to be processed will be of the order of 400 if respiratory pads are included.

8. CALCULATIONS AND PRESENTATION OF RESULTS

8.1 Calculation of percentage toxic dose received by each man

8.1.1 Dermal exposure

This is calculated from the exposure pads, relating the quantity of pesticide in a pad of known area to the area of the limb or part of the body as modified from Berkow (1931) Amer. J. Surg., 2, 315. These are added to give a total amount expressed in mg/day or per hour.

Body surfaces are as follows if not covered by clothing. Based on:

Head neck area = 1115 cm^2: reduce by a quarter if hat worn = 837 cm^2	Head pad
Upper chest, V of neck = 149 cm^2	Sternal pad
Top of shoulders near neck = 298 cm^2	Head pad
Back just below neck = 100 cm^2	Back pad
Forearms = 1208 cm^2	Arm pad
Hands = 808 cm^2	Arm pad or hand wash
Legs from knees down = 2322 cm^2	Lower leg pad
Upper legs from knees up = 3477 cm^2	Thigh pad

These areas may need adjustment according to area unclothed.
Add results from exposed parts and substract from 18301 cm^2: the remainder is based on the skin pad

8.1.2. Respiratory exposure

If done, this is calculated from the respirator pad and expressed in mg/day or mg/hour.

[1] With dimethoate, benzene was found to be the best solvent.

8.1.3. Percentage toxic dose per hour

This is calculated from these indices according to the method of Durham & Wolfe (1962) Bull. Wld Hlth Org., 26, 75-91, using the formula:

$$\frac{\text{Dermal exposure (mg/day or hour)} + (\text{Respiratory exposure (mg/day or hour)} \times 10)}{\text{Dermal } LD_{50} \text{ mg/kg (rat)} \times 70} \times 100$$

If respiratory tests have not been carried out, this formula can be modified as follows:

$$\frac{\text{Dermal exposure (mg/day or hour)} + 10\%^{1}}{\text{Dermal } LD_{50} \text{ mg/kg (rat)} \times 70} \times 100$$

8.1.4. This index of exposure is likely to give conservative results which might be less conservative in some tropical countries due to the lower average body weight. The use of the index is discussed by Hayes (1971) Bull. Wld Hlth Org., 44, 277-288.

8.2 Comparison of sites

All the results for a particular exposure pad site are grouped together and the mean and standard deviation (SD) are calculated. If any results fall outside the mean ± (SD x 3), these are excluded and a new calculation made of the mean and SD of those that remain. This is repeated until all results fall within the adjusted mean ± (SD x 3). The purpose of this is to give an idea of the "normal" distribution after those results that represent some unusual contamination have been excluded. The chance of such exclusions being made in error is 1%. Results from the adjusted means can be used to rank the sites and compute their ratio to the skin pad = 1.

[1] This is based on the fact that respiratory exposure does not usually exceed 1% of the dermal exposure.

FORM 1

OPERATIONAL DETAILS

Day_____

To be completed on Day 1, and reviewed daily. Any changes should be entered on a blank form, entering only those details which have changed.

Site:

Administration of spraying:

Crop:

Height of crop:

Pesticide:

Batch No.:

Concentration - before mixing:

- as applied:

Type of spray equipment:

Capacity of sprayer and tank:

Nozzle type:

Total personnel engaged - supervisors:

- mixers:

- spraymen:

Washing facilities provided:

Protective equipment provided:

Particle size distribution (if known):

FORM 2

DAILY RECORD

	Day of spraying														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Time spraying commenced	X	X														
Time spraying finished	X	X														
Quantity of pesticide used in whole operation (before dilution)																
Estimate of area covered																
Minimum temperature °C																
Maximum temperature °C																
Relative humidity																
Rain during spraying period (hours)	X	X														
Rain outside spraying period (hours)																
Wind force																
Wind direction in relation to general direction of spray[1]	X	X														

[1](S = side, B = wind behind, F = wind in front, V = variable)

DIARY: Note below any relevant events, sickness of spraymen, accidents to equipment or man, etc.

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

DAY

FORM 3.

DAILY PERSONAL RECORD

Name Personal No.

Details of exposure to any pesticide during preceding month

			Day														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	21
Type of work[1]	X	X															
Time started work	X	X															
Time finished work	X	X															
Total working hours	X	X															
Number of pump charges	X	X															
Clothing worn[2] / Material																	
Overall	X	X															
Scarf	X	X															
Hat or helmet	X	X															
Boots	X	X															
Shoes	X	X															
Thongs	X	X															
Face mask	X	X															
Gloves	X	X															
Apron	X	X															
Number of times washing during day: hands	X	X															
face	X	X															
Time (minutes) between end of work and bathing	X	X															
changing clothes	X	X															
Smoking (while spraying)	X	X															
Eating or drinking	X	X															
Cholinesterase level: before work																	X
after work	X	X															
Haemoglobin G/100 ml		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
If parathion used, p-nitrophenol level: before work																	X
after work	X	X															
Tick days exposure pads worn	X	X															X
Tick days respirator worn	X	X															X

[1] (S = spraying; M = mixing)

[2] (N = not warn; W = worn, newly washed; X = worn, not washed since last worn for spraying.)