RESIDUE REVIEWS

RESIDUES OF PESTICIDES AND OTHER
FOREIGN CHEMICALS IN FOODS AND FEEDS

RÜCKSTANDS-BERICHTE

RÜCKSTÄNDE VON PESTICIDEN UND ANDEREN
FREMDSTOFFEN IN NAHRUNGS- UND FUTTERMITTELN

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Preface

Residues of pesticides and other "foreign" chemicals in foodstuffs are of concern to everyone everywhere; they are essential to food production and manufacture, yet without surveillance and intelligent control some of those that persist could at times conceivably endanger the public health.

The object of "Residue Reviews" is to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of residues of these chemicals in foods, in feeds, and in transformed food products. These reviews are either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology; certain affairs in the realm of food technology that are concerned specifically with pesticide and other food-additive problems are also appropriate subject matter. The justification for the preparation of any review for this book-series is that it deals with some aspect of the many real problems arising from the presence of residues of foreign chemicals in foodstuffs.

The scope of "Residue Reviews" is international. It encompasses those matters, in any country, which are involved in allowing pesticide and other plant-protecting chemicals to be used safely in producing, storing, and shipping crops. Added plant or animal pest-control chemicals or their metabolites that may persist into meat and other edible animal products (milk and milk products, eggs, etc.) are also residues and are within this scope. The so-called food additives (substances deliberately added to foods for flavor, odor, appearance, etc., as well as those inadvertently added during manufacture, packaging, distribution, storage, etc.) are also considered suitable review material.

Manuscripts are contributed by invitation, and may be in English, French, or German. Suggestions for reports on specific subjects will always be welcomed, but preliminary communication with the editor is recommended before volunteered reviews are submitted in manuscript form.

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The experimental induction of cancer by pesticide residues and food additives: its rationale and interpretation*

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The desirability of excluding potentially carcinogenic substances from the human environment is unquestionable. However, those who rise in indignation over the apparent resistance or dilatory attitude of industry toward subjecting every suspected carcinogen to exhaustive animal tests are generally unaware of how recently there has been even provisional agreement among scientists as to what tests are appropriate, how much time and effort are involved in such studies, and the uncertainties associated with relating the findings in animals to practical conditions of human exposure.

For more than a century after 1775, when an abnormally high incidence of scrotal cancer was recognized among chimney sweeps as reported by POTTS (1778), evidence for a relationship between cancer and occupational exposure to chemicals rested largely upon epidemiological grounds. The association of the products of the coal tar industry with certain forms of skin and bladder cancer was based upon clinical observations in industrial workers. With the growth of the chemical and dye industries from the mid-nineteenth century, and the concomitant advances in the biological sciences, it was inevitable that interest should develop in animal experimentation as a means of evaluating potential occupational hazards. Indeed much of our present knowledge of chemical carcinogens is now derived from animal studies which, except for a relatively few cases, are not supported by human experience.

Research in the field of carcinogenesis derived particular impetus from the work of YAMAGIWA and ICHIKAWA (1914) who reported that cancer could be induced by painting the ears of rabbits with tar extracts. The active components of these extracts were subsequently shown by KENNAWAY (1924) and LACASSAGNE (1950) to be polycyclic aromatic compounds, more specifically derivatives of anthracene and phenanthrene. These discoveries led to the investigation of a large number of polycyclic hydrocarbons as well as of other aromatic and aliphatic compounds. Of the thousands of compounds catalogued in the surveys published by HARTWELL (1951) and SHUBIK and HARTWELL (1957) of the National Institutes of Health, some

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2000 were tested for 30 days or more. About one-fourth were reported to have induced tumors, approximately one-third of them resulting from the administration or application of derivatives of benzanthracene and related polynuclear compounds.

In recent decades, scientists and writers on the subject of cancer have hardly overlooked a single aspect of the human environment from the viewpoint of the possible causal relation of chemicals to this disease. Attention has been directed to atmospheric smoke, smog, and dust, to occupational exposure, to medicinal agents, to tobacco smoke, to pesticide residues and additives in food, and to polluted water supplies. Many students of the evidence believe that alleged carcinogenic hazards of certain substances, like estrogens, arsenic compounds, and food colors, have received a degree of emphasis out of proportion to their true significance. Broad generalizations and speculations concerning chemical carcinogenesis are often based on exceedingly fragmentary and unsubstantiated evidence. Exaggeration of hypothetical, suspected or potential hazards is not in the best public interest since it not only induces unwarranted fears and doubts, but tends to divert the efforts of scientists and legislators away from areas where they more properly belong.

There can be no denial however that this is a subject where more research is sorely needed to gain better perspective. The motivation for investigations in experimental carcinogenesis is by no means limited to the discovery and evaluation of potential hazards. The possibility that a carcinogenic response to a known chemical substance may reveal the existence of etiologic agents in cancers of unknown origin, or that it may shed light on the mechanism of action of factors responsible for the initiation or stimulation of cancerous growths, has intrigued many workers in this field. The so-called known carcinogens have furnished a useful tool for the study of species and strain variations in susceptibility and resistance. Much of the work on cancer chemotherapy has involved the use of test animals in which tumors have been induced by transplantation. However, chemicals are proving increasingly important in the investigation of carcinogenetic mechanisms and the discovery of anti-cancer agents.

Current interest in the study of chemical carcinogenesis is due largely to the public health aspects of air pollution, tobacco smoking, and the use of chemicals in food production and processing. There is no question of the need for preventing, insofar as possible, any human contact with potent carcinogens. However the problem of evaluating the potential risks of weak or borderline carcinogens is beset with many difficulties. Well-meaning legislators in this and other countries have placed upon the shoulders of scientists a responsibility which can be met only by the application of reasonable judgement, since the factual bases upon which to design truly critical studies, and to extrapolate experimental findings from animals to man with absolute certainty, do not yet exist.

The criterion which distinguishes potent from weak carcinogens is the magnitude of the total dose necessary to elicit the effect, regardless of the route of administration. Potent carcinogens are considered to be substances which, after a single dose, or after repeated administration of extremely

minute doses, induce a high incidence of malignant tumors. In contrast, weak carcinogens require continuous dosing in relatively large amounts and for periods as long as an entire lifetime; their effects may be only marginal or of extremely low incidence. When the evidence indicates the potency of a carcinogen to be of a high order, there is generally no dispute as to the decision to be taken from the standpoint of health protection. But in the case of weak carcinogens, certain questions arise: first, as to the suitability of the testing procedure, particularly with respect to the dosage levels compared to the predicted exposure level; second, as to the validity of the interpretation of minimal histopathological changes; and third, as to the degree of probability to be assumed in the statistical analysis of the data, with particular reference to the significance of differences between test and control groups.

In the past five years many toxicologists, pathologists, and cancer experts have individually and collectively, in both national and international committees, weighed the problems of methodology and interpretation and have recommended certain guidelines in the appraisal of the potential carcinogenicity of orally ingested substances. While there is general agreement as to the indispensibility, in the present state of our knowledge, of chronic feeding studies in animals, the multiplicity of factors to be considered and the differences in the recommendations of various expert groups illustrate the complexity of the problem. Table I lists the major factors which must be taken into account in designing a study of the carcinogenic potential of a food component.

Tabe I. *Major factors and important parameters involved in experimental carcinogenesis*

Factor	Parameters
Test substance:	Identity Purity (freedom from contaminants) Physical state Diluents, vehicles
Animals:	Number, species, strain, sex, generations
Conditions:	Basal diet, housing, sanitation, pest control
Dosage:	Amount, route, frequency, duration
Observations:	Physical examinations Functional tests, biopsies Necropsy Histopathologic examinations
Statistical evaluation:	—
Interpretation:	—

Table II illustrates the variations in the design of carcinogenic feeding studies published recently by the U. S. Food and Drug Administration (1959), the Food Protection Committee of the National Academy of Sciences-National Research Council (1959), the British Ministry of Health (1960), and the Joint Expert Committee of Food and Agricultural Organization and the World Health Organization of the United Nations (1961). The use of the dog (or other non-rodent species) as a test animal is stressed

only in the United States. All groups recommend both the rat and the mouse, one strain of each being generally considered sufficient, unless "pure" strains are used. Other non-rodent species are considered optional. Among the reasons why the dog is not favored as a test subject by many investigators is the fact that four or even seven years represent too small a fraction of its normal life cycle, whereas the full span of 12 or 15 years, not to mention a statistically sufficient number of animals, would be quite impracticable.

Table II. *Recommendations for carcinogenicity tests of food additives*

Source (see text)	Species (No. of strains)	No. of groups (control vs test)	No. of animals (Control group/ Test group)		Route of administration	Duration of	
			Beginning	End		Dosage period (Years)	Total test period (Years)
FDA	Rat (2)	1 vs 3	100/50	q.s.[1]	Oral	2	2
	Mouse (2)	1 vs 3	100/50	q.s.	Oral	2	2
	Dog	1 vs 3	6/6	q.s.	Oral	7	7
FPC	Rat	1 vs 3	25/25	q.s.	Oral	2+	2+
	Mouse	1 vs 3	25/25	q.s.	Oral	2	2+
	Dog	1 vs 3	8/8	q.s.	Oral	4+	4+
BMH	Rat	1 vs 1+	25/25	12/12	Oral[3]	1	2+
	Mouse	1 vs 1+	25/25	12/12	Oral[3]	1	1.5+
FAO/WHO	Rat (2)[2]	1 vs 3	q.s.	20/20	Oral	— Lifetime —	
	Mouse (2)[2]	1 vs 3	q.s.	20/20	Oral	— Lifetime —	

[1] *Quantum sufficit*, a sufficient number.
[2] If pure strains are used.
[3] Parenteral also, whenever possible.

Except for the British Ministry of Health, all agencies specify the use of multiple test levels, although a finding of cancer at any dosage would — as officially interpreted under the U. S. Food, Drug, and Cosmetic Act — preclude the use of the substance in question. It will be noted from Table II that there are substantial differences in the proposals for the size of each test and control group, varying in combined totals from 200 to 500 rats of each strain. However, in all cases it is required that there be a sufficient number of survivors at the termination of the test to permit statistically valid comparisons of tumor incidence among the test and control groups. Also, the recommended duration of the dosage period in rodent studies ranges from a minimum of one year to the full life span of the animals. The U. S. Food and Drug Administration has regarded two years as a sufficient period of observation in both chronic toxicity and carcinogenicity investigations in rodents. The trend is definitely toward continuing carcinogenicity studies for the full life span of these species, however, since cancer in animals as well as in man is associated with advancing age.

All expert groups agree on the necessity for the oral route of administration of test substances which may become components of food. On the

subject of parenteral administation, however, opinions vary somewhat as shown in Table III. Most of the evidence for the more potent carcinogens, such as the polycyclic aromatic hydrocarbons and certain of the azo dyes, has been based on dosage by either the topical or the subcutaneous routes.

Table III. *Opinions on parenteral administration of test substances*

Authority	Opinion[1]
BMH	"Tests by the oral route will invariably be required in the case of substances proposed as food additives. ... Although tests by parenteral injection *should be performed whenever possible*, it is recognized that there are some materials which are not suitable for administration in this way."
FAO/WHO	Induction of local sarcomas by subcutaneous administration is not proof that substance will be carcinogenic by oral route. ... Some countries, however, consider this sufficient basis to reject such substances for use in food pending more proof of safety. More research needed.
FDA	"Carcinogenesis ... by one route of administration does not imply carcinogenesis ... by another route of administration."
FPC	"When large doses of material must be given repeatedly, the subcutaneous route of testing substances for their carcinogenic activity is of *limited value* and of *dubious interpretation*." More research needed.

[1] Italics supplied by author.

Little basis exists for predicting from such findings the potentiality of each compound for inducing cancer by ingestion. Moreover wide variations exist in the choice of species, conditions of dosage, and duration of observation in parenteral studies. Subcutaneous tests are futher complicated by trauma resulting in sarcomata at the site of injection. Space will not permit detailed discussion of more than a few of these test recommendations. Suffice it to say, however, that in addition to the use of normal animals on normal diets, it has been proposed that metabolically or nutritionally unbalanced animals, and pregnant and lactating female animals, be employed in evaluating potential carcinogens; and that the studies be extended for the full life span of descendant, as well as parent generations of animals. However, most investigators agree that some practicable limitations must be placed on the extent and expense of these investigations if they are to be done at all.

The Delaney anti-cancer clause in the Food Additives Amendment in the U.S.A. forbids the establishment of a tolerance for any substance which "is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal; ..." At least for the present, this clause is being applied by the U. S. Food and Drug Administration only to substances which are, or may be, ingested. However no restriction is contained either in the statute or in the regulations on the level, frequency, or duration of dosage in animal tests. Obviously, therefore, the degree of exaggeration of dosage levels and other conditions employed in an experimental evaluation of a potential weak carcinogen

are of critical importance. On the subject of maximum dosage, various authorities have made the recommendations stated in abbreviated form as follows: as high as possible without inducing inanition or early mortality (FDA); maximum that can be tolerated without affecting long-term survival (FPC); as high as can be administered without materially reducing the life span (BMH); one which produces a minimum to moderate amount of short-term toxicity and does not materially decrease life span (FAO/WHO). One might ask "Why feed more than a single dosage level?" Under present interpretations, a positive response at any level would preclude the promulgation of a tolerance even if no tumors were observed at lower dosages. On the other hand, a negative response at a single dosage, assuming it to be the highest tolerated level, might justify the presumption of no effect at lower levels.

The answer to this query usually is that the information accumulated from multiple dosage studies is needed in order, some day, to establish a basis upon which safe levels of dietary carcinogens may be permitted. It should be noted that any dose high enough to bring about the initial organic or functional effect produces a metabolically stressed test animal. The continued addition of the causative insult to the primary injury, throughout the lifetime of the animal, represents an exaggeration of experimental conditions far beyond that simulating use conditions. There are reports of swamping effects of normal metabolic pathways with subsequent overflow into replacement pathways. The consequences of continuous saturation with toxicants throughout the life of an animal may differ in a qualitative sense in large part depending on the functional capacity of an organ system at various stages of its life cycle. Excessive dosage levels may thus induce difference in metabolites, sites of storages, or routes of excretion, and hence may alter toxicological manifestations to a degree that the parameters being evaluated no longer represent the same physiological mechanisms.

Some investigators believe that the principle of the "safety margin" can be applied to potential carcinogens just as well as to any other type of toxic substances, with the reservation that the ratio between the no-effect level and use level be substantially greater than the arbitrary 100 : 1 factor generally proposed. If, for example, a food color intended for use at a dietary level of 20 ppm (mg./kg.) induced no effect in chronic feeding studies at 1000 times this level, it might be considered safe, even though tumors were observed at a level above 2 percent (20,000 ppm). This concept was considered reasonable and legally acceptable prior to enactment of the Delaney clause.

Another, less arbitrary, approach to the setting of safe levels for weak carcinogens could be predicated upon the fact that precancerous (i.e., non-cancerous) changes frequently precede the actual appearance of cancer. Such precursory symptoms were associated with certain of the substances recently regarded as carcinogens by the U. S. Food and Drug Administration, for example, thyroid depression in the case of the herbicide amino-triazole [3-amino-1,2,4-triazole] or liver hyperplasia in the cases of the acaricide Aramite [2-(*p-tert*-butylphenoxy)-1-methylethyl-2-chloroethyl sulfite], the soft-drink flavoring agent safrole [4-allyl-1,2-methylenedioxy-

benzene], and the synthetic food color FD & C Red No. 1 [Ponceau 3R, or the disodium salt of 1-pseudocumylazo-2-naphthol-3 : 6-disulfonic acid]. In each case there was experimental demonstration of no-effect dosage levels in the test animals but, what is more significant, the precancerous effects induced at higher dosages were probably of a regressive nature. Substances such as these have been designated „indirect secondary" carcinogens because their action results from metabolic disturbances in certain target organs, rather than directly, as in the case of aromatic amines or polycyclic hydrocarbons, or through the production of endogenous carcinogens such as certain metallic compounds, radiations, etc. In fact, one of the more common findings in toxicological tests in rats is liver damage, including hyperplasia, cirrhosis, and hepatoma, which, under conditions of chronic feeding, may result in liver cancer. Similar changes can also be induced by dietary means.

In any event it would seem that the maximum dosage level which induced no evidence of a precursor effect could serve as a reasonable basis for arriving at a safe tolerance level, assuming of course, the judicious application of a safety factor. This would be entirely consistent with the view expressed by Dr. G. Burroughs Mider (1960) of the National Cancer Institute in this country in his report to the U. S. Secretary of Health, Education, and Welfare, viz. "some arbitrary decision must be made as to the margin of safety to be used in translating data obtained from animal experimentation to man, taking into consideration all known facts concerning the carcinogen in relation to its proposed use in the human environment". This statement appears to offer some promise for the extrapolation of safe levels for man from animal data. Hope vanishes, however, when this statement is contrasted with the same author's oft-quoted assertion that "No one at this time can tell how much or how little of a carcinogen will be required to produce cancer in any human being". One wonders whether such an objective can ever be attained short of quantitatively controlled, lifetime studies in human subjects.

Those who are called upon to interpret and apply the results of animal experiments thus find themselves on the horns of a dilemma from which only corrective legislation can extricate them. Legislation which would permit the exercise of reasonable scientific discretion is now denied by the Delaney clause in the U.S.A., according to its many critics.

In all fairness it should be pointed out that some scientists do not accept the view that the absence of a precancerous lesion precludes the ultimate appearance of a malignant tumor. Some support the idea that even a minimal exposure to a potential carcinogen, e.g. ultraviolet radiation, will induce some degree of cellular damage which, given enough time, will inevitably lead to cancer. The fact that extrapolation of time:response data indicates that it would require several times the normal life span to induce the carcinogenic response seems to be ignored in translating such observations to man.

Reference has been made to the problem of the histopathological lesions induced by weak or borderline carcinogens. The key specialist in the assessment of the results of a carcinogenicity experiment is the pathologist.

Training and experience in human pathology is important but it is not enough. The pathologist should be familiar with the particular species of laboratory animal, with strain variations in the types of so-called spontaneous tumors and, on the basis of past experience or of periodic observations, he should be able to predict or extrapolate progressive histopathologic changes. There is often the need for expert appraisal of pathology in specific organs, particularly where species variations are known to exist.

Pathologists seldom disagree on what they see but they not infrequently differ in their interpretation of its significance. The following scheme shows the classification of neoplasms as defined in the recent report of the Food Protection Committee (1959):

Neoplasms (tumors)
(Non-regressive, in contrast with hyperplasia)

Benign	*Malignant (cancers)*
Structure resembles tissue	Structure atypical
Growth slow, expansive, encapsulated	Growth rapid with mitosis, infiltrative, progressive
Non-metastatic	Metastatic (often transplantable)
Non-lethal	Lethal
Examples:	Examples:
Adenoma (gland-like)	Carcinoma (epithelial)
Papilloma (wart-like)	Sarcoma (mesenchymal)
Chondroma (cartilage)	Lymph sarcoma (lymphatic)
Osteoma (bone)	Leukemia (hematopoietic)

It will be noted that a distinction is drawn between hyperplasia, a localized increase in cell numbers which is regressive, and tumors or neoplasms which generally are persistent and progress to fatal termination. Benign tumors are distinguished in various ways from malignant tumors or cancers but because a benign tumor may, if given enough time and favorable pathogenetic conditions, become malignant, many pathologists attach as much weight to the one as to the other. For this reason some cancer research workers are dubious about the classification of the ubiquitous insecticide DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] as a non-carcinogen on the basis of the observation that at the dietary level at which tumors resulted "there was only a minimal and late tendency in the formation of hepatic cell tumors" described by FITZHUGH and NELSON (1947) as benign.

The entire subject of environmental carcinogenesis, from experimental methodology to practical interpretation, is one where sound scientific judgement and discretion must be applied. Without animal studies the evaluation of potential carcinogens is impossible; with animal studies unequivocal proof of non-carcinogenicity is impossible. The application of animal tests to the control of safety of foods, drugs, and other aspects of our external as well internal environment demands not only objectively determined facts but the considered judgement of qualified scientists. To the extent that these are precluded under present laws in the U.S.A., repeal or amendment will be essential to progress.

Summary

Prior to the experimental induction of tar cancers in 1915, certain types of cancer were associated with occupational exposures. Interest in chemical carcinogenesis as an environmental health problem has grown as a result of increased air and water pollution, tobacco smoking, and the use of pesticides and food additives. Difficulties have arisen at both the experimental and regulatory levels owing to matters of definition (e.g. benign vs malignant tumors, weak vs potent carcinogens), to lack of agreement as to laboratory procedures for the evaluation of carcinogenic potentiality, and to differences concerning administrative policy in interpreting and applying the results of such studies. Recent attempts of expert committees to agree upon the experimental approach as regards animal species, dosage and route of administration, duration of test periods, etc., are described.

Résumé*

Avant la cancérisation expérimentale par les goudrons obtenue en 1915, certains types de cancers avaient été associés à des activités professionnelles. L'intérêt porté par les hygiénistes aux cancers d'origine chimique s'est accru du fait de la pollution croissante de l'air et de l'eau, du tabagisme et de l'emploi des pesticides et des additifs. Des difficultés ont surgi aussi bien pour l'expérimentation que pour la réglementation au sujet des définitions (par ex.: différence entre tumeur bénigne et maligne, cancérigènes faibles et forts), au sujet du choix des techniques de laboratoire pour évaluer le pouvoir cancérigène et au sujet des différences dans l'interprétation et la mise en pratique par l'Administration des résultats de telles études. On rapporte enfin des tentatives récentes des commissions d'experts pour s'entendre sur le choix des espèces animales, le dosage et la voie d'administration, la durée des périodes d'essai, etc.

Zusammenfassung**

Bevor es 1915 gelang, Teerkrebs experimentell zu erzeugen, verband man bestimmte Krebsarten mit der Exposition in gewissen Berufen. Dadurch, daß die Verunreinigung der Luft und des Wassers, das Tabakrauchen, die Anwendung von Schädlingsbekämpfungsmitteln und Nahrungszusatzstoffen zunahmen, ist das Interesse für chemische Carcinogenese als gesundheitliches Umweltsproblem gewachsen. Schwierigkeiten sind sowohl auf dem Gebiete der Forschung als auch der Gegenmaßnahmen entstanden infolge von Definitionsfragen (z. B. gutartige oder bösartige Tumoren, schwache oder starke Carcinogene), oder aus Mangel an Übereinstimmung in bezug auf die zur Feststellung der carcinogenen Wirksamkeit notwendigen Laboratoriumsversuche oder infolge unterschiedlicher Auffassung über das administrative Vorgehen bei der Deutung und Verwertung derartiger Versuchsergebnisse. Neuere Versuche fachwissenschaftlicher Gremien in der experimentellen Bearbeitung dieser Fragen zu einer Übereinstimmung, z. B. hinsichtlich der Tierarten, Anwendungsdosis und -art, Versuchsdauer etc., zu kommen, werden beschrieben.

* Traduit par R. MESTRES.

** Übersetzt von O. R. KLIMMER.

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Flavor and quality changes in fruits and vegetables in the United States caused by application of pesticide chemicals*

By
CHARLES H. MAHONEY**

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I. Introduction

The chemical industry in the United States has spent millions of dollars in the development of insecticides, fungicides, rodenticides, herbicides, and soil fumigants. These agricultural chemicals have been largely responsible both for increased yields and for the high quality of fruits and vegetables produced for both fresh market and processing. These commodities have also been free of insects and insect parts, by virtue of applied insecticides. Due to the diligence and compliance of both the chemical manufacturer and the farmer, these foods have been free of chemical residues. The amount of time, money, and energy involved in having a new pesticide accepted for use by the Pesticide Division of the U. S. Department of Agriculture and acceptable to the U. S. Food and Drug Administration, with or without a tolerance, is of course a matter of history. However, a number of years of testing are required by chemists, entomologists, toxicologists, horticulturists, and food technologists before an agricultural pesticide is acceptable for registration.

There are many citations in the literature which show quite conclusively that certain pesticide chemicals have caused and will cause flavor changes in fruits and vegetables, and more particularly when they are processed by heat. These examples, together with known instances where food has had to be destroyed because of off-flavors, bring us to the conclusion that a

* The common names of all pesticide chemicals in this review with their appropriate chemical names are given in Table I.

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definite flavor problem may exist and that therefore any new pesticide must be critically evaluated for flavor changes before it is recommended for use on food crops. There are a number of other instances of significant modifications due to physiological changes in the fruit or vegetable which are not classified as off-flavor but which nevertheless affect the quality and marketability of the crop.

II. Review of literature

No attempt will be made to present a comprehensive review of all the literature pertaining to flavor changes in foods attributed to applied chemicals, but enough examples will be presented in order to illustrate species selectivity, varietal variation, and method of application as it may affect the flavor of the food product.

Technical benzene hexachloride when used as a soil insecticide has been shown by MACLINN *et al.* (1950), GREENWOOD and TICE (1949), and JAMESON and TANNER (1951) to cause off-flavor in potatoes. GOULD *et al.* (1951) have reported off-flavors in canned potatoes, carrots, tomatoes, lima beans, peaches, and plums when treated with low gamma-BHC formulations. STITT and EVANSON (1949) found that soil surface treatments of benzene hexachloride definitely caused an off-flavor in fresh peas and pole beans but less definite responses for bush beans, carrots, and cucumbers. Off-flavor in fresh samples of vegetables sprayed with the high gamma-type BHC was less noticeable than with ordinary BHC.

Lindane, the pure gamma-isomer of BHC, also has been reported by many of these workers to have caused off-flavor when used as a soil insecticide, although not to the same degree as the technical BHC. KIRKPATRICK *et al.* (1951) clearly evaluate the comparative differences between the effect of BHC and lindane on the flavor of potatoes. They state "palatability tests show that both 1 lb. of lindane per acre and about 8 lbs. of technical BHC (1 lb. gamma isomer) per acre applied to soil prior to planting imparted an objectionable flavor to cooked potatoes. The untreated sample had no impairment of odor or flavor. Each treatment mean was found significantly different from the other at the 5% level for both odor and flavor. Technical benzene hexachloride treatment gave a stronger off-flavor to cooked potatoes than did the lindane treatment".

Extensive use of BHC formulations for insect control on non-food crops grown in rotation with food crops has also created some problems. FOSTER (1950) has found that one-half the amount of BHC applied to the soil was still present after three years. POOS *et al.* (1951) have shown that peanuts are capable of accumulating considerable amounts of BHC from soils. At rates of application of BHC or lindane at 1 to 1½ lbs. per acre, the corresponding value of the nuts ranged from 2 to 8 p.p.m. Taste tests indicated that both raw and roasted peanuts may develop off-flavors from both BHC and lindane under field conditions with applications around one pound per acre. This apparently preferential species absorption of a chemical from the soil emphasized the potential hazard of soil accumulation of chemicals applied to non-food crops grown in rotation with susceptible food crops. There are also several instances where sweet corn after process-

ing had to be destroyed when grown on land which had been in alfalfa the year before and treated with a half pound of lindane per acre.

REED and MACLINN (1952) have done comprehensive work on the effect of insecticides applied to the soil for wireworm control, and the subsequent effect of these applied chemicals on the flavor and acceptability of potatoes grown on the treated plots. They made flavor evaluations the first, second, third, and fourth years following treatment.

They emphasized that the levels of application for effective wireworm control for a period of at least four years are as follows: technical BHC, 1/2 lb. per acre; lindane, 1 lb. per acre; chlordane, 8 lbs. per acre; parathion, 10 lbs. per acre; and toxaphene, 25 lbs. per acre. It is necessary to take these application levels into consideration when interpreting sensory tests. Insecticide treatments were made in the spring of 1948, and flavor evaluations on the tubers were made during the next four years. The results might briefly be summarized as follows: after the first year treatment, technical BHC gave very strong off-flavor; refined BHC produced definite off-flavor but not to the extent caused by crude BHC; lindane gave some off-flavor but was less apparent than the two preceding treatments; parathion and toxaphene gave no off-flavors; chlordane at the 8-lb. level produced no off-flavor, but off-flavors were present at the 2-, 4-, and 16-lb. levels. At the end of the fourth year, technical BHC still produced a strong off-flavor in tubers; refined BHC gave undesirable off-flavors at the 1/4- and 1/2-lb. treatment level; lindane, parathion and toxaphene produced no off-flavors, and chlordane caused an off-flavor at the 16-lb. treatment level only.

HENING *et al.* (1954) used DDT, aldrin, dieldrin, and chlordane as soil treatments each at two levels of application on replicated plots. They grew peas, snap beans, beets, carrots, squash, and tomatoes in these plots and samples of each were processed by heat and then evaluated for flavor changes. They found a significant off-flavor difference in the pureed squash from the aldrin-treated plots, but not from the chlordane plots. There were no significant flavor changes with the other crops grown, but they found a significant color difference in squash and carrots which was influenced by all of the treatments except DDT. This is an illustration of a quality change not necessarily associated with off-flavor.

GILPIN *et al.* (1959) conducted palatability studies on carrots, green beans, and turnips over a period of four years, and reported that undesirable off-flavors were present in carrots, turnips, and green beans which were grown without insecticide treatment in soils contaminated with residues of technical BHC or lindane applied to preceding crops. Soil residues of the alpha-, beta-, and delta-isomers of BHC also resulted in off-flavors in carrots. Heavy residues of aldrin (both technical and purified), dieldrin, heptachlor, dilan, toxaphene, chlordane, endrin, isodrin, TDE, technical DDT, and methoxychlor did not cause significant flavor changes.

BIRDSALL *et al.* (1956) report on taste tests made by some 200 persons over a period of four years on samples from pesticide-treated plots, and present the following conclusions: "Snap beans and tomato juice seemed to be impervious to flavor changes. Carrots, onions and potatoes also were fairly free from flavor changes. Only lindane and chlordane caused trouble.

Lindane in the soil affected all three, chlordane only potatoes. Lindane on foliage caused flavor changes in potatoes, and chlordane on foliage caused an off-flavor in canned onions.

"Canned squash was affected by soil applications of dieldrin and endrin and by foliage applications of endrin and lindane. Pumpkin, sauerkraut and beets were most sensitive. Soil applications of endrin affected all three; of lindane, beets and sauerkraut; heptachlor all three; chlordane, pumpkin. On foliage treatment endrin affected beets; lindane, both beets and sauerkraut; chlordane, aldrin and dieldrin harmed the taste of canned pumpkin."

BHC applied to peach trees has caused off-flavors especially in the canned product as reported by SMITH *et al.* (1949), DAVIS (1949), CHANDLER (1949), VAN BLARICOM (1950), TRESSLER (1950), and BAILEY *et al.* (1949). TRESSLER (1950) has pointed out the importance of storage life as it may affect the change in off-flavor of canned peaches. He found that samples from the South Carolina Experiment Station treated with lindane appeared to be normal five months after canning, but twenty-one months after canning when stored at room temperatures all samples were inedible due to off-flavor, even the sample in which the last use of lindane was a first cover spray.

KIRKPATRICK *et al.* (1960) treated cantaloupes with 0.35 lb. of lindane per acre either as a dust, wettable powder or emulsion, and these were compared with untreated controls. Four applications were made at weekly intervals, the last one a day prior to harvesting. Palatability evaluations indicated that as a dust lindane did not affect flavor, as a wettable powder it slightly affected flavor, and as an emulsion it adversely affected flavor and also aroma.

KIRKPATRICK *et al.* (1955), however, in other work studying the flavor of Sebago potatoes grown in soil treated with chlordane, heptachlor, dieldrin, aldrin, and endrin over a period of three years reached the following conclusions: "In the first year's tests no significant amount of off-flavor was found in potatoes grown in soil treated with chlordane, heptachlor or dieldrin. The use of a 2 lb. dosage of aldrin in three tests resulted in flavor scores lower than those for other treatments but significantly low in only one test. In the second year significant off-odor and off-flavor were present in the case of the 2 lb. dosage of heptachlor on a commercial farm where chlordane, dieldrin and endrin were applied. At another location the second year neither aldrin nor heptachlor caused a significant amount of off-flavor. Potatoes grown for the third consecutive year in soils treated with aldrin, heptachlor or chlordane resulted in no injurious effect on the flavor when cooked. Significant differences were not apparent between sound and wireworm-injured potatoes from untreated plots in the third year."

Although there is some variation in these results it is clearly evident that many chemicals can be used as soil treatments without causing adverse flavor in food crops, even those that are processed by heat. BOSWELL (1955) presented the cooperative results of the work of the U. S. Department of Agriculture in cooperation with the states of Washington, New Jersey, Georgia, and Illinois on a basic set of eleven soil treatments involving single applications at different levels and degrees of purity of DDT and

BHC and levels of chlordane. As a result of these studies he reports: "Technical BHC in any form is likely to impart off-flavors or otherwise impair acceptability of products exposed to it; therefore it should not be used on food crops. Lindane is nearly as bad and is recommended only for certain seed treatments and for control of pickleworm on cucumbers for slicing."

HINREINER and SIMONE (1956 a) reported on the extensive work done in California on the effects of soil insecticides on the flavor of vegetable crops. They substantiated the effect of BHC and lindane as far as affecting the flavor of foods, particularly those processed by heat. They found that neither aldrin nor dieldrin presented flavor problems in their experiments when used at reasonable dosages. They also found that none of the three root crops grown in soil treated with endrin at five pounds per acre were affected in flavor when tasted either fresh or canned. Isodrin-treated fresh sweet potatoes in 1954 and canned sweet potatoes and canned carrots in 1953, however, had a detectable difference and an apparently undesirable flavor to the panel. Somewhat surprisingly, fresh white potatoes grown in soil treated with this pesticide seemed to be affected very little, if at all. They also found that chlordane and heptachlor at even relatively high levels of 20 and 10 lbs. per acre, respectively, did not produce serious flavor difficulties on crops tested, and they also found that DDT and parathion on white potatoes caused no significant differences in flavor.

HINREINER and SIMONE (1956 b) have also reported on their rather extensive tests on the effect of acaracides on the flavor of almonds and canned fruits. They found that genite 923 seemed to be the most likely to cause flavor difficulties on peaches, and they also found the undesirable flavor of the genite-treated samples to intensify during storage. They also found that ovotran appeared to cause more flavor difficulties than most of the other acaracides on pears, but tests the next year did not bear out this conclusion, and the interpretation was that the controls at the high level of mite infestation which caused defoliation of the untreated trees and therefore the flavor of the pears from the untreated controls. They also found than an application at petal fall of ovotran to peaches was more encouraging than for similar early genite application in that one year.

Almonds apparently were not affected by the application of either genite 923 or ovotran.

The use of Aramite and sulphenone gave unfavorable organoleptic results on pears in one year, but there was no significant difference in the year following. When sulphenone was used on peaches the difference was actually in favor of the acaracide. They attributed this possible better flavor in fruit trees to keeping the mite infestation under control.

They found that malathion caused few, if any, flavor difficulties in extensive tests on fresh grapes, melons and dried figs, and apples and pears.

They also made organoleptic evaluations of other fruits treated with five other acaracides, but it covered only one year's work. Dimite, for example, appeared to produce no change when tested soon after canning, but the pears and apple sauce treated with Dimite seemed to develop some off-flavor upon longer storage. The same is true for chlorobenzilate and perhaps also for diazinon. In the latter case only pears and peaches were

evaluated and while the treated samples apparently were initially better tasting than the controls, they became about equal or slightly worse after eight months. They did not feel they could draw any definite conclusions for the effect of systox and OMPA on the basis of the small amount of work done.

HARD and ROSS (1954) conducted extensive organoleptic tests on beans, peas, sweet cherries, apples, peaches, pears, and strawberries treated with malathion in the state of Washington. They did not find any flavor changes in any of these crops as a result of applying this insecticide.

TICHENOR *et al.* (1959) studied the effect of 21 insecticides and five fungicides on the flavor of frozen strawberries. This work covered two years, and although they found some significant difference in flavor in frozen strawberries treated one day before harvest, those that were treated two to four days before harvest showed no significant difference in flavor of the frozen fruit.

Food technologists who cooperated with entomologists, plant pathologists or chemical companies have observed quality changes as a result of applied chemicals when none of the original chemical could be detected in the product with present chemical methods. This poses some very interesting biochemical problems of a fundamental nature which should be of interest to the chemists and food technologists and plant physiologists. Whether these quality changes are truly physiological or whether they are due to the metabolites of the original applied chemical has not been determined, but the use of the arsenates to increase sugar content of grapefruit in some of the southern states is well known, and also it is known that the application of lead arsenate has a tendency to flatten somewhat the flavor of peaches.

Probably one of the most significant studies on the effects of fungicides and insecticidal spray treatments on the physical-chemical properties of fresh and processed cherries has been reported by BEYER and WECKEL (1960). In most cases fungicides have not caused any appreciable differences in quality of fruits and vegetables where they have been used. However, these workers in Wisconsin found that the fungicide spray treatment had a considerably greater effect than insecticide spray treatment on the various quality factors. Cherries sprayed with Bordeaux mixture were the smallest, had the greatest drained weight, and had the greatest percent of acidity and soluble and total solids. Their flavor was preferred to that of cherries given other fungicide spray treatments. Canned cherries sprayed with Bordeaux mixture had the least lost-can vacuum while in storage. Cherries sprayed with ferbam, nabam or cyprex were the largest in size, had the poorest color, the greatest pH, and had the least drained weight, percent acidity, and percent soluble and total solids. Their flavor was flat and less desirable than that of cherries sprayed with Bordeaux mixture or with actidione plus ferbam. Canned cherries sprayed with ferbam or nabam had the greatest loss of can vacuum while in storage. Cherries sprayed with actidione had the lowest pH, and the greatest amount of color, and their flavor tended to be somewhat bitter. Cherries sprayed with actidione plus ferbam had a low pH, a great amont of color, and were large in size. Their flavor was generally preferred to that of cherries sprayed only with either actidione or

ferbam. They also found that cherries sprayed with Bordeaux mixture were the most desirable and cherries sprayed with ferbam, nabam, and cyprex were the least desirable from the processed quality standpoint. Cherries sprayed with other fungicide spray treatments tended to be intermediate in overall quality. They also found that cherries sprayed with lead arsenate tended to have the greatest drained weight, the most color, and the greatest percent soluble and total solids. Cherries sprayed with parathion and with methoxychlor tended to have the lowest pH and the greatest percent titratable acidity. The insecticide spray treatments used had no effect on flavor.

Another interesting quality change is reported by JOHNSTON (1957). He found that lindane residues in cucumber fermentation retarded or inhibited the yeast in the microflora and appeared to reduce the competition for the available nutrients in the cucumber brine which the fastidious lactic acid-producing bacteria require for reproduction, growth, and fermentation.

A more recent comprehensive study of the effect of insecticides and fungicides on the flavor and quality of fruits and vegetables was conducted during six years by seven experiment stations and the Bureau of Human Nutrition of the U. S. Department of Agriculture. They tested by organoleptic methods the effect of pesticides on the flavor of twenty vegetables and three fruits. Common samples were exchanged and then were evaluated by a taste-test method developed by the group. Some pesticides were tested enough times (11 to 68 tests) to draw a picture of the distribution of flavor effects in terms of controlled samples: that is, better, equal, poor, slight, and definite off-flavor. These results are reported by MURPHY *et al.* (1961). They found that five single insecticides and one fungicide were associated with a notable degree of poor flavor quality. Six combinations of pesticides had adverse effects on flavor. Some of these seemed to be related to specific components of the multiple treatment while others were ascribed to interaction. No ill effects on flavor or quality were noted in association with ten insecticides, three fungicides on potatoes, and three fungicides on apples.

They found that 25 percent of the 24 samples treated with endrin and 16 percent of the 68 samples treated with malathion were judged to be in the poor flavor category.

Single insecticides which in general did not induce poor flavor quality as based on fourteen or more evaluations were chlordane, DDT, dibrom, dilan, dimethoate, heptachlor, phosphamidon, Sevin, thiodan, and trithion.

During the past few years there has been a large number of new chemical herbicides introduced which are applied either pre-emergence or post-emergence, and many of them are selective in nature. It has been only within the past few years, however, that much attention has been given to the effect of chemical herbicides on the quality of the crop where the chemical is used to control weeds either before emergence or after the crop is up. MCARDLE *et al.* (1961) recently reported on the work done cooperatively by the Pennsylvania and Maryland Experiment Stations on the influence of herbicides on the flavor of processed fruits and vegetables. This was a part of a regional research project and included 28 herbicides applied to

major processing crops. Manufacturers' suggested rates were used with all chemicals, and some were applied in excess of the suggested rate to increase effectiveness of weed control. Flavor evaluation of the processed products by experienced taste panels indicated that eleven herbicides reduced product flavor scores, two produced slight off-flavors when applied at the suggested rates, three produced slight off-flavors when applied in excess, and seventeen of the chemicals studied did not reduce flavor scores of any products treated. The flavor changes observed were of low magnitude and might not have been detected by a consumer panel. They grouped these herbicides into two groups. Group one included herbicides which produced tests rated poorer than their standard sample. In this group were ACP 103, benzac 103 A, chlorozene, diuron, emid, FW 450, neburon, Niagara 5521, randox, simazin, and 2,4-D amine. Group two included herbicides for which no test scored lower than the standard sample. In this group were ACPM 118, ACPM 119, ACPM 622, atrazine, chloro IPC, Crag-Sesone, dalapon, endothal, EPTC, monuron, natrim, premerge, salt, Stoddard Solvent, trietazine, URAB, and vegedex.

Very little work has been done on the effect on flavor of the crop and possible quality changes caused by most of the nematocides, but there is some indication that in some areas under certain methods of application undesirable quality changes may occur.

Many scientists in the fields of chemistry, entomology, plant pathology, and food technology may be rather apprehensive as to the value of organoleptic or subjective tests for making flavor evaluations. Some scientists who are very exacting as to experience and equipment for making chemical determinations contend that because people vary so much in their sensory capacities, some even having no taste, there is no such thing as an average person, and that therefore precision in dealing with human reactions cannot be expected. However, it is possible in the field of flavor measurement to obtain objectively, by test methods, results which will permit interpretation at a significantly higher level than pure speculation.

No attempt will be made to go into detail on methods of making organoleptic flavor difference tests since they have been reviewed by PERYAM and SWARTZ (1950), HARRISON and ELDER (1950), LOCKHART (1951), MAHONEY *et al.* (1957), KRAMER and DITMAN (1956 a), KRAMER (1956 b), and KRAMER *et al.* (1961). These workers have shown that flavor difference evaluations can be made objective and scientific. Methods are presented for selection of panel members who can detect slight differences and are able to obtain similar results on comparable samples in replicated trials. Through careful selection and a certain amount of training, small panels of ten to twelve judges can obtain quite similar scores on flavor differences on the same samples in different laboratories in the country (MAHONEY *et al.*, 1957 and MURPHY *et al.*, 1961).

III. Discussion

These illustrations point out, as stated in the beginning of this article, that the food industry feels very strongly that well-coordinated research programs must be developed to obtain sound factual information on which

to base recommendations for the use of chemicals for the control of insects and diseases, weeds, and nematodes, which will have the confidence of the consumer, the grower, and the entire food industry. The farmer should be given an adequate selection of chemicals for pest control, but the consumer must be supplied with food which is free from hazardous residues and off-flavors.

Detailed data on acute and chronic toxicity for all pesticides is the first phase of any coordinated research program, and responsibility for this phase is that of the chemical manufacturing industry. The chemical industry likewise develops suitable and approved chemical methods for detecting residues on or in food products. A majority of the chemical companies are spending several years und large sums of money to screen chemicals for insect control under field conditions, obtaining residue data, and making sensory tests for flavor effects. Most of the work is well planned but because tests are made over widely scattered areas much of it lacks the complete coordination which is really needed to satisfy the requirements of regulatory agencies, research personnel, and the food industry. There are a number of agricultural experiment stations where this type of work could be done under a much more thoroughly coordinated research program, and probably more cheaply than by many of the companies themselves. This is clearly illustrated in the cooperative Northeastern Project reported by MURPHY *et al.* (1961), and this type of research could be run concurrently with the necessary toxicological and chemical methods research.

The food industry, and more particularly the canning industry, believes that most of the present research programs should go one step further to include a study of the physiological effects of these chemicals on the general quality of fruits and vegetables. The experiment station horticulturist could cooperate with the entomologist or plant pathologist to determine the effects of applied chemicals on phytotoxicity, growth and development, and on maturity or date of ripening. At harvest time the experiment station food technologist should take over and process those crops which are normally processed, prepare and cook those crops which are normally cooked in a home, and make critical sensory tests of treated food crops. At the completion of the experiments, the research staff in each department should join in an evaluation of all the data and make recommendations for use based upon all evidence. Such an approach would be extremely helpful in eliminating, insofar as possible, the confusion and apprehension, not necessarily among entomologists, chemists, and food technologists, but mostly among consumer groups which are quite concerned about chemicals in food.

Summary

The effects of applied insecticides, fungicides, herbicides, and nematocides on flavor and quality changes in fruits and vegetables are discussed. This review presents research data obtained from experiment station and U.S. Department of Agriculture studies in different ecological areas of the country.

A comparatively small number of pesticide chemicals when applied to either the soil or the growing crop cause definite off-flavors, particulary in

fruits and vegetables processed by heat. Others cause physiological changes which also affect the quality of the finished product such as changes in pH, titratable acidity, soluble solids, and color. The effects of 61 pesticide chemicals are presented in the review.

Résumé*

On envisage les effets de l'application des insecticides, fungicides, herbicides et nematocides sur l'arôme et la qualité des fruits et des légumes. Cette mise au point présente des résultats obtenus par le Centre d'expérimentation et par l'U.S.D.A. dans différentes conditions écologiques aux Etats Unis.

Un nombre relativement faible de pesticides chimiques provoque une perte définitive d'arôme lorsqu'ils sont appliqués soit dans le sol, soit sur les plantes, particulièrement avec les fruits et les légumes préparés par cuisson. D'autres pesticides provoquent des changements physiologiques qui affectent également la qualité du produit fini, tels que des variations du pH, de l'acidité, de la solubilité et de la couleur. Cette mise au point indique les effets de 61 pesticides chimiques.

Zusammenfassung**

Die Wirkungen angewandter Insecticide, Fungicide, Herbicide und Nematocide auf Geschmacks- und Qualitätsveränderungen in Früchten und Gemüse werden besprochen. Der Beitrag berichtet über Forschungsergebnisse, die durch Untersuchungen der Versuchsstation und den U.S. Departments of Agriculture in verschiedenen ökologischen Bezirken des Landes gewonnen wurden.

Eine verhältnismäßig kleine Zahl von chemischen Schädlingsbekämpfungsmitteln rufen, wenn sie im Boden oder auf wachsende Kulturen angewandt werden, bestimmte Geschmacksveränderungen, vor allem bei Hitzeverarbeitung der Früchte und Gemüse, hervor. Andere Mittel verursachen physiologische Veränderungen, die ebenfalls die Qualität der Endprodukte beeinträchtigen, z. B. durch Veränderung des pH, des titrierbaren Säurewertes, der löslichen Stoffe und der Farbe. Die Wirkungen von 61 chemischen Schädlingsbekämpfungsmitteln werden in der Übersicht dargelegt.

Table I. *Common and chemical names of pesticides mentioned in text*

ACP-103	Experimental — no information
ACPM 118	Experimental — no information
ACPM 119	Experimental — no information
ACPM 622	Experimental — no information
Actidione	Cycloheximide
aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene
Aramite	2-(*p-tert*-butylphenoxy)-1-methylethyl-2-chloroethyl sulfite
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine

* Traduit par R. MESTRES.
** Übersetzt von O. R. KLIMMER.

Table I. (continued)

Common or trade name	*Chemical name*
Benzac-103A	2,3,6-trichlorobenzoic acid
BHC, technical	1,2,3,4,5,6-hexachlorocyclohexane
Chlorazine	2-chloro-4,6-bis-(diethylamino) *s*-triazine
chlordane	1,2,3,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Chlorobenzilate	ethyl 4,4′-dichlorobenzilate
chloro-IPC	isopropyl *N*-(3-chlorophenyl) carbamate
Crag-sesone	Sodium 2,4-dichlorophenoxyethyl sulfate
Cyprex (Dodine)	Dodecylguanidine acetate
Dalapon	2,2-dichloropropionic acid
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane
2,4-D amine	2,4-dichlorophenoxy acetic acid
Diazinon	O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dibrom	O,O-dimethyl O-1,2-dibromo-2,2-dichloroethyl phosphate
dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-4,8-dimethanonaphthalene
Dilan	mixture of 2-nitro-1,1-bis(*p*-chlorophenyl) propane and 2-nitro-1,1-bis(*p*-chlorophenyl) butane (1 to 2 ratio)
dimethoate (Rogor)	O,O-dimethyl *S*(*N*-methylcarbamoylmethyl) phosphorodithioate
Dimite	1,1-bis(*p*-chlorophenyl) ethanol
diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Emid	2,4-dichlorophenoxyacetamide
Endothal	3,6-endoxohexahydrophthalic acid
endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro 1,4-*endo*,*endo*-5,8-dimethanonaphthalene
EPTC	ethyl *N*,*N*-di-*n*-propylthiolcarbamate
Ferbam	ferric dimethyl dithiocarbamate
FW-450	2,3-dichloro-2-methylpropionic acid, sodium salt
Genite-923	2,4-dichlorophenyl ester of benzenesulfonic acid
heptachlor	3a,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
isodrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene
lindane	Gamma-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
malathion	O,O-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate
methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl) ethane
monuron	3-(*p*-chlorophenyl)-1,1-dimethylurea
Nabam	Disodium ethylene bisdithiocarbamate
Natrin	2-(2,4,5-trichlorophenoxy)ethyl sulphate, sodium salt
neburon	1-*n*-butyl-3-(3,4-dichlorophenyl)-1-methylurea
Niagara 5521	Experimental — no information
OMPA (Schradan)	bis-*N*,*N*,*N*′,*N*′-tetramethylphosphorodiamidic anhydride
Ovotran (ovex)	*p*-chlorophenyl *p*-chlorobenzenesulfonate
parathion	O,O-diethyl O-*p*-nitrophenyl phosphorothioate
Phosphamidon	2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate
Premerge (DNBP)	4,6-dinitro-*o*-*sec*-butylphenol
Randox (CDAA)	2-chloro-*N*,*N*-diallylacetamide
Sevin	1-naphthyl *N*-methylcarbamate
simazine	2-chloro-4,6-bis(ethylamino)-*s*-triazine
Stoddard solvent	light fractions of petroleum oil
Sulphenone	*p*-chlorophenyl phenyl sulfone and related sulfones
Systox (demeton)	O,O-diethyl O(and *S*)-(2-ethylthio)ethyl phosphorothioates
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane

Table I. (continued)

Common or trade name	*Chemical name*
Thiodan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
toxaphene	chlorinated camphene containing 67% to 69% chlorine
Trietazine	2-chloro-4-diethylamino-6-ethylamino-*s*-triazine
Trithion	*S*-[*p*-(chlorophenylthio)methyl] *O,O*-diethyl phosphorodithioate
URAB	tenuron-T.C.A.
Vegedex (CDEC)	2-chloroallyl diethyldithiocarbamate

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The use of fluorometric measurements for determination of pesticide residues*

By
D. Mac Dougall**

With 6 figures

Contents

I. Introduction

The requirement for detection and accurate determination of increasingly small quantities of pesticide residues in animal and plant parts is forcing the residue chemist to explore every possible means of physical detection which is applicable to submicrogram quantities of material. The use of fluorometric methods is of value in this field because it is possible to measure accurately quantities of material which are onlv a tenth, a hundredth, or even a thousandth of the smallest amounts measurable by classical colorimetric procedures.

For a detailed discussion of the theory of fluorescence the reader is referred to one of the comprehensive treatises on the subject (Förster 1951, Pringsheim 1949, West 1956, Wotherspoon and Oster 1960). No really adequate theory explaining the relationship between fluorescence and molecular structure has been developed. One such attempt was made by Oda and Yoshida (1951).

A few generalizations regarding the occurrence of fluorescence can be made. It is well known that fluorescence occurs when a substance is maintained in an excited electronic state for 10^{-8} to 10^{-9} seconds or longer. It is obvious that to fluoresce a substance must absorb radiation. In general,

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fluorescence does not occur among compounds whose absorption bands are in the short wavelength ultraviolet region of the spectrum.

Most aliphatic compounds are not fluorescent. This is true of saturated hydrocarbons, materials with isolated double bonds, or compounds with short chains of conjugated double bonds. It is also true of the corresponding alcohols, acids, ethers, esters, halides, etc. In some cases aliphatic compounds with long conjugated chains fluoresce. An example is the dimethyl ester of decapentaene-1,10 dicarboxylic acid.

Most aromatic compounds fluoresce. Phenyl substitution for hydrogen in condensed ring hydrocarbons tends to increase the intensity of fluorescence and to displace the absorption and fluorescence bands to longer wavelengths. The fluorescence of condensed ring hydrocarbons like benzene, naphthalene, anthracene, or fluorene in hexane solution is considerably diminished by the presence of oxygen. Oxygen-free solvents should be used in measuring the fluorescence of these compounds. The saturation of linearly condensed ring hydrocarbons at sites which reduce the number of rings in conjugation may cause visible fluorescence to disappear. In such cases the absorption bands are displaced into the ultraviolet region.

Substitutions in aromatic hydrocarbons can change both the position and the intensity of fluorescence bands. Amino and phenolate groups cause a shift of the fluorescence bands to longer wavelengths. Alkyl substitution in benzene and naphthalene has little effect on the general position of the bands but somewhat increases their intensity. Substitution of fluorine has little effect on the fluorescence of aromatic hydrocarbons. However, chlorine substitution causes a decrease in fluorescence intensity. Brominated compounds are less fluorescent than chlorinated ones and iodinated materials are not fluorescent. The internal quenching caused by the halogen increases as the carbon-halogen bond strength diminishes. Amino and dimethyl amino groups cause the appearance of relatively intense absorption bands with a relatively high yield of fluorescence. Hydroxyl and methoxyl groups increase the fluorescence of aromatic compounds while carboxyl and nitro groups depress it. The sulfonic acid group has little effect on fluorescence and may be used to solubilize otherwise insoluble material. In benzene and naphthalene the nitrile group increases fluorescence. The quinone structure is not conducive to fluorescence.

Fusion of a benzene ring to a heterocyclic compound increases fluorescence. Thus quinoline, isoquinoline, indole, etc. fluoresce in the ultraviolet region. Compounds with oxygen-containing heterocyclic rings often fluoresce visibly.

In general, ring closure increases the tendency to fluoresce.

II. Instrumentation

In the past the complete utilization of the fluorescence principle was hampered by lack of satisfactory instrumentation. Most older fluorometers made use of one of the commercially available mercury arc lamps as radiation source. These lamps give very intense line spectra but are in-

adequate where a systematic survey of excitation radiation needs to be made. In addition, the older instruments all made use of glass filters to isolate the desired wavelengths of both the exciting and emitted radiation. These have obvious limitations. Several years ago two commercial instruments which circumvent both of these difficulties became available in the U.S.A. These were manufactured by the Farrand Company of New York

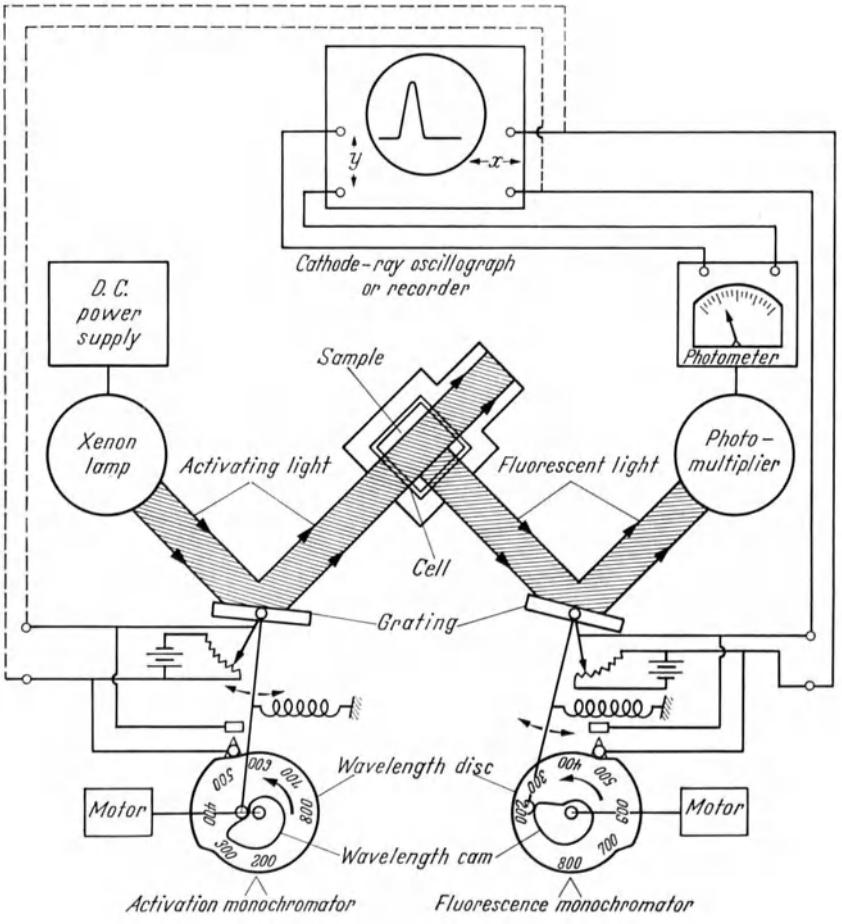


Fig. 1. Schematic diagram of aminco spectrophotofluorometer (Courtesy American Instrument Company)

and The American Instrument Company of Silver Spring, Maryland. Both of these instruments utilize a Xenon arc lamp as radiation source. This type of lamp emits intense continuous radiation from wavelength 200 to 800 mμ. In addition, both of these instruments are equipped with grating

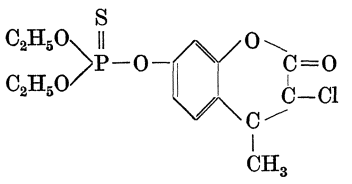
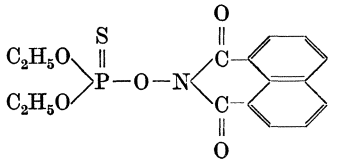
monochromators in both the excitation and emitted light paths. Thus it is possible to isolate any desired wavelength for excitation purposes and to measure any particular emitted band. These two instruments are essentially equivalent in performance. The Aminco-Bowman instrument can be equipped for spectral ranges of 400—1000 mμ and 600—1200 mμ. With these, the near infrared portion of the spectrum can also be utilized. Slit widths for both beams can be varied with both instruments. In addition, the monochromators are motor driven so that it is possible to scan the fluorescence spectrum using a given excitation wavelength or to scan the excitation spectrum for a given fluorescent wavelength. A schematic diagram of the Aminco-Bowman instrument is shown in Fig. 1.

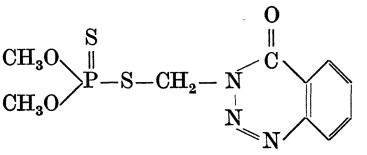
In use in the author's laboratory for pesticide residues the Aminco-Bowman instrument has performed very well. The principal problem encountered was some instability during periods of high humidity. The manufacturer recommends storage of the phototube in a desiccator during periods when the instrument is not in use. In actual practice we have found that better results can be obtained by building a plastic housing around the phototube and placing silica gel in it. In this way the phototube can be kept dry permanently.

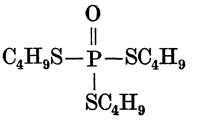
Instruments of the types described above have greatly expanded the possible analytical uses of fluorescence. It is obvious that the double spectral requirement greatly increases the selectivity of the procedure. With equipment of this type multicomponent analyses of the type commonly used in connection with ultraviolet and infrared absorptiometric methods are possible. Multicomponent analyses could be based on differences in the optimum excitation wavelengths, in the fluorescent spectra, or in both.

III. Application

The pesticides on which fluorescence has been used for residue analyses are shown in Fig. 2.

Name	*Structure*	*Source of method*
Co-Ral[1]		Chemagro
Bayer 22 408		*U. S. D. A.*

Guthion [1]  Chemagro

DEF [2]  Chemagro

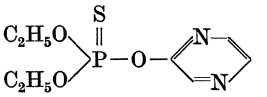
Zinophos  Oregon State University (Kiigemagi and Terriere, 1960)

Fig. 2. Pesticides for which fluorescence methods have been developed

It is interesting to note that all of the pesticides to which this technique has been applied are organophosphorus compounds. Hornstein (1958) has indicated that fluorescence can be used as a basis for residue analysis of several other pesticides, however.

The optimum activation wavelengths and wavelengths of maximum emission of the compounds listed in Fig. 2 are shown in Table I. These

Table I. *Activation and fluorescent wavelengths used in fluorescent methods for pesticides*

Compound	Activation (mμ)	Fluorescence (mμ)
Co-Ral	330	410
Bayer 22408	372	480
Guthion	330	425
DEF	365	500
Zinophos	315	375

show that there are real differences in these respects between compounds. This illustrates the possibility of applicaiton of multicomponent techniques.

As with other techniques the application of the fluorescence method can be made in a variety of ways.

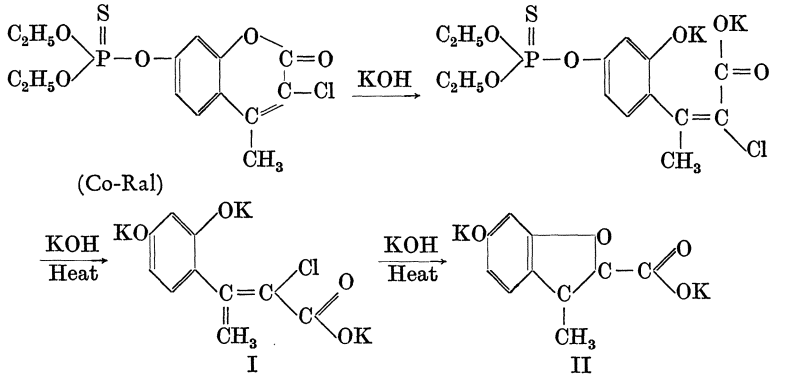
It is obvious that direct measurements can be made if the compound to be measured is highly fluorescent.

In many instances the compound in question can be readily converted to a highly fluorescent substance. This may be accomplished by hydrolysis as by Anderson *et al.* (1959) in the case of Co-Ral (O,O-diethyl O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) or by Adams and MacDougall (1961) with Guthion (O,O-dimethyl *S*-4-oxo-1,2,3-benzotrazin-3(4H)-ylmethyl phosphorodithioate). As shown in

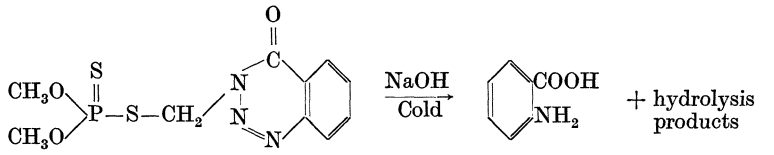
[1] Registered Trademark — Farbenfabriken Bayer A.G., Chemagro Corporation licensee.

[2] Registered Trademark — Chemagro Corporation.

the equations below, Co-Ral is hydrolyzed in alkali to produce a highly fluorescent compound which may be either the coumaric (I) or the coumarilic acid (II). Both of these compounds are highly fluorescent.



On the other hand, Guthion can be readily hydrolyzed to anthranilic acid which is very highly fluorescent.

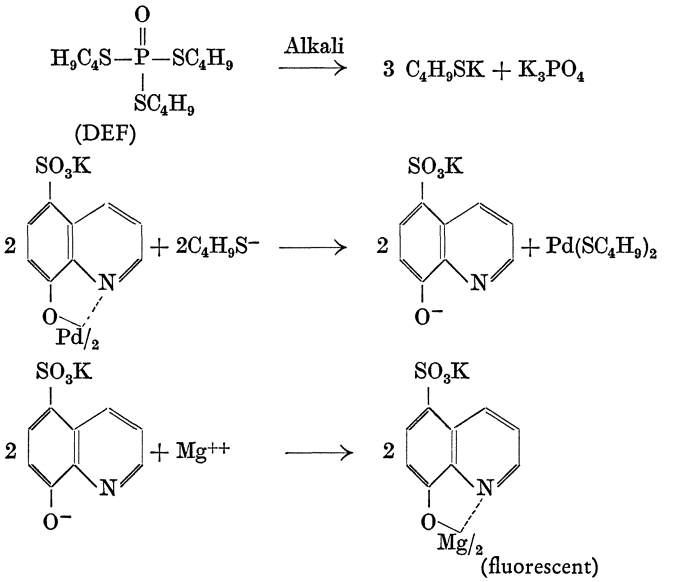


Other techniques are also possible although they have not been used for pesticides. Oxidation has long been used to produce the fluorescent compound thiochrome from thiamine. This type of procedure may be applicable to some pesticides. Fluorescent compounds may be formed by chemical reaction of the material with another compound. A recent publication by COHN and SHORE (1961) described a procedure in which the amine agmatine was reacted with *o*-phthalaldehyde in alkali to from a highly fluorescent derivative.

A third type of procedure is one in which the material to be measured is used as the limiting factor in a reaction which produces a highly fluorescent material. Although this procedure has not been applied to pesticides, GEHAUF and GOLDENSON (1957) have applied it to the war gas, sarin (isopropyl methyl phosphorofluoridate). In their procedure perborate oxidizes the sarin to a peracid which in turn oxidizes indole to indoxyl. The latter compound is highly fluorescent.

A fourth type of fluorescent reaction utilizes the high fluorescence of some metal chelates. This techniques was used indirectly by LOEFFLER and MACDOUGALL (1960) in the development of a photofluorometric method for DEF (*S,S,S*-tributyl phosphorotrithioate). The method was suggested for mercaptans by HANKER *et al.* (1958). In the procedure for DEF the compound is hydrolyzed to form butyl mercaptan which is distilled into a solution of a palladium chelate of 8-hydroxy-5-quinoline sulfonic acid. The

mercaptan ties up part of the palladium freeing a corresponding amount of the complexing agent. On addition of magnesium chloride a fluorescent magnesium chelate is formed. The palladium chelate is not fluorescent. These reactions are shown in the equations below.



These examples illustrate some of the types of reactions which can be used as a basis for fluorescence methods.

IV. Advantages and precautions

The principal advantage of fluorescence methods over classical type procedures such as colorimetry is their great sensitivity. In general, fluorescence methods are 10 to 100 times more sensitive than colorimetric techniques. The relative sensitivity of colorimetric and fluorometric methods for some pesticides is shown in Table II. The approximate ultimate sensiti-

Table II. *Ultimate sensitivity of fluorescence and colorimetric methods*

Compound	Colorimetric[1]	Fluorometric[2]	Ratio
Co-Ral	2.5 μg	0.002 μg	1250
Guthion	1.6 μg	0.01 μg	160
DEF	10 μg	0.02 μg	500

vities which can be attained with the Aminco-Bowman spectrophotofluorometer and the Beckman DU spectrophotometer are shown. For the

[1] Micrograms required for absorbance of 0.1 using 2 ml. of solution in a Beckman DU spectrophotometer.

[2] Micrograms required for 10% galvanometer reading at maximum sensitivity with 1 ml. of solution (slit arrangement # 3).

fluorometric values the actual amounts of compound required for a 10% galvanometer deflection using a total volume of 1 ml. of solution and slit arrangement # 3 are shown. For the colorimetric methods the values shown are the number of micrograms of substance which, in 2 ml. of solution, will give an absorbance of 0.1. The fluorometric methods referred to are those in use in the author's laboratory. The colorimetric methods referred to are those developed by CLABORN *et al.* (1958) for Co-Ral and MEAGHER *et al.* (1960) for Guthion. The colorimetric Co-Ral method is based on hydrolysis and reaction of the liberated hydroxy coumarin with 4-amino-antipyrene. The colorimetric DEF procedure was based on a nitroprusside test for butyl mercaptan. More sensitive colorimetric methods for mercaptans are available.

The ratios in Table II illustrate the sensitivity of fluorescence over colorimetric procedures. It must be emphasized that these data show the ultimate sensitivity possible with the various procedures. In actual practice the sensitivity may be limited by other factors.

Extreme sensitivity of this type allows the residue chemist to adjust conditions for whatever sensitivity is required. With colorimetry or other techniques it is often necessary to use very large samples and concentrate greatly to obtain the desired final sensitivity. With fluorescence the same effect can be accomplished with much smaller samples. The consequent decrease in volumes of extraction solvents, etc., effects a great saving of both time and reagents. In addition, the Food and Drug Administration in the United States has recently required residue methods for milk which are sensitive to 0.01 ppm. For this sensitivity, colorimetric methods will usually not function without using samples so large as to be impractical.

In the investigation of the fluorescent properties of a compound, it is very important that a screening technique be developed. The effects of solvent and pH are particulary important in this connection. To illustrate the effect of solvent on intensity of fluorescence, the relative fluorescence of anthranilic acid in a number of solvents was determined. The results are shown in Table III.

Table III. *Relative fluorescence of anthranilic acid in different solvents*

Solvent	Fluorescence	Relative intensity
Carbon tetrachloride	0.04	1
Water	0.96	24
Hexane	1.05	26
Acetone	1.43	36
Ethanol	1.64	41
Diethyl ether	2.12	53
Methylene chloride	2.19	55
Chloroform	2.41	60
Benzene	2.42	61
Methanol	2.71	68
Toluene	3.97	99
Dichloroethane	7.29	182

It is obvious from these results that solvent has a great effect o[n] rescence intensity. In addition, there does not appear to be any [re]

sequence in the relation of fluorescence intensity to solvent type. It is noteworthy that this acid is least fluorescent in dichloroethane, while chloroform gives an intermediate value. The solvents used for this test were all "Reagent Grade" but were not especially purified. These results may be due partially to impurities in the solvents which may greatly effect the fluorescence either directly or by increasing the solubility of the compound in the solvent.

Three examples will serve to illustrate the effect of small changes in solvent composition on fluorescence. In the fluorometric method for Guthion anthranilic acid is extracted into benzene from an aqueous isopropyl alcohol solution. In this extraction enough isopropyl alcohol passes into the benzene to allow solution of the anthranilic acid. If the isopropyl alcohol were not present in the benzene, the solubility of anthranilic acid would not be great enough to allow determination in this medium.

A second illustration of this can be taken from the Giang (1961) method for determination of Bayer 22 408 (O,O-diethyl-O-naphthalmido phosphorothioate). In this procedure hydrogen peroxide is added to the dioxane which is used as solvent for the final fluorescence measurement. The hydrogen peroxide apparently causes a great increase in fluorescence. The reason for this is not explained in the paper.

The effect of methanol concentration on the fluorescence of the magnesium chelate of 8-hydroxy-5-quinoline sulfonic acid is shown clearly in Fig. 3.

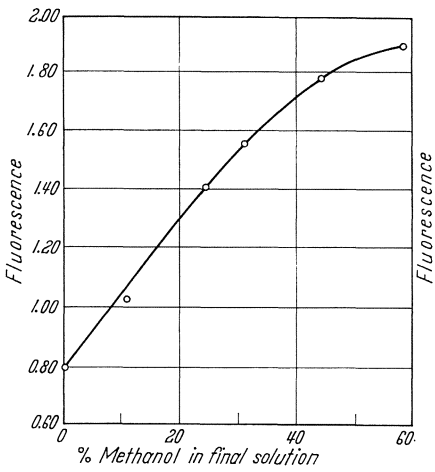


Fig. 3. Effect of methanol concentration on fluorescence of magnesium chelate of 8-Hydroxy-5-quinolinesulfonic acid

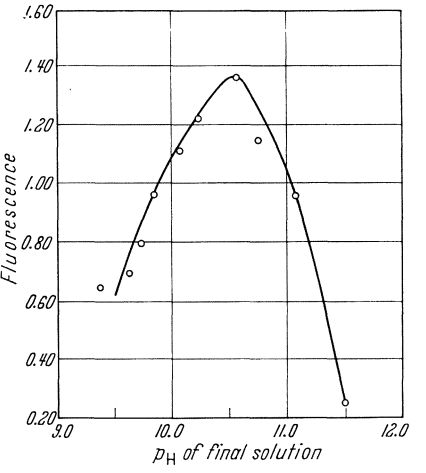


Fig. 4. Effect of pH fluorescence of magnesium chelate of 8-Hydroxy-5-quinolinesulfonic acid

The effect of pH on fluorescence is also very marked. This is illustrated for the magnesium chelate of 8-hydroxy-5-quinoline sulfonic acid in Fig. 4. It is obvious that in this case pH is very critical. A similar critical effect

can be shown for anthranilic acid in aqueous buffers (Fig. 5). One further example will serve to illustrate the critical nature of pH. THOMMES *et al.* (1958) showed that simultaneous determinations of *o*- and *m*-hydroxybenzoic acids could be made. At pH 5.5 only the ortho-isomer is fluorescent while at pH 12.0, both compounds fluoresce. Furthermore, the fluorescence of the ortho-isomer is the same at pH 5.5 and 12.0.

For a compound with unknown fluorescence properties it is obvious that a very careful survey must be made under a wide variety of conditions of solvent and pH to insure that the fluorescent properties of a compound will be discovered.

The relationship between fluorescence and concentration is usually only linear over a limited concentration range. Great care must be taken to ensure that measurements are being made in the range where linearity exists. The graph in Fig. 6 shows the linearity range for the DEF determination

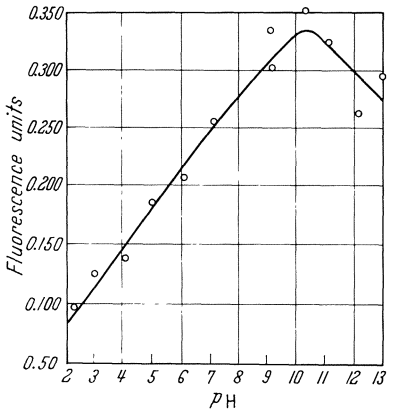


Fig. 5. Effect of pH on fluorescence of anthranilic acid in aqueous buffers

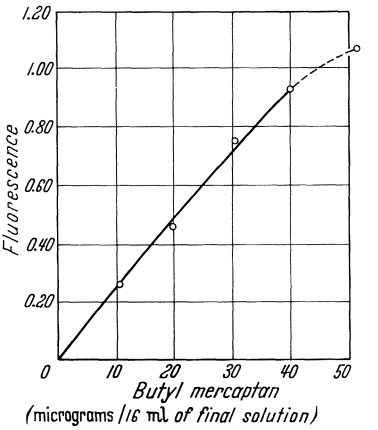


Fig. 6. Standard curve of magnesium chelate of 8-Hydroxy-5-quinolinesulfonic acid

discussed earlier. The deviation from linearity is caused by concentration quenching.

The exact reasons for concentration quenching are not well understood. The phenomenon is probably due to intermolecular collisions in which the electronic excitation energy is transferred either to solvent or to other solute molecules. The probability of such collisions occuring increases greatly as concentration increases. It is also possible that the fluorescent radiation may be partially absorbed by color or foreign materials present in the solution. In many cases the presence of foreign materials will alter the slope of the concentration fluorescence line. This is not a serious problem provided that the relationship is still linear over the concentration range with which one is concerned. To overcome the problem of a shifting calibration line, it may be necessary to use the internal standard technique. In this method known increment amounts of the compound to be measured

are added to aliquots of the extract being analyzed. In this way it is possible to determine the slope of the concentration fluorescence line for each sample analyzed. This amounts to developing a separate calibration for each sample.

The greatest problem in completely utilizing the sensitivity possible with fluorescence is in preparing an extract of biological material with sufficiently low fluorescence. It is a general analytical principle that quantitative analyses cannot be made unless the value obtained for a treated sample is at least twice that obtained for an untreated sample. The inherent variability of biological material is such that difference values which are less than 50% of the total measurement should not be used. This means that if a sensitivity of 0.01 ppm is required then clean-up procedures must be developed which will lower the values obtained on untreated samples to at least this value. In most cases in the author's experience this has been the limitation on sensitivity obtainable with fluorometric methods.

V. Clean-up

In order to obtain the desired degree of clean-up, special procedures have been necessary in many cases. In the Anderson *et al.* (1959) fluorescence method for Co-Ral residues the initial extraction and clean-up steps are quite standard. However, in development of this method it was found that the residues remaining after the chromatography step produced fluorescent material upon hydrolysis. A great deal of difficulty was experienced in separating this interfering material from the fluorescent product which it was desired to measure. It was found after considerable time that if the hydrolysate is extracted with *n*-amyl alcohol the interfering fluorescence goes into the amyl alcohol and the Co-Ral fluorescence remains in the aqueous phase. By this means the background fluorescence for meat and meat products was lowered to that equivalent to 0,02 ppm Co-Ral. For milk the value was equivalent to 0,01 ppm of Co-Ral. These are the sensitivities of the method.

In the fluorescent method for Guthion Adams and MacDougall (1961) made use of the fact that anthranilic acid can be extracted with benzene from a solution buffered to the isoelectric point of the acid. In the case of Guthion the hydrolysis is carried out in potassium hydroxide in anthranilic acid is extracted into dilute hydrochloric acid. The pH of the isopropyl alcohol. After hydrolysis benzene is added to this mixture and the hydrochloric acid solution is then buffered to 4.0—4.2; and on extraction with benzene, the anthranilic acid passes into the benzene-isopropyl alcohol phase. The fluorescence measurement is made in this solution. The background fluorescence for milk is equivalent to about 0.004 ppm of Guthion.

For the DEF procedure of Loeffler and MacDougall (1960), the mercaptan was distilled from the hydrolysis mixture. This, of course, provides excellent clean up. Under these conditions background values were equivalent to approximately 0.02 ppm of DEF.

VI. Conclusions

To summarize, then, it is apparent that the full potential of spectrofluorometric methods has not yet been realized. In general, these methods are among the most sensitive available and there is usually no instrumental limitation on the sensitivity. The effects of solvent and pH must be very carefully investigated for each compound and great care must be taken to insure that the fluorescence concentration relationship is linear in the range in which measurements are being made. The biggest problem is to lower the background fluorescence sufficiently so that actual use can be made of the sensitivity possible with this technique. However, in spite of the problems involved this technique will undoubtedly find many new uses in the pesticide residue analysis field.

Summary

The use of fluorometric methods for pesticide residue analyses is discussed. The main advantage of this technique over colorimetric methods is increased sensitivity. The principal disadvantage is the difficulty in obtaining clean-up procedures which will remove interfering fluorescent materials. The uses which have already been made of this technique for residue purposes are discussed. The effects of variables such as solvent composition, solvent purity, and pH on the fluorescence yield are illustrated by examples from established pesticide residue methods. The general scope of the procedure and possibilities for future utilization of this technique for residue purposes is reviewed.

Résumé*

L'emploi de la fluorescimétrie pour l'analyse des résidus de pesticides est examiné. Le principal avantage de cette technique sur les méthodes colorimétriques est une sensibilité accrue. Le principal inconvénient est la difficulté d'obtenir des techniques d'isolement capables d'éliminer les substances fluorescentes interférentes. Les applications existantes de cette technique pour les analyses de résidus sont examinées. Les effets de la composition du solvant, de sa pureté et l'effet du pH sur le rendement de la fluorescence sont illustrés par des exemples tirés de méthodes connues d'analyse de résidus d'insecticides. Une vue générale du procédé et des possibilités futures qu'il ouvre dans l'étude des résidus est rapportée.

Zusammenfassung**

Die Anwendung fluorometrischer Methoden zu Pesticid-Rückstandsanalysen wird erörtert. Der Hauptvorteil dieser Technik vor colorimetrischen Methoden ist die höhere Empfindlichkeit. Der wesentliche Nachteil ist die Schwierigkeit, Reinigungsverfahren zu entwickeln, die störende fluo-

* Traduit par R. MESTRES.
** Übersetzt von G. HECHT.

rescierende Verunreinigungen beseitigen. Die Anwendung, die diese Technik bereits für Rückstandsbestimmungen erfahren hat, wird besprochen. Der Einfluß solcher Veränderlichen wie Lösungsmittel-Zusammensetzung und -Reinheit und des pH auf die Fluorescenzausbeute werden an Beispielen von eingeführten Pesticidrückstandsmethoden illustriert. Es wird eine Übersicht gegeben über den allgemeinen Anwendungsbereich dieser Methoden und über die Möglichkeiten der künftigen Auswertung dieser Technik für Rückstandszwecke.

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Pesticide residue analysis by microcoulometric gas chromatography

By
CARROLL C. CASSIL*

With 8 figures

Contents

I. Introduction

The principles of gas chromatography, combustion, and coulometry are well recognized and have been used in chemical analysis for several years but the combination of these methods has not been used previously. COULSON *et al.* (1960) have developed a singularly rapid and sensitive method through instrumentation for the determination of pesticide residues containing halogen or sulfur, making use of these principles. The microcoulometric gas chromatograph [1], hereafter referred to as MCGC, was not designed to be an answer to all pesticide residue problems nor to supplant other methods of analysis. It does provide a ready means of analyzing a large number of samples with a minimum of time, manipulation, apparatus,

* Niagara Chemical Division FMC Corporation, Richmond, California.

[1] This instrument is manufactured commercially by the Dohrmann Instrument Company in Palo Alto, California.

and reagents. A desirable degree of accuracy and sensitivity is obtained with this instrument with little or no cleanup of sample extracts. Residues of 0.05 to 100 p.p.m. are easily found by varying the size of sample but the MCGC is not limited to this range.

GUNTHER *et al.* (1962) have studied the gas column and combustion section of the instrument in order to determine the reason that DDT[1] gives multiple peaks and low theoretical recoveries. They have contributed a means of avoiding decomposition of DDT and some other organic halogen compounds in the injection block and column by adding tris(*p*-phenyl phenyl) phosphate to the system. This phosphate blocks decomposition by covering active sites on the column packing and exposed metal which would be contacted by the DDT. These workers explain this increased recovery of DDT type compounds as a blocking of the Friedel-Crafts or Ullman type reaction.

BURKE and JOHNSON (1962) have found that over seventy halogenated pesticides can be determined in the MCGC and many of these can be determined when two or more are combined in a single solution.

CASSIL (1962) has shown how Thiodan can be determined in the presence of DDT in the halide detector cell even when these two materials have the same relative retention time from a silicone column. This was done by finding the total area under the curve for Thiodan plus DDT from one aliquot and the area for DDE from a second identical aliquot after a dehydrochlorination treatment. With these values, the amount of DDT and Thiodan can be calculated.

CAVANAGH and COULSON (1961) have discussed various ways of forming halogenated derivatives of micro quantities of compounds not containing halogen or sulfur. This is a means of determining compounds in the MCGC that could not otherwise be analyzed by this instrument.

II. Description of instrument

The MCGC consists of a Model C-100 Coulometer, a Model T-100 Titration Cell, a Model P-100 Furnace Control Unit, a Model G-100 Gas Chromatograph, and a 10-millivolt Recorder equipped with a Disc Integrator. Different recorders may be used since provision is made for matching the output impedance of the coulometer with the input impedance of the recorder in the circuitry of the coulometer.

The gas chromatograph provides a means of separating complex mixtures. The coulometer is an extremely sensitive electrochemical detector with the necessary specificity for analyzing chloride, bromide, iodide, or sulfur dioxide coming from compounds that are eluted from the gas column and formed in the combustion tube of the furnace. The total electrical charge (current $\times$ time) used for the coulometric titration is a quantitative measure of the element being determined.

The coulometer and titration cell may be used independently of the gas column and with a combustion furnace to give a rapid microanalytical

[1] All pesticide names and their precise chemical designations are given in Table VIII.

method for organic halides or sulfur-containing organic compounds. Inorganic halides, sulfites, or sulfides may also be determined quantitatively by direct injection into the titration cells.

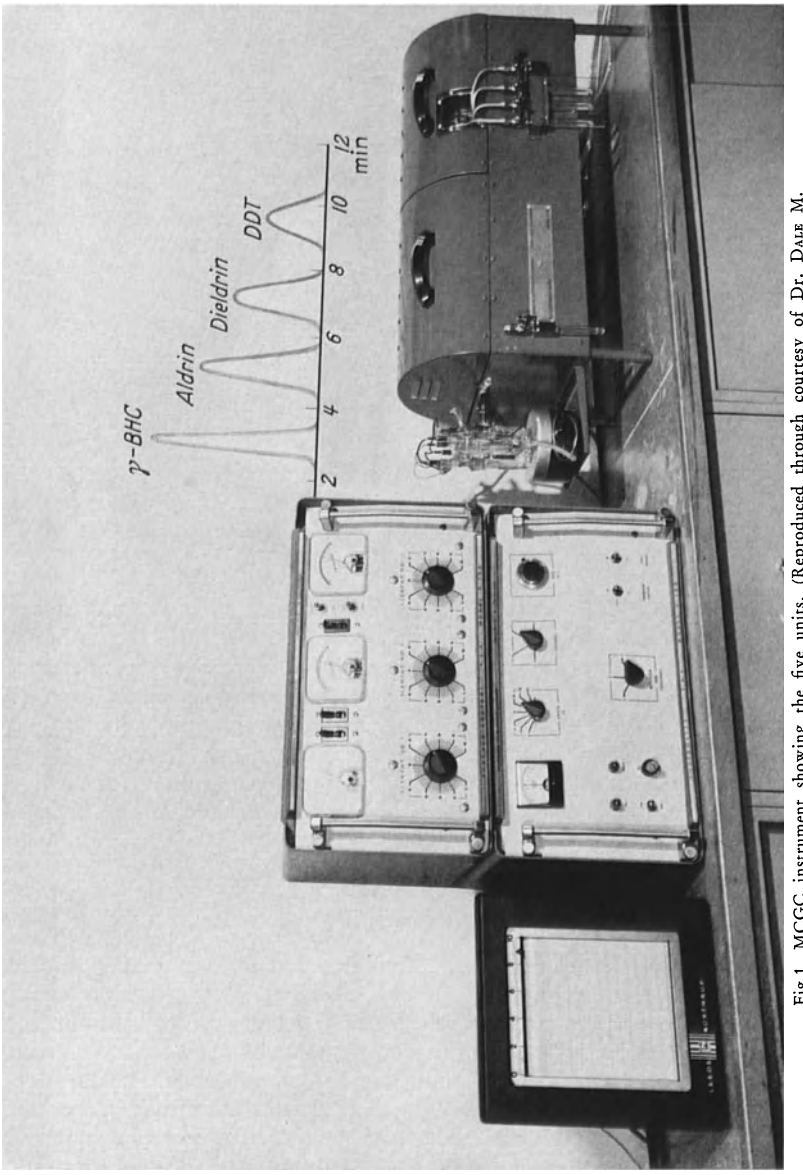

Fig 1. MCGC instrument showing the five units. (Reproduced through courtesy of Dr. Dale M. Coulson, Stanford Research Institute, Menlo Park, California.)

The instrument is illustrated in Fig. 1 and each of the following units is shown therein.

a) *Gas chromatograph and combustion section*

This is a unit comprising a sample injection block, a gas chromatographic column, a quartz combustion tube as in Fig. 2, and built-in heating

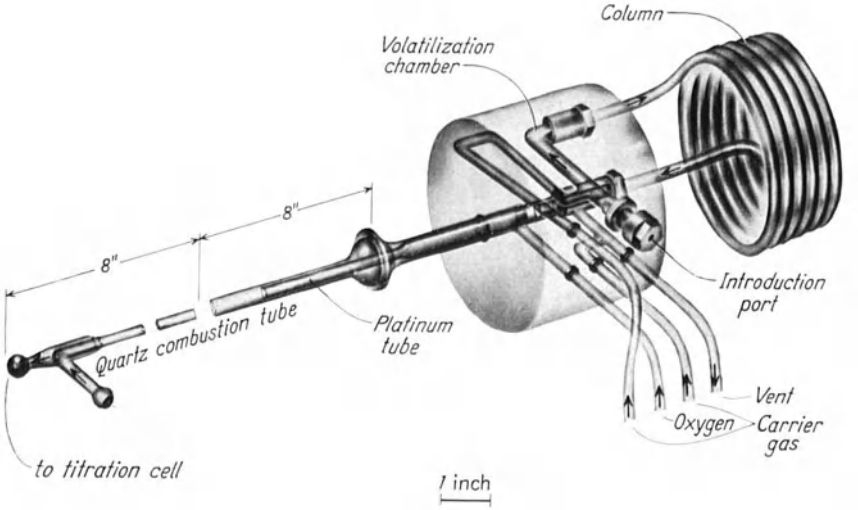

Fig. 2. Gas chromatograph and quartz combustion tube from furnace unit. (Reproduced through courtesy of Dr. DALE M. COULSON, Stanford Research Institute, Menlo Park, California.)

elements. A separate compartment is provided for the high temperature (800° C) combustion furnace to minimize heat transfer to the injection block and column oven which are operated at a much lower temperature (180—270° C). A circulation fan is attached inside the column oven to maintain a uniform temperature of the chromatographic column and injection block. The necessary gas flow meters and regulating valves are located at the front of the unit. Carrier gas, oxygen, water (for cooling coils), and electrical connections are conveniently located at the back of the unit.

b) *Titration cells for halide and sulfur*

The bodies of the titration cells for halide and for sulfur are identical but the electrodes differ and are described separately. The cell is connected to the exit end of the combustion tube which protrudes through the furnace housing. The cell has four compartments separated by fritted glass discs. A four-electrode system is used, consisting of a silver-silver acetate reference, two platinum cathodes in separate compartments, a silver generator anode, and a silver sensor electrode in the center titration compartment. All four compartments are filled with electrolyte (70 to 85 percent acetic acid). A magnetic stirrer is provided in the titration compartment to insure adequate agitation of the titrating solution at all times. This cell is illustrated in Fig. 3.

The sulfur cell differs from the halide cell in that the sensor and generator anode in the center compartment are platinum or gold-plated, and the reference electrode is a platinum-elemental iodine couple. The

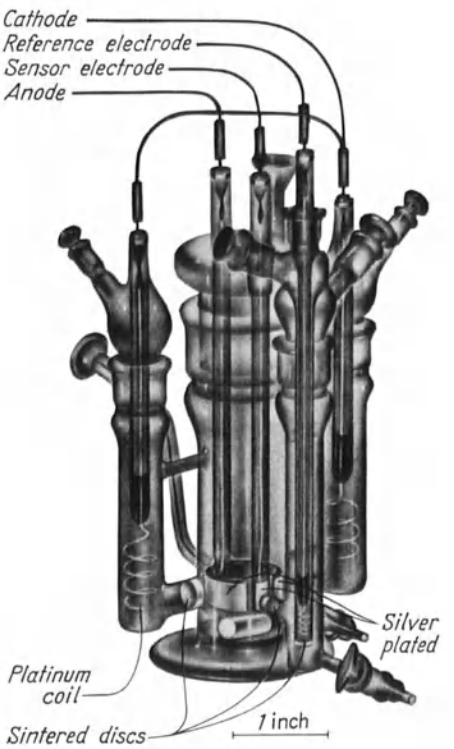

Fig. 3. Titration cell showing arrangement of electrodes. (Reproduced through the courtesy of Dr. Dale M. Coulson, Stanford Research Institute, Menlo Park, California.)

cathodes are platinum and are shielded from the fritted discs by small test tube inserts. The electrolyte used in all compartments is water containing 0.4 percent glacial acetic acid and 0.04 percent potassium iodide.

c) *Furnace control module*

This module houses three variable resistors to control the electrical AC input into the combustion furnace, the injection block, and the column oven. Each of these units is equipped with a pyrometer so that the operating temperatures can be observed and recorded at any time. The switch for the fan in the column oven is also located in this module.

d) *Coulometer module*

The conditions within the titration cell (Model T-100) are electronically controlled by this module to achieve optimum operating efficiency. It is the device which controls the amount of silver ion generated in the solution

during the titration of the halide. The system operates with a servo-motor on a null balance principle; hence, the current required to maintain a constant silver ion concentration during a titration, after amplification, becomes the input to a recorder. An integral part of the coulometer is the sensitivity range switch. This consists of a group of resistors, each of which is in series with the generator electrode system. The switch is calibrated at 1, 4, 16, 64, 128, and 512 ohms. Maximum sensitivity is obtained at 512 ohms with a practical working range of 0.05 to 0.30 μg. at 128 ohms, 0.5 to 2.0 μg. at 64 ohms, and 1.5 to 8.0 μg. of halide ion at 16 ohms. It is not recommended that the 1- or 4-ohm range settings be used with the current titration cell for halide ion. The 4-ohm range switch has been found useful for the sulfur cell when determining 3.0 to 10 μg. of sulfur. The other working ranges for sulfur are 0.5 to 2.5 μg. at 16 ohms, 0.1 to 0.5 μg. at 64 ohms, and 0.05 to 0.25 μg. at 128 ohms. The limit of detection is about 0.02 μg. of sulfur. Operation of the sulfur cell is based on the oxidation of sulfur dioxide to sulfur trioxide by triiodide ion which is formed in the potassium iodide-acetic acid electrolyte.

e) *Recorder and Integrator*

Several types of recorders and integrators are available. A 10-millivolt strip-chart recorder[1] equipped with a disc integrator[2] has been used satisfactorily to translate the signal from the coulometer into graphical form. The areas under the peaks are readily measured by the integrator.

The area under a peak is directly proportional to the amount of halide ion being precipitated or sulfur dioxide being oxidized. Peak height is not necessarily proportional to the amount of silver being precipitated or sulfur dioxide being oxidized, especially if the gas chromatographic conditions are varied.

The complete apparatus is schematically depicted in Fig. 4.

III. Operation of the MCGC

Optimum operating conditions for individual compounds are achieved only by experimentation. It is necessary to adjust the carrier gas flow rate and temperatures of the chromatographic column and injection block to yield the greatest efficiency and resolution for a single compound or combination of compounds. Lengths of columns and different types of packings can be selected to give the desired results. Chromatographic columns are discussed under a separate section.

When the instrument is used every day or is needed for stand-by operation, furnace, block, and column temperatures are continuously kept within a few degrees of optimum to save time in heating the oven unit. Thus the instrument should be installed in such a manner that the water can be left flowing through the cooling coils and the variable resistors set with current flowing on a 24-hour basis.

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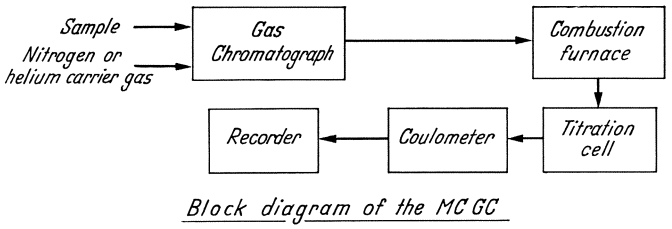
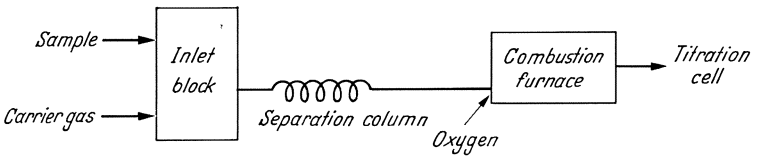
[1] Brown Electronic High Speed Recorder manufactured by Minneapolis Honeywell Regulator Co., Brown Instruments Div., Philadelphia 44, Pennsylvania.

[2] Disc Chart Integrator manufactured by Disc Instruments, Inc., 12 671 Bubbling Well Road, Santa Ana, California.

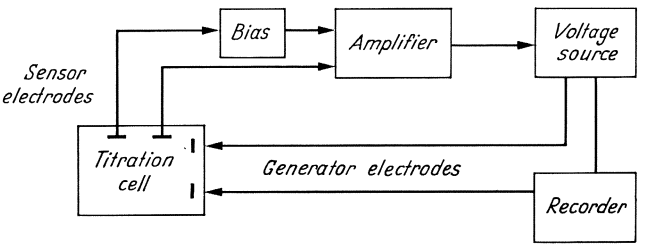
To start the instrument for halogen determination, all compartments of the titration cell are rinsed at least three times with the acetic acid electrolyte to make certain that no colloidal silver is in the fritted discs.



Block diagram of the MC GC



Block diagram of gas chromatograph



Block diagram of coulometer

Fig. 4. Block diagrams of MCGC instrument

Adjust carrier gas (helium or nitrogen) and oxygen flow to predetermined levels. Adjust all temperatures to optimum and switch on power to coulometer and recorder. The instrument is ready for operation as soon as an even base line has been established on the recorder. When using the sulfur cell, the same procedure is followed but the potassium iodide-acetic acid electrolyte is used.

With the column vent valve open and a desired range setting, inject 100 microliters or less of a standard solution, containing a known amount of pesticide, into the block. Mark the recorder chart at the time of injection by means of the Recorder Time Constant Switch on the coulometer. Close the column vent 30 to 60 seconds after the injection or when the bulk of

the solvent has escaped into the air. Most of the solvent must be vented since there is not sufficient oxygen in the furnace to burn it all, and partially burnt solvent may foul the titration cell.

GUNTHER (1961) has found that venting is not necessary for quantities of solvent up to 100 microliters if the combustion tube is packed with small quartz chips and more oxygen is passed into the combustion zone. An extra silica tube for oxygen is built into the exit end and into the middle of the combustion tube which allows the introduction of extra oxygen for more difficult combustions. A roll of platinum gauze is placed at each point of oxygen entrance into the combustion tube and the remainder of the tube is packed with quartz chips. One hundred milliliters per minute of oxygen is passed into the tube where solvent enters combustion tube and 200 milliliters per minute of oxygen is passed into the middle of the combustion zone.

After volatilizing from the block, the sample is eluted through the chromatographic column. Upon leaving the column, it mixes with oxygen and is burnt in the quartz furnace tube to form hydrogen halide or sulfur dioxide which subsequently passes into the titration cell, the results being recorded on the chart paper. Furnace, block, and column temperatures, retention times and areas of peaks are recorded in a notebook along with the size of sample injected for purposes of calculations as well as future reference.

IV. Determination of pesticide residues

Extracts prepared from the desired crops are used for injection into the instrument. The solvent used for extraction should have a boiling point below 100° C. for purposes of easy concentration in a Kuderna-Danish evaporator[1] on a steam bath. Solvents, such as Skellysolve B, pentane, hexane, or benzene, have been found satisfactory if pretested — after a concentration of 100-to-1 — in the instrument to insure the absence of solvent contributed halide and sulfur. In many cases, an aliquot of 20 to 80 microliters of plant extract will contain from 0.5 to 16 μg. of the pesticide and no concentration is necessary.

If the pesticide residue is present in a lesser amount, a suitable aliquot must be concentrated in a Kuderna-Danish evaporator fitted with a Snyder column and a calibrated collection ampule.

Surface extracts of fruits, such as apples, pears, peaches, cherries, or plums, have been injected directly into the block for 400 to 500 determinations without fouling the system. It is necessary when injecting routine samples into the instrument to make frequent checks with standard solutions and fortified samples to make certain that consistent recoveries are being obtained and that neither the column nor the block has been fouled. The frequency of this operation is dependent upon the nature of the extracts being injected into the instrument.

Experience with a given type of crop extract will indicate the number of samples that can be put through the instrument and the number of

[1] Available from Kontes Glass Company, Vineland, New Jersey.

standards and recoveries needed. In actual practice for routine work, it has been found that 30 injections can be made per 8-hour day. This group of 30 injections may include 5 standards without crop extract, 3 to 5 untreated crop extracts fortified with the pesticide being determined, and 20 to 22 unknown samples. These 30 injections may be distributed as follows:

(a) Standard solution injections are generally distributed throughout the 8-hour period, 2 at the start, 2 in the middle, and one at the end of the period.

(b) The fortified recovery samples are distributed at random throughout the period. These untreated crop extracts are fortified at a level comparable to the amount of pesticide being found on the unknown samples.

Three or four injections of a leafy vegetable extract, such as lettuce, alfalfa, cabbage, etc., may foul the block system so it may have to be cleaned thoroughly with acid and alkali before it will again give normal shaped peaks and recoveries. A cleanup procedure for leafy vegetable extracts is strongly recommended if the instrument block is not fitted with a quartz injection liner tube (to be discussed later).

Cleanup procedures are available that will remove waxes and chlorophyll. COULSON *et al.* (1960) have described a liquid column cleanup using a special grade of aluminum silicate and A.O.A.C. "Official Methods of Analysis" (1960) describes a cleanup procedure that works well under some conditions. GUNTHER and BLINN (1955) have described several cleanup procedures.

V. Calculation of results

The halide ion or sulfur dioxide titration cell, as a detector in combination with the coulometer module and recorder, is a system that is quantitative and peak areas can be precalculated for a specific compound. Such precalculated areas are correct in practice if a chromatographic column is used that will pass this compound quantitatively.

Very few compounds will elute from a gas column quantitatively, or with 100 percent efficiency. However, a constant percentage of most compounds will elute giving good reproducibility within plus or minus 3 to 5 percent. This becomes a correction factor called "column efficiency".

The generation of silver ion or triiodide ion to maintain the null balance of the titration system proceeds according to Faraday's Law. The amount of silver ion used is directly proportional to the amount of halide ion coming from the combustion tube and the amount of triiodide ion used is proportional to the sulfur dioxide coming from the combustion tube. Thus the theoretical area for an organic compound containing sulfur or halide can be determined by a simple equation derived as follows:

(a) Assume recorder has been driven one inch per millivolt.

(b) Assume recorder has chart travel of one inch per 3 minutes.

(c) Assume Equivalent Weight defined as $\frac{\text{Molecular weight of compound}}{\text{Number of halide atoms}}$

or $\frac{\text{Molecular weight of compound}}{2\text{ (number of sulfur atoms)}}$

(d) Faraday's Law

$$\text{Equivalents} = \frac{\text{Q (change in coulombs)}}{\text{96,500 (coulombs/equivalent)}}$$

(e) $$\text{Q} = \text{Area (inches)}^2 \times \frac{3 \text{ min.}}{\text{inch}} \times 60 \frac{\text{sec.}}{\text{min.}} \times \frac{\text{mv}}{\text{inch}} \times \frac{10^{-3} \frac{\text{v}}{\text{mv}}}{\text{R}}$$

(f) $$\text{Equivalents} = \frac{\text{Area} \times 3 \times 60 \times \frac{\text{mv}}{\text{inch}} \times 10^{-3}}{\text{96,500} \times \text{R of range switch in ohms}}$$

(g) $$\text{Micrograms compound} = \frac{\text{Area} \times 3 \times 60 \times 10^{-3} \times \frac{1}{1} \text{ inch} \times 10^{6} \times \text{eq. wt.}}{\text{96,500} \times \text{R of range switch in ohms}}$$

(h) $$\text{Micrograms compound} = \frac{1.865 \times \text{A} \times \text{eq. wt.}}{\text{R in ohms}}$$

Where A is area under peak, eq. wt. is molecular weight of compound divided by halide or sulfur equivalent, and R is the range resistance in ohms.

Typical factors for several pesticides are shown in Table I.

NOGARE and SAFRANSKI (1960) have discussed the analytical interpretation of chromatograms and various means of evaluating the areas of peaks obtained in gas chromatography.

The area under the peak is measured to the base line of the peak and not necessarily to the absolute recorder zero line. At times the base line representing the titration cell may be considerably above the recorder zero base line especially at ranges of 64 to 512 ohms. This discrepancy is usually caused by small amounts of materials which titrate and bleed from the chromatographic column. Since the disc integrator usually is adjusted to the recorder zero base line, the area between this base line and the cell base line must be subtracted from the total peak area to give net area.

In actual practice, most organic compounds will not yield theoretical areas because knowledge of gas chromatographic columns has not yet progressed far enough to always obtain 100 percent efficiency. Some compounds like aldrin and DDE do pass through silicone columns with practically 100 percent efficiency. For this reason, known amounts of the compound are run through the instrument and the column efficiency determined by dividing 100 times the peak area found by the theoretical area to obtain percent column efficiency. This factor is then used to correct all unknown results.

GUNTHER *et al.* (1961) evaluated certain aspects of efficiency and response of the microcoulometer cell and found it to have very good linearity throughout the range of 16 to 512 ohms. These workers reported percent recoveries of 103.4 ± 3.4 at 16 ohms, 106.0 ± 2.3 at 64 ohms, 97.6 ± 4.7 at 128 ohms, and 92.1 ± 13.3 at 512 ohms when sodium chloride was added directly to the cell.

Table I. *Typical theoretical factors*[a] *for several pesticides in terms of micrograms per square inch on recorder*

Compound	Percent		Range in Ohms									
			Halide cell					Sulfur cell				
	Cl	S	4	16	64	128	512	4	16	64	128	512
Chloride (Cl)	100	—	16.6	4.14	1.03	0.52	0.13	—	—	—	—	—
Sulfur (S)	—	100	—	—	—	—	—	7.46	1.86	0.47	0.23	0.058
Aldrin	58.4	—	28.4	7.09	1.77	0.89	0.22	—	—	—	—	—
DDE	44.7	—	37.1	9.26	2.30	1.16	0.29	—	—	—	—	—
DDT	50.1	—	33.1	8.26	2.06	1.04	0.26	—	—	—	—	—
Ethion	—	33.3	—	—	—	—	—	22.4	5.59	1.41	0.69	0.17
Lindane	73.2	—	22.7	5.66	1.41	0.71	0.18	—	—	—	—	—
Malathion	—	19.4	—	—	—	—	—	38.5	9.59	2.42	1.19	0.30
Parathion	—	11.0	—	—	—	—	—	67.8	16.9	4.27	2.10	0.53
Tedion	39.9	9.0	41.6	10.37	2.58	1.30	0.33	82.9	20.7	5.22	2.56	0.64
Thiodan	52.3	7.9	31.7	7.92	1.97	0.99	0.25	94.4	23.5	5.95	2.91	0.73
Trithion	10.4	28.0	160	39.8	9.90	4.95	1.25	26.6	6.64	1.68	0.82	0.21

[a] Based on recorder having paper travel of one inch per three minutes and pen travel of one inch per millivolt input. Each factor will change proportionally if different recorder characteristics are used according to formula given under "V. Calculation of Results".

Varying quantities of Tedion and Thiodan were analyzed with range switch settings of 16, 64, and 128 ohms to demonstrate the linearity of the range scale as well as the sensitivity and reproducibility of the MCGC. Results of these tests are given in Table II. A very good relationship exists

Table II. *Data showing the linearity of the range scale of the MCGC from actual determinations of Thiodan and Tedion standards*

Compound	μg. added	Range (Ohms)	Peak area (Sq. In.)	μg. found	Percent recovery	C[a]
Thiodan . . .	12	16	1.34	11.6	97	0.112
	8	16	0.86	7.44	93	0.108
	4	64	1.72	3.70	93	0.108
	2	64	0.91	1.95	98	0.113
	1.2	128	1.05	1.13	95	0.109
	0.8	128	0.72	0.78	98	0.113
Tedion . . .	12	16	1.01	11.4	95	0.084
	8	16	0.63	7.2	90	0.079
	4	64	1.35	3.82	96	0.084
	2	64	0.64	1.82	91	0.080
	1.2	128	0.79	1.11	93	0.082
	0.8	128	0.52	0.73	92	0.081

[a] $C = \dfrac{\text{Area (in.}^2\text{)}}{\mu\text{g.} \times \dfrac{R}{16}}$

between quantity of compound and area at all ranges. It is convenient to use this linear function "C" in the calculation of actual residues so that known amounts of standard solutions do not have to be run at more than one sensitivity range.

VI. Maintenance

The continuous operation of one MCGC for 18 months with about 15,000 individual injections has required little abnormal maintenance. All normal maintenance problems are thoroughly discussed in the operational manual supplied by the manufacturer. The titration cell, coulometer module, furnace control module, column and furnace ovens, and combustion tube have required a very minimum of maintenance. The gas chromatograph (block and column) have required more attention because many plant extracts have been put into the system without prior cleanup. Even these components would require little maintenance if all extract solutions were submitted to prior rigid cleanup techniques.

a) *Care of halide titration cell*

When this cell is in constant use with 20 to 35 titrations per day, it gradually becomes coated on the inside with unburned organic matter and should be thoroughly cleaned every 7 to 10 days. This is done by disconnecting all wire leads to the cell, removing all electrodes from their compartments, washing the cell with soap and water, rinsing it with water,

flushing the fritted glass discs with 90 percent warm (70—90° C.) nitric acid to remove metallic silver and silver chloride particles, and rinsing it with water. The platinum cathodes need no particular cleaning. The reference electrode is cleaned by rinsing it with acetic acid electrolyte. The sensor and anode electrodes are carefully polished with a pumice-type cleanser to a bright silver finish and rinsed with water. The cell is reassembled, filled with electrolyte, connected to the instrument, and the sensor electrode adjusted to the correct damping sensitivity by rotating it to obtain one-half full-scale deflection of recorder pen by a change of 10 millivolts on the bias with range switch at 64 ohms. The bias should be set between 270 and 240 millivolts and at that particular setting which gives a minimum deflection of the pen for a 10-millivolt change on the bias. Counterclockwise motion causes lesser damping. In the interim between major cell cleanups, the cell compartments are rinsed with electrolyte night and morning to remove silver chloride. If recorder pen tends to ride below zero base line, the fritted glass discs in the cell should be cleaned. A single silver-plating of the sensor and anode electrodes of the cell will last up to one year with continuous use.

b) *Care of sulfur titration cell*

This cell is cleaned in the same manner as outlined for the silver cell except for the electrolyte used. This contains 0.4 percent acetic acid and 0.04 percent potassium iodide (no free iodine). When the cell is clean, assembled, and connected to instrument, turn the AC current on. Find the null point of the cell with the generator voltage switch off by changing the bias setting to point where the servo light indicator is off between 140 and 160 millivolts. Retard gain on the amplifier in the coulometer from that used in the chloride titration cell, and switch on the generator voltage. If there is excess iodine in the electrolyte, volt meter and recorder pen will ride negatively. If this condition persists, iodine will form on the cathodes and electrolyte must be changed in all compartments of the cell to correct this condition. Do not polish the gold electrodes with any material that will scratch the gold surface. Electrolyte in all cell compartments, except that in the interior of the reference electrode, must remain colorless for the cell to operate properly.

VII. Discussion of gas chromatography unit

Several theories have been advanced as to why theoretical recoveries are not obtainable with all pesticides in this unit, as follows: (a) thermal decomposition or isomerization in the block or column, (b) catalytic activity of the aluminum alloy used in making block and column, (c) catalytic activity of the solid support in the chromatographic column, and (d) the liquid substrate in the column is not ideally suited for all pesticides. All these factors undoubtedly contribute to the low recoveries of some compounds. Many variables have been tested with these basic theories, and experience gained thereby is discussed below.

a) *Chromatographic columns*

A standard chromatographic column consists of a 6-foot, 1/4" (O.D.) aluminum tube wrapped in a coil to fit the oven and filled with 16 to 17 grams of a standard column packing material. The standard packing material contains 80 percent of 30/60 mesh granular Chromosorb P[1] (a calcined diatomite) treated with 20 percent high molecular weight methyl silicone (vacuum silicone stopcock grease). Liquid fractionation of the grease as described by CROPPER and HEYWOOD (1954) is accomplished by dissolving 50 grams in 300 ml. of ethyl acetate, diluting with 300 ml. of ethanol, stirring, allowing high molecular weight fractions to sedimentate, decanting mixed solvent, and washing three times with 50 ml. portions of ethanol. Last traces of ethanol are removed on the steam bath by evaporation. Twenty grams of the high molecular weight fraction is dissolved in 200 ml. of ethyl acetate and added to 80 grams of 30/60 mesh Chromosorb P (acid-washed) granules. The ethyl acetate is then removed under reduced pressure while rotating the container to leave a uniform coating of the grease on the Chromosorb P granules.

This method of making the column packing material does not remove the silica filler normally present in the grease. SMITH (1961) described a method of preparing the grease in a similar manner except the silica filler was removed by careful filtration before precipitating with ethanol. Column packing material made in this way not only conditions more rapidly but also significantly improves the theoretical recovery of compounds being eluted through the column.

The following procedure for preparing a silicone column has proven most satisfactory in the author's laboratory:

Slurry 50 grams of silicone grease with 50 ml. of ethyl acetate in a 100-ml. beaker, dilute with 250 ml. of ethyl acetate and stir into homogeneous mixture. Filter through a Buchner funnel with vacuum (requires 6 to 8 hours, because of the gelatineous nature of the solids present) to remove the inorganic solids. After filtration, add sufficient ethyl acetate to the filtrate to give a total volume of about 300 ml., then add 300 ml. of ethanol while stirring. After about 5 minutes, decant the ethanol-ethyl acetate containing low molecular weight silicones from the precipitated higher molecular weight silicones. Wash the latter material twice with 50 ml. of ethanol, decant the washings, and remove the last traces of ethanol on a steam bath. Approximately 25 grams of higher molecular weight silicones are obtained by this procedure.

Redissolve the higher molecular weight silicones in sufficient ethyl acetate so that each 28 grams of solution will contain 5 grams of silicone grease. Place a small wad of glass wool in the lower part of a 50 ml. burette, add 20 grams of Chromosorb (acid-washed, 30/60 mesh), and pour 28 grams of the ethyl acetate-silicone solution onto the column of Chromosorb and allow the solution to filter through it by gravity (time required is approximately 20 minutes). When solution has penetrated the entire length of column, attach stream of air to the upper opening of the burette and purge all the remaining ethyl acetate in gaseous form. When dry (about 30 minutes), pour packing material out of the burette and check the weight (25 grams) as an added precaution that all ethyl acetate has been removed. After a 6-foot column of 1/4-inch aluminum tubing is packed with 16 to 17 grams of this silicone-coated Chromosorb, place it in the MCGC. When the column oven has reached a temperature of 250° C. (about 30 minutes), the column should give a satisfactory base line at a range setting of 16 ohms.

[1] Chromosorb P is manufactured by Johns-Manville Co., 22 E. 40th St., New York 16, New York.

It has been found that high molecular weight methyl silicone oil[1], when fractionated with the ethyl acetate-ethanol procedure gives the same results as the vacuum silicone stopcock grease without silica filler. The use of the oil is preferred over the grease to avoid the filtration step to removing the silica filler. A high molecular weight silicone gum[2] dissolves in ethyl acetate to the extent of about 15 percent. When it is incorporated at the rate of 20 percent in granular diatomaceous silica, it also gives excellent results with many pesticides. A typical chromatogram for Thiodan ether, Thiodan isomers I and II, and Tedion on a silicone column is shown in Fig. 5.

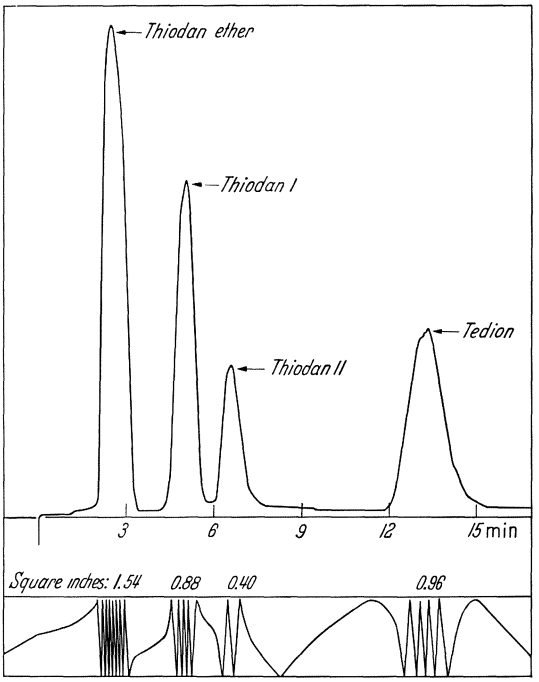


Fig. 5. Chloride chromatogram, 10 μg. each of Thiodan ether, Thiodan I and II, and Tedion, using a 3-foot Vycor quartz column packed with 20 percent silicone grease on Chromosorb P. Column temperature 220° C., block temperature 270° C., helium flow rate 160 ml./minute

Goodwin *et al.* (1961) have reported the use of epoxy resin[3] as a liquid substrate for column packings. Epoxy resin has been applied at the rate of 2.5 percent by weight on Chromosorb P (30/60 mesh, acid-washed) and found to give excellent results in the MCGC. This type of column will separate the isomers of DDT from the two isomers of Thiodan, as shown

[1] DC-200 Oil manufactured by Dow Corning Corp., Midland, Michigan.
[2] Silicone SE-30 manufactured by General Electric Co., Silicone Products Dept., Waterford, New York.
[3] Epon Resin 1001 manufactured by Shell Chemical Co., San Francisco, California.

in Fig. 6, when using the halide detector. It will also separate ethion from the Thiodan isomers when using the sulfur detector as shown in Fig. 7.

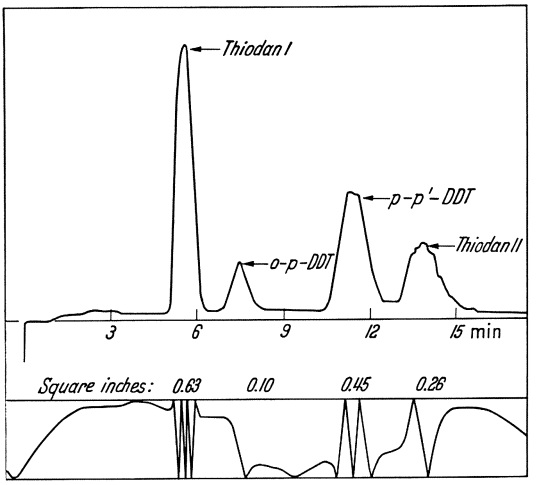


Fig. 6. Chloride chromatogram of 10 μg. each of Thiodan (I and II) and technical DDT (*o,p'*- and *p,p'*-isomers) using a 3-foot Vycor quartz column packed with 2.5 percent Epon resin on Chromosorb P. Column temperature 192° C., block temperature 262° C., and a helium flow rate of 150 ml./minute

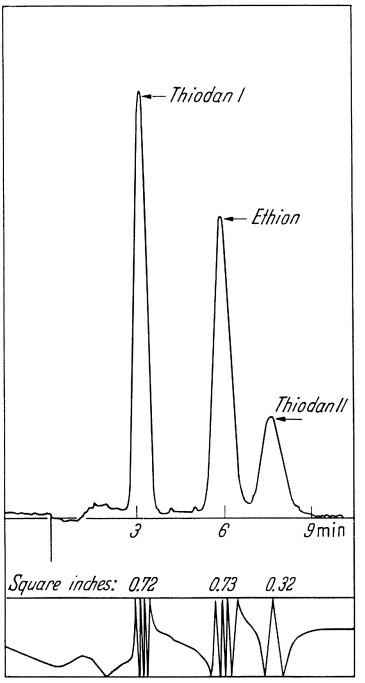


Fig. 7. Sulfur chromatogram of 16 μg. of Thiodan I, 6 μg. of ethion, and 8 μg. of Thiodan II using a 3-foot Vycor quartz column packed with 2.5 percent Epon resin on Chromosorb P. Column temperature 190° C., block temperature 260° C., and a helium flow rate of 160 ml./minute

A large number of liquid substrates and solid supports have been described in the literature. Only a few of these liquid substrates will function at temperatures in excess of 240° C. without decomposition or excessive bleeding from the column. Most pesticides will elute from a 6-foot column in 5 to 15 minutes at temperatures of 240 to 260° C. Therefore, it is not practical to use liquid substrates that are not stable at temperatures of 240° C. or above.

Much more research is needed to find better solid supports and more kinds of high temperature liquid substrates. Theoretically, it would be possible to identify a compound within a class by finding its retention time first on one substrate and then on another substrate at a given temperature and carrier gas flow rate. For example, Thiodan has a low and a high melting isomer. These two isomers have retention times of 5 and 6.5 minutes on a silicone column, and 4 and 10 minutes on the epoxy resin column at a given temperature and gas flow rate.

COULSON (1962) has shown through thermodynamic considerations and calculations that aluminum metal should be the most inert of all common metals as a catalyst in decomposing organic compounds at high temperatures. It is difficult to find pure aluminum tubing for making columns. Observations in making and using aluminum columns have shown that best results are obtained with the softer grades of aluminum tubing when working a few micrograms of pesticides. It has also been noted by many that better results are obtained with a new column after 2 to 4 milligrams of DDT have been passed through it. The DDT appears to serve as a conditioning agent which is interpreted as the DDT reacting with active impurities of the metal or covering the impurities (see also GUNTHER *et al.*, 1962). If a new column is prepared from the aluminum tubing of an old used column, the conditioning treatment is often not necessary. Columns made from pure quartz do not appear to need any conditioning or use to obtain optimum results after they have been heated to the desired temperature.

Length of columns does not appear to be critical since temperature and gas flow can be adjusted to give a desired retention time. Good resolution of pesticides has been obtained with columns varying in length from 3 to 12 feet with a diameter of 1/4 inch (O.D.). Six-foot columns are in general use.

b) Injection block

The injection port (vaporization chamber) in the instrument block is probably the most critical part of the entire MCGC. The aluminum block is well designed with perhaps the best heat reservoir of any gas chromatograph. The interior of this chamber is aluminum metal. Experience with four separate blocks has shown the need to condition this vaporization chamber before consistent and high theoretical recoveries can be obtained. This conditioning of the chamber can be accomplished by heating it to 250—270° C., and injecting four 2-milligram portions of DDT at 2-minute intervals with the vent open to avoid large contamination of the titration cell. Approximately 4 to 6 hours are required to obtain a normal base line

on the recorder because a portion of the DDT is decomposed and resulting chloride compounds bleed out of the system slowly. Once the block is conditioned in such a manner or with trisbiphenyl phosphate (GUNTHER *et al.*, 1962) earlier mentioned, no further treatment is necessary until it becomes fouled with excess organic material or carbon. This condition is detected by malformed peaks and low recoveries after the installation of a new column. The block can be cleaned out by removing it from the oven, washing it with 5 percent alkali, concentrated nitric acid, water, and a final rinse with acetone.

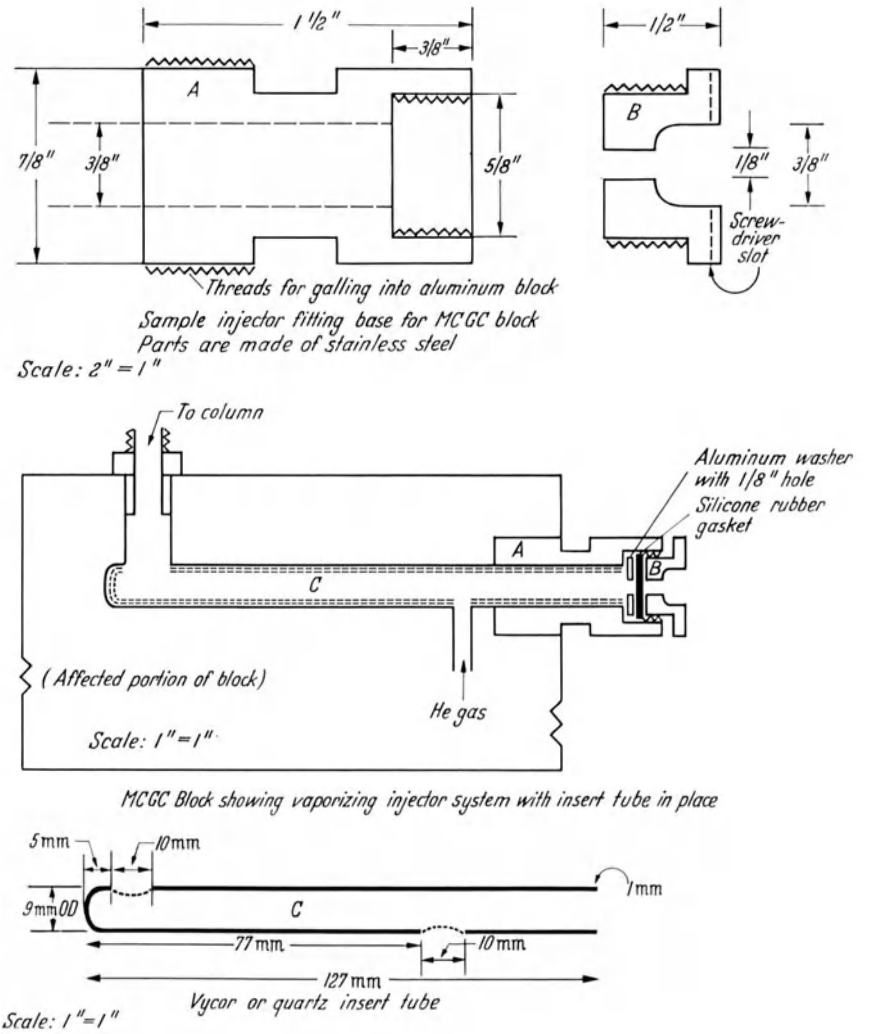


Fig. 8. Detailed drawing of quartz injection tube and necessary changes in MCGC block for use of the tube

Many plant extracts cannot be injected into the block without contamination to the point of obtaining atypical peaks and recoveries. Leafy vegetable extracts must be subjected to prior cleanup procedures to avoid block contamination. New and cleaned aluminum blocks generally must be conditioned as above before normal recoveries are obtained consistently. Therefore, is was concluded that the instrument could be made even more useful for routine analysis if an easily replaceable tube could be developed for the block in the gas chromatographic section of the MCGC. CASSIL (1961) has developed such a tube that has proven to be successful in routine analysis on many samples of leafy vegetable extracts without prior cleanup.

Aluminum tubes, with and without silicone coating, were used first but these were no more inert than the aluminum block without the tube and also required conditioning. Platinum tubes yielded close to the theoretical values of many compounds but are extremely costly. Vycor[1] or quartz tubes have proven to be extremely satisfactory and yield practically theoretical values on compounds such as aldrin, Tedion, Thiodan, DDE, etc. These tubes are relatively low in cost so that several can be available for replacement as often as they become contaminated. The contaminated tube is cleaned by allowing it to stand in dichromate-sulfuric acid cleaning solution and rinsing it with water.

When this replaceable tube is used in the MCGC block, no prior cleanup procedures are necessary for extracts of alfalfa, lettuce, cabbage, or other leafy vegetables. Atypical peaks and low recoveries occur on the recorder as soon as an excessive amount of extract builds up in the tube. The tube can be changed in about 2 minutes. A new tube will immediately give good resolution of the compounds and good recoveries.

The necessary change in block design and the design of the quartz tube are given in Fig. 8.

VIII. Types of extracts analyzed

Surface extracts of apples, pears, peaches, plums, prunes, cherries, artichokes, oranges, lemons, apricots, strawberries, melons, peppers, and figs have been analyzed, without prior cleanup, for pesticides including Thiodan, Tedion, ethion, and DDT. Extracts of macerated alfalfa, broccoli, cabbage, Brussels sprouts, watercress, tomatoes, hops, beans, bean straw, celery, carrots, lettuce, sugarbeet tops, and mint have been analyzed for such pesticides as Tedion, Thiodan, ethion, DDT, and dieldrin. Lemon oil, orange oil, and mint oil have been analyzed for Tedion without prior cleanup by injecting 100 microliters of pure oil into the injection tube.

Extracts from unsprayed surface stripped fruits, such as apples, pears, prunes, peaches, and cherries, do not show any measurable reading on the recorder. Extracts from leafy vegetables and root crops have measurable amounts of halides. The level of the natural organic halides ranges from 0.05 to 1.0 p.p.m., alfalfa having the largest amuont (see also GUNTHER and BLINN, 1955). These organic halides generally do not show on the recorder as definite peaks but as a continuous elevated base line or mound

[1] Vycor manufactured by Corning Glass Works, Corning, New York.

over a period of about 10 minutes. Since this measurable amount of halide does not show as one or more definite peaks, it is thought that there are many organic halides present in minute amounts in vegetable crops. Inorganic chlorides do not gas chromatograph under the conditions of the method.

Definite peaks on the recorder have been obtained many times from so-called "unsprayed fruit" but these have generally been found to be due to spray drift or pesticides being used other than the particular one under investigation.

The general practice has been to subtract the amount of halide in the elevated base line over the period of the base of the peak sought. No correction has been made if a definite peak at the correct retention time has been found since further investigation generally shows that this is not due to natural-occurring substances.

Table III. *Expected percentage recovery through a standard chromatographic column using silicone or epoxy liquid substrates*

Compound	Percent Expected range of column efficiency
Aldrin	90—100
Casoron	80— 90
Chlorendic Acid	60— 70
DDD	85— 95
DDE	90—100
DDT	60— 80
Dichlone	60— 70
Dicryl	70— 80
Dieldrin	70— 80
Endrin	90—100
EPN	70— 80
Ethion	90—100
Kelthane	70— 80
Malathion	80— 90
Methoxychlor	80— 90
Mitox	85— 95
Ovex	80— 90
Parathion	80— 90
Solan	65— 75
Tedion	80— 95
Thiodan I isomer	85— 95
Thiodan II isomer	85— 95
Thiodan diol	85— 95
Thiodan ether	40— 60
Trithion	80— 90

Experience to date with the sulfur cell has been primarily with fruit surface extracts with no measurable amount of natural sulfur-containing organic compounds. Some vegetable crop extracts may show natural-occurring sulfur compounds.

Analysis by the MCGC is limited in the pesticide field to those organic compounds containing chlorine, bromine, or sulfur. Approximately 90 percent of the currently available pesticides contain these halides or sulfur and

can be analyzed satisfactorily. A list of twenty-five compounds analyzed with approximate column efficiencies are shown in Table III. BURKE and JOHNSON (1962) listed a large number of pesticides that gas chromatograph in the MCGC as given in Table IV.

Table IV. *Relative retention ratios of chlorinated pesticides in the MCGC*[a] *column: 20% silicone Grease and 80% Chromosorb.* Carrier gas flow rate: 120 ml./min. Injection block temperature: 250° C. Column temperature: 220° C.

Pesticide	Ratio	Pesticide	Ratio
DDVP	0.1	α-Chlordane	1.39
Dipterex	0.1	Ovex	1.40
Phosphamidon	0.1	Thiodan	*1.42*[b], 1.86
Dichloropropene	0.12	β-Chlordane	1.51
Ethylene dichloride	0.12	DDE	1.58
Methylene chloride	0.12	Ethyl hexyl ester 2,4-D	1.61
Dichloral Urea	0.13	Dieldrin	1.65
Chlorpicrin	0.13	Aramite	1.66
Ethylene dibromide	0.14	Tech. DDT	1.71, 2.0, *2.5*[b]
Ethide	0.15	Perthane	1.78
p-Dichlorobenzene	0.16	Iso-octyl ester 2,4-D	1.84
Monuron	0.17	Chlorobenzilate	1.89
Neburon	0.19	DDD	1.90
Diuron	0.19	Prolan	2.0, *2.3*[b]
CIPC	0.37	Endrin[c]	*2.0*[b], 2.9
Me Ester 2,4-D	0.38	*o, p'*-DDT	2.0
Isopropyl ester 2,4-D	0.46	Butoxy ethanol ester 2,4,5-T	2.1
Hexachlorobenzene	0.51	Bulan	2.3, 2.6
PCNB	0.54	Dilan	2.3, 2.7
Lindane	0.55	Trithion	2.3
BHC	0.55	Iso-octyl ester 2,4,5-T	2.5
Pentachlorophenol	0.58	*p, p'*-DDT	2.5
Dichlone	0.59	BEP ester 2,4-D	2.9
Iso butyl ester 2,4-D	0.63	Methoxychlor	3.2
n-Butyl ester 2,4-D	0.68	Tedion	3.8
Mixed butyl esters 2,4-D	0.69	Chlordane (tech.)	0.54, 0.73, 0.79, 0.94, 1.12, 1.34, 1.47, 1.80, 2.25
Isopropyl ester 2,4,5-T	0.70		
Ronnel	0.78		
Heptachlor	0.81		
Chlorthion	0.96	Toxaphene	No definite peaks
Kelthane	0.96		
Aldrin	1.00		Continuous from 2—25 min.
Butyl ester 2,4,5-T	1.02		
Dyrene	1.09	BEP ester 2,4,5-T	0.69, 1.05, 2.12, 2.51, 4.42
Captan	1.12		
Sulphenone	1.15	Co-Ral	6.4
Phaltan	1.16		
Heptachlor epoxide	1.18		
Chlorobenside	1 22		
Butoxy ethanol ester 2,4-D	1.37		

[a] From preprint of J. BURKE and L. JOHNSON, Division of Food, Food and Drug Administration, U.S. Dept. of Health, Education, and Welfare, Washington, D.C. (Reproduced by permission of the authors).

[b] In case of more than one peak, the major peak is underlined.

[c] Endrin's ratio appears to shift occasionally from about *1.85*—2.6 to *2.1*—3.0.

Results obtained on surface extracts of apricots, lemons, grapes, pears, and apples for Tedion by the MCGC procedure as compared to those by a colorimetric method by FULLMER and CASSIL (1958) are given in Table V. The values found by the two methods are in close agreement.

Table V. *Tedion residues found on five crops as obtained by both MCGC and colorimetric methods* [a]

Sample No.	Crop	Field treatment	p.p.m. Tedion found	
			MCGC	Colorimetric [b]
1	Apple	Sprayed	1.2	1.0
2	Apple	Sprayed	1.4	1.5
3	Apricot	Unsprayed	<0.05	<0.05
3[c]	Apricot	Unsprayed	9.7	8.3
4	Apricot	Sprayed	8.3	9.0
5	Apricot	Sprayed	11.0	11.0
6	Grape	Unsprayed	<0.05	<0.05
6[d]	Grape	Unsprayed	1.9	1.5
7	Grape	Sprayed	1.6	1.7
8	Grape	Sprayed	1.6	1.8
9	Lemon	Sprayed	1.2	1.2
10	Lemon	Sprayed	1.1	1.3
11	Pear	Sprayed	1.5[e]	2.4
12	Pear	Sprayed	1.8	2.0

[a] Percent average mean deviation between methods = 8.1.
[b] FULLMER and CASSIL (1958).
[c] 10 ppm Tedion added.
[d] 2 ppm Tedion added.
[e] MCGC method showed presence of another compound which interfered with colorimetric method.

A similar set of data for Thiodan residues found on apples, cabbage, and peppers by the MCGC procedure and a sulfur-dioxide colorimetric method by GRAHAM and KALDON (1962) is shown in Table VI. These results also demonstrate the reliability of the instrumental method.

Table VI. *Thiodan residues found on three crops by both MCGC and sulfur-dioxide colorimetric methods* [a]

Sample No.	Crop	p.p.m. Thiodan found	
		MCGC	Colorimetric [b]
1	Apple	2.2	2.1
2	Apple	0.75	0.58
3	Cabbage	0.40	0.44
4	Cabbage	2.9	2.8
5	Cabbage	8.3	12.0
6	Cabbage	0.80	0.35
7	Peppers	0.30	0.29
8	Peppers	0.15	0.20
9	Peppers	0.10	0.16
10	Peppers	2.7	2.3
11	Peppers	4.4	4.1
12	Peppers	0.41	0.38

[a] Percent average mean deviation between methods = 22.
[b] GRAHAM and KALDON (1962).

Ethion spray residues on pears as determined by MCGC and cholinesterase methods on the same extract are given in p.p.m. in Table VII. The compound was determined by the sulfur cell since there is no chlorine present in the molecule. The results obtained by the two methods are in excellent agreement.

Table VII. *Ethion residues found on pears as obtained by both MCGC and cholinesterase methods* [a]

Sample No.	p.p.m. Ethion	
	MCGC	Cholinesterase
1	0.46	0.38
2	0.54	0.68
3	0.55	0.74
4	0.68	0.63
5	0.82	0.96
6	0.88	0.86
7	0.88	0.92
8	0.96	1.04
9	0.96	0.91
10	1.01	0.93
11	1.03	0.87
Average	0.80	0.81

[a] Percent average mean deviation between methods = 11.

The inability of the silicone-Chromosorb gas chromatographic column or other known types of column packing materials to effect separation or resolution of all pesticides makes it necessary to identify the peaks on the recorder either by history of the sample or by some other method of analysis. Compounds containing no sulfur will not analyze or show peaks in the sulfur cell and those containing no halide will not analyze in the silver cell. Therefore, two compounds (e.g. parathion and DDT), which may not resolve in the column, can be separated by the use of two detectors because parathion contains sulfur with no halide, and DDT contains chlorine with no sulfur.

Some compounds (e.g., Thiodan and Tedion) contain sulfur and chloride, and can be determined equally well in both the sulfur and silver titration cells. This offers a means of identifying these two compounds which contain a different percentage of sulfur and chloride. It is unlikely that any two pesticide compounds would have the same percentage of sulfur and chloride. Thiodan has 7.9 percent sulfur and 52.2 percent chloride; whereas Tedion has 9.0 percent sulfur and only 39.8 percent chloride.

There are at least seven variables which can be used to provide resolution or separation of mixed pesticides in a single solution.

(1) The length of the chromatographic columns can be varied from 2 to 12 feet. If short columns are used, two compounds having similar retention times cannot be resolved. In many cases, where it is desirable to analyze a single compound or a group that is easily resolved, it is expedient to use a short column so that more samples can be analyzed per unit time. As the length of the column is increased from 2 to 12 feet better resolution of the compounds, which tend to overlap in retention time, can be obtained.

(2) The carrier gas flow can be varied from about 50 to 200 milliliters per minute. Fast rates of gas flow decrease retention time of compounds, conversely slow rates of flow increase retention time and resolve the compounds that tend to overlap.

(3) The temperature of the chromatographic column oven can be varied from 50 to 300° C. This is another means of varying the retention time of compounds. Although the column oven is normally operated at 250° C. with a 6-foot column, and 210° C. with a 3-foot column, temperatures can be increased or decreased to obtain the desired retention times.

(4) Column packing materials may be varied as to type of solid support and liquid substrate to obtain different retention times of compounds. For example, Thiodan and DDT can be separated on an epoxy resin type liquid substrate but they cannot be separated on a silicone liquid substrate.

(5) Injection block temperatures can be varied at times to give sharper and narrower peaks which will aid in resolution. It is often advantageous to carry the block temperature 10 to 15 degrees (or even more) higher than the column temperature which serves to flash vaporize the compound and get it all into the column over a very short period, thus leading to narrow peaks. If peaks are narrow, there is less tendency for the peaks to overlap one another.

(6) Detector cells may be changed from the halide to sulfur type to separate compounds effectively. Trithion has a retention time similar to that of DDT on a silicone column. Both compounds have chloride but only Trithion contains sulfur. Therefore, the Trithion can be determined accurately in the presence of DDT by use of the sulfur cell.

(7) If two compounds cannot be separated by any of these means, chemical derivatives of one of the compounds can possibly be made and in this way effect separation of two or more compounds.

Table VIII. *Common and chemical names of pesticides mentioned in text*

Common or trade-name designations of compounds mentioned in the text are listed alphabetically below, with the chemical name conforming to Chemical Abstract's nomenclature of the major ingredient.

Aldrin 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene

Aramite® .... 2-(*p-tert*-butylphenoxy)-1-methylethyl-2-chloroethyl sulfite

BHC .... Mixture of stereoisomeric 1,2,3,4,5,6-hexachlorocyclohexanes

Bulan® .... 1,1-bis(*p*-chlorophenyl)-2-nitrobutane

Captan *N*-trichloromethylmercapto-4-cyclohexane-1,2-dicarboximide

Casoron® 2,6-dichlorobenzonitrile

Chlordane .... 2,3,4,5,6,7,8,8-octachloro,2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (technical material contains both α and β isomers)

Chlorendic acid .... 1,4,5,6,7,7-hexachloronorbornene-2,3-dicarboxylic acid

Chlorobenside® .... *p*-chlorobenzyl *p*-chlorophenyl sulfide

Chlorobenzilate® .... ethyl 4,4′-dichlorobenzilate

Chloro IPC .... Isopropyl *N*-(3-chlorophenyl) carbamate

Chloropicrin .... trichloronitromethane

Chlorthion® .... O-(3-chloro-4-nitrophenyl) O,O-dimethylphosphorothioate

Table VIII (continued)

Co-Ral® .... O,O-diethyl O-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate
DDD .... 2,2 bis-(*p*-chlorophenyl)-1,1-dichloroethane
DDE .... 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene
DDT .... 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-isomer)
DDT .... 1,1,1-trichloro-2(*p*-chlorophenyl)2-(*o*-chlorophenyl)ethane (*o,p'*-isomer)
DDVP .... O,O-dimethyl-2,2-dichlorovinyl phosphate
Dichlone® 2,3-dichloro-1,4-naphthoquinone
Dichloral Urea .... 1,3-bis(2,2,2-trichloro-1-hydroxyethyl) urea
p-Dichlorobenzene 1,4-dichlorobenzene
2,4-Dichlorophenoxy acetic acid (butoxyethoxypropyl ester of)
2,4-Dichlorophenoxy acetic acid (butoxyethanol ester of)
2,4-Dichlorophenoxy acetic acid (ethyl hexyl ester of)
2,4-Dichlorophenoxy acetic acid (isobutyl ester of)
2,4-Dichlorophenoxy acetic acid (isooctyl ester of)
2,4-Dichlorophenoxy acetic acid (isopropyl ester of)
2,4-Dichlorophenoxy acetic acid (methyl ester of)
2,4-Dichlorophenoxy acetic acid (mixed butyl esters of)
2,4-Dichlrophenoxy acetic acid (*N*-butyl ester of)
Dichloropropene 1,3-dichloropropene
Dicryl® .... *N*-(3,4-dichlorophenyl) methacrylamide
Dieldrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene
Dilan® .... one part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and two parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane
Dipterex® .... O,O-dimethyl(1-hydroxy-2,2,2-trichloroethyl) phosphonate
Diuron .... 3-(3,4-dichlorophenyl)-1,1-dimethylurea
Dyrene .... 2,4-dichloro-6-(*o*-chloroanilino)-s-triazine
Endrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene
EPN .... O-ethyl O-*p*-nitrophenyl phenylphosphonothioate
Ethide® 1,1-dichloro-1-nitroethane
Ethion .... O,O,O',O'-tetraethyl *S,S'*-methylene bisphosphorodithioate
Ethylene dibromide 1,2-dibromo-ethylene
Ethylene dichloride 1,2-dichloro-ethylene
Heptachlor 1,4,5,6,7,8,8-heptachloro-3a,4,5,5a-tetrahydro-4,7-*endo*-methano-indene
Heptachlor epoxide .... 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,7a-tetrahydro-4,7-methanoindene)
Hexachlorobenzene .... hexachlorobenzene
Kelthane® .... 4,4'-dichloro-α-(trichloromethyl)benzhydrol
Lindane .... Gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane
Malathion .... O,O-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate
Methoxychlor .... 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane
Methylene chloride .... dichloromethane
Mitox® .... *p*-chlorobenzyl-*p*-chlorophenyl sulfide
Monuron .... 3-(*p*-chlorophenyl)-1,1-dimethylurea
Neburon .... 1-*n*-butyl-3-(3,4-dichlorophenyl)-1-methylurea
Ovex .... *p*-chlorophenyl *p*-chlorobenzenesulfonate

Table VIII (continued)

Parathion .... O,O-diethyl O-*p*-nitrophenyl phosphorothioate
PCNB .... pentachloronitrobenzene
Pentachlorophenol .... pentachlorophenol
Perthane® .... 2,2-dichloro-1,1-bis(*p*-ethylphenyl)ethane
Phaltan .... *N*-(trichloromethylthio)phthalimide
Phosphamidon® .... dimethyl diethylamido-1-chlorocrotonyl(2)phosphate
Prolan® .... 2-nitro-1,1-bis(*p*-chlorophenyl)propane
Ronnel® .... O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate
Solan® .... *N*-(3-chloro-4-methylphenyl)-2-methylpentanamide
Sulfenone® (Sulphenone) .... *p*-chlorophenyl phenyl sulfone
Tedion® .... *p*-chlorophenyl-2,4,5-trichlorophenyl sulfone
Thiodan® .... 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide (isomers I and II)
Thiodan diol® .... 1,4,5,6,7,7-hexachloro-2,3-bis(hydroxymethyl)-bicyclo-(2.2.1)heptene-5
Thiodan ether® 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran
Toxaphene .... Essentially a mixture of isomers of octachlorocamphene (67—69 percent chlorine)
2,4,5-Trichlorophenoxy acetic acid (butoxyethoxypropyl ester of)
2,4,5-Trichlorophenoxy acetic acid (butoxyethanol ester of)
2,4,5-Trichlorophenoxy acetic acid (butyl ester of)
2,4,5-Trichlorophenoxy acetic acid (isooctyl ester of)
2,4,5-Trichlorophenoxy acetic acid (isopropyl aster of)
Trithion® .... *S*-(*p*-chlorophenylthiomethyl)O,O-diethylphosphorodithioate

® Trademark

Summary

Routine use of the microcoulometric gas chromatograph has shown it to have a greater degree of sensitivity for many pesticides than other known residue analytical methods. It is capable of determining from 0.25 to 50 μg. of a pesticide, depending on the percentage of sulfur or chloride in the compound. The chloride is determined by silver precipitation and the sulfur by oxidation of sulfur dioxide to sulfur trioxide with iodine. The precision and accuracy of the instrument is well within plus or minus 5 percent in the range of 0.5 to 50 μg. The linearity of the coulometer is excellent over its entire range of 4 to 512 ohms. A greater number of residue samples (20 to 30) per man day can be analyzed by this procedure than any other method formerly used. Combinations of pesticides can be analyzed by a single injection into the instrument provided they can be resolved at a specific temperature, rate of carrier gas flow, on a single column packing, and in a single detector. For example, when Thiodan, DDT, and lindane are all present in a single extract, all can be determined with a single injection in about 15 minutes.

Isomers of some pesticides can also be resolved and determined quantitatively. Identity of peaks must be made through experience, standard solutions, history of sample, or by other methods of analysis. It is a method that does not require rigid cleanup of sample if instrument is fitted with a replaceable quartz or platinum injection tube.

Aluminum tubing that has been used as a chromatographic column, when repacked with new packing material, gives better results immediately than new aluminum tubing. Chromatographic columns, made from pure quartz and packed with either epoxy resin or silicone packing materials, give excellent results as soon as they are brought to the desired temperature. Methods are given for the preparation of silicone and epoxy resin column packing materials that give good resolution and recoveries of many pesticide compounds.

The microcoulometric gas chromatograph offers a ready-made method of analysis for many new compounds where it might take many weeks to develop a chemical method.

Résumé*

L'utilisation quotidienne du chromatographe à détection par microcoulométrie a montré qu'il était plus sensible pour de nombreux pesticides que toute autre méthode connue d'analyse des résidus. Il peut doser de 0,25 à 50 μg de pesticide, selon le pourcentage de soufre ou de chlore du composé. Le chlore est dosé par argentimétrie et le soufre par iodométrie. La précision de l'instrument est de plus ou moins 5% entre 0,5 et 50μg. La linéarité du coulomètre est excellente sur tout le domaine compris entre 4 et 512 ohms. Cette technique permet l'analyse d'un plus grand nombre d'échantillons de résidus (20 à 30) par journée de travail que autre méthode employée jusqu'içi. Des mélanges d'insecticides peuvent être analysés en une seule injection dans l'appareil pourvu qu'ils puissent être séparés à une certaine température, avec une vitesse déterminée du gaz éluant, sur une seule colonne garnie et un seul détecteur. Par exemple, lorsque le Thiodan, le DDT et le Lindane existent simultanément dans un seul extrait, ils peuvent être tous dosés grâce à une seule injection en 15 minutes environ.

Les isomères de quelques pesticides peuvent aussi être séparés et dosés. L'identité des pics doit être déterminée expérimentalement grâce à des solutions étalons, l'historique de l'échantillon ou par d'autres méthodes d'analyse. C'est une méthode qui n'exige pas une purification rigoureuse de l'échantillon si l'appareil est équipé d'un tube d'injection interchangeable en quartz ou en platine.

Les tubes d'aluminium utilisés comme colonnes donnent de meilleurs résultats lorsqu'ils sont regarnis avec un nouveau remplissage que lorsqu'ils sont neufs. Les colonnes à chromatographie en quartz pur et garnies de matériaux impréneés de silicone ou de résine époxy donnent d'excellents résultats aussitôt qu'elles sont portées à la température désirée. Des méthodes sont données pour la préparation des garnissages de colonnes avec la silicone et la résine époxy qui donnent une bonne séparation et un bon rendement pour de nombreux composés pesticides.

Le chromatographie à gaz et microcoulométrie offre une méthode toute faite d'analyse pour beaucoup de composés nouveaux pour lesquels des semaines de au point seraient nécessaires pour élaborer une méthode chimque d'analyse.

* Traduit par R. MESTRES.

Zusammenfassung*

Routinegebrauch der mikrocoulometrischen Gaschromatographie hat gezeigt, daß diese für viele Pesticide eine höhere Empfindlichkeit aufweist als irgendeine andere bekannte Rückstandsanalysenmethode. Sie ist in der Lage, 0,25—50 μg Pesticid zu erfassen, je nach dem Schwefel- oder Chlorgehalt der Verbindung. Chlor wird als Chlorid mittels Silberfällung und Schwefel durch Oxydation von Schwefeldioxyd zu Schwefeltrioxyd mittels Jod bestimmt. Die Fehlergrenze des Instrumentes beträgt allerhöchstens ± 5% im Meßbereich von 0,5—50 μg. Die Linearität des Coulometers über seinen ganzen Meßbereich von 4—512 Ohm ist ausgezeichnet. Von einer Arbeitskraft kann mit diesem Verfahren an einem Tage eine größere Anzahl von Rückstandsproben (20—30) analysiert werden als mit irgendeinem früher benutzten. Pesticid-Kombinationen können durch eine einzige Injektion in das Instrument analysiert werden, vorausgesetzt sie können bei einer spezifischen Temperatur und Trägergasgeschwindigkeit auf einer einzigen Säulenfüllung und in einem einzigen Detektor getrennt werden. Wenn z. B. Thiodan, DDT und Lindan alle gleichzeitig in einem einzigen Extrakt zugegen sind, können sie alle mit einer einzigen Injektion von 15 Minuten bestimmt werden.

Isomere von Pesticiden können auch getrennt und quantitativ bestimmt werden. Die Identifizierung der einzelnen „peaks" setzt Erfahrung, Vergleich mit Standards, Kenntnis der Vorgeschichte der Analysenproben oder Anwendung anderer Analysenverfahren voraus. Es ist eine Methode, die keine strenge Vorreinigung der Probe erfordert, falls das Instrument mit einem auswechselbaren Quarz- oder Platin-Injektionsrohr ausgerüstet ist.

Aluminiumrohr, das schon einmal für eine chromatographische Säule verwendet wurde, gibt sofort bessere Resultate als neues Aluminiumrohr. Chromatographische Säulen, die aus reinem Quarz hergestellt und entweder mit Epoxydharz- oder mit Siliconfüllkörper gefüllt sind, geben, sobald sie auf die gewünschte Temperatur gebracht sind, ausgezeichnete Ergebnisse. Es werden Methoden für die Herstellung von Silicon- und Epoxydharzfüllkörpern angegeben, die für viele Pesticid-Verbindungen gute Trennungen und Ausbeuten geben.

Die mikrocoulometrische Gaschromatographie bietet fertige Analysenmethoden für viele neue Verbindungen, für die man sonst manche Wochen zur Ausarbeitung einer chemischen Methode benötigen würde.

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Some important properties of pesticide* deposits on various surfaces

By
W. M. Hoskins**

Contents

I. Introduction

Interest in the details of insecticide application has gone through an interesting cycle within the past thirty years. It was in the 1930's that certain problems in insect control with the materials then available became acute. Scale insects on citrus had developed resistance to hydrogen cyanide in various areas and oil sprays were employed in its place. But the margin was narrow between the amount needed for insect control and that which injured the trees. Hence much attention was paid to formulations and dosages and deposits upon various parts of a sprayed tree. To a somewhat lesser degree, the same situation had come about in the use of lime-sulfur solution for control of other scale insects on deciduous trees.

Other pressing problems were concerned with lepidopterous larvae, especially that of the codling moth on apples and pears. Lead arsenate had been the standard control for over a generation, but steadily increasing dosages were needed until ten to twelve applications at the rate of four pounds per 100 gallons of spray were needed in Colorado in the 1920's (Hough, 1928) and in other states soon thereafter. Instead of relying upon a certain sequence of spraying, it became the practice to maintain a minimum load of deposit, usually expressed in μg. lead arsenate per square inch of fruit surface, and analyses were made to check on the deposit as laid

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* Chemical names of pesticides mentioned in text are in Table II.

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down at each application and at intervals between applications. To make matters worse, tolerances, arising in the first place from the refusal of certain European countries to accept American fruit with large amounts of lead arsenate upon it, were imposed by the federal Food and Drug Administration. It is easy to understand that this situation of being caught between contrary requirements forced orchardists and the apple industry in general to pay much attention to the nature and magnitude of deposits and residues. For instance, there was a lively argument about the relative merits of continuous versus spotty deposits of lead arsenate. SMITH (1926) reported that a uniformly distributed type of deposit such as it given with a casein-lime spreader was more efficient, whereas MARSHALL (1937) strongly supported the spotty deposit resulting from the socalled invert or "dynamite" sprays containing loosely emulsified mineral oil along with the arsenical.

With the advent of DDT and other organic insecticides, all this was changed. These compounds were so much more effective than most former materials that even the most careless application gave good control. And the general feeling, based on the behavior of the few organic insecticides already in use, that these substances would soon be decomposed or evaporate and hence leave no residue, gave little incentive to study the nature or magnitude of deposits as had been done earlier. Consequently, interest in these matters lagged and little constructive work was done for several years.

The events that shattered this complacent attitude are too well known to need elaboration. First, the development of resistance on the part of many important pest species and secondly, the imposition of strict tolerances, sometimes zero, for nearly all widely used insecticides, have brought the amount and nature of insecticide deposits back into importance. The full cycle has been made but on a much grander scale than before, and many studies have been and are being made on the subjects of application of insecticides and the nature and persistance of deposits. Much the same story may be told about acaricides, fungicides and weedkillers, except that most of the modern developments came on later than in the case of insecticides, and the numbers so far developed are much smaller. It should be understood in the further discussion that all pesticidal deposits have much in common, and while, for simplicity of expression, most examples are drawn from insecticides, the same principles apply to the pesticides used for other purposes.

The use of an insecticide in the form of a dust or a spray consists of several successive processes. Assuming that the active material has been formulated und placed in the apparatus for use, the first step is from the apparatus to the surface, e.g., of a plant, on which the pest is present or to which it is expected to come. The term "application" is often used to denote the process of forming a deposit upon the surface. Of course, to some degree the application usually is made also directly to the insect, but this usually is of minor importance with persistent toxicants. The amount and nature of the deposit have been the subjects of well-nigh innumerable studies ranging from visual observation of gross distribution to the most refined study of the effects of nozzle design, pressure (of diluting liquid or air), relative

amounts of active and inert constituents, wetting and other accessory agents, and electrostatic charge on particle or surface.

The second step is transfer of a part of the deposit to the surface of some part of the insect's body. This may depend upon the tendency of a liquid to spread, as in case of an oil spray used for scale control or, more usually, upon activity of the insect as when it walks upon the treated surface. In case of a stomach poison, eating of the plant or cleaning of legs by drawing them through the mouth parts will be most significant. Unfortunately, relatively little attention has been paid to this extremely important second step, which may be designated by the convenient term "pickup".

The third step is passage of the toxicant from the surface of the insect's body into the tissues and ultimately to wherever the toxic action occurs. The factors concerned in this penetration and distribution are pertinent to the present discussion only insofar as the nature of the deposit affects them. For example, the state of subdivision of the toxicant will affect the area of the insect's integument which it reaches, or incorporation of a non-volatile solvent for the toxicant may be expected to alter the rate of passage through the integument. Specific examples of such effects will be mentioned later. For influence of the integument upon wetting and pickup, cf. HOSKINS (1958).

A pesticidal deposit may be described in simplest terms as a coating, either continuous or discrete, placed on a surface for the purpose of killing or disabling a pest organism which may be present already or which may arrive at a later time. Chemicals of the most diverse nature are used in accordance with accumulated experience in the control of several hundred kinds of pests. This coating, which usually is so light that it escapes notice unless examined with care, is the chief means for protecting both quality and quantity of most agricultural products. Accordingly, its nature and behavior have received considerable attention, but many important properties are as yet imperfectly understood. The present account aims first to treat of insecticidal and fungicidal deposits as a physical system whose behavior is controlled in part by the supporting surface and the surrounding atmosphere. Secondly, the phenomena which control the contact between pest organism and pesticide and the uptake of the latter will be examined briefly. In practical pest control the deposit may be upon the pest, or upon a surface which the pest is likely to contact. The present discussion will be limited to the latter case with occasional reference to phenomena common to both situations.

Application of a pesticide as a spray or dust involves subdivision into fine droplets or particles whose behavior both during flight from the nozzle and upon impact with the receiving surface is largely controlled by the size of the droplets or particles. It is understood, of course, that there is always a range of size on either side of the mean, the range varying considerably with the nozzle and pressure used to form the spray or the type of grinding and screening equipment used to produce the dust. Because of the well-nigh universal experience that for a given amount of pesticide higher toxicity is obtained as subdivision is made finer, there has been much emphasis upon extremely fine dispersion. This in turn has aggravated the problem of drift from the point of release, especially from aerial application (POTTS, 1946;

BROOKS, 1947; COURSHEE, 1959; YATES, 1960). The ramifications of this matter are not pertinent to the present discussion, except insofar as the state of subdivision affects the nature and distribution of the resulting deposit as will be mentioned in later sections.

II. Deposits as a physical system

Probably the simplest and most convenient classification of pesticidal deposits is into solid, liquid and solid-liquid combinations. Historically, dusts doubtless came first since ancient manuscripts occasionally mention use of sulfur or even road dust on diseased vines. Pesticidal dusts are always finely divided, but the actual size range and statistical average diameter vary widely and any theoretical optimum size is entirely governed by the purpose and condition of use. Both size and shape of particles are largely controlled by the method of preparation. Data on the more important materials will be given in the section on factors influencing pesticidal action.

Dusts. Dusts are classified primarily by the chief toxicant but with the exception of sulfur and certain arsenic and fluorine insecticides, from 80 to 99+ percent usually is so-called "inert" materials. These are used to give enough bulk for proper action of the application machines, and sometimes to prevent injury to treated plants from overdose of active ingredients. The diluents or fillers are of the most diverse nature but they seldom are truly inert (CHIU, 1939, 1940; HUNT, 1947; WATKINS and NORTON, 1955). In many cases activity of the toxicant is controlled by the choice of diluent, e.g., a basic substance such as lime will give a "fast" dust with nicotine sulfate since this is all converted to volatile free nicotine. On the other hand, lime decomposes DDT to the harmless ethylene derivative, DDE. Even when a definite chemical reaction does not occur, the degree of sorption of the toxicant has a controlling effect upon its action. Thus, to cite nicotine again, bentonite holds this substance so tightly upon its large external and internal surfaces that volatility is negligible and the dust is active only as a stomach poison.

Any chosen combination of toxicant and diluent may be made into a number of widely differing dusts by altering the method of mixing. For example, benzene hexachloride and a mineral diluent may be ground separately and mixed or they may be ground together to give dusts in which the particles of the two components are more or less independent of one another. On the other hand, if the benzene hexachloride dissolved in a volatile solvent is added to the whole amount of diluent, each particle of the latter will have benzene hexachloride upon its surface and the toxicant presents a very large area. Usually it is easier in the formulation process to impregnate a portion of the diluent in this way and then add the remainder, which may be a different solid, so only a portion of the total surface of the finished dust bears the toxicant.

The diluents used in pesticidal dusts are extremely varied in physical and chemical nature but may be classified primarily according to botanical or mineral source. Thus wood or bark flour, walnut shell flour, tobacco dust, and soybean flour illustrate the first group and are especially useful when low density is needed. A very considerable number of oxides, carbonates,

and silicates of mineral origin are the most widely used diluents, however, and they provide a wide range of properties (WATKINS and NORTON, 1947). Among the more important physical properties are density, abrasiveness, and flowability which primarily affect behavior of a dust while it is being applied. Range in sorptive capacity may be illustrated by bentonite on the high end and perhaps by sulfur on the low end. Probably the most important chemical property is the basic or acidic nature which is usually measured by the pH and total titratable base or acid liberated by contact with water.

Much attention has been given to the area of a given amount of dust for this affects the amount of toxicant that can contact a pest. With non-porous solids a knowledge of the distribution of size among the particles enables the surface area to be calculated. Numerous methods varying from measurement under a microscope to air permeation have been devised for determining this property. No method is universally applicable since the ultimate particles of various toxicants and diluents vary greatly in shape as is illustrated by electron microscope pictures of attapulgite, bentonite and other clays (HAUSER, 1945; WATKINS and NORTON, 1955). Individual particles often coalesce into aggregates which do not break up during application. This decreases the total area. If the particles are porous, i.e., have pits or internal channels, the available area is much more difficult to measure, and such procedures as adsorption of a dye from solution must be used. Attapulgite is an example of such a clay, the total area of a well processed sample of this fibrous substance being about 125 square meters per gram (MCCARTER, KRIEGER and HEINEMAN, 1950).

In order to improve the behavior of a dust various "conditioners" may be added. Finely divided sulfur, especially that formed by grinding, has a tendency to agglomerate into clumps that are hard to apply and do not adhere well. This may be prevented by inclusion of a few percent of very finely divided rosin, talc, magnesium carbonate or similar mineral substance (KUNKEL, 1950). These materials may be added to any dust which, because of particle shape or other reason, does not flow well. In case of pyrethrum or derris dusts, antioxidants are sometimes added to prolong the period of effectiveness. The wide use of organic phosphates which readily hydrolyze to form innocuous degradation products has led to formulation of various stabilized dusts, often of secret composition.

The modified dusts mentioned previously are all dry but doubtless the most commonly used conditioner is petroleum oil, or some other non-volatile liquid. Addition of such a substance changes the physical state of a dust radically. The outer surface of each particle is covered by a liquid film whose thickness depends upon the amount of oil used. Pits in the surface and internal passages become more or less completely filled. In such impregnated dusts, the surface liquid acts as an adhesive between particles, so agglomeration is increased, with consequent difficulty in securing uniform distribution. When applied to a plant, contact is chiefly by means of the liquid in which the active ingredient may be partly or wholly dissolved. As long as the oil remains there is little possibility for any considerable part of the preparation either to blow off or be washed away.

An important recent development is use of so-called "liquid dusts", which are mixtures of toxicant, diluent and sufficient oil to give a slurry which can be applied in a blast of air from either ground equipment or airplane. Enough water and emulsifying agent may be added to give a concentrated emulsion which is applied in the same way. Or the toxicant may be dispersed in a minimal amount of water plus a wetting or deflocculating agent, or both. It is obvious that these formulations are complex systems but a simplifying feature is the fact that they are used sparingly and there is little or no runoff, so each particle or clump of particles that reaches the surface remains where it strikes.

The application of a dust to a surface practically never results in the latter becoming covered completely. On the contrary, particles of the dust are scattered about in random fashion though there is a tendency for the deposit to be heavier near edges, e.g., of leaves, on stems or small twigs, and especially on hairs and other fine projections (HURTIG, 1948; YEOMANS *et al.*, 1949; GOOSSEN, 1958). Examination under the microscope will reveal that particles adhere to the surface in a most irregular manner so that it is impossible to calculate accurately the actual area that is covered. A prime consideration in controlling any pest is to apply enough toxicant so a lethal amount will reach each organism but excess must be avoided to keep cost down, avoid injury to the host, and escape from excessive residue at harvest.

Granules. The many problems resulting from the tendency of finely divided dusts to drift in air currents far from the point of application have led to interest in coarser formulations to which the name "granules" is often applied. It was found during mosquito control work during the second world war that coarsely divided Paris Green was more effective than the traditional fine dust for larval control when the breeding water lay beneath a thick canopy of leafy plants or trees. The reason was that heavier particles could bounce or roll off the upper surface of leaves and find their way downward while fine ones remained on the leaves. From this it was but a step to formulate many other insecticides with various carriers in pellet form. A very large literature has appeared on this subject in connection with the control of various pests that spend some part of their lives in the water. Thus WHITEHEAD (1951) found that control of *Psophora confinis* and *P. discolor* mosquito larvae in rice fields of Arkansas was especially effective with dieldrin or DDT in a pelleted mixture of bentonite and rice hulls since the intact pellets reached the water efficiently and sank, but upon their disintegration the rice hull portion rose again, thus distributing the toxicant throughout the water.

To a considerable degree the remarks concerning the relation of porosity of the carrier in ordinary dusts apply to granulated formulations since with these it is also possible to have the toxicant as separate particles or on the surface of the carrier pellets or absorbed to some degree within them.

A great variety of toxicants lend themselves to pellet formulation. A point of much interest is the release of the insecticide from the carrier. WEIDHAAS (1957) studied the release of parathion from attapulgite (Attaclay) granules and found uniform distribution in beaker tests after 24 hours, during which time most of the parathion entered the water. Chlorothion

was released more slowly and EPN, which is less than one-hundredth as soluble as parathion, never reached a measureable concentration. Not much difference was found with bentonite, vermiculite or pyrophylite, but montmorillonite released considerably less. Incorporation of kerosene or Triton X-100 had little effect upon release but Velsicol AR-60 cut it by one-half.

A study of the effect of forty-four petroleum solvents upon the release of parathion from 30/60 mesh attapulgite granules has been reported by MULLA and AXELROD (1960). Analyses were made by bioassay with fourth instar larvae of *Culex quinquefasciatus* mosquitoes. An inverse relation was found between distillation range and rate of release of parathion, expressed by the formula: rate = f (Flash point $\times$ initial boiling point $\times$ distillation range). Of course, this relation was found only for certain petroleum solvents and parathion on one carrier, but the work indicates how similar studies may be of value with other systems.

Granulated insecticides are useful also against other aquatic pests than mosquitoes, e.g., horsefly larvae, *Tabanus nigrovittatus* and *T. lineola,* by dieldrin and other chlorinated hydrocarbons on Attaclay granules (JAMNBACH and WALL, 1957). Sand fly larvae in the mangrove marshes of Florida were not controlled satisfactorily by aerial sprays because little reached the water, but 16/30 mesh bentonite granules impregnated with BHC gave excellent control for over twelve weeks and a commercial granulated dieldrin was almost completely effective for at least twenty-four weeks (LA BRECQUE and GOULDING, 1954).

Granular formulations are useful for control of certain agricultural pests. An early report by FARRAR (1953) mentioned good results with dieldrin or toxaphene on 30/60 mesh clay for control of green June bugs, corn root worms, sod webworms, ants, chinch bugs, white-fringed beetles, and cutworms. Use of granules is not limited to soils for good results have been secured with malathion, DDT or heptachlor against European corn borer larvae. Total residue was much lower than from comparable dust application, but distribution of the granules was more efficient since they were held largely on the whorl leaves where first brood larvae feed and the leaf axils where second brood larvae enter the stalks (COX *et al.,* 1956, 1957). Other situations in which the habits of the pest and nature of the infested plant are such that granules are advantageous have been reported in recent literature. Thus, parathion or malathion granules for control of seedcorn maggot on spinach foliage (WALTON and KANTACK, 1958), phorate (THIMET) on sugar beets for aphids, mites and leafhoppers (STARKER, 1960), and aldrin, dieldrin, heptachlor or toxaphene on 30/60 mesh Attaclay granules for the seed weevils, *Hypera meles* and *H. nigrirostris,* on crimson clover (TIPPINS, 1958) have all been reported to give good results with these widely varied pests on several crops.

It is obvious that deposits of granular formulations must be very different in character from those of ordinary finely divided dusts. The latter are intended to cover the exposed surfaces with many very small particles, but granules being fewer in number must leave much bare space. If the carrier swells and disintegrates in the presence of moisture, e.g., bentonite, coverage will be more complete after dew or rain have had their effect. If contact

with a widely distributed pest is important as in case of aphids, mites, mosquito larvae or soil-inhabiting species, such a breakup of the original granules is necessary. The distribution of granules upon a plant tends to be very uneven for they will roll off open areas of leaves and have little tendency to adhere to leaf edges, fine stems or plant hairs as dusts do, but slide into crevices and pockets. This is advantageous, of course, when the pest happens to frequent such locations. But probably above all other reasons, granules are advantageous because use of them avoids the difficulties associated with drift. In addition, simultaneous use with fertilizer in commercial fertilizer spreaders frequently reduces the cost of application.

Sprays. The traditional agricultural sprays are dilute solutions, emulsions or suspensions of a toxicant in water. The application of a solution to a surface, such as the leaves and branches of a tree, is intended to leave a film from which the water will evaporate and leave a more or less continuous deposit of toxicant. If the toxicant is a liquid its final distribution will coincide, except for slight spreading, with the distribution of the spray, which is governed by efficacy of wetting during application and the extent of drainage to lower areas. Little is gained by use of so much spray that it runs off, for preferential deposit of a solute occurs to only a minor degree. The volume of spray adhering controls the amount of toxicant that may be applied and hence the latter may be increased in only two ways: a) by increasing the concentration, or b) by adding some kind of thickening agent which enables a heavier film of spray to adhere. Starch, proteins, soluble gums and various polymerized substances are of some value for this purpose (WOODMAN, 1924), though in practical trials little or no benefit was found in case of copper sprays (SOMERS, 1957). Any leaf hairs or similar protuberances assist in retention of a thicker film.

Petroleum spray oils, either used straight or as solvents for toxicants such as the chlorinated hydrocarbons, form the dispersed phase in typical pesticidal emulsions. These sprays leave liquid droplets which spread on almost any surface to which they may be applied, and thus form a continuous film. During the spraying process the dispersed phase competes with the water for space and builds up what has been called "primary deposit" (SMITH, 1933; HOSKINS and BEN-AMOTZ, 1938; MOILLIET and COLLIE, 1951). Later, during evaporation of the adhering water film, an additional "secondary" deposit is formed which corresponds to the total deposit from a solution discussed above. Subsequent changes dependent upon the nature of the underlying solid, as will be discussed in a later section.

A truly enormous amount of theoretical and practical study has been devoted to the problem of securing the most efficient deposition of a finely divided liquid or solid from a water spray. Much of this is not pertinent to the present account of the nature of pesticidal deposits, but the water softeners, emulsifiers, deflocculators, wetting agents, and stickers which are almost always included all have effects upon the deposit of toxicant and its subsequent behavior. These phenomena have been ably discussed by FAJANS and MARTIN (1937, 1938) and by SOMERS (1956), and the special case of the effect of formulation of acaricides for control of citrus mites has been examined by EBELING and PENCE (1953).

In the case of a suspension, such as lead arsenate or DDT, the deposit resembles that from a dust, since individual particles or clumps are scattered at random over the surface. A characteristic feature is the heavy piles or streaks at the bottom edge of leaves or in other areas to which the water film has drained and left a heavy secondary deposit. Since these are difficult to remove they frequently complicate the residue problem very seriously. If a diluent is used with the toxicant as in the case of fifty percent wettable DDT or twenty-five percent wettable parathion, a mixed deposit is left unless the diluent was impregnated with the toxicant as discussed for the case of impregnated dusts.

Stickers, which usually are oils or gums, are added for the purpose of binding the solid toxicant more firmly to the sprayed surface. Some of the other accessory materials have a similar effect, so solids deposited from a water spray are almost always more adherent and persistent than dusts. However, inclusion of excess wetting agent will facilitate the washing effect of rain or dew as illustrated by attempts to improve Bordeaux mixture by various additives (KOVACHE and FICHEROULLE, 1945). Small amounts of wetting agents had no effect upon either initial deposit or persistence, but larger amounts resulted in heavy loss during damp weather. Similar results were obtained with lindane deposits on elm leaves (HORNSTEIN *et al.*, 1954). At one time an oil-lead arsenate combination was widely used for control of codling moth larvae. Minimum emulsifying and wetting agent was used so a very unstable system was formed in the spray tank. The affinity of lead arsenate for water was overcome by the surrounding oil and when the oil-soaked particles struck a leaf or apple the water ran away completely, whereas the lead arsenate and oil stuck tightly. Thus, by continued application to any area an extremely heavy deposit could be built up. By this method it was possible to obtain the heavy loads, e.g., up to 200 micrograms of As_2O_3 per cm^2. (MARSHALL, 1937), which were used in some regions prior to the advent of DDT. Unless applied with extreme care to ensure that all parts of a tree were sprayed equally, this formulation resulted in very unequal distribution and the heaviest deposits could not be brought down to the legal tolerance for residual lead and arsenic. Similar procedures may be used with modern insecticides, but usually are not practical because of limits on the permissible residue at harvest.

In addition to acting as a sticker for a solid toxicant, oils are sometimes used in larger amounts for control of another pest, e.g., DDT and oil for caterpillars and aphids. Such systems may act more like emulsions than suspensions since the liquid visibly engulfs the solid. Resistance to rain may make such combinations very valuable as in the winter control of peach blight and leaf curl (WILSON, 1942). Addition of four percent spray oil to Bordeaux mixture gave a heavier initial deposit and reduced loss during the rainy season by fifty percent or more. Similarly, addition of oil to DDT sprays led to slower loss of the deposit of DDT on grapes (TASCHENBERG and AVENS, 1960).

A very important question is how long before harvest a given insecticide may be applied without the residue exceeding a predetermined tolerance level. An immense body of data has been published on the rate of decrease

of deposit with time. Quantitatively, the decrease depends upon such phenomena as evaporation of the chemical, loss by wind and rain, or growth of the underlying surface, but in general the fraction remaining at various periods may be plotted along a descending curve of decreasing slope. If the only factor operating is evaporation and this is proportional to the amount present, the situation is an example of the usual law of decay, i.e., log fraction remaining $= -k \times$ time. The same rule holds fairly well when other factors are operating and makes possible the calculation of such useful quantities as half or three-quarters or nine-tenths life periods once the rate of loss has been established for a given situation. Knowing the original deposit needed to control the pest it is then possible to calculate how long before harvest this may be applied. For a more complete discussion of the rate of loss of insecticidal residues, the influence of the nature of the deposit, of weather factors, and of plant growth, reference may be made to the following reports: DORMAL and CAUSSIN (1957); HOSKINS (1961).

It should be emphasized that while one may speak of a certain weight of deposit resulting from an application of dust or spray, the figure is at best an average of widely varying deposits on the various surfaces concerned. Thus in practical work, the average deposit upon the upper leaves of a large tree may be only half that on the lower leaves. If individual leaves or fruit are considered, the variation is very much larger. Perhaps an extreme case is that of alfalfa hay dusted with five percent DDT dust. Samples taken from various parts of a field in as uniform a manner as possible contained from a fraction of one p.p.m. to twenty p.p.m. (SMITH, FULLMER and MESSENGER, 1948).

Even visual examination of any dusted or sprayed tree will reveal great variation in deposit among various leaves or even from point to point on a single leaf. It is to be expected that the parts nearest the nozzle will receive the largest amount. In case of a dust, separation of particles because of different size or density may result in varying ratio of toxicant to diluent. This is especially marked in case of application from an airplane. Similar separation of spray droplets of different sizes leads to deposits which at one extreme are thick and continuous and on the other are made up of small discrete droplets not large enough to unite and form a continuous film. Many laboratory devices have been constructed to give highly uniform deposits of both sprays and dusts but in any practical application, large variation is the rule. Not only the initial condition of the deposit but its subsequent behavior will vary from one region to another, depending upon exposure, amount of wind, and other conditions. Unfortunately, in many cases the pest occurs in least accessible locations, such as bud mites in the narrow spaces between immature petals or codling moth eggs and larvae near the tops of apple trees. Perhaps the most general example is given by the numerous insects that stay on the lower side of leaves, which is very difficult to reach with either a dust or a spray. This is one reason for the great interest at the present time in systemic toxicants which are carried throughout the plant by movements of its circulating fluids.

Charge on dust or spray. Considerable attention has been paid to the effect of electrostatic charge upon the retention of particles upon a surface. The existence of such charges upon various kinds of pesticidal dusts has been noticed for many years. Determinations made by catching a multitude of particles upon an electroscope leaf or other detecting surface give only the excess charge of one sign but yield no information regarding the condition of individual particles. The magnitude of overall charge varies with the method of producing a cloud of dust (MACLEOD and SMITH, 1943), and even may reverse in sign as in the case of lead arsenate (MOORE, 1925). A commonly used diluent, diatomaceous earth, acquires a greater average negative charge if sent through a constricted glass tube than when an open tube is used (FISHER, 1939). This could result from either increased rubbing of particles upon one another or upon the discharge tube, or both. A charging device suitable for attaching to a knapsack duster has been described by SHREEVE (1957) who cited data showing several-fold increase in deposit of copper dusts on several plants. GÖHLICH (1957) has stressed the improved uniformity of deposit from charged dusts because agglomerates do not form. They also adhere better because of the fine subdivision of the deposit.

Partial analysis of particles according to sign of charge has been accomplished by sending a cloud between two parallel plates of differing potential, as in the apparatus used by MACLEOD and SMITH (1943). With several dozen samples of dry insecticides and diluents, in all cases some powder deposited on each plate. Lead and calcium arsenates consistently favored the negative plate by about four-to-one, but different lots of sulfur varied from 61.2 to 18.7 percent on the negative plate. The ratio of positively to negatively charged powder was influenced somewhat by the nature and length of the discharge tube but apparently the nature of the material itself was most influential. Combinations of powders gave unpredictable segregation upon the collecting plates and it is of possible practical importance that addition of a very small amount of a second powder often strongly increased deposit of the predominant dust.

A detailed investigation of the charge on dust particles has been made by HANSEN (1948), by KUNKEL and HANSEN (1950), and by KUNKEL (1950) who photographed individual particles as they settled downward between vertical charged plates. Mass was determined by Stokes' law and charge by deflection in a known potential field. In every cloud both positive and negative particles occurred and whenever a substance had contacted only itself, i.e., sulfur dust from a sulfur-lined tube or quartz powder from a quartz tube, the total charges balanced. When the discharge tube was a different material the electrobalance was shifted in agreement with ordinary contact potentials, e.g., a sulfur cloud was strongly negative when passed through a metal discharge tube, but the effect was chiefly on the larger particles, since small ones very seldom contact the tube due to the ease with which they follow the air stream (SELL, 1931; POTTS, 1946). The charge per particle was roughly proportional to the surface area.

An important feature of this work is the failure to observe any deviation of a particle as it fell toward a slightly inclined uncharged plate. If a charged particle was attracted by the induced charge of opposite sign on the

plate, the distance through which an effect was exerted was less than the small part of a millimeter observable with low power magnification. The charge seldom exceeded a few electrons per particle for sizes up to one or two microns, but if a much stronger charge could be put on the particles by subjecting them to ionizing radiation an effect upon deposition might be brought about.

For the present it seems that electrostatic effects are more likely to be concerned with adhesion of a dust particle than with its initial deposition. Separating two materials always imparts to them equal and opposite charges whose magnitude depends upon the difference in dielectric properties. When a pesticidal particle severs contact with a surface upon which it has been resting, a difference in charges is set up in the same way as when a cloud is dispersed or moved along a discharge tube. The difference, however, is that these charges are proportionally greater upon very small particles and they come into being when the separation is only of molecular dimensions and hence have a strong ability to prevent the particle from escaping. The residual deposit which no amount of shaking or blowing can remove doubtless is held in part by this mechanism.

Since plant surfaces usually are waxy and of low electrical conductivity, it is obvious that local charges resulting from the settling of dust particles will introduce local effects upon deposition and retention of insecticidal dusts. Atmospheric humidity and especially rain will decrease or abolish such "hot spots". Much is still to be learned about the electrical forces involved in the application and retention of dusts.

Droplets of spray often become charged during shattering of a stream of liquid and this can be very greatly increased by putting a high potential upon the nozzle (WAMPLER and HOSKINS, 1939). However, deposition of suspended particles is not affected when a strongly charged spray is directed against the receiving surface, since the charge does not affect contact between the surface and the film of spray formed by impinging droplets. In case of a "spray dust" it is probable that imposition of a strong charge on individual spray droplets would result in more complete deposition of the droplets and hence of the pesticide in them.

The foregoing discussion will indicate that placing a pesticide in the proper place in a suitable condition is a matter of considerable difficulty. Usually the time of application must be synchronized with normal activity of the pest and this may extend over a considerable period during which the original effective condition may alter radically. Deposits applied for control of household or storage pests such as flies, mosquitoes, clothes moths or grain beetles are ordinarily exposed to conditions that prevail in houses or other buildings. Since extremes of temperature, moisture or air movement are seldom encountered, these indoor deposits are relatively long lived unless placed upon a surface which absorbs strongly or is chemically reactive. Of course, deposits put near an electric light, above a stove or near any warm object are soon volatilized. It may be noted that the phenomenally persistent DDT deposits have usually been indoors. On the other hand, outdoor deposits are subjected, to varying degrees, to rain and dew, sunlight, wind and dust, and motion of leaves, etc., all of which either

wash off, volatilize, oxidize or mechanically remove the adhering toxicant. Growth of leaves or fruit also has the effect of decreasing the density of deposit. These factors all influence the persistence and effectiveness of various toxicants in accordance with the physical state and chemical nature of the chemical concerned. There is another set of conditions, namely the nature of the solid upon which the deposit is placed, that also influences the behavior of the toxicant. This subject must be examined in some detail.

III. Effect of the underlying solid upon the behavior of a deposit

It has been pointed out already that the ease with which a spray liquid wets the surface has a marked effect upon the amount and uniformity of deposit. The nature of the surface and underlying solid also affect behavior of the deposit after it is initially laid down. Particles of a dust seldom move about or penetrate into a solid surface except as they are blown by wind or carried by rain, heavy dew or overhead irrigation water. The same may be said for a solid deposited from a spray. On the other hand, if open passages extend inward from the surface, a liquid spray or some component of it will flow into these passages if the angle of contact is less than 90 degrees. The rate of entry is controlled chiefly by the viscosity of the penetrating liquid and the size of the openings (HOSKINS, 1933). A familiar example is entrance of oil sprays into the stomata of leaves. Any toxicant dissolved in the penetrating liquid will accompany it into open passages, though separation may occur when intact membranes, such as cellular membranes, are encountered.

Diffusion through the cuticle of a plant or the skin of an animal is, of course, not entirely dependent upon the presence of actual holes. Organic toxicants usually are somewhat soluble in plant wax and diffuse into it from surface deposits. The polarity of the toxicant has a determining influence upon penetration into the plant, e.g., the relatively nonpolar systox thiol-isomer readily penetrates into lemon leaves but the polar methyl sulfonium salt does so only do a very slight extent (METCALF *et al.*, 1956). Another example is afforded by schradan, of which 69.6 percent of the amount placed upon bean leaves by dipping penetrated within 24 hours, whereas with the more polar dimefox only twenty-eight percent penetrated under similar conditions (DAVID, 1951, 1952). A considerable literature on the absorption of pesticides, especially systemic compounds, into plants has accumulated within the past few years. This process is essential for distribution of the toxicant to untreated parts of the plant, e.g., roots to leaves, but is pertinent to the present discussion only because it decreases the amount of deposit remaining on the treated part.

Since control of many insect pests is best accomplished by use of residual deposits upon areas which they frequent, there has been much interest in the persistence of toxicants upon various natural and artificial surfaces. One of the most obvious means by which a chemical can disappear from a surface is by penetration into underlying solid material if the latter is permeable. Results with a considerable number of construction materials have been fairly consistent with the obvious structure. Thus, glass, glazed

tile and metal sheets are totally impermeable and deposits can be lost only by processes already mentioned. The nature of the deposit from solutions or emulsions is highly dependent upon volatility of the solvent, e.g., aldrin, dieldrin and lindane, which form well-defined crystalline deposits from solvents such as benzene, give only oily masses of solids from commercial emulsions (TEOTIA and DAHM, 1950). DDT, especially crude samples, may remain on glass or similar surfaces for weeks without crystallizing, though all odor of the solvent has disappeared. If a needle is dragged across the surface a line of crystals will form almost instantly. Even a fly walking about will have the same effect (HOSKINS and MESSENGER, 1950).

Woods are penetrated readily by light solvents which carry in a toxicant. In one experiment, a five percent solution of DDT in kerosene was applied to poplar wood at the rate of 200 mg. per square foot. After four days layers were removed and analyzed (SCHMITZ and GOETTE, 1948). The recovery at various depths was: 0.001 inch (30%, an undetermined part of which was completely on the surface), 0.006 inch (60%), 0.010 inch (65%), 0.025 inch (70%), and 0.040 inch (74%). With a series of solvents, the most volatile (xylene) left 38 percent in the 0.001-inch layer and 73 percent in the 0.006-inch layer, whereas the least volatile (Velsicol AR-50) left 16 percent and 35 percent in these layers. It is obvious that the less volatile solvents, provided they are fluid enough to diffuse, will carry more DDT far into the wood and leave less on the surface. Different woods are not penetrated in proportion to their apparent hardness, for hard white oak and soft yellow polar behave about the same. Probably open pores such as can be seen easily in oak are more important than hardness of fibers.

Wallboard which is made from wood fibers is penetrated by solutions of toxicants in the same manner. Films of DDT left from a volatile solvent may not crystallize until disturbed. If stroked gently with a soft brush, long crystals are formed (PARKIN and GREEN, 1947). A rough rule is that crystallization can occur from application of kerosene solutions on wallboard if the product of percent DDT multiplied by deposit in mg. per square foot exceeds 150. Probably this figure applied only to one kind of wallboard.

Much research has been done on the use of residual insecticides on the mud or sun-dried brick walls that are characteristic of buildings, including human habitations, in many tropical parts of the world. A very complex situation exists when an aqueous emulsion or suspension is applied to such surfaces. In the first place, part of the water carrier quickly soaks into the wall, but this process is soon reversed as the water evaporates from the surface until a moisture balance is established in accordance with the prevailing relative humidity of the atmosphere (and of the air in the interior of the building). The deposited toxicant is subject to two influences: a) diffusion into deeper layers of the wall, and b) evaporation into the atmosphere. Both processes are favored by high vapor pressure. Data on the penetration of DDT into blocks of air-dried Uganda mud, 12 mm. thick, have been given by BARLOW and HADAWAY (1955). After application of the DDT as a wettable powder at the rate of 100 μg./ft.^{2}, the blocks were kept at 25° C. and at intervals layers 1 mm. thick were removed and analyzed

for DDT content. Expressed as percent of total applied the contents of layers were as shown in Table I.

Table I. *DDT content (as percent of total applied) of Uganda mud*

Time after application	Depth of layer (inner side) in mm.							
	1	2	3	5	7	10	11	12
2 weeks.	80	16	4	—	—	—	—	—
3 months	33	27	18	8	—	—	—	—
6 months	16	14	13	10	6	7	—	—
13 months	10	11	11	10	7	6	6	7

Since total recoveries were 119, 100, 92 and 117 percent at the four intervals, it follows that loss of DDT by evaporation was negligible. At least a year was needed for the distribution to approach equilibrium within the blocks. Instead of the chemical determination used by BARLOW and HADAWAY, a rapid procedure for measuring penetration by radioactive DDT was used by MILES and PEARCE (1957) and, of course, is applicable to any insecticide which may be prepared in labelled form. Perhaps it is to be expected that the chemical and physical nature of the soil from which a brick or mud plaster is made would affect the amount of insecticide available to an insect alighting upon the surface. However, in the case of four Indian soils, two from Africa and one synthetic product, there was no good correlation between surface DDT determined from one half-hour to six weeks after spraying and such properties as pH, percent clay, exchangeable ions, pore space, moisture-holding capacity, and rate of penetration of carbon tetrachloride (BAMI, 1961). A soil giving a hard, smooth surface to a block made from it was least permeable to the DDT.

Even in case of more volatile compounds, little loss by evaporation occurs while diffusion into the inner layers is proceeding. Thus BARLOW and HADAWAY (1958 a) found that within eight weeks gamma-BHC applied as a fifty percent wettable powder had become uniformly distributed in similar blocks of the Uganda mud, and only after that period was there appreciable loss by volatilization, though half a similar dosage was lost in one week from glass plates. Dieldrin, whose volatility is intermediate between those of DDT and gamma-BHC, also lies between them in the periods for distribution equilibrium to be reached in porous blocks and for a given loss from glass surfaces.

The insecticidal efficiency of chemicals applied to porous surfaces is strongly influenced by humidity. Thus the average kill of female *Anopheles pseudopunctipennis* mosquitoes had fallen to about five percent nine months after DDT was applied in huts in Mexico, but it rose to over seventy percent when the rainy season started (BORDAS *et al.*, 1953). The same change was found with DDT, dieldrin and gamma-BHC by BARLOW and HADAWAY (1958 b). In a continuation of the experiments mentioned earlier, they found that dieldrin became uniformly distributed within a 12-mm. mud block within four weeks when the relative humidity was maintained at ninety percent but required a year or more at fifty percent relative humidity. The apparently contradictory phenomena of increased departure from the surface by diffusion and heightened toxic effects may be explained on the basis that

the strong adsorptive power of the dried mud holds much of the original deposit right near the surface so tightly that it is scarcely available for either toxic action or diffusion. Moisture partially satisfies this binding power, thereby releasing the insecticide. The effect of loss of insecticide from the outermost layer through inward migration is more than compensated by greater availability of that which is still present (VAN TIEL, 1961). How long this dynamic shift can persist obviously depends upon the humidity of the environment, the nature of the insecticide formulation and that of the mud and other material composing the treated surface. While laboratory tests have shown decidedly higher insecticidal activity of dieldrin-Aroclor (a synthetic resin) wettable powder deposits than that of dieldrin alone, in practical tests there is little difference. The reason apparently is that the dieldrin-Aroclor is superior only under comparatively dry conditions, but the interior of mud huts in the tropics usually is humid and under this condition the combination has little or no advantage.

There has been much interest in pretreatment of sundried brick or mud walls in the tropics. Application of a lime wash, the so-called *encalado* finish, to adobe in Venezuela partially prevented penetration of DDT emulsions or solutions (MAIER *et al.*, 1948), but there is danger of hastened decomposition because of the alkalinity. Several nonreactive materials were studied by HEWLETT and PARKIN (1945, 1947). These included starch paste, five percent gelatin solution, ten percent size paste, polyvinyl acetate emulsion, and a sealing paint made especially for use on bricks. In agreement with usual experience, the paint was penetrated readily, but the size and gelatine prolonged effectiveness of the DDT very markedly. Application of size to the Uganda mud blocks largely prevented diffusion of DDT into the blocks (BARLOW and HADAWAY, 1955), but the toxicant was held so tightly that effectiveness was low toward adult mosquitoes confined upon the treated surface. Urea-formaldehyde resins acted similarly.

Furniture of floor wax on a porous solid aids in retaining an insecticidal deposit placed upon it if the toxicant is a relatively nonpolar substance, but uniform application is difficult because wax is very hard to wet. However, solutions or emulsions penetrate into a wax layer. Of course, application of wax, e.g., to wallboard or linoleum after the latter has been treated with a toxicant, will cover this over more or less completely (FAY *et al.*, 1948; PRADHAN, 1949). Unless the exacting conditions of solubility needed for diffusion to the surface are accidentally satisfied, there will be little chance of effective contact action being restored.

Waxes in which insecticides are incorporated have aroused considerable interest and several preparations have been patented. e.g., KENNEDY'S (1954) which contains five percent chlordane. Lindane is the toxic ingredient in at least two floor waxes especially recommended for control of roaches, silverfish and ants (WASSERMANN, 1955). The same conditions of solubility and diffusion that affect passage *through* an overlying layer apply to this case of incorporated toxicants.

Paints are of interest in two connections. They are penetrated readily, especially when fairly fresh, by DDT solutions and hence are of little use in keeping the toxicant at the surface (CLAPP *et al.*, 1947). Paint or varnish

on an impermeable solid such as a metal will absorb DDT and then release it later as a "bloom" of fine crystals on the surface (BLOCK, 1948). If the bloom is wiped off it will reappear after a time. Paints may be used as vehicles for contact insecticides and a considerable number of DDT paints, kalsomines and enamels have appeared on the commercial market. The successful formulation of such a product is a matter of considerable difficulty. Only the chemical actually at the surface at any given time can be effective. Since loss by evaporation, mechanical abrasion and chemical degradation is occurring continuously, there must be constant migration from the underlying layer. This will occur most readily if a supersaturated solution is maintained within the body of the paint layer.

Initiation of crystallization at the surface may be due to accidental dust particles which serve as seed in the same way as they sometimes do in an ordinary solution. This crystallization should be confined to the surface, for if it extends throughout the layer of paint, further migration as evidenced by renewed bloom does not occur (BLOCK, 1948). A multitude of small crystals will offer more active surface than fewer large crystals, but non-crystalline toxicants such as chlordane, toxaphene or pyrethrins have been incorporated successfully. It is probable that a different formulation should be used for each toxicant since solubilities differ widely and a self-renewing surface can be maintained only if there is a proper gradient from interior to surface.

Various synthetic resins have come into use in paints in recent years and insecticidal formulations were a logical development (PRICE, 1960). When a toxicant such as DDT or dieldrin is incorporated in a urea-formaldehyde formulation, the resulting dry film is so hard that migration from the interior cannot take place and the only effective insecticide is that left originally at the surface. By use of oil-modified alkyd resin as a plasticizer (optimum ratio, 50 : 50), a somewhat softer film results in which migration is possible. The insecticide comes out as a bloom which is restored whenever it is removed. Liquid toxicants such as malathion or diazinon have been incorporated and are effective though a visible bloom does not form (DYTE, 1960).

IV. Uptake of toxicant from a deposit

In all cases in which contact action is the predominant process leading to toxic effects, the determining factor is how much insecticide is transferred from the deposit to the insect's body. All the matters considered previously having to do with the formation and nature of deposits are of importance in connection with insect control only as they influence this one step in the whole procedure. Hence it is appropriate to consider in some detail how pickup may come about and how its magnitude is affected by the nature of the deposit.

The normal activities of a pest organism very largely control the kind of deposit which will give effective control. The toxicant must be brought to a motionless organism such as a fungus spore or a scale insect after it has settled, and the deposit must be either a very finely divided and uniformly scattered solid or a liquid which will spread to a uniform film. There was

considerable dispute at one time regarding the relative merits of a uniform "smooth" versus a spotty deposit of lead arsenate for control of codling moth larvae. This tiny insect makes a hole through the skin of an apple or pear shortly after hatching. SMITH (1928) changing his earlier opinion (1923) concluded that thin, uniform deposits often were penetrated safely, but that in getting through thicker spots, which seemed to give the larva a "toehold", fatal amounts of lead arsenate were usually ingested. He proposed that spotty deposits be used. However, such thick masses caused discoloration of the underlying skin and were almost impossible to remove at harvest. The answer to the problem was development of improved wetting agents and addition of a little oil which resulted in very heavy and continuous deposits. These prevailed until the advent of DDT. Leaf-eating insects usually consume any toxic deposit along with the leaf, and hence a good deposit is one that kills the pest with minimum defoliation of the plant. The tendency of dusts and sprays to collect at the edge is often of benefit. On the other hand, leaf-miners usually enter at any point, and hence a uniform deposit is required.

The development of very powerful contact insecticides within the past several years has lessened the importance of stomach poisons, for most insects crawl around enough to pick up a fatal dose before they begin eating. Gross distribution should be as even as the method of application will permit, but strict smallscale uniformity is not important. Contact poisons usually must penetrate into some part of the body before exerting their characteristic effects, though the site of action is known in very few cases. Penetration is determined by the ease of entry, the area contacted and the duration of contact. Only the barest beginning has been made in measuring the permeability of insect integument, and nothing quantitative is known regarding penetration through the coverings of sensory organs which some workers consider to be the ports of entry of DDT (HAYES and LIU, 1947) and other chlorinated hydrocarbons, but this has been disputed by FISHER (1952) who found that the first tarsomere of the housefly's leg, which has no sensilla, is a more effective point for application of DDT than the third, fourth or fifth tarsomeres which bear many sensilla. No correlation between presence of sensory structures on the legs and sensitivity to DDT was found by SARKARIA and PATTON (1949) with several insect species. It, therefore, is impossible at present to interpret the effects of toxic deposit in terms of penetration through the integument except in a most general way.

The area of contact with a solid toxicant is influenced by the state of subdivision. The minimum example probably is large flat crystals over which an insect may move without attaching any to the body. The penetration occurring under this condition may be called primary. On the other hand, a finely divided toxicant not only reaches a larger area of the body but many fine particles remain attached and thus add a later secondary penetration.

Final toxicity depends upon the total amount of toxicant available for entry as well as upon the rate of absorption, and hence "pickup" from a surface deposit is an important characteristic. An efficient deposit allows an insect to transfer a maximum amount of toxicant to its body so that

absorption may continue after the surface has been deserted. Small, easily detached crystals, a liquid toxicant, or a solution of a solid toxicant have this property to a marked degree. With mosquitoes exposed to crystals of DDT on plaster blocks, BARLOW and HADAWAY (1952 a) found maximum rate of pickup during short exposures to be from those of particle size 10 to 20 microns, though in terms of amount of pickup for toxic effects the finer crystals, 0 to 10 microns, were much more effective. In a more detailed study on the significance of shape of crystalline deposits, these same workers (BARLOW and HADAWAY, 1952 b) found that the crystals of DDT deposited upon glass from a kerosene solution lay close to the plane of the surface and were not readily detached by walking mosquitoes. But the bloom on wallboard treated with the same solution consisted of needles projecting from the board. These were readily detached by moving mosquitoes and remained attached to their bodies. Hence the toxicity was much greater, in agreement with the results of HOSKINS *et al.* (1952) who showed that lindane crystallized from petroleum ether in a chunky form which was highly toxic to house flies, whereas chloroform solutions gave flat plates which were but slightly toxic. If the surface contains many fine holes or other recesses, a finely divided deposit may be less effective than a coarser one because so much of the total enters the holes and is not accessible to an insect walking about, e.g., on clay bricks (ROTH *et al.*, 1957), or on coarse filter paper (GRATWICK, 1957). A marked irritant effect of several chlorinated hydrocarbons causes insects to leave a deposit hurriedly and unless some toxicant is carried away very little can be absorbed. Under the name of "behavioristic resistance" this phenomenon has assumed great importance in recent years, especially with anopheline mosquitoes (TRAPIDO, 1952; COLUZZI, 1958). Also much of an irritating substance may be thrown off during cleaning motions (LEWIS and HUGHES, 1957).

A further effect of the nature of the surface on which a deposit is located must be mentioned. It is obvious that deposits, no matter how laid down, adhere to surfaces with considerable tenacity for otherwise in any practical use all would soon slide off or be blown away. The chlorinated hydrocarbons, plant-derived toxicants and numerous other insecticides are lipophilic and hence have an affinity for waxy surfaces. Hence they tend to stick to the waxy layer that covers many plant surfaces, especially young leaves and fruits. The feet and other parts of insect bodies have a waxy covering too. Hence when an insect walks about on a treated plant and disturbs a deposit there may be said to be competition between the two somewhat similar surfaces for the insecticide. A quantitative study of this matter has been reported by GRATWICK (1957) who deposited DDT particles of mass mean size twenty-five microns on several surfaces and determined the weights picked up within fixed times by adult *Phormia terraenovae* flies. DDT is quite lipophilic and accordingly was picked up much more readily from a dry, fibrous surface (filter paper) than from one previously treated with a small amount of oil. Similarly, the presence of a dried film of wetting agent, which is necessarily semi-lipophilic, reduced the pickup of DDT by the flies. If the DDT particles were placed on the surface before the wetting agent had dried entirely, they were held still more firmly.

Another very important aspect of this subject is the differing tendencies of various plant surfaces to hold the several insecticides. This largely controls the effective length of time that a deposit exerts its toxic action and also the residue that persists to harvest time.

Since all insects appear to have a lipoid layer on or near the surface, chemicals that penetrate must dissolve in this layer (WEBB and GREEN, 1945). Small crystals will dissolve more rapidly than large ones. Reduction of the toxicant to colloidal size magnifies the effect, e.g., with flies sprayed with an aqueous suspension of DDT (KIDO and ALLEN, 1947) or grain beetles dipped in suspensions of rotenone (MCINTOSH, 1951). It would seem that a toxicant in solution in an oil or an oil-soluble solvent would have maximum opportunity to penetrate into the lipoid layer of the integument and hence to other parts of the body. The nature of the solvent is very important in this connection as FERGUSON (1939, 1949, 1951) has emphasized. According to his theory, the toxic action of a chemical is controlled by a) its intrinsic toxicity, b) its equilibrium distribution coefficient between the sensitive body region and the external solution, and c) the rate of penetration. The latter is itself a function of the equilibrium distribution ratios of the toxicant between the external phase and various body phases. It follows that if a given toxicant is applied to the same species of insect at a given concentration in a number of solvents of different dissolving power, the escaping tendency (into the body as well as in any other direction) will be greatest from the poorest solvent and vice versa. Strictly speaking, this holds rigidly only if the solvents do not penetrate also. Practically no application has been made of this theory as yet but it promises to put the selection of solvents for toxicants upon a more rational basis than now prevails.

Table II. *Common and chemical names of pesticides mentioned the text*

aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene
benzene hexachloride	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Chlorothion[1]	O,O-dimethyl-O-(3-chloro-4-nitrophenyl) phosphorothionate
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane
Diazinon[1]	O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothionate
dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene
dimefox	*N,N,N',N'*-tetramethylphosphorodiamidic fluoride
EPN	O-ethyl-O-*p*-nitrophenyl phenylphosphorothioate
gamma-BHC	gamma isomer of benzene hexachloride
heptachlor	3a,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
lindane	99+percent gamma isomer of benzene hexachloride
malathion	O,O-dimethyl-*S*-(1,2-dicarboethoxyethyl) phosphorodithionate
parathion	O,O-diethyl-O-*p*-nitrophenyl phosphorothioate
phorate (Thimet[1])	O,O-diethyl-*S*-ethylthio methylphosphorodithioate
schradan	octamethylpyrophosphoramide
Systox thiol isomer[1]	O,O-diethyl-O-2-(ethylthio) ethylphosphorothioate
toxaphene	chlorinated camphene containing 67—69 percent chlorine

[1] Trade name

Summary

The nature and persistence of pesticide deposits are of great interest because of the development of resistance on the part of many important pests and because of legal restrictions on the amount of residue permitted on foodstuffs. As physical systems, deposits differ according as they are formed by application of dusts, granules, or sprays of suspensions or emulsions. Dusts are often highly charged and their adhesion to a surface is partially controlled by electrostatic forces. This is the case to a much smaller degree with particles from a spray.

The surface upon which a deposit is placed has an important influence upon its persistence. If it is impermeable, the whole deposit must be entirely at the surface. On the contrary, a permeable surface such as mud, brick, wood or wallboard permits penetration of a solvent carrying the pesticide with it and also of the pesticide alone as it volatilizes from the surface. Moisture facilitates loss from the surface both to the atmosphere and to underlying regions. Various waxes, resin, paints, etc., have been recommended to prolong the effectiveness of deposits by reducing their movement from the surface.

Pickup from a surface deposit is largely dependent upon an insect's movements. The need for an uniform deposit depends upon the pest to be controlled. Size and shape of the particles is important since large, flat plates seldom become attached to an insect's body, but small chunks or needles are easily picked up. The presence of oils or other nonvolatile liquids affects distribution of a pesticide between the surface and the body of an insect on it.

Résumé*

La nature et la persistance des dépots de pesticides ont un grand intérêt en raison de la création de la résistance de la part de nombreux parasites importants et en raison des limites légales de résidus tolérés sur les matières alimentaires. En tant que systèmes physiques, les dépots diffèrent selon qu'ils proviennent de poudrages, de granules ou de pulvérisations de suspensions ou d'émulsions. Les poudres sont souvent fortement chargées et leur adhérence à la surface dépend en partie des forces electrostatiques. C'est le cas à un degré moindre avec les particules données par la pulvérisation.

La surface sur laquelle est placé un dépot a une influence importante sur sa persistance. Si elle est imperméable, le dépot se trouve entièrement sur la surface. Au contraire, une surface perméable comme la boue, la brique, le bois ou un mur de planches, permet la pénétration d'un solvant amenant le pesticide avec lui et aussi du pesticide seul lorsque le solvant se volatilise à la surface. L'humidité favorise le départ de la surface, à la fois vers l'atmosphère et vers les régions sous-ja centes. Diverses cires, résines, peintures, etc., ont été recommandées pour prolonger l'efficacité des dépots en réduisant leur départ de la surface.

La prise d'un insecticide déposé sur une surface dépend beaucoup des mouvements de l'insecte. Le besoin d'un dépot uniforme dépend du para-

* Traduit par R. MESTRES.

site à controler. La taille et la forme des particules sont importantes puisque des plaques grandes et plates se lient rarement au corps d'un insecte tandis que de petites parcelles ou des aiguilles sont aisément captées. La présence d'huiles ou d'autres liquides non volatils affecte la distribution d'un pesticide entre la surface et le corps d'un insecte qui s'y trouve dessus.

Zusammenfassung*

Großes Interesse gehört der Natur und Persistenz von Schädlingsbekämpfungsmittel-Belägen, da viele wichtige Schädlinge Resistenz entwickelt haben und zudem die zugelassenen Rückstandsmengen auf Lebensmitteln gesetzlich eingeschränkt sind. Die physikalischen Systeme der Beläge sind verschieden, je nachdem sie von Stäubemitteln, Granulaten, Spritzmitteln aus Suspensionen oder Emulsionen gebildet werden. Stäubemittel sind oft stark geladen und elektrostatische Kräfte regeln zum Teil ihre Adhäsion an eine Oberfläche. Bei den Partikeln eines Spritzmittels ist dies weniger der Fall.

Die Persistenz des Belages hängt stark von der Oberfläche ab, auf die der Belag sich absetzt. Ist die Oberfläche undurchlässig, so muß der Belag ganz auf ihr sein; ist die Oberfläche hingegen durchlässig, wie z. B. Schlamm, Ziegel, Holz oder Pavatex, kann das Lösungsmittel, mit dem das Schädlingsbekämpfungsmittel formuliert ist, oder auch das Schädlingsbekämpfungsmittel allein eindringen, während es sich an der Oberfläche verflüchtigt. Feuchtigkeit erleichtert die Wirkstoffabgabe von der Oberfläche sowohl an die Atmosphäre als auch an die Unterlagen. Verschiedene Wachse, Harze, Anstriche etc. wurden empfohlen, weil sie die Wirkung der Beläge durch Verminderung ihrer Bewegung von der Oberfläche verlängern.

Wirkstoffaufnahme von einem Oberflächen-Belag hängt weitgehend von der Bewegung des Insektes ab. Der zu bekämpfende Schädling bestimmt auch, ob ein gleichförmiger Belag benötigt wird. Größe und Form der Partikel sind sehr wichtig, da große, flache Platten selten am Insektenkörper haften bleiben, während kleine Klumpen oder Nadeln leicht aufgenommen werden. Das Vorhandensein von Ölen oder anderen nicht-flüchtigen Flüssigkeiten beeinflußt die Verteilung des Schädlingsbekämpfungsmittels zwischen der Oberfläche und eines auf ihr weilenden Insektes.

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Insecticide residues in olive oils and table olives from efforts to control the olive fly

By
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With 3 figures

Contents

I. Introduction and history

Olive oil occupies a leading position among the agricultural products of the nations of the Mediterranean Basin, such as Italy, France, Greece, Spain, Portugal, Turkey, Yugoslavia, Tunisia, Libya, Israel, Morocco, Algeria, etc. This oil is both a basic component of the diets of the people of these nations and a major item in their foreign trade. Together with special qualities of whole olives used for direct consumption, the oil is exported by these nations throughout the world, and for some of them it constitutes one of the most important economic resources. As an illustration, it will suffice to say that Italy alone has 165 million olive trees capable of producing each year about 350,000 tons of oil, worth around 155 billion Lire or 250 million dollars (Min. Agr. Foreste-Italy, 1960).

It is therefore readily understandable why, for many centuries, efforts have been made to protect the olive crop against the attacks of various pests, and especially against the olive fly *Dacus oleae* Gml.

Thus, the problem is an ancient one. Damages caused by the *Dacus* have been known and described since the time of Pliny (GRANDI, 1951). More recently, for example, NAPOLEON I and FRANCIS BOURBON (BERTELLI, 1938) ordered that olives be harvested ahead of time in an effort to prevent more serious losses from this fly.

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In the light of present knowledge of the ecology of this parasite and of the biology of the olive tree, damages caused by *Dacus* can be summarised as follows:

1. Quantitative damages, due to reduction in the weight of affected olives and to stoppage of the oil-producing process in fallen olives, the yield in oil being lower than that obtainable in the absence of infestation.

2. Qualitative damages, due to the high acidity and altered organoleptic properties of the oil caused by the presence of the pre-imaginal stages of the insect, of the excrements of the larvae, and of bacteria and fungi (NACAMULI, 1955).

The extent of such damages can be easily visualised if we consider that, in Italy, the annual loss used to amount to about one third of the total oil production. In the years prior to 1955, when oil production was considerably smaller than it is now, this meant a loss of 25 to 30 billion Lire or 40 to 48 million dollars. Not infrequently the loss was as much as 50 percent of the entire crop; furthermore, in monetary terms, it would be much greater if we consider that infestation lowers the quality of the oil produced (NACAMULI, 1955).

In Italy, the *Dacus* scourge was at its worst in 1955, when it caused damage estimated at 50 to 80 billion Lire or 80 to 130 million dollars. The small quantity of oil produced was characterised by extremely high acidity, in most cases 10 to 12 percent and frequently as high as 20 to 25 percent (BONCI, 1956). If we consider the entire Mediterranean Basin, we find that the damage caused annually by the olive fly can be estimated at around 400 billion Lire or 645 million dollars (LOGOTHETIS, 1953).

It is natural that, since the most remote times, olive-growing specialists and responsible authorities in various nations should make real efforts to eradicate such a harmful pest. The first experiment in *Dacus* control dates back to 1790, with the use of honey poisoned with potassium cyanide (PELLEGRINI, 1955). Since that time and up to World War II, studies and research led to the development of control methods which can be classified into the following four basic groups:

1. Biological control, i. e., action of other insect species on *Dacus*.

2. Spraying olives with "protective compounds" which, on drying, form a sort of film on the surfaces of the olives which is supposed to prevent the fly from laying its eggs.

3. Catching the adult pests by "trap bottles".

4. Killing the adult pests by "poisoned sugar baits".

These control methods found many and different applications in the Mediterranean countries, but they afforded only partial and limited relief, and practically were confined to relatively small areas.

After World War II, the advent of synthetic organic insecticides opened new prospects for pest control based on sounder and more effective scientific methods.

In 1945 GRANDORI conducted in the Liguria Region of Italy the first tests with DDT, followed in subsequent years by MARTIN in Spain, AYOUTANTIS in Greece, MELIS on the Tuscan Coast, HADJINICOLAU in Greece, RUSSO in the Province of Salerno, GOMEZ and DEL RIVERO in Spain, PERETZ and

PLANT in Israel, and other experimenters. In such attempts various chlorinated hydrocarbons were used, such as DDT, Chlordane, Aldrin, Dieldrin, BHC, and methoxychlor, either singly or combined in mixtures (PELLEGRINI, 1955).

These were isolated attempts, conducted with differing techniques and not coordinated along logical lines. Since these earlier tests, however, it appeared that all chlorinated hydrocarbons could be used effectively to kill adult pests only, but not their larvae. Furthermore, the lethal action was not instantaneous and allowed the pests hit by the insecticide to keep laying their eggs (PELLEGRINI, 1955). On the other hand, such a control system created a new and most important problem: that of the residues of the insecticides or their metabolites left in varying quantities on and in the olives and in the oils of the olives from treated trees, which could have constituted a health hazard. In effect, it was found that chlorinated hydrocarbons, if used in quantities sufficient to ensure a modicum of effectiveness, due to their solubility in oils remain dissolved in the oil obtained from the treated olives in substantial and sometimes very large quantities.

II. Five-year program

For an adequate solution of this important problem, which would reconcile the interests of production through an effective control method with those of public health, between 1954 and 1955 an agreement was reached in Italy by the Ministry of Agriculture and the "Istituto Superiore di Sanità" (Ministry of Health), calling for a five-year program which would effectively attack — and if possible solve — the serious problem of the effective control of *Dacus Oleae.*

In particular, the problem was to be considered under two principal aspects:

1. Infestation was to be controlled either by preventing it or by combatting it, if already in progress, by the most suitable and effective means among those commercially available. This was to be a responsibility of the Ministry of Agriculture and its field agencies.

2. Investigations were to be conducted to make sure that the oils produced from the olive trees treated with the insecticides did not contain toxic residues, or at least that such residues were kept within such limits as to afford a reliable guarantee of harmlessness for the consumer's health. This was to be a responsibility of the "Istituto Superiore di Sanità".

From this cooperation came a working plan which has led to the fully satisfactory solution of this centuries-old problem.

The Ministry of Agriculture, through its field agencies (the Regional Plant Disease Observatories), conducted extensive experiments in the various olive-growing areas of Italy, in an initial stage on several hundred thousand trees, and later extending the treatment to several million trees: 4.8 million in 1958, about 9 million in 1959, and 8 million in 1960 (Min. Agr. Foreste-Italy, 1960). In these experiments, the active principles made available by the industry were applied with different techniques, procedures, and treatment times.

From each lot of trees, samples of olives were systematically collected and sent to the "Istituto Superiore di Sanità", either as such and from which oil was obtained through a standard process of grinding, pressing, decanting, and subsequent centrifugation and filtration, or already in the form of oil, to be tested for residues of the several insecticides tried.

The five years that followed can be divided into two stages. There was a two-year period of general orientation work, during which many insecticides were used with different methods and times of treatment in order to obtain general information as to the choice of the most suitable products and processes. There followed a three-year period of more thorough research on the insecticides which had proved more satisfactory in terms of both effectiveness and amounts of residues.

Even though by then the use of chlorinated hydrocarbons appeared to be undesirable in controlling the *Dacus*, above all because of the large quantity of residues left in the oil, certain experimenters did include them in the earlier testing. Meanwhile particular attention was given to synthetic organophosphorous compounds because of their greater effectiveness and because of the fact that, while being generally more toxic than chlorinated hydrocarbons in regard to mammals in terms of acute toxicity, their residual action is much shorter and they do not, generally speaking, appear to have a cumulative action.

In the first two years, experiments were conducted with the following insecticides:

a) Chlorinated Hydrocarbons: *DDT* [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane], *aldrin* (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene), *dieldrin* (1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene), and *heptachlor* (1,4,5,6,7,8,8-heptachloro-3a,4,5,5a-tetrahydro-4,7-*endo*-methanoindene).

b) Organophosphorus Compounds: *parathion* (O,O-diethyl O-*p*-nitrophenyl phosphorothioate), *diazinon* [O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate], *malathion* [O,O-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate], *Dipterex* [O,O-dimethyl(1-hydroxy-2,2,2-trichloroethyl) phosphonate], and *Rogor* (a trade mark of Montecatini Co., also known in the U.S.A. as *dimethoate*) [O,O-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate].

Experiments were also run with *Phosdrin* (O,O-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate), but this compound was soon dropped because of its very short duration of action.

As a first step, the "Istituto Superiore di Sanità" was concerned with lining up suitable methods of extraction, purification, and testing for and determination of the residues of the various active principles. Some of these methods were suggested by the manufacturers of the individual insecticides, but for the most part they were studied and developed by the author and collaborators, and disclosed in several publications.

Specifically, the methods adopted for residue determination were the following:

DDT: method by M. E. Alessandrini and V. Amormino (1954)

Aldrin and Dieldrin: method by L. BONIFORTI and M. DORETTI (1959)

Heptachlor: method by M. E. ALESSANDRINI and G. F. LANFORTI (1957 b)

Parathion: method by M. E. ALESSANDRINI *et al.* (1955, 1957 f)

Diazinon: method by M. DORETTI (1960)

Malathion: method of the American Cyanamid Co., adapted for determination in olive oil

Dipterex: method by M. E. ALESSANDRINI and G. F. LANFORTI (1957 c)

Rogor: method by B. BAZZI *et al.* (1956, 1958)

By the end of the first two-year period, the following conclusions could already be drawn (ALESSANDRINI, 1957 e, 1958, 1959):

1. For all chlorinated hydrocarbons, the residues were always large and sometimes very large, up to a maximum of 320 p.p.m. for DDT, 58 p.p.m. for aldrin, 322 p.p.m. for dieldrin, and 260 p.p.m. for heptachlor, making it quite clear that their use for *Dacus* control was definitely unacceptable;

2. As it appears from Table I, in 1955 in only 18.8 percent of the samples tested and containing parathion residues this insecticide had been found to be present in quantities not exceeding 1 p.p.m. or 1 mg./kg., which is the limit of tolerance recommended in Italy by the "Technical-Advisory Experts' Committee on the Use of Pesticides in Food Products and Crops". In 1956 this percentage showed a definite improvement, rising to 54 percent.

Considering the large number of oil samples in which parathion residues were substantial, and the fact that, because of its high effectiveness and low cost, many entomologists rated it (and still do) as one of the best weapons in the fight against *Dacus*, the possibility was considered of eliminating from the oil the residues of that insecticide by some special process.

Entomologists experimented with associating parathion with copper salts, based on the catalytic action which such salts are supposed to have on the decomposition of that product in the plant, while the author sought to eliminate them by refining and developed a special process by which as much as 200 p.p.m. of parathion may be eliminated from the oil. This process consists in adding to the oil, before neutralization, a small quantity of certain reducing substances, such as sodium hydrosulphite, containing special stabilizers, and following the exact description stated (ALESSANDRINI, 1957 a, d). In this way parathion is reduced to the corresponding amine derivative, which is soluble in the oil but is partially hydrolyzed with the formation of *p*-aminophenol. This is soluble partially in the oil, partially in the aqueous-alkaline liquid and mostly in the formed soap. During the decolorizing process, the mixture of fuller's earth and charcoal used for this purpose, which does not retain parathion as such, completely retains both the amine derivative of parathion and *p*-amino phenol. The other part of the molecule, that is the sodium salt of diethylthiophosphoric acid, remains dissolved in the aqueous-alkaline phase. The oils obtained do not contain either parathion or the transformation and hydrolysis products, and are thus non-toxic. Moreover, the organoleptic characters and the physical and chemical properties of the oils obtained by such process are normal and the same as those of the oils obtained by the usual process.

Table I. *Residues of Parathion, Diazinon, and Rogor found in the samples of olive oil from the experiments carried out in Italy by the Ministry of Agriculture during the years 1955 and 1956*

Year	Parathion						Diazinon						Rogor	
	No. Samples analyzed	Samples containing residues					No. Samples analyzed	Samples containing residues					No. Samples analyzed	Samples containing residues
		none	up to 1 p.p.m.	from 1 to 3 p.p.m.	from 3 to 10 p.p.m.	from 10 to 30 p.p.m.		none	up to 1 p.p.m.	from 1 to 2 p.p.m.	from 2 to 10 p.p.m.	from 10 to 20 p.p.m.		none or less than 0.5 p.p.m.
		%	%	%	%	%		%	%	%	%	%		%
1955	170	6.9	11.9	28.3	32.3	20.6	56	5.4	12.6	10.7	55.3	16	4	100
1956	35	17	37	40	6	—	15	—	27	33	40	—	12	100

The method outlined above was patented both in Italy (Brev. No. 567,346 of Feb. 15, 1957) and in the U.S.A. (No. 2,937,193 of May 17, 1960).

Consequently, it was ruled by Italian authorities that olive oils containing parathion residues in excess of 1 p.p.m. are to be rated unfit for human consumption, and in order to be made edible must either be appropriately mixed with oils free from that insecticide or be subjected to the special refining process outlined above so as to lower the parathion content below 1 p.p.m.

3. Diazinon, malathion, and Dipterex caused far less concern, because the residues had proved to be quite small, and because such insecticides are far less toxic to mammals than is parathion.

However, the effectiveness of malathion proved to be quite limited and subject to much dispute, while Dipterex had an action too limited in time. In effect, these two insecticides were subsequently abandoned in the *Dacus* control program, at least in Italy.

4. Residues of Rogor had always been found absent or present in quantities under 0.5 p.p.m., the maximum limit of the sensitivity of the chemical method then available (BAZZI, 1956). In this connection, it can be added that later the sensitivity of that method was increased to 0.1 p.p.m. (BAZZI, 1958), and residues of Rogor in the various oil samples were always found to be either absent or present in quantities under 0.1 p.p.m. (see Table I).

III. *Dacus* control in other countries

At the same time, more or less extensive experiments in *Dacus* control had also been conducted in other countries of the Mediterranean Basin, using various active principles, almost all of which were organophosphorous compounds. Mention should be made of the experiments (FAO, 1957) conducted in:

Greece, with Rogor, parathion, malathion, diazinon, nexion, ekatin (O,O-dimethyl-*S*-ethylthioethyl-phosphorothiolthionate), and Dipterex.

Spain, with diazinon, Dipterex, chlorthion [O-(3-chloro-4-nitrophenyl) O,O-dimethyl phosphorothioate], and malathion.

Portugal, with diazinon and Dipterex.

Yougoslavia, with parathion, diazinon, Rogor, Phosphamidon (2-chloro-2-diethylcarbamoyl-1-methylvinyl-dimethylphosphate), Dipterex, nexion [O-methyl-O,O-(1-carbethoxy-1-methyl-2,2-dichloro) × 1,3-propylenphosphate], and malathion.

Israel, with dieldrin and methoxychlor [1,1,1-trichloro-2,2-bis-(*p*-methoxyphenyl)ethane].

Libya, with DDT, dieldrin, chlordane (2,3,4,5,6,7,8,8-octachloro-2,3,3a, 4,7,7a-hexahydro-4,7-methanoindene), malathion, Dipterex, and Phosphamidon.

Some of these countries sent also to the "Istituto Superiore di Sanità", either directly or through FAO, several samples of oils to be tested for residues of some of the insecticides tried (ALESSANDRINI, 1957 e, 1958). These experiments gave highly interesting results, having been conducted

under different climatic and environmental conditions, with different methods and techniques, and the conclusions which could be drawn assumed a more general and reliable value.

With the data gathered from such experiments, the countries of the Mediterranean Basin participated in the III FAO Meeting on Olive Fly Control, held in Florence November 25—30, 1957.

Based on residue data and on entomological considerations, the meeting found that the experimenters' interest was concentrating on a limited number of insecticides belonging to the class of organophosphorus compounds, and specifically parathion, diazinon, and Rogor. However, with respect to each of these insecticides, special recommendations and reservations were made, stressing the need for promoting chemical and toxicological experiments and research, in order to gather information which would definitely clarify the advantages and hazards connected with the use of those products.

IV. Results in Italy, olive oil

These recommendations by FAO guided the whole subsequent development of the *Dacus*-control drive in the various countries of the Mediterranean Basin. In particular regard to Italy, the "Istituto Superiore di Sanità" — in addition to continuing to test for insecticide residues all samples from the *Dacus*-control experiments run by the Ministry of Agriculture and the results of which, with respect to parathion, diazinon, and Rogor, are shown in Table II — also conducted extensive chemical and toxicological research. Since the latter has yielded results which are particularly clear and definitive, they are summarized briefly below.

a) Parathion. Because of its high toxicity and because of fact that, being liposoluble, it remains in the oil if still present in the olives at the time of pressing, the III FAO Meeting recommended that parathion be used with the greatest caution.

This insecticide has been used in Italy either alone or associated with copper salts.

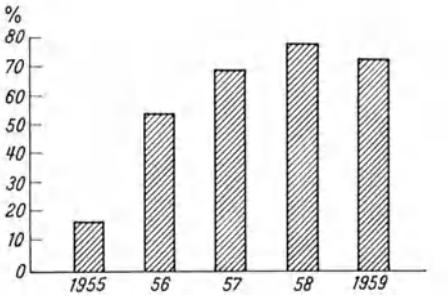

Fig. 1 — Samples of olive oil from the experiments carried out in Italy by the Ministry of Agriculture during the years 1955, 1956, 1957, 1958, and 1959, containing less than 1 p.p.m. of Parathion

Fig. 1 shows that over the last three years of the experiments (1957, 1958, and 1959), thanks to the greater caution exercised in treatments, to improved methods, and to time and mode of application (with or without

Table II. *Residues of Parathion, Diazinon and Rogor in the samples of olive oil from the experiments carried out in Italy by the Ministry of Agriculture during the years 1957, 1958, and 1959*

Year	Parathion							Diazinon						Rogor	
	No. Samples analyzed	Samples containing residues					No. Samples analyzed	Samples containing residues					No. Samples analyzed	Samples containing residues	
		none	up to 1 p.p.m.	from 1 to 3 p.p.m.	from 3 to 10 p.p.m.	from 10 to 30 p.p.m.		none	up to 1 p.p.m.	from 1 to 2 p.p.m.	from 2 to 10 p.p.m.	from 10 to 20 p.p.m.		none or less than 0.1 p. p. m.	
		%	%	%	%	%		%	%	%	%	%		%	
1957	50	46	22	22	10	—	3	—	67	33	—	—	30	100	
1958	42	61.8	14.3	16.7	7.2	—	5	40	—	60	—	—	18	100	
1959	37	56.7	16.2	16.2	5.6	5.6	7	—	28.6	42.8	28.6	—	54	100	

copper salts), the percentage of oils containing quantities of residues under 1 p.p.m. has risen from the 18.8 percent registered in 1955 to about 73 percent.

b) Diazinon. At the III FAO Meeting, a report on Diazinon prepared by the Hygiene Institute of the University of Florence, Italy, stated that the chronic toxicity of this product in the rat is about four times lower than that of parathion (MELIS, 1955). Consequently, the above-mentioned Committee recommended for Italy a tolerance limit with respect to diazinon residues in olive oil of 2 p.p.m.

Judging by the limited number of oil samples submitted to the "Istituto Superiore di Sanità" from olive groves treated with diazinon, it would appear that in the last three years not much experimental work has been done on this insecticide. The results obtained, therefore, have a limited value in that they relate to a small number of samples.

In any event, as it appears from Fig. 2, the percentage of oil samples

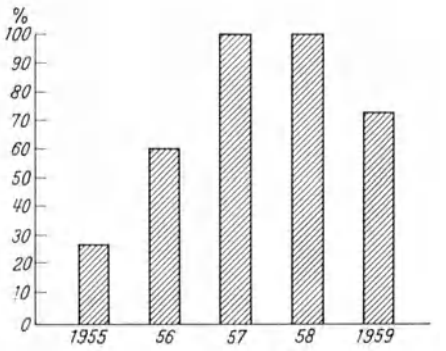

Fig. 2 — Samples of olive oil from the experiments carried out in Italy by the Ministry of Agriculture during the years 1955, 1956, 1957, 1958, and 1959, containing less than 2 p.p.m. of Diazinon

containing quantities of diazinon residues under 2 p.p.m. varied between 70 and 100 percent.

c) Rogor. The III FAO Meeting recognized that Rogor (Dimethoate) is one of the insecticides which, in addition to being highly effective, presents the lesser danger, first of all since its acute toxicity in rats is about one fiftieth that of parathion (Rogor: LD50, acute, oral, in rats 320 to 380 mg./kg.; parathion: LD50, acute, oral, in rats 4.5 to 5.5 mg./kg.), and then because in the oils from olive groves treated with it the residues of the active principle had always appeared to be either absent or present in quantities not detectable with the chemical methods available.

On the other hand, this was justified by the fact that this insecticide is much more water-soluble than liposoluble, so that any residues pass into the aqueous tissues unlike many other products such as parathion. The hope was expressed, however, that more thorough studies could be conducted on the behavior of this active principle in order to obtain more complete information sufficient to remove any possible reservation as to the toxicological aspects of its use in the control of the *Dacus* pest.

The results of analytical tests conducted over the last few years have confirmed the findings of the previous experiments, i.e., as it appears from Fig. 3, that in none of the 118 samples of oil examined in the five years

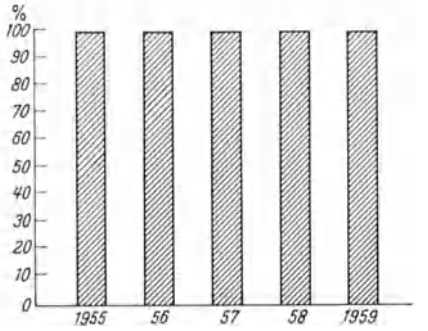

Fig. 3 — Samples of olive oil from the experiments carried out in Italy by the Ministry of Agriculture during the years 1955, 1956, 1957, 1958, and 1959, containing less than 0.1 p.p.m. of Rogor

were found quantities of residues detectable with the available chemical methods. Moreover, an extensive study has been conducted to determine whether more or less toxic metabolites are formed and whether such metabolites are present in the olives and in the oil. This study was summarized in a paper presented at the XVIII International Congress of Pure and Applied Chemistry, Section C 3 on "Analysis, Degradation, and Metabolism of Pesticides", in Montreal in August, 1961 (ALESSANDRINI, 1961), as follows:

A paper chromatographic (one-dimensional descending) method was developed to differentiate between the active principle of Rogor and its oxygenated analog [O,O-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate]. This latter had been identified by other authors with other means, and chiefly with the use of radioactive isotopes, in the metabolism of Rogor in the liver of mice and in cherry and horse-bean plants (SANTI, 1959 a and b), in bovine rumen fluid (DAUTERMAN, 1959), and in certain cultures (DAUTERMAN, 1960). This metabolite, which possesses an acute oral toxicity in rats about 10 times higher than that of the parent product, is most important from the toxicological viewpoint and our research was therefore concentrated on it. Consequently, samples of oil and olives to which the above two compounds were added in the laboratory, after appropriate processes of extraction and purification, were tested for parent compound and metabolite with the above-mentioned chromatographic process.

Subsequently, tests for the oxygenated product were conducted on the olives from groves treated with Rogor and collected at different intervals after treatment. The metabolite was found to be present, but in quantities never exceeding 1 p.p.m.

Its coefficient of distribution between water and oil was then investigated, with the finding that it is very highly soluble in water so that, even when deliberately large quantities of the metabolite are added to the oil, when the latter is simply washed with water the metabolite passes entirely into solution in the water.

Finally, tests for the metabolite were conducted on oil obtained from olives treated with Rogor, which contained small quantities of it, and as could be expected from the foregoing it was found that the oil was completely free of this metabolite.

The findings of this study were confirmed by tests conducted independently at the Montecatini Co. laboratories, using Rogor marked with P^{32} tracer (ALESSANDRINI, 1960).

It was thus possible to reconstruct the various phases of transformation and decomposition of Rogor in the olive, and to establish theoretically as well as experimentally the absence of toxic residues of Rogor or of its metabolites in the olive oil.

These chemical and paper-chromatographic researches have led to the ascertainment of the following facts:

1. In the plant, Rogor undergoes a gradual process of oxidation and hydrolysis, as a result of which, within about 10 days of the time of treatment and operating under the severest conditions possible (much heavier dosage than usual, application of the insecticide at a time much closer to the picking of olives than is normally required, etc.), the metabolite is present in the olives in quantities ranging from 2 to 3 p.p.m., while after 20 days this quantity is reduced to very low values, at most around 1 p.p.m. Because of its high solubility in water, Rogor passes mostly into the aqueous plant tissues, and the practical absence of its residues in the oil is thus accounted for.

2. As a result of the process of oxidation and hydrolysis of the active principle of Rogor, various metabolites are formed but only one of these might cause concern from the toxicological standpoint. This is the oxygenated analog which has an acute oral toxicity in rats 10 times higher than that of the parent active compound and 5 times lower than that of parathion (acute oral LD_{50} in rats 30 mg./kg.). This compound, however, undergoes a further decomposition, being converted into non-toxic products of the types of phosphoric, thio- and dithiophosphoric acids, and their mono- and dimethyl derivatives, so that in the olives it cannot exceed 1 p.p.m.

3. Because of its very high solubility in water, the oxygen analog of Rogor passes completely into aqueous plant tissues, and the oil expressed from olives containing it, even in deliberately large quantities, is absolutely free from it.

In general, as regards the oil expressed from olives treated with Rogor at normal dosages, it can be concluded that the concentration of its residues in the olives at the time of picking will be at most 0.1 to 0.2 p.p.m. It appears entirely logical and explainable why the very many analytical tests so far conducted on oil samples from a wide variety of olive-growing areas have consistently shown the practical absence of residues of Rogor and its oxygenated metabolite.

Experience based on the findings of the tests run on many samples and on the information collected leads to the conclusion that it could even be unnecessary to test for Rogor or Rogor-metabolite residues in the oils obtained from Rogor-treated olives, provided at least 15 days have been allowed to elapse between the last treatment and the picking of the olives.

V. Results in Italy, table olives

The problem with table olives may appear to be different since the entire pulp of the fruit is eaten, but in practice certain factors prevail which allow us to view this problem, too, without concern, for the following reasons:

1. Since table olives need to be protected for a time interval shorter

than oil olives, the recommended dosages are lower and consequently the initial deposits of the active principle are smaller.

2. In any event, when table olives are picked not less than one month after the last treatment with Rogor, the residues in the olives are not large enough to constitute a toxicological hazard.

3. The treatment of table olives with an alkaline solution, which is normal practice in the industry, causes a further degradation and a high rate of extraction of the residues. It has been proved that at the end of the period of soaking in the alkaline solution 85 to 90 percent of the residues originally in the olives remain in the alkaline solution.

VI. Chronic toxicities of insecticides in olive oil

In addition to the chemical research outlined above, the "Istituto Superiore di Sanità" has conducted studies on the chronic toxicity of parathion in monkeys, and of parathion, diazinon, and Rogor on quail, in the Institute's Therapeutic Chemistry Laboratory, directed by Prof. BOVET (in press).

Such studies have led to the conclusion that the limit of tolerance to parathion in monkeys, when the insecticide is administered orally in olive oil, is about 0.1 mg./kg. and perhaps less. In quail, the chronic toxicity of parathion was found to be about 5 times higher than that of diazinon and about 40 times higher than that of Rogor (ALESSANDRINI, 1960).

VII. Other compounds and other countries

Also, in the other countries of the Mediterranean Basin, subsequently to the III FAO Meeting, i.e., from 1957 to 1960, experiments were conducted with some newer organophosphorous compounds in anticipation of other possible requirements. Reports on the findings of such experiments were submitted in the course of the IV FAO Meeting in December, 1960 at Tel Aviv, Israel on the "Control of Olive Pests" (FAO, 1960).

We will mention the experiments conducted in:

Greece, with Rogor, Lebaycid [O,O-dimethyl-O-4(methylmercapto) 3-methylphenyl phosphorothioate], Phosphamidon, Fac [O,O-diethyl-*S*-(*N*-isopropylcarbamoylmethyl) phosphorodithioate], Pardol [O-methyl, O-ethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate], parathion, diazinon, ekatin, morphothion [O,O-dimethyl-*S*-(morpholinocarbonyl-methyl) phosphorothiolthionate], and Dipterex. The best results were obtained with the first four products.

Israel, with Phosphamidon, Rogor, ekatin, Lebaycid, Dipterex, and diazinon. The best results were obtained with the first four products.

Portugal, with Rogor, diazinon, Lebaycid, Phosphamidon, and ekatin. The best results were obtained with Rogor.

Cyprus, where satisfactory results were obtained with Rogor.

Turkey, with parathion, diazinon, chlorthion, Dipterex, and Lebaycid.

VIII. Conclusions

The conclusions set forth in the Report of the said FAO meeting open with the following words:

"Having heard reports on the chemical control of the olive fly, the meeting concluded that insecticides, which give both satisfactory control and leave harmless amounts of residues or no residues at all in the resulting olives or olive oil, are now available."

These words conclude centuries-old efforts and research towards complete control of the olive fly. The best proof of this provided by the fact that while the previous meetings called by FAO were concerned with the specific theme "Olive Fly Control", the IV Meeting held at Tel Aviv discussed the theme "Control of Pests", on which further meetings will be held by FAO.

In effect, the study of the means for complete protection of the olive cannot be considered concluded because of the existence of other pests, even though of lesser importance than *Dacus*, and because of the possibility that *Dacus* may develop a degree of resistance to the insecticides which are now giving such satisfactory results even though no sign of such resistance has yet been observed.

It is therefore necessary to continue the study with a view to developing new olive-protection weapons which must be both effective and safe.

The foregoing can provide an effective illustration of the progress which can be achieved, and also in recovering the large quantities of food which pests destroy, when empirical efforts are replaced by a soundly planned and implemented research program. Such a program is the product of a fruitful cooperation among all sectors concerned, such as *Agriculture* for the increase in production, *Health* for the protection of the public health, and *Industry* for the development of new products.

Summary

The paper starts with a brief discussion of the severe damages caused by the olive fly (*Dacus oleae* Gml.) in all countries of the Mediterranean Basin since remotest times, and of the more or less effective methods used through the centuries to control that pest, until the advent of modern synthetic insecticides.

The author then reports on the first sporadic experiments made with chlorinated hydrocarbons, and later with organophosphorous compounds, in various parts of the world, and principally in Italy where, starting in 1954—55, a sound five-year testing plan was adopted in cooperation between the Ministry of Agriculture and Forests and the "Istituto Superiore di Sanità" (Ministry of Health).

In particular, the problem was to be considered under two aspects:

1. Infestation was to be controlled either by preventing it or by combating it, if already in progress, by the most suitable and effective means among those commercially available. This was to be a responsibility of the Ministry of Agriculture.

2. Investigations were to be conducted to make sure that the oils produced from the olive trees treated with the insecticides do not contain

toxic residues, or at least such residues are kept within such limits as to afford a reliable guarantee of harmlessness for the consumer's health. This was to be a responsibility of the "Istituto Superiore di Sanità".

In connection with the foregoing, tests were conducted with the following chlorinated hydrocarbons: DDT, aldrin, dieldrin, heptachlor, and the following organophosphorous compounds: parathion, diazinon, malathion, dipterex, and Rogor (Dimethoate).

For the testing for and determination of the residues of the various active principles it was necessary to use suitable analytical methods which, for the most part, were studied and developed by the "Istituto Superiore di Sanità" while some were suggested by the manufacturers of the individual insecticides.

The experiments conducted in the first two years proved that, apart from the effectiveness, the chlorinated hydrocarbons were definitely not to be recommended because of the large and sometimes very large residues found, and therefore the subsequent research was concentrated solely on organophosphorous compounds, and chiefly on parathion, diazinon, and Rogor.

Some tables in the paper show the percentages of the residues of the said three pesticides found in the many samples of olive oils tested in the five years of the experimental program. Also briefly described is a process patented in Italy and in the U.S.A. for the refining of oil containing parathion residues, by which as much as 200 p.p.m. can be extracted from the oil.

The author then describes research conducted on Rogor to determine whether more or less toxic metabolites are formed, and to test for their presence in the olives or in the oil by either paper chromatography or radioactive isotopes. This research has indicated that oils from treated olive plants do not contain residues of Rogor or toxic metabolites thereof.

Reports are also given on research on the chronic toxicity of parathion in monkeys and of parathion, diazinon, and Rogor in quails.

Lastly, a brief report is given of the results obtained from some experiments conducted by several countries in the Mediterranean Basin, with the use of several new active principles also belonging to the group of organophosphorous compounds, as reported at the IV FAO Meeting at Tel Aviv, Israel, in December, 1960.

Based on the findings of the various experiments conducted, the Report of the FAO Meeting concluded:

"Having heard reports on the chemical control of the olive fly, the meeting concluded that insecticides, which give both satisfactory control and leave harmless amounts of residues or no residues at all in the resulting olives or olive oil, are now available."

These words conclude centuries-old efforts and research towards complete control of the olive fly pest.

Résumé*

On rapporte avant tout, brièvement, les graves dommages causés par la mouche de l'olive (Dacus oleae Gml.) dans tous les Pays du Bassin de la

* Traduit par M. E. ALESSANDRINI.

Méditerranée dès les temps les plus reculés, et sur les méthodes, plus ou moins rationnelles, qui ont été employées pendant des siècles pour la combattre, jusqu'à la découverte des insecticides modernes de synthèse.

On rapporte ensuite les premières tentatives sporadiques réalisées à l'aide des hydrocarbures chlorés et, successivement, des insecticides phosphorés en différentes partie du monde et, en premier lieu, en Italie, où, dès 1954—55, un plan rationnel quinquennal d'expérimentation a été organisé en collaboration entre le Ministère de l'Agriculture et des Forêts et l'Istituto Superiore di Sanità (Ministère de la Santé).

Le problème, notamment, devait être considéré sous deux aspects principaux:

1. la lutte contre l'infestation, soit par la prévention, soit, le cas échéant, par l'extermination à l'aide des moyens les plus convenables et efficaces disponibles sur le marché.

Cela était du ressort du Ministère de l'Agriculture et de ses organes périphériques.

2. des recherches pour s'assurer que les huiles produites par les oliviers traités avec les insecticides ne contenaient pas de résidus toxiques, ou que tout au moins ces résidus se maintenaient entre des limites de securité tolérables en vue de la santé des consommateurs.

Cela était du ressort de l' Istituto Superiore di Sanità.

En conséquence, les hydrocarbures chlorés suivants ont été expérimentés: DDT, Aldrine, Dieldrine, Heptachlore, et les insecticides phosphorés suivants: Parathion, Diazinon, Malathion, Dipterex et Rogor (Dimethoate).

Pour la recherche et la détermination des résidus des différents principes actifs il a été nécessaire de disposer de méthodes d'analyse efficientes, qui pour la plupart furent étudiées et mises au point dans l'Istitut Supérieur de Santé, tandis que quelques-unes furent pourvues par les Firmes productrices de chaque insecticide.

L'expérimentation effectuée pendant les deux premières années démontra que, faisant abstraction de l'efficacité, les hydrocarbures chlorés étaient sans doute à déconseiller en consideration de leurs résidus toujours élevés et souvent même très élevés; par conséquence les recherches successives furent conduites exclusivement avec les insecticides phosphorés, notamment Parathion, Diazinon et Rogor.

Quelques tableaux sont présentés montrant les pourcentages des résidus des trois insecticides susdits qu'on a trouvés dans les nombreux échantillons d'huile d'olive examinés pendant les 5 années d'expérimentation. Un procédé aussi est décrit, sommairement, pour le raffinage des huiles contenant des résidus de Parathion: ce procédé a été breveté en Italie et dans les U.S.A., et il permet d'éliminer jusqu'a 200 p.p.m. de résidus, et peut-être plus.

Ensuite on décrit les recherches effectuées sur le Rogor, conduites dans le but d'établir la formation éventuelle de métabolites plus ou moins toxiques, et leur présence possible dans les olives et dans l'huile, soit par chromatographie sur papier, soit au moyen d'isotopes radio-actifs; ces recherches ont permis d'établir l'absence de résidus de Rogor ou de ses métabolites toxiques dans les huiles provenant des oliveraies traitées.

On reporte aussi quelques recherches sur la toxicité chronique vis-à-vis des singes du Parathion, et vis-à-vis des cailles du Parathion, Diazinon et Rogor.

Finalement on rapporte brièvement les résultats obtenus par quelques expérimentations effectuées dans plusieurs Pays du Bassin de la Méditerranée, par l'emploi de nombreux principes actifs nouveaux, appartenant également au groupe des insecticides phosphorés, et communiqués au cours de la IV Réunion de la F.A.O., tenue à Tel Aviv (Israël) du 5 au 12 décembre 1960.

D'après les résultats obtenus par les expérimentations effectuées, le rapport de la susdite Réunion de la F.A.O. a ainsi conclu:

«Ayant écouté les rapports sur le contrôle chimique de la mouche de l'olive, la Réunion conclut que des insecticides, qui en même temps donnent un contrôle satisfaisant et laissent des quantités tolérables de résidus, ou bien ne laissant pas de résidus du tout, sont maintenant à disposition.»

Ces mots concluent les efforts et les recherches séculaires pour le contrôle complet de la mouche de l'olive.

Zusammenfassung*

Zu Beginn der Abhandlung werden die schweren Schäden, die die Olivenfliege (*Dacus oleae* Gml.) den Kulturen des Mittelmeerbeckens schon seit ältester Zeit zufügt, und die mehr oder weniger wirksamen Bekämpfungsmethoden erörtert, die schon seit Jahrhunderten angewandt wurden bis zur Entdeckung der modernen synthetischen Insecticide.

Die Autorin berichtet dann von den ersten, vereinzelten Versuchen, die in verschiedenen Teilen der Welt mit chlorierten Kohlenwasserstoff- und später mit organischen Phosphor-Verbindungen unternommen wurden, und zwar hauptsächlich in Italien. Seit den Jahren 1954/1955 wurde ein wohlfundierter 5-Jahres-Versuchsplan in Angriff genommen in Zusammenarbeit mit dem Landwirtschafts- und Forstministerium und dem „Istituto Superiore di Sanità" (Gesundheitsministerium).

Das Problem mußte insbesondere von zwei Seiten aus betrachtet werden:

1. Ein Befall sollte verhütet oder, falls schon zu fortgeschritten, bekämpft werden mit den geeignetsten und wirksamsten Mitteln der vorhandenen Handelspräparate. Das Landwirtschaftsministerium war dafür verantwortlich.

2. Untersuchungen mußten vorgenommen werden, die sicherstellten, daß Öl von Oliven bespritzter Bäume keinerlei giftige Rückstände enthält, oder daß zumindest solche Rückstände innerhalb der Grenze liegen, die eine völlige Harmlosigkeit für den Verbraucher zuverlässig garantiert. Für diesen Punkt blieb das „Istituto Superiore di Sanità" verantwortlich.

Gemäß dieser Voraussetzungen wurden Versuche mit den chlorierten Kohlenwasserstoffverbindungen, DDT, Aldrin, Dieldrin, Heptachlor, und den organischen Phosphorverbindungen, Parathion, Diazinon, Malathion, Dipterex und Rogor (Dimethoat), durchgeführt.

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* Übersetzt von H. MARTIN.

Für die Versuche und die Rückstandsbestimmungen der verschiedenen Aktivsubstanzen brauchte man geeignete Analysenmethoden, die meistens vom Istituto Superiore di Sanità erforscht und entwickelt, zum Teil allerdings auch von den Herstellern der einzelnen Insecticide vorgeschlagen wurden.

Die Versuche der ersten zwei Jahre bewiesen, daß die chlorierten Kohlenwasserstoffverbindungen, ganz abgesehen von ihrer Wirksamkeit, entschieden nicht empfehlenswert waren wegen ihrer großen, oft sogar sehr großen Rückstände. Deshalb beschränkten sich die späteren Untersuchungen nur noch auf die organischen Phosphorverbindungen, insbesondere auf Parathion, Diazinon und Rogor.

Die Rückstände der drei genannten Insecticide, die in den vielen während der fünf Versuchsjahre geprüften Olivenölproben bestimmt wurden, sind in einigen Tabellen der Abhandlung als Prozentwerte aufgeführt. Ferner ist ein Verfahren zur Raffinierung von Öl, das Parathionrückstände enthält, kurz beschrieben. Dieses Verfahren ermöglicht es, bis zu 200 p.p.m. aus dem Öl zu extrahieren.

Wie die Autorin weiter ausführt, sind Untersuchungen mit Rogor gemacht worden, um die Bildung von mehr oder weniger giftigen Metaboliten zu erforschen. Mit der Papierchromatographie oder mit radioaktiven Isotopen kann das Vorhandensein von Metaboliten in Oliven oder in Öl bestimmt werden. Die Forschungsergebnisse haben gezeigt, daß Rogor keine Rückstände oder giftige Metaboliten im Öl, das von behandelten Oliven gewonnen wird, hinterläßt.

Berichte von Untersuchungen über chronische Toxicität von Parathion bei Affen und von Parathion, Diazinon und Rogor bei Wachteln werden aufgeführt.

Schließlich werden noch kurz Resultate beschrieben, die in mehreren Mittelmeerländern mit verschiedenen, zur Gruppe der organischen Phosphorsäureverbindungen gehörigen Aktivsubstanzen erzielt worden und an der IV. FAO Konferenz in Tel-Aviv, Israel, im Dezember 1960 bekannt gegeben worden sind.

Der FAO-Bericht, der sich auf die Ergebnisse der verschiedenen Versuche stützt, schließt folgendermaßen:

„Auf Grund der Berichte über die chemische Bekämpfung der Olivenfliege stellt der Kongreß fest, daß nun über Insecticide verfügt wird, die sowohl befriedigende Wirkung als auch harmlose Mengen oder sogar überhaupt keine Rückstände in den behandelten Oliven und im Olivenöl ergeben."

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The chemical and physical nature of plant surfaces in relation to the use of pesticides and to their residues

By
A. S. CRAFTS* and C. L. FOY *

With 2 figures

Contents

I. Introduction

One aspect of the agricultural revolution of the twentieth century that is proving to be a mixed blessing is the widescale use of pesticides. On the beneficial side are the great many instances of increased production that have resulted. On the other side are the many misuses that have resulted in injuries and death, and the wide array of residue problems of varying degrees of seriousness. The nature of plant surfaces is important from both aspects; effectiveness of many pesticides, particulary the systemic insecticides, fungicides, and herbicides is related to their penetration and subsequent translocation; the amounts and nature of pesticide residues on or in plant parts on the other hand may be intimately related to the composition of plant cuticle. Pesticides now include a wide variety of organic and inorganic compounds such that generalizations and accurate predictions with respect to their distribution and metabolic fate in plants is extremely difficult. It is apparent that many of the phenomena which are involved in the retention, absorption, accumulation and persistence of pesticides are not well understood. Not only do the physical and chemical properties of compounds and mixtures differ widely but plant surfaces are known to be highly complex and variable as well, having protuberances of many forms, wax crystals and rodlets of innumerable types, cutin layers of varying amounts and composition, including wax platelets of crystalline nature, pectic and cellulosic components.

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The literature dealing with plant surfaces is very extensive. Comprehensive review of all facets is beyond the scope of this chapter, wherein general principles will be emphasized and the chemical and physical nature of plant surfaces will be discussed mainly in relation to pesticide residues. Various aspects of the broad subject have been treated in texts, theses, and other reviews as follows: (a) waxes: KREGER (1948) and SCHIEFERSTEIN (1955); (b) cuticle: ORGELL (1954), PRIESTLEY (1943) and SCHIEFERSTEIN (1957); (c) retention and penetration of pesticides and growth regulators: CURRIER and DYBING (1959), MITCHELL *et al.* (1960), VAN OVERBEEK (1956) and WOODFORD *et al.* (1958); (d) plasmodesmata: MEEUSE (1957). Also of recent interest and debatable importance to pesticide researchers is the occurrence of ectodesmata, protoplasmic strands protruding from the protoplasts of epidermal cells for varying distances into the exterior walls (FRANKE, 1960 a and b, 1961; LAMBERTZ, 1954; SCHUMACHER, 1942; SCHUMACHER and LAMBERTZ, 1956).

Differences in form and wettability of plant cuticles are known to determine the deposition of insecticides and fungicides and in some instances to affect the selectivity of herbicides (BLACKMAN, 1959; CURRIER and DYBING, 1959; EBELING, 1939; ENNIS *et al.*, 1952; LINSKENS, 1950; STANIFORTH and LOOMIS, 1949). Root surfaces in contrast are known to be specialized in relation to the uptake of water and nutrient ions from soils. Many modern pesticides are applied through the soil and are known to be readily absorbed and translocated within plants. Thus many of the special features of plants that enable them to live and thrive in their various terrestrial and aquatic environments may serve in the uptake and distribution of pesticides. They also relate to the persistence of these compounds on or within plants and to their ultimate fate. Some pesticides volatilize and leave plant parts quite rapidly; some persist as adsorbed residues on plant surfaces; some penetrate and are metabolized within plants; and some remain relatively stable within plants and may even pass from one generation to the next unchanged (FOY, 1961 b; MCILRATH and ERGLE, 1951, 1953; MILLER *et al.*, 1958; PRIDHAM, 1947). With such a wide array of known behaviors, and with the increasing awareness of the implications of residues in food, it is important to know what happens to agricultural chemicals, fertilizers, micronutrients, growth regulators, pesticides and all other materials used on crops from the time they are applied until the plant products are ready for consumption. The properties of plant surfaces as these relate to the above problems will be considered here, in so far as they determine the fate of toxic chemicals.

II. Phylogenetic and ecological considerations

It is commonly assumed by botanists that plants originated in water as simple unicellular organisms, and that by specializations of many sorts they have attained their present complexity of form and organization. Algae have increased in size until by specialization of vascular tissues for conduction of foods they have been able to grow to a hundred meters in length

and thus to occupy waters of great depth. The brown algae have phloem tissue as specialized as that of many lianas and this enables them to produce the kelp beds common to many coasts. Another direction of specialization was in the increasing complexity of reproductive structures as exemplified by the red algae. These specializations and others have enabled algae to occupy waters of varying depths, temperatures, and salt contents. They did not, however, enable them to migrate onto the land and it was probably extinct species of the green algae that first occupied truly terrestrial environments.

Several new features were needed before plants could permanently move from the aquatic environments of their origin. Whereas vascular tissues enabled brown algae to occupy deeper and deeper water, and complex reproduction cycles enabled red algae to attain a wide distribution and to endure great differences in light, temperature, pressure, day length, etc., it was not until plants were able to acquire a cutinized surface that they could grow permanently in a terrestrial environment with foliar organs exposed to the air. The aerial exposure was undoubtedly of great advantage, for gaseous diffusion is tremendously faster than diffusion through liquid; the provision of adequate CO_2 for rapid growth was a definite gain from the standpoint of potential dry weight synthesis but the very exposure that enabled the plant to absorb CO_2 also provided for rapid water loss, and thus the plant could not endure for long.

The cuticle, a layer laid down of products of metabolism of cells of laminar organs, was the answer to this problem. Whereas, in water these compounds were largely washed away, in the air they oxidized and polymerized and formed a coating that greatly inhibited water loss. And because this coating also prevented rapid uptake of CO_2 the terrestrial plant developed a second new feature, the stoma. This opening which enables the CO_2 molecules to penetrate to the interior of the leaf and the O_2 molecules to diffuse out, is under control of the protoplasm and plants, through evolution, have specialized this mechanism so that stomatal opening takes place during the light hours when photosynthesis is at a maximum, and closing occurs at night so that water loss is minimized.

Another essential feature of the land plant is the root system that allows for uptake of water and minerals from the soil, thus guaranteeing a water supply to replace that which is necessarily lost during the period when CO_2 is being absorbed. The root hairs represent a great extension of the root surface that enables the plant to quickly tap the total soil mass that is occupied by its roots.

Finally the plant developed the xylem system by which the water and salts taken up by the roots are quickly and effectively distributed throughout the aerial portion. By this combination of specializations green plants were able to migrate to the land and to occupy deserts, arctic regions, alpine sites and marshes. And by even more elaborate specialization some plants occupy epiphytic environments, some grow on rock or metal surfaces, and others are parasites on or in other plants. Throughout this great array of growing conditions two needs are uppermost; mineral nutrients are essential to growth and water is necessary for survival.

III. The chemical nature of plant cuticle

Both the chemical nature and the physical structure of cuticle are important to its function of water conservation. Chemical composition will be considered first.

Since terminology in the literature on plant cuticle has become badly confused, perhaps some clarification is necessary before proceeding. BRONGNIART (1830, 1834) first described the thin membrane that covers the aerial parts of the higher plants. He named it "cuticle" and showed that it was noncellular in structure. In this review, most definitions of terms will follow those adopted by PRIESTLEY (1943) in his review on cuticle, by KREGER (1948) in his comprehensive study of plant waxes, and each as redefined by ORGELL (1954) and SCHIEFERSTEIN (1957). Cuticular layers are the several lamellae of the surface-covering that have become embedded with wax and cutin. This term may include the cellulose of the outer walls of epidermal cells when this layer has become impregnated with lipid substances. Cuticle refers to the outermost of the cuticular layers consisting of semilipoidal materials exterior to the outer epidermal cell wall, including cutin layers and wax incrustations. Cutin refers to the semi-lipoidal polymer, insoluble in most organic solvents, which results from the oxidation and polymerization of various unsaturated lipid compounds and which constitutes the matrix of the cuticle. Cuticular wax refers to the petroleum-ether soluble mixture of more saturated lipid substances embedded in the cuticular layers. Finally, surface wax refers to the usually irregular deposits of similar material found on the cuticle surface of some species. All leaf and young stem surfaces are covered by cuticle.

PRIESTLEY (1924) proposed that the lipoidal substances composing cutin and suberin are not simple chemical entities but rather classes of acidic substances resulting from metabolism of growing cells. LEE and PRIESTLEY (1924) concluded that cuticle formation is associated with synthesis of fatty compounds by dividing protoplasts. They considered cutin to be an oxidative polymer of unsaturated fatty acids and other lipoidal compounds. They found that treatment with potassium hydroxide led to the solubilization of a portion of the cutin but with mature cuticle an unsaponifiable residue remained. BONNER (1950) suggests that fats in plants are synthesized from carbohydrates. Some of this substrate is oxidized to CO_2 while the remainder is reduced to fatty acids. BONNER proposes that acetate is an intermediate in the process and that acetyl phosphate with its high-energy bond is an essential component of the system; acetyl phosphate and acetic acid condense to form acetoacetate plus phosphate. The acetoacetate by reduction gives butyric acid which condenses with more acetate to form butyroacetate that is subsequently reduced to caproic acid; by a continuation of this process long chains having even numbers of carbon atoms are synthesized, high energy phosphate being required for each condensation, and a hydrogen donor being required for each reduction.

Long chain fatty acids and alcohols formed by these condensations migrate to the ectoplasmic surfaces during differentiation and thence via the cell walls to the outer epidermal wall. The fatty acids and alcohols in these migrating products of differentiation contain unsaturated linkages

as do all vegetable fats (PRIESTLEY, 1943). At the air-water surfaces in the epidermal wall they are finally deposited with their polar groups in the water phase and their hydrocarbon chains in the air. In the presence of oxygen these oxidize and gradually condense to form a more or less continuous film over the outer surface of the plant. As water is lost and reaction with oxygen proceeds, the outer layer becomes "varnish-like" and constitutes a relatively water proof, protective coating. LEE and PRIESTLEY (1924) were therefore justified in drawing an analogy between cutin and varnish.

In more recent studies, ROELOFSEN and HOUWINK (1951) concluded that cutin is a polymolecular network of various unsaturated fatty acids, dicarboxylic acids, and hydroxycarboxylic acids connected by ester, ether and diether bridges. They considered this framework to be impregnated with wax, some of which may be in chemical combination with the cutin. Plant scientists have long recognized the almost universal presence of such a coating on land plants, and they emphasize that this cuticle, along with the suberized bark of older stems, constitute a continuous layer that materially reduces water loss.

The outer cuticular layer is readily recognized and it may be selectively stained with the dye Sudan III. Careful studies have shown that fatty substances may coat and even impregnate cell walls of sub-stomatal chambers inside the leaf or stem. Investigations by ARTZ (1933), FREY-WYSSLING and HAUSERMANN (1941), HAUSERMANN (1944), SCOTT (1950), THODAY (1933), URSPRUNG (1925) and others indicate that the walls of mesophyll cells and spongy parenchyma, and particularly of sub-stomatal chambers are cutinized. LEE and PRIESTLEY (1924) have suggested that sodium, potassium, and magnesium ions carried into mesophyll cell walls in the transpiration stream may facilitate migration by forming water-soluble soaps. Calcium ions, forming insoluble soaps would inhibit movement. This may partly explain the failure of radioactive calcium, applied to leaves as a tracer, to move out in the phloem (CRAFTS and YAMAGUCHI, 1960 b).

Studies by X-ray methods have shown that even primary walls of young cotton hairs, *Avena* coleoptiles and many meristematic tissues are impregnated with wax, that is, esters of fatty acids and long chain monohydric alcohols. Chain lengths of these vary between 24 and 32 carbon atoms (HESS *et al.*, 1936).

FREY-WYSSLING (1948) has summarized the chemical nature of cutinized plant cell walls; they are composed of four distinct substances all of which may vary in distribution within the wall. Submicroscopic anatomical relationships have been represented diagrammatically by ORGELL (1954) in Fig. 1 as modified from FREY-WYSSLING (1948), MUELLER *et al.* (1954) and ROELOFSEN (1952). These substances are: (1) cutin, (2) cutin waxes, (3) pectin, and (4) cellulose.

Cutin is isotropic in polarized light, insoluble in ordinary reagents, stainable in basic lipid dyes, saponifiable with sodium hydroxide and it absorbs ultraviolet radiation strongly. This last property probably effects considerable protection to the photosynthetic mechanism of plant cells that

are subject to solarization in strong light. Particularly is this possible in the case of alpine plants at high altitudes.

Cutin waxes are optically negative, soluble in pyridine, stainable in lipid dyes, and they melt above 220° C. They do not absorb ultraviolet radiation.

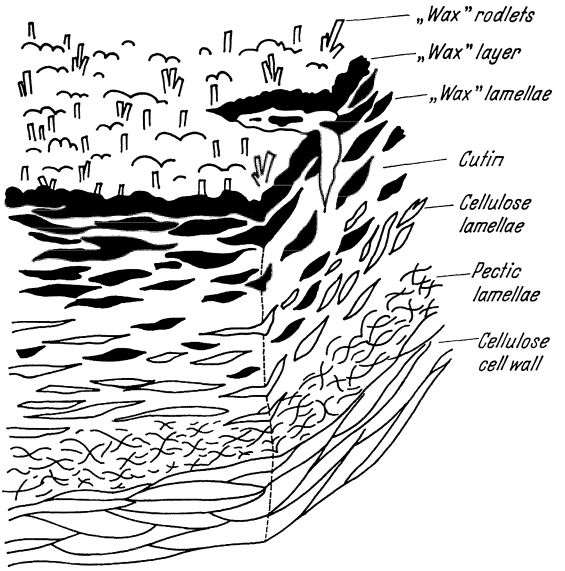

Fig. 1. Submicroscopic structure of the cuticle. Lamellae are interlinked and cracks may occur in the outer surface. From ORGELL (1954), as modified from FREY-WYSSLING (1953), ROELOFSEN (1952), and MUELLER *et al.* (1954)

Pectins are formed of long chain polygalacturonic acid molecules having side carboxyl groups. These are capable of forming salts; they impart to pectins base exchange properties.

Polygalacturonic acid is soluble in water but its calcium salt is insoluble. Some of the carboxyl groups of pectin are methylated through oxonium oxygen; these methoxy groups do not lower the water solubility. Pectic substances have little tendency to crystallize, they occur in an amorphous state in plant cell walls, and they are responsible for the strong water holding properties of the walls. Pectins are isotropic, soluble in picric acid and hydrogen peroxide, stainable in Ruthenium red; they break down upon hydrolysis, are disintegrated by pectic enzymes, and they do not absorb ultraviolet light.

Cellulose is optically positive, soluble in Sweitzer's reagent, stainable in iodine-zinc chloride and readily hydrolyzed. It does not absorb ultraviolet radiation. Cellulose is composed of very long chain molecules that are relatively stable. These molecules in turn are organized into micelles and the micelles are associated into microfibrils. Thus the cellulose structure of the cell walls because of its fibrillar organization imparts tensile strength and elasticity. It is this property of cell walls that resists expansion and hence results in turgor. Turgor in turn results in form, enabling the plant

to grow erect against the force of gravity, to extend its roots into the soil and tap a large water and nutrient supply, and to maintain its foliar organs in positions favorable for maximum absorption of light and CO_2.

Cutin waxes consist of short rod-shaped molecules having no reactive end groups; being unable to polymerize they are of low molecular weight. Suberized and cutinized wall layers contain unsaturated high molecular weight ketones, alcohols or esters of unsaturated alcohols; their reactive end groups enable these compounds to form esters and thus to develop high polymeric chains of complex structure. Since cutin has a pronounced negative charge, has selective cation permeability, and is stainable with basic dyes, it must be only partially esterified, with free carboxyl groups exposed at the surface. Since it is optically isotropic, cutin must possess a reticular linkage structure like lignin.

As described by FREY-WYSSLING (1948) the molecules of the membrane waxes are optically positive rodlets. However, since the waxes referred to the tangent of the cuticular layers produce negative birefringence, their molecules must be perpendicular to the membrane. After extraction of the wax, the cuticle exhibits textural birefringence which, when referred to the optical axis of the layer, is negative. This constitutes lamellar birefringence indicating submicroscopic lamellae, presumably of cellulose. The wax platelets are interposed between and perpendicular to these cellulose chains. Since the hydrophobic wax molecules repel the hydrophyllic cellulose it seems probable that the polar cutin molecules are interposed between them with their hydrophyllic hydroxyl, carboxyl, and methoxy groups bounding the cellulose, and their hydrophobic hydrocarbon chains in contact with the waxes.

VAN OVERBEEK (1956) states that the wax rods of sugar cane stems are composed of higher alcohols of chain length of 28 carbon atoms. These molecules are visualized as lying perpendicular to the longitudinal axis of the rods. The rods are pushed up from below and crystallization may take place near the surface or above the surface. The latter situation gives rise to the waxy bloom common on many plant structures (i. e. "blue" plums, cabbage leaves, etc.). Cotton cuticle contains C_{28} and C_{30} alcohols; rose petal wax has primary alcohols, saturated ketonic acids, and hydroxy acids in the C_{24} to C_{30} range (VAN OVERBEEK, 1956). Many such waxes have even numbered carbon chains indicating their origin by condensation of acetate; paraffins, ketones, and secondary alcohols however may contain odd numbered chains indicating formation by decarboxylation of even numbered acids.

Wax is synthesized throughout the life of the plant; young structures have acid wax indicating condensation of cellular fatty acids to form high molecular weight acid molecules. Alcohols may result from reduction of such molecules; esters may then be formed between acid and alcohol molecules. Cutin may contain appreciable quantities of polymerized dicarboxylic and hydroxy carboxylic acids (ROELOFSEN, 1952). Having many polar groups such cutin may absorb water and swell. This increases permeability to polar molecules and promotes absorption of water soluble pesticides (see later).

IV. The physical nature of plant cuticle

Probably the most characteristic feature of plant cuticle is its extreme variability. This involves not only chemical variability as indicated in the above pages, but variability in distribution over the plant surface, in continuity and degree of perforation, in thickness on various parts of a single leaf and in chemical and physical properties as between different plants and plants of different environments. Since in its early stages of deposition cutin may be liquid, it tends to follow the laws of capillarity making thick deposits in crevices and being thinner over convex surfaces. Thus cuticle may vary in thickness in relation to the cellular structure of the plant leaf; it may vary as between the covering over veins and in interveinal regions; and it is usually thicker on the upper than on the lower leaf surface (see reviews cited earlier).

Considerable evidence from electron micrographic studies supports the hypothesis that during the expansion of leaves that have a rapid growth rate, the cuticle is extended at unhardened zones near the margin of individual epidermal cells (SCHIEFERSTEIN and LOOMIS, 1956). Secondary cuticle thickening may then proceed to such an extent even on herbaceous annual leaves that the cuticle becomes anchored directly in the cellulose of the outer walls of epidermal cells. It is apparent that areas directly over anticlinal cell walls, veins, and otherwise where cuticle has not yet been deposited may serve as preferential sites for absorption of pesticides.

Cuticle varies in composition and structure between plant species; succulents such as cacti have thick cuticle; trees often have medium to thick cuticle; herbs vary widely and those that characteristically grow in shade have thin cuticle. Environment also has great influence on cuticle development, high insolation as in desert areas and at high altitudes being conducive to heavy cuticle whereas shade and moisture are conducive to thin cuticle.

Although many instances of strong correlation between cuticle thickness and habitat might be cited, attempts to correlate cuticle thickness with xerophytism often fail and here stomatal regulation of gaseous exchange may be crucial. Only when stomata are closed may cuticle thickness be instrumental in water conservation. However, cuticular transpiration — that is evaporation of water through the cuticle — may range from 10—25 percent of total transpiration when stomata are open. FREY-WYSSLING (1953) believes that cuticular transpiration takes place through hydrophilic lamellae of cellulose and pectin. HÄRTEL (1951) and ROUSCHAL and STRUGGER (1940) postulate that transpiration occurs preferentially through pores. Although cuticular transpiration can be shown to correlate rather strictly with cuticle thickness, a few examples may also be cited where cuticle thickness was not correlated with rate of water loss (KAMP, 1930). Thus cuticle quality as well as cuticle thickness must be considered. Also, rates of movement of organic substances and even of water may not be the same when they are escaping as when they are entering the leaf. "Coefficients of asymmetry" have been calculated for movements of water inward and outward through insect cuticles and through synthetic model membranes (HURST, 1948). Using similar techniques, SCHIEFERSTEIN (1957)

found that stomateless cuticular preparations from the upper surface of ivy leaf had 1.44 ± 0.14 times greater permeability to water in the inward direction than the outward direction. This coupled with results of experiments on the direction of shrinking and swelling of cuticle (ORGELL, 1954; SCHIEFERSTEIN, 1957) may be considered further evidence of a gradient from low polarity on the exterior to relatively high polarity in the layers bordering the epidermal cell wall. Other features of the environment than humidity enter the problem; salinity and alkalinity as they relate to both water availability and plant composition may alter the cuticle, and bog plants of acid environments may well have as heavy cuticle as xerophytes of alkaline soils.

Although the cuticle layer is often viewed as a continuous layer over the surface of the foliar portions of the plant much evidence indicates that it varies widely in perviousness. As would be expected, cracks and perforations increase cuticular permeability (ORGELL, 1957). Cuticular transpiration is evidence for movement of water molecules through the cuticle and cases have been cited (RUDOLPH, 1925) where as much as 33 percent of total water loss may be cuticular in the case of shade plants.

Further evidence for permeability of the cuticle to polar compounds is the salty residues found on the outer leaf surface of leaves of plants of saline environments (e.g. *Disticlis spicata*). This salt evidently moves in solution to the outer leaf surface where it crystallizes out as the water evaporates.

Irrefutable evidence for permeability of cuticle to polar compounds is found in work with radioactive tracers. Many of these, applied in aqueous solution to upper leaf surfaces of hypostomatous leaves, penetrate into the vascular tissues where they are translocated to other parts of the plant (CRAFTS and YAMAGUCHI, 1958; FOY, 1962). Alternate polar or apolar pathways across the cuticle have been suggested (ROBERTS *et al.*, 1948). And comparative studies prove that penetration may be determined very largely by the properties of the tracer molecule; some apparently penetrate rapidly via the lipoid phase of the cuticle (CRAFTS, 1956) but others of highly polar nature, for instance amino triazole (amitrol), maleic hydrazide (MH), PO_4^{-3}, and K^{+1} apparently enter the leaf via an aqueous route (CRAFTS, 1961 a and b).

By spectrophotometric analysis, SCHIEFERSTEIN (1957) showed that the rate of penetration of 2,4-dichlorophenoxyacetic acid (2,4-D) through ivy cuticle varied with age and hence with thickness of the cuticle. Relative penetration rates of different cuticle thicknesses indicated, however, that thickness was not the only factor involved. The relative abundance of cutin, waxes, and other minor constituents and their order and arrangement of deposition are undoubtedly important and may be expected to vary among species and with changes in environment. RICHMOND and MARTIN (1959), for example, found that levels of both cutin and wax increased in cuticle of apple during development, that N deficiency led to reduced wax, and that N, P, and K deficiencies resulted in a higher content of phenolic compounds.

Thus the cuticle of plants is a heterogenous material of highly variable

properties. It must vary not only in composition but in physical structure as well. And like other plant structures it undergoes ontogenetic change being fluid and reactive when first laid down, but gaining in thickness, in degree of oxidation, and in viscosity with age. Finally, the cuticle becomes subject to weathering and degradation from exposure to sunlight, rain, buffeting by wind, and the activities of insects. All of these factors relate to the permeability of plant surfaces to pesticides, to the persistence of their residues on or in plant organs, and to the loss of such residues by leaching, by decomposition, and by weathering away of the surface layers.

V. The nature of the epidermal wall

Underneath the cuticle there is a layer of pectin which is intercalated to varying degrees with wax and cutin. According to RAWLINS and TAKAHASHI (1952) and to VAN OVERBEEK (1956), a distinct layer of unimpregnated pectin is often present just outside the cellulose of the epidermal cell wall. This is probably most often true in the early stages of cuticle maturation.

The cellulose of the outer wall of epidermal cells is normally embedded in pectins. This layer may eventually become impregnated also with cutin and waxes (HUELIN and GALLOP, 1951; KREGER, 1948; VAN OVERBEEK, 1956), and when this happens it becomes the innermost of the cuticular layers.

The above description indicates the disposition of the cell wall substances in the outer epidermal wall; hydrophilic lamellae of cellulose and pectins adjoining and containing the symplast, layers of radially oriented wax molecules arranged in rodlets, and between and around these amorphous cutin in random arrangement. In a way this resembles lignified walls of the inner plant structures. In these, amorphous lignin is intercalated between cellulose rodlets or lamellae; in both cases the cellulose is masked by incrustation with sharing of valence bonds. Cellulose cannot be dissolved from the cutinized wall with Schweizer's reagent; it is more easily liberated by saponification of the cutin. According to SCHIEFERSTEIN (1957), the degree to which wax and cutin are present depends upon the species and probably upon the age of the leaf. Apparently the cellulose of the outer epidermal wall does not react chemically the same as cellulose elsewhere (HUELIN and GALLOP, 1951; SCHIEFERSTEIN, 1955). It is uncertain, however, whether such behavior is due to chemical combination between cellulose and wax and cutin or to a physical masking of the cellulose by lipid substances.

With respect to the relative location of the four principal constituents of the epidermal wall, cellulose and pectins predominate in the inner regions where they lend strength and water retaining properties. Waxes are in predominance toward the outside; the outer layers contain only wax and cutin while the isotropic cutin proper comprises only a thin pellicle of amorphous material. In all probability the cutinic acids released from the surface of the protoplasts migrate outward along the cellulose-pectin apoplast in a simple state; waxes may migrate as acids and alcohols. As these substances move outward they are subject to progressive dehydration (from

99 + percent relative humidity to a much lower value) and to oxidation. Together they form a relatively watertight, varnish-like layer that effectively limits water loss. This is the structure that has enabled plants to survive a terrestrial environment and thrive in many habitats of medium to low water availability.

VI. Root surfaces of plants

With the development of roots primitive plants were able to move out of the marshes, bogs, and sloughs up onto well drained soils and thence to inhabit the varied terrestrial habitats that we know today as favorable for plant growth. Roots of plants are specialized by potentialities for rapid growth for the purpose of exploring thoroughly large masses of soil. They are highly subdivided (branching) and their surfaces are elaborated (root hairs) to provide great absorbing surface. And they grow continuously into new soil so long as soil moisture is adequate and the foliar organs provide a food supply. Absorbing root surfaces are composed of polyuronides, polar compounds permeable to water and salts, and in the case of the pectic wall substances, dissociable, providing charged surfaces along which ions may migrate in the process of contact absorption. Impregnation of the radial walls of the endodermis with suberin renders them water tight which limits radial movement of water and salts to the symplast. This is an important feature of the young root enabling it to absorb water and salts actively and to provide a pumping mechanism for the distribution of inorganic nutrients into the top of the plant during the early life of the seedling.

Two features of the root system are of interest in the problems of the use of pesticides and the occurrence of residues. The same absorption mechanism that takes up water and salts will absorb some pesticides from the soil. And so there have been developed a great many fumigants and soil borne pesticides that are useful for controlling insects, fungi, nemotodes or weeds. Examples of these are methyl bromide; carbon disulfide; ethylene dibromide; mixed 1,2-dichloropropane and 1,3-dichloropropene (D,D mixture); chloropicrin; sodium *N*-methyldithiocarbamate (Vapam); ethyl-*N*,*N*-di-*n*-propylthiocarbamate (Eptam); propyl ethyl-*n*-butylthiolcarbamate (Tillam); hexachlorocyclohexane; the substituted ureas and triazines; 2-chloroallyl diethyldithiocarbamate (Vegedex); *alpha*-chloro-*N*,*N*-diallylacetamide (Randox); 2,4-D; isopropyl *N*-phenyl carbamate (IPC); isopropyl *N*-(3-chlorophenyl) carbamate (CIPC); etc. All of these chemicals are effective in controlling one or more pests; all of them may ultimately be present in plants or plant parts used for animal or human food and all of them present residue problems in the soil, although some volatilize or decompose so rapidly as not to constitute a serious problem. And even pesticides that are applied only as foliar sprays may reach the soil in appreciable quantities.

Some of the soil-borne systemic pesticides undergo chemical change before and/or after their absorption by plants and so the residues within the plant may have a different chemical composition than the applied

material. It is problems such as these that the residue chemist has to face when he attempts to determine the significance of residues in relation to new pesticides. Some compounds such as Vegedex and IPC break down very rapidly in moist soils and so present very little difficulty from a residue standpoint; others such as arsenicals, selenium, and the more resistant organic molecules as for example the substituted ureas, the symmetrical triazines, sodium 2,2-dichloropropionate (dalapon), and the chlorinated benzoic acids may persist in soils for a long time and hence cause considerable difficulty as residues. In the case of some pests, particulary weeds, it is highly desirable to eliminate all growth for long periods and so soil sterilizing doses are applied. Chemicals that sterilize soils are widely used on railroad ballast, on irrigation canal banks, on air fields, and on many industrial sites. While such chemicals are highly desirable for such use it should be evident that their use on crop land is fraught with danger. There is no such thing as a universal weed killer and pest control operators must learn to use the many pesticides that are available in the proper places and at proper dosages. Only in this way can serious residue problems be avoided.

A second aspect of root function is the recognized ability of roots to secrete compounds into the soil, and to leave residues as a result of root decay. Natural compounds secreted by roots may be important in plant succession (MARTIN and RADEMACHER, 1960). Some examples are the inhibition of potatoes and flax by *Polygonum persicaria,* the residues of quackgrass that inhibit growth of alfalfa and other crops (OHMAN and KOMMEDAHL, 1960), and the secretions from roots of corn and other cereals that trigger seed germination in witch-weed (SUNDERLAND, 1960 a and b).

Of similar importance are secretions of systemic herbicides by roots. It is recognized that *alpha*-methoxyphenylacetic acid (MOPA) (LINDER *et al.*, 1957), 2,4-D (CLOR, 1959; CRAFTS, 1956 b), dalapon (FOY, 1961 a), benzoic acids (MASON, 1960), MH (CRAFTS, 1962), and other herbicides may be secreted into soils or nutrient solution and that some such as the chlorobenzoic acid herbicides may affect the growth of subsequent crops (LINDER *et al.*, 1958). Thus residues from roots constitute a recognizable problem in the use of pesticides.

VII. Plant surfaces in relation to uptake and distribution of pesticides

Most foliar applied substances appear to enter leaf tissue by (a) diffusion as a vapor through the stomata, (b) mass movement as a liquid through the stomata, or (c) diffusion through the cuticle and epidermal walls. Limited amounts of pesticides probably also enter through hydathodes, lenticels, and fissures in the bark. Some believe cuticular diffusion is the usual mode of foliar entry. For example, ROBERTS *et al.* (1948) believe that water soluble substances penetrate through pectic lamellae interspersed with cutin lamellae in the cuticle. Opinion is divided, however, on the relative importance of cuticular penetration and stomatal penetration; both are known to occur (cf. references cited by CURRIER and DYBING, 1959). Although not directly the subject of this chapter stomatal

penetration deserves brief mention. Stomata are penetrated easily by vapors, oils, or aqueous solutions containing suitable surfactants, but are not penetrated readily by water. Completely closed stomata can apparently exclude all fluids (VAN OVERBEEK, 1956). It should be pointed out, however, that pesticide solutions entering the leaf via the stomata still encounter a lipid layer lining the sub-stomatal cavity, the so-called internal cuticle. This layer is normally thinner than, but somewhat similar in properties to, the external cuticle. Its exact nature and permeability, however, are less well understood; histochemical tests indicate a suberin-like nature (SCOTT, 1950; SCOTT *et al.*, 1948). Plasmodesmata are believed to be absent in walls exposed to intercellular spaces (SCOTT and LEWIS, 1953).

Since penetration by pesticides is ultimately controlled by adhesion between molecules the chemical composition and the surface chemistry of the cuticle become of paramount importance. Both chemical and physical properties are involved and the two are not easily separable. ORGELL (1954) visualized cuticular penetration to occur in three phases as follows: (a) adsorption of the penetrant at the cuticle-spray solution interface; (b) absorption of the penetrant into the bulk of the cuticle, either by adsorption on internal surfaces or by solution within the cuticle; (c) desorption into the plasma membrane of the cell. VAN OVERBEEK and BLONDEAU (1954) considered hydrocarbons and surfactants to "solubilize" into the plasma membrane displacing the fatty molecules and increasing the permeability of the membrane. Here ectodesmata may play a role (FRANKE, 1960 a and b, 1961; LAMBERTZ, 1954; SCHUMACHER and LAMBERTZ, 1956), i. e., desorption from the cutin reservoir into these strands may occur. However the general prevalence and importance of ectodesmata and/or cuticular pores in the accumulation of pesticides remains obscure. The above concept applies mainly to organic pesticides in aqueous solution since inorganic salts, acids, and bases apparently do not penetrate intact cuticle very rapidly. It is to be expected that oils and pesticides of a lipophilic nature will be absorbed by cuticle more readily than those that tend to be polar. For example, as reviewed by MITCHELL *et al.* (1960), regulating substances in the form of esters, nitriles, and others with lipophilic radicals are thought to pass through the cuticle and into cells more readily than comparable compounds of a more polar nature (BEEVERS *et al.*, 1952; CRAFTS, 1953; OSBORNE *et al.*, 1955; VAN OVERBEEK *et al.*, 1955).

Movement of substances through cuticle involves diffusion, which is conditioned by particle size, pH, molecular structure of the penetrant, the prevalence of water (HURST, 1948) which may spread platelets apart and thereby alter permeability, and possibly other factors. Interactions existing between cuticle and applied substances may be mechanical (relation of penetrant particle to pore size), physico-chemical (competition for adsorption sites, etc.). or chemical (chemical or electrical reactions). The nature of the cuticle and penetrating substances, and their physical and chemical environments, determine the extent of these interactions and whether they will help or hinder penetration. The steric, polar, electrical (ion charge), and chemical properties of the penetrant molecule will influence its inter-

action with cuticle (MITCHELL *et al.*, 1960; ORGELL, 1954). Also the physical environment is known to influence cuticle-penetrant interactions. Sorption is the first phase in the cuticular penetration process. Initially it is rapid, and is influenced by pH, polarity of solvent and solute, charge on penetrating particles and additive particles, and concentration (ORGELL, 1957). In experiments with acid 2,4-D and both acid and basic dyes electrostatic effects of penetrants dominated polar effects. ORGELL (1954) found (a) that adsorption to isolated cuticle surfaces increased in a parabolic fashion and approached a maximum value (surface saturation) with increasing concentration; (b) that the effect of temperature on amount of penetrant dissolved in cuticle was difficult to predict, but sorption generally decreased as temperature increased; (c) that time of adsorption and absorption varied with the sorbing substance and penetrant; and (d) that aqueous solutes sorbed by the cuticle were generally desorbed only with difficulty in distilled water. As regards the influence of the physico-chemical environment, ORGELL (1954) found that sorption of a substance by cuticle was large when (a) the polarity of the cuticle and penetrant were similar, (b) the polarity of the solvents and penetrant were different, and (c) the cohesive forces between the molecules of solvent are large. The polarity of pesticide molecules determines their solubility in the carrier solution, cuticle, cell wall, and plasma membrane. The less polar the molecules, the more lipid soluble they are. Undissociated solutes are relatively non-polar, and therefore are oil-like and penetrate more readily. Oils can penetrate both stomata and cuticle; they also move through intercellular spaces by capillarity. Penetration is often noted first over midrib and leaf margins. FOGG (1948) also found water soluble substances to move chiefly by diffusion within cell walls. Oils and apolar substances in oil also penetrate the cuticle directly, apparently through the waxy portion of the cuticle with subsequent contact with pectic portions. Ions or polar substances, after sometimes being aided in coming in contact with the leaf surface by the use of a surfactant, penetrate the cuticle through cracks or punctures or areas of leaf not completely covered by waxy lamellae. Ions thus presumably penetrate via the hydrophilic pectic and cellulosic portions of the wall.

It has long been recognized that surfactants may increase penetration of pesticides into plants (CURRIER and DYBING, 1959; JANSEN *et al.*, 1961). Surfactants are known to enhance both stomatal penetration, presumably by reducing surface tension of solutions, and cuticular penetration (FOY, 1962). Surfactants have a large effect on sorption which is greatly influenced by pH. ORGELL (1957) found that both cationic and anionic surfactants hindered the sorption of acid dyes and 2,4-D at low pH values. Nonionic surfactants had little effect. At high pH values, however, cationic surfactants caused sorption of acid dyes and 2,4-D whereas anionic and nonionic surfactants had little effect. Desorption of a substance occurred best under the opposite conditions.

Factors to be considered when studying the effects of surfactants as spray additives on the cuticle and penetrant are (a) wetting and spreading, (b) sorption of the surfactant to the cuticle changing its charge and polarity,

(c) solubilization of the penetrant, and (d) complex formation or reaction with either the penetrant or cuticle. Interpretation of surfactant action is still obscure. Certain hygroscopic additives such as glycerine, Carbowax, molasses, etc., keep the penetrant in a moist condition upon the leaf for a longer period.

Only rarely are many of the major factors affecting penetration and performance of pesticides evaluated in a given practical study on intact plants. One pertinent example is cited. For the translocatable growth regulator maleic hydrazide, SMITH *et al.* (1959) found that absorption, rather than translocation, chemical instability, or volatility, was the important factor limiting its effectiveness. Light, temperature, and application rate were not critical in influencing absorption rate within the normal range. Plant species and plant conditions had significant effects, however. Relative humidity and formulation were also very important. All formulations were absorbed poorly at low relative humidity. At moderate and high relative humidity formulation differences were evident.

Considering further the two major components of the cuticle, cutin and waxes, these may exert decidedly different influences upon penetration. Some of these aspects will be discussed in the following paragraphs.

SKOSS (1955) found that the wax content of isolated cuticle samples increased with age of the leaf and that cuticle from sun grown leaves contained more wax than cuticle from shade grown leaves. Cuticular and surface wax were not differentiated in these studies. JUNIPER (1959) also found a more dense wax layer occurs on plants exposed to higher light intensities and wind speeds. The cutin layer of young cuticle is probably more hydrophilic than that of older cuticle, since wax impregnation increases with age. Functionally, cuticular wax and surface wax deposits differ with respect to their influence on water loss and retention and penetration of pesticides. For example, SCHIEFERSTEIN (1957) found the amount of cuticular wax per unit area to be correlated with the degree to which plants could withstand dry habitats for four species that ranged from a mesophyte to an extreme xerophyte. No such correlation was obtained for amount of surface wax. Wax deposition with age varied for the different species. Surface wax deposition where present usually ceased with termination of leaf expansion. Cuticular wax deposition usually continued for a longer period. Massive and more or less continuous deposits of cuticular wax undoubtedly restrict the movement of aqueous solutions both inward and outward. Irregular surface wax deposits, however, apparently exert their greatest influence on wetting and the contact angle between droplet of solution and leaf surface. Adhesion between liquid and solid molecules and the degree of speading are characterized by the contact angle (Fig. 2). The magnitude of contact angle, and hence the degree of spreading of a given solution, is determined by three properties of the solid surface: (a) the nature of the chemical groups at the surface, (b) its degree of roughness, and (c) the presence or absence of air film below the drop (ADAMS, 1958; FOGG, 1948). From a knowledge of binding strengths it is apparent that the contact angle between water and the waxy component of cuticle will be larger than between water and the cutin component, i.e., water wets cutin

better than waxes. Thus large differences in wettability are known to exist among plant species due to variations in amount and patterns of deposition of cuticular components (FOGG, 1948; LINSKENS, 1950; and others). In general, evidence indicates that aqueous solutions have higher contact angles and show poorer wettability on young leaves that on older leaves (CRAFTS, 1933; EBELING, 1939; FOGG, 1947; LINSKENS, 1950). The ventral (upper) surface of leaves is more easily wetted than the dorsal (lower) surface; also wettability is usually greatest above veins (EBELING, 1939; LINSKENS, 1950). In other studies on cuticle wettability, FOGG (1944) found that contact angle increased as leaf hydration decreased. This is consistent with the observation that leaf wettability was greatest in the early morning and least just before sunset.

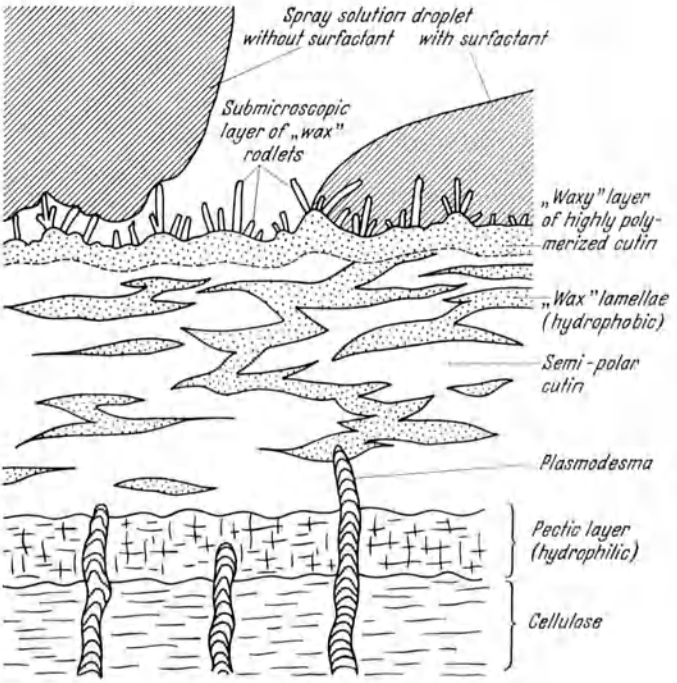

Fig. 2. Hypothetical structure of the functional aspects of the plant cuticle. The waxy rodlets of leaves having "bloom" may prevent contact of a spray droplet with the leaf surface. The use of dinitro contact sprays selectively in peas depends upon this mechanism. From ORGELL (1954)

Oil or oil-like sprays already exhibit a low contact angle with plant cuticle. The contact angle of aqueous pesticides is often markedly reduced, and therefore the degree of wetting markedly improved, by the addition of a suitable surfactant (see earlier discussion). The most effective substance for lowering the contact angle between water and a leaf surface (when this is desirable) depends upon the nature of the plant surface. And the most effective spray additive to enhance penetration may depend on several factors of the plant, environment, and spray formulation, either related to

or possibly independent of wettability properties (CURRIER and DYBING, 1959; others).

Although the wax components are not elastic as is the spongy cutin frame work, it is evident, as reviewed by VAN OVERBEEK (1956), that swelling of the cutin by water will spread the wax components farther apart, and this will have the effect of increasing the permeability of the cuticle. High turgidity of the underlying tissue would achieve a similar result. Conversely, dehydration of the cuticle causes shrinkage, thus decreasing permeability. This proposed "valve system" may have real significance with respect to the absorption of pesticides *via* an aqueous (polar) route as contrasted to a lipoidal (apolar) route. Various chemicals are known to penetrate the cuticle most efficiently in a saturated atmosphere (CLOR, 1959; ENNIS *et al.*, 1952; HURST, 1948; WEINTRAUB *et al.*, 1954). On the basis of accumulated evidence, the view that everything external to the epidermal cells is static can no longer be held. Another example men tioned earlier is that surface wax may be continuously passed through the cuticular layers until leaf expansion has ceased and perhaps even longer (JUNIPER, 1959, 1960; MUELLER *et al.*, 1954; SCHIEFERSTEIN and LOOMIS, 1956). Interestingly, the prior history of pesticide use in crops may influence cuticle development. For example, certain soil-applied herbicides markedly alter wax deposition in peas, thereby reducing contact angle and increasing wettability (DEWEY *et al.*, 1956; JUNIPER, 1959 b; WOODFORD *et al.*, 1958). In certain floating aquatic species, a similar interference with surface wax deposition by dalapon tends to cause submergence (PRASAD, 1961): electron micrographs showed a marked alteration in the sub microscopic anatomy of the frond surface of *Lemna minor* due to dalapon treatment.

Various pathways by which pesticides may enter plants have been reviewed (CRAFTS, 1961 b; CURRIER and DYBING, 1959; MITCHELL and LINDER, 1957; MITCHELL *et al.*, 1960; VAN OVERBEEK, 1956). The precise routes and mechanisms by which a pesticide penetrates plant surfaces may directly influence the likelihood or level of residue found in various plant parts. Whereas some pesticide residues may be superficial, and hence be readily removed by wind, rain, etc., others may persist almost indefinitely.

In summary, several properties of the plant cuticle are of significance in relation to the absorption of spray chemicals and the retention of pesticide residues: (1) Being fat-like in nature the cuticle is permeable to non-polar compounds. (2) The fatty acids, alcohols and unsaturated esters are dissociable and non-mobile, hence they impart to the cuticle surface a negative charge in the presence of water; this charge repels anions and attracts cations. (3) The waxy and fatty constituents constitute an important pool for holding fat-soluble compounds such as 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and 2,4-D esters in solution. Such solution may effectively prevent partition into the symplast and transport to roots of systemic herbicides and insecticides. It may also hold such compounds intact for long periods and thus create residue problems where hazardous chemicals are involved. (4) By hydration the cuticle may be swelled; by certain surfactants it apparently may be made more permeable (JANSEN

et al., 1961); by sequestering agents its cation content may be altered, cross links weakened, and penetrability improved.

The oil-like or oil-soluble properties of many pesticides which enable them to dissolve in and penetrate the cuticle are important with respect to phytotoxicity. In the case of insecticides and fungicides phytotoxicity is not desirable; compounds must be screened to avoid undue plant damage. With herbicides phytotoxicity is important but in the case of systemic materials contact injury should be low or lacking so that uptake and transport are not hindered by injury to the photosynthetic apparatus. Thus it is important that systemic materials move slowly and steadily into leaves and on through stems to roots; too rapid penetration may result in excessive contact injury; too slow uptake may fail to bring about sufficient accumulation in roots for death to be brought about. Fat solubility also makes for persistence in the cuticle of hazardous materials that constitute residue problems. For leaves that abscise and decompose this is not so important but for leafy vegetables, fruits, and forage crops and for chemicals having zero tolerance this is of great import. It may be impossible to wash such residues off and they do not readily volatilize from, nor decompose in, the cuticle. Cutin solubility of all hazardous pesticides should be determined so that their persistence in vegetable, fruit, and forage crops may be anticipated and formulations so devised that residues are held to a minimum.

In the pesticide residue problem the nature of the plant surface is important but the nature of the pesticide and of the formulation in which it is applied are also deserving of consideration. Often it is the relation between the cuticle and the chemical that is paramount. Pesticidal chemicals may be classified in many ways, for example (1) as organic or inorganic, (2) in relation to their physical properties such as gases, volatile liquids, nonvolatile liquids, and solids, (3) with respect to mode of application such as those applied pure as vapors, liquids or solids, those applied in aqueous solution, those applied in a solution of surfactant, those applied in a cosolvent or in an emulsion, those applied in oil, and those applied dry as dusts or pellets, and (4) with respect to their action pesticides may be effective as surface deposits as in the case of stomach poisons and fungicides, as contact killers as in the case of toxic herbicidal oils, or as systemic poisons as in the case of translocated herbicides, insecticides and fungicides. Related to each of these methods of application and mode of action is one or more residue problems. No matter how selective a material may be nor how low the required dosage, if it will kill insects, or fungi, or weeds there is always the possibility that it will injure or kill the careless applicator or the person who uses the treated product. All pesticides are inherently toxic and selectivity is a relative matter.

It should be apparent from these considerations that pesticide residues may present many complex problems, most of which involve absorption through or persistence on plant surfaces. To solve these problems we need to understand the development of these surfaces throughout the lifetime of the plant or animal organ, we need to know the changes that occur with maturity of the organs, and we need to be aware of the final disposition of the organs and the chemical residues that they carry. The latter knowledge

is important in the case of pesticides involving metals such as arsenic, lead, copper, zinc, and thallium. We should also recognize the importance of non-metallic materials particularly compounds containing chlorine, bromine, iodine, and selenium.

Although surface deposits are often the more important forms of residues, for an intelligent approach to residues in plants it is necessary to realize that many chemicals penetrate the cuticle, migrate to the vascular tissues and translocate to other parts, particularly to centers of growth or high metabolic activity. Reference has been made to the two possible routes of entry of chemicals through the cuticle, namely the lipoid route and the aqueous route (CRAFTS, 1961 a and b, 1962; ROBERTS *et al.*, 1948). Undoubtedly the cutin itself constitutes the lipoidal pathway and oily and fat soluble pesticides undoubtedly move into leaves *via* this route. Early work with the well-known dinitro compounds, pentachlorophenol, 2,4-D, and disodium 3,6-endoexohexahydrophthalate (endothal) proved that buffering a spray solution on the acid side to maintain a supply of undissociated molecules of the toxicant greatly enhanced penetration. In all of these cases the undissociated acids are more soluble in plant cuticle than are the ions.

Work with maleic hydrazide and amino triazole has indicated just as definitely that these highly polar, water-soluble molecules enter plant leaves at relatively slow rates except in the presence of a high degree of water saturation; in this latter situation they enter rapidly and in much greater quantity. Since under these conditions of water saturation the water continuum of leaves would by capillarity approach the outer wall surfaces it seems evident that a spray solution upon application would immediately make contact with the water continuum so that diffusion into the walls and thence to the symplast would be unimpeded. Likewise penetration of the stomata would allow the solution entering the stomatal chambers access to cell walls in a virtually saturated state and, hence promote ready uptake.

Cuticular pores and ectodesmata are subjects of controversy at present. Pores, canals, or fissures in plant cuticle have long been observed (cf. references cited by ORGELL, 1954). Some workers have reported pores in the epidermal cell wall (SCOTT, 1950; SCOTT *et al.*, 1948) lined with cytoplasm and plugged by cutin covered with wax. Others have reported a wax layer supported by pegs of cutin. Electron micrographs of cuticle and cellulose indicate a certain porosity (FREY-WYSSLING, 1948; SCOTT *et al.*, 1958). No plasmodesmata have been demonstrated in cuticle, but similar structures exist in the cutinized outer epidermal wall (LAMBERTZ, 1954; SCHUMACHER, 1942). These ectodesmata reportedly push into and, alternately, withdraw from pores in the wall, a process which is stimulated by histidine and other chemical substances (SCHUMACHER and LAMBERTZ, 1956). As stated earlier, latest views are that fatty substances are secreted and polymerize to form cuticle. Most ideas center around the epidermis containing pores in the outer cell wall through which lipids or cutin are secreted. Still nebulous are (a) the distance outward to which such pores normally extend, (b) whether "cuticular pores" correspond generally to ectodesmata, (c) the prevalence of ectodesmata in various parts of a given leaf, among species, and under different environmental conditions, and (d) their role and importance in the

absorption of pesticides. Information on the microscopic anatomy is generally sketchy and quite widely scattered; little is known of the submicroscopic anatomy of plant cuticle. Some success has been obtained with electron microscopy using plastic replica (MUELLER *et al.*, 1954; SCHIEFERSTEIN and LOOMIS, 1956) and carbon replica techniques (BRADLEY, 1954; JUNIPER, 1959 a and b, 1960; PRASAD, 1961; and others). Supposed merits of the latter technique are that "carbon is completely inert, non-crystalline, can replicate detail down to the 1 mμ level, and unlike plastics, can replicate biologically moist surfaces" (JUNIPER, 1959 a). Radial canals that extend all the way to the cuticular surface and might serve either as direct routes of entry of pesticides or as excretory ducts concerned with the formation of surface rodlets and wax protuberances have not been definitely identified (JUNIPER, 1959 a; MUELLER *et al.*, 1954; SCHIEFERSTEIN, 1955; SCOTT *et al.*, 1957; WATSON, 1943; WOOD *et al.*, 1952).

Finding no pores by the carbon replica technique, JUNIPER (1959 a) concluded that wax projections must have been extruded as liquids. From SCHIEFERSTEIN's studies (1955) also, diffusion of the wax in liquid solution or fluid paste would seem to be indicated. JUNIPER (1960) considered it unlikely that the process of wax extension is even indirectly connected with plasmodesmata. The surface replica technique reflects only surface structure, of course. It seems unlikely that any surface replica technique will reveal extrusion pores. As yet no truly successful attempts at ultrathin sectioning of the cuticle have been made. Much work of this type still needs to be done in order to provide information concerning cuticular porosity as a general phenomenon. Cuticular transpiration and the demonstrated penetration of polar tracer molecules provide circumstantial evidence for such porosity. Electron microscope pictures giving definite evidence for known plant species will be much more gratifying. Possible new approaches that might be applicable to the study of ectodesmata, cuticular pores, fine structure, and routes of nonstomatal entry of pesticides are (a) the combined use of autoradiography and electron microscopy (VAN TUBERGEN, 1961) and (b) impregnation of ultrathin sections with silver or other heavy metal for electron microscopy (MARINOZZI, 1961).

Developmentally the cuticle progresses from a thin highly hydrophobic covering of young stems and leaves to a considerably thicker layer at leaf maturity. Plant species, environment, and age all make differences. Succulent plants like cacti may have very thick impervious cuticle; fruits often develop such cuticle. With age cuticle oxidizes becoming less permeable in a chemical sense but often weathering and cracking to become physically imperfect as a layer. Insect punctures, abrasion, and physical stresses may also deteriorate the cuticle covering.

The cuticle may have qualitative differences from species to species. For example, the cuticle of nutgrass is readily wet by water; that of many grasses and broad leaved weeds is not wet by water but oils wet it and spread rapidly. Purslane, on the other hand, has a cuticle that is not wet by either oil or water and here accumulation of spray liquid in leaf axils may play an important rôle in contact injury. These qualitative differences in leaf surfaces may provide for selective control of weeds in crops by the

simple process of differential wetting; sulfuric acid, iron sulfate, and copper salts kill certain weeds in cereal crops by this means.

Turning to the root absorption process, recent work with labeled tracers and radioactive mineral ions prove that most chemicals adsorb to roots readily. Many penetrate to the symplast in a short time, some enter the vascular channels and translocate to the foliar organs within less than an hour. Some, on the other hand, may not move to the tops in days (CRAFTS and YAMAGUCHI, 1960 a). Like movement of systemic spray materials, this distribution or lack of distribution from roots is important with respect to residues. For example 3-(*p*-chlorophenyl)-1,1-dimethylurea (monuron) or 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) applied through the soil may distribute thoroughly throughout plants in a matter of a few hours; applied to leaves they may never translocate to roots. Amino triazole and 2,4-D applied to roots may move to tops only in minute quantities and then only after days; applied to leaves they may pass downward into the stems and roots in quantity within a few hours. Differences of this type may readily explain the presence or the absence of a given pesticide in plant organs and they may account for wide changes in pesticide concentrations with time. Although the ability of plant roots to discriminate between different ions—for example, Na^+ and K^+ in the soil solution—may be definitely beneficial to their proper nutrition and hence survival, little or nothing is known concerning the differential uptake of pesticide molecules. Many toxic materials such as arsenic, chlorates, borates, 2,4-D, IPC, CIPC, etc., are readily absorbed by roots resulting in the ultimate death of the plant. It seems quite obvious that the plant has no built in defense mechanism against new pesticide molecules. For this reason we have no way of predicting or anticipating the quantities of a new pesticide compound that may be absorbed *via* either the foliage or the root. Only by exhaustive research may we hope to gain the knowledge that will enable us to work safely and intelligently with these materials. Much hard work lies ahead.

Summary

Plant surfaces are important both with respect to the increase of agricultural production through use of pesticides and with respect to health hazards connected with application of pesticides and persistence of pesticide residues.

Plant surfaces vary in chemical composition, in physical structure, and in the manner in which they are formed. Plant foliage is universally covered with fatty or waxy non-polar substances that inhibit water loss in the vapor form. Root cells have walls made of pectins including polyuronides that are polar and possess base-exchange properties. These are highly permeable to water and salts in the primary regions.

Cuticle, the common covering layer of foliage, is made up of cutin and cutin waxes. The cutinized plant cell wall also contains pectin and cellulose. Cutin is described as an oxidative polymer of long chained fatty acids and alcohols. With age these condense to form a "varnish-like" layer over the surface of the plant. Cutin waxes are made up of short rodshaped mole-

cules having no reactive end groups; they contain mainly esters and saturated alcohols.

From evidence on cuticular transpiration and pesticide penetration, cuticle is presumed to be permeable. Since both polar ions and non-polar fat-soluble molecules are known to penetrate cuticle it is assumed that there are two routes of entry into the plant leaf, a polar or aqueous route and a non-polar or lipoid route. Both of these are important in the penetration of pesticides; both are important with respect to pesticide residues.

Since the fatty acids, alcohols, and unsaturated esters of the cuticle are dissociable, in the presence of water the plant surface has a negative charge that attracts cations and repels anions. The lipoidal constituents of cuticle constitute an important pool for holding fat-soluble pesticides in solution. This restricts further penetration and translocation of such pesticides; it also presents residue problems.

By hydration the cuticle may be swelled. By surfactants the cuticle may be made more permeable. These effects aid in penetration and distribution of systemic pesticides.

In the use of pesticides the nature of plant surfaces and the composition of the pesticide formulation are both of great importance. Every constituent of a formulation may affect the penetration of the toxicant and hence the distribution and concentration in a given organ. This is important in residue considerations.

With age cuticle oxidizes becoming less permeable but often weathering to become physically imperfect. Insect punctures, abrasion, and physical stresses tend to deteriorate the cuticle layer. Some cuticles are readily wet by water; many are not wet by water; some are not even wet by oils. These qualitative differences provide for selective effects of pesticides.

All water soluble pesticides are readily adsorbed or absorbed by roots; some may move rapidly to vascular tissues and translocate to tops; others may remain for days in the roots with very little moving to the tops.

The plant has no built-in mechanisms to protect itself from new pesticide molecules. Thus we have no basis for predicting or anticipating the quantities of a new compound that may be absorbed *via* the foliage or the root.

Résumé*

Les propriétés des surfaces végétales présentent de l'importance à un double point de vue: celui de l'accroissement de la production agricole en relation avec l'usage des pesticides et celui de la santé publique qui est lié à l'application des pesticides et à la persistance de leurs résidus.

Les surfaces végétales ont une composition chimique, une structure physique et une morphologie variables. Le feuillage est entièrement recouvert de substances non polaires, grasses ou cireuses, qui inhibent l'évaporation de l'eau. Les cellules des racines sont pourvues de membranes qui contiennent des pectines comprenant des polyuronides polaires doués de propriétés d'échanges. Elles sont très perméables à l'eau et aux sels dans les régions de structure primaire.

* Traduit par SIMONE DORMAL VAN DEN BRUEL.

La cuticule, qui constitue le recouvrement habituel du feuillage, est faite de cutine et de cires végétales. Les parois des cellules cutinisées contiennent aussi de la pectine et de la cellulose. La cutine est définie comme étant un polymère d'oxydation d'acides gras et d'alcools à longues chaînes. En vieillissant, ces molécules se condensent pour former un revêtement semblable à un vernis sur la surface de la plante. Les cires de la cutine sont faites de courtes molécules en forme de bâtonnets qui n'ont pas de groupes terminaux réactionnels; elles contiennent principalement des esters et des alcools saturés.

La transpiration cuticulaire et la pénétration des pesticides font présumer que la cuticule perméable. Comme on sait que les ions polaires et les molécules non polaires liposolubles pénètrent dans la cuticule, on en déduit qu'il y a deux voies d'accès dans la feuille, une voie polaire ou aqueuse et une voie non polaire ou lipoïdique. Ces deux voies sont importantes pour la pénétration des pesticides; elles le sont également pour ce qui concerne les résidus.

Etant donné que les acides gras, les alcools et les esters non saturés de la cuticule sont dissociables, la surface végétale possède, en présence d'eau, une charge négative qui attire les cations et repousse les anions. Les substances lipoïdiques de la cuticule constituent un excellent milieu pour retenir en solution les pesticides liposolubles. Il limite leur pénétration ultérieure et leur translocation, mais soulève, de ce fait, un problème de résidus.

La cuticule peut gonfler par hydratation. Elle peut être rendue plus perméable sous l'action des agents tensio-actifs. Ces effets aident la pénétration et la distribution des pesticides systémiques.

La nature des surfaces végétales et la composition des formulations de pesticides ont, toutes déux, une grande importance pour l'usage des pesticides. Chaque constituant de la formulation peut influencer la pénétration du toxique et, par conséquent, sa distribution et sa concentration dans un organe déterminé. Ceci est à prendre en considération dans l'étude des résidus.

Avec le temps, la cuticule s'oxyde; elle devient moins perméable, se dégrade sous l'influence des agents atmosphériques, ce qui altère ses propriétés physiques. Les piqûres d'insectes, l'érosion et les actions physiques tendent à la détériorer. Certaines cuticules sont facilement mouillées par l'eau; beaucoup ne le sont pas; d'autres ne sont même pas mouillées par les huiles. Ces différences qualitatives interviennent dans les effets sélectifs des pesticides.

Tous les pesticides solubles dans l'eau sont facilement adsorbés ou absorbés par les racines; certains peuvent circuler rapidement dans les tissus vasculaires et migrer dans les sommets végétatifs, d'autres peuvent se maintenir pendant plusieurs jours dans les racines et se déplacer très peu vers la pousse aérienne.

La plante ne possède pas de mécanisme interne de défense contre les molécules de pesticides. Par conséquent, il n'existe pas de base pour prédire ou prévoir quelles seront les quantités d'un nouveau produit que les feuillages ou les racines pourraient absorber.

Zusammenfassung*

Die Oberfläche der Pflanzen ist in zweierlei Hinsicht von Bedeutung, einmal im Hinblick auf die steigende Produktion der Landwirtschaft durch den Einsatz von Schädlingsbekämpfungsmitteln, zum anderen hinsichtlich der Gefahren für die Gesundheit, die mit der Verwendung der Schädlingsbekämpfungsmittel und ihren Rückständen zusammenhängen.

Die Oberflächen der Pflanzen zeigen Unterschiede in ihrer chemischen Zusammensetzung, ihrer physikalischen Struktur und ihrer äußeren Form. Die Blätter der Pflanzen sind im allgemeinen mit einer fett- oder wachsartigen, unpolaren Substanz bedeckt, die Wasserverluste durch Verdunsten verhindert. Die Zellwände der Wurzeln bestehen aus Pektinen, einschließlich Polyuronsäuren, die polar sind und basenaustauschende Eigenschaften besitzen. Sie sind in hohem Maße für Wasser und Salze in den äußersten Schichten durchlässig.

Die Cuticula, die im allgemeinen die Deckschicht der Blätter bildet, besteht aus Cutin und Cutin-Wachsen. Die Cutinschicht enthält außerdem Pektin und Cellulose. Cutin wird als ein oxydiertes Polymeres langkettiger Fettsäuren und Alkohole beschrieben. Mit zunehmendem Alter kondensieren dieselben und bilden einen „lackartigen" Überzug auf der Pflanzenoberfläche. Die Cutinwachse bestehen aus kurzen, stäbchenförmigen Molekülen, die keine reaktionsfähigen Endgruppen besitzen; sie bestehen hauptsächlich aus Estern und gesättigten Alkoholen.

Aus der cuticulären Transpiration und dem Eindringen von Schädlingsbekämpfungsmitteln ist zu schließen, daß die Cuticula durchlässig ist. Da bekannt ist, daß sowohl polare Ionen als auch unpolare, fettlösliche Moleküle in die Cuticula eindringen, wird angenommen, daß es 2 Möglichkeiten gibt, um in das Blatt einzudringen, einmal auf polarem oder wäßrigem Weg und zweitens auf unpolarem oder lipoidem Weg. Beide Möglichkeiten sind wichtig im Hinblick auf das Eindringen der Schädlingsbekämpfungsmittel und im Hinblick auf ihre Rückstände. Da die Fettsäuren, Alkohole und ungesättigten Ester der Cuticula dissoziierbar sind, trägt die Pflanzenoberfläche in Gegenwart von Wasser eine negative Ladung, die Kationen anzieht und Anionen abstößt. Die lipoiden Bestandteile der Cuticula bilden einen wichtigen Faktor, um fettlösliche Schädlungsbekämpfungsmittel in gelöster Form festzuhalten. Dies hemmt das weitere Durchdringen und Weiterwandern solcher Schädlingsbekämpfungsmittel; es ergeben sich dadurch Rückstandsprobleme.

Durch Wasseraufnahme kann die Cuticula quellen. Durch oberflächenaktive Substanzen kann sie durchlässiger werden. Diese Effekte erleichtern das Eindringen und die Verteilung der systemischen Schädlingsbekämpfungsmittel.

Bei Anwendung der Schädlingsbekämpfungsmittel ist sowohl die Natur der Pflanzenoberfläche, als auch die Zusammensetzung der Pesticidmischung von großer Wichtigkeit. Jeder wesentliche Bestandteil einer Mischung kann das Eindringen des toxischen Stoffes und damit die Verteilung und Konzentration in einem bestimmten Organ beeinflussen. Dies ist bei der Betrachtung des Rückstandsproblems von Bedeutung.

* Übersetzt von S. W. Soucı.

Mit zunehmendem Alter wird die Cuticula oxydiert und undurchlässiger; durch Witterungseinflüsse wird ihre physikalische Struktur beschädigt. Insektenstiche, Abschilferung und physikalische Beanspruchung führen zu einer Verschlechterung der cuticulären Schicht. Manche cuticulären Schichten werden leicht von Wasser benetzt, andere wiederum nicht; manche werden nicht einmal von Ölen benetzt. Diese qualitativen Unterschiede bedingen die selektiven Wirkungen der Schädlingsbekämpfungsmittel.

Alle wasserlöslichen Schädlingsbekämpfungsmittel werden leicht durch die Wurzeln ad- oder absorbiert; manche können rasch zu den Gefäßzellen wandern und bis zu den Vegetationspunkten gelangen; andere können tagelang in den Wurzeln verbleiben, wobei sich nur ganz geringe Mengen zu den Vegetationspunkten bewegen.

Die Pflanze hat keinen Mechanismus, um sich vor neuen Molekülen von Schädlingsbekämpfungsmitteln zu schützen. Wir haben daher keinen Anhaltspunkt, um vorauszusagen, welche Mengen einer neuen Verbindung durch das Blattwerk oder die Wurzel aufgenommen werden können.

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Principles for the establishment of pesticide residue tolerances

By
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Contents

I. Introduction

Several countries have already taken steps to control amounts of pesticide residues that may remain in or on food, while others are considering the need for appropriate action. The concept of establishing safe limits for chemicals, both those produced by man and those of natural origin is not new or novel. In recent years the increase in the amount and number of pesticide chemicals introduced into the environment of man and his animals for use in agriculture and public health has resulted in an intensive study of the associated problems in many parts of the world. The scientific and regulatory procedures for establishing and enforcing pesticide residue tolerances are relatively new. Reviewers of the literature of the past five years will be impressed by the dynamic state of scientific knowledge surrounding pesticides and their use. Numerical tolerances in some form are eventually required for law enforcement purposes. Unfortunately, when published, these numerical values induce an hypnotic attachment to their significance, while by contrast the principles and procedures by which they are established are not universally understood. Since knowledge in many aspects of this field of pesticides and their use is in an extremely fluid state of development, any approach adopted to the regulation of pesticide residues

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in food should recognize that both the tolerances which have been established and the procedures of establishment employed to date should be subject to modification as new information becomes available.

In spite of the differences in the scientific approaches and legal mechanisms that have been applied, the general philosophy apears to be similar. *The presence of some pesticide residues in selected foodstuffs is allowed in amounts demonstrated to be no higher than those resulting from "good agricultural practice", provided that the final amount of residue in the daily food is no greater than the amount accepted as safe for long-term consumption by man.*

The measures that have been taken to ensure the safety of the consumer differ between countries, depending on their ability to administer a system and provide the required technical services. No attempt is made in this report to review comprehensively the procedures employed by all the countries mentioned. Rather, it is expected that *Residue Reviews* will provide this detailed information through experts in each country [1]. We have selected a few examples here and have described them briefly in order to adduce the emerging principles that appear to be clear at this time.

II. Basic requirements

The United Kingdom has adopted a system based on the voluntary registration of pesticides and the publication of official recommendations for their use based on *unpublished "administrative" residue tolerances.* It should be noted that a series of statutory tolerances for residues of arsenic have been published and provision exists in the Food and Drugs Acts (1955) under which regulations can be made to establish tolerances or prohibit a particular residue in food.

Austria, Belgium, Canada, Denmark, France, Germany, Luxemburg, the Netherlands, Norway, Sweden, Switzerland, the U.S.A., and the U.S.S.R. require pesticides to be officially registered for sale, and control their use by means of regulations or official recommendations. In the Western, Southern, and Victoria states of Australia, in Canada, New Zealand, the U.S.A., and the U.S.S.R. the amount of pesticide residue that may remain in or on raw agricultural commodities is restricted by *official tolerances.* In the Netherlands and in Switzerland the control is based on *unpublished tolerances.* In all the countries mentioned, minimum intervals between the final application of the pesticide and harvesting are either recommended or prescribed. Regardless of the system adopted, four requirements have been recognized as basic to them all (FAO/WHO 1961). These are:

(a) Qualified official agricultural advisers who know the agricultural practice of the country, the chief crops, and the pests and diseases of these crops, and who have an adequate working knowledge of the different types of available pesticides.

(b) Analytical facilities for determining any residues on a crop grown, treated, and harvested in that country.

[1] Added by editor: reviews in progress include those for Australia, Belgium, Canada, England, France, Israel, Italy, Japan, Switzerland, The Netherlands, U.S.A., U.S.S.R., and W. Germany.

(c) Toxicological advice on the amount of a pesticide that may be consumed daily in the human diet without effect on the consumer.

(d) Joint consultation at all stages between agricultural, health, analytical, and nature conservation authorities in order to obtain agreement on the conditions for the use of a pesticide before that use is implemented.

The most effective systems to protect the health of the consumer are those based on tolerances and enforced by means af adequate inspection services and control of market samples. The necessary corollary is an adequate educational service to users of pesticides to ensure that they understand how pesticides can be used effectively and safely and the importance of observing the recommended intervals between last application and harvest. These steps permit the establishment of a tangible basis to take punitive measures against those who fail to observe the official advice and produce food containing harmful residues.

The various modalities that have been used for establishing tolerances and permissible levels differ from one country to another. Full information on how these values are arrived at has not been published by all the countries concerned. The basic principles that are employed have been reported by LEHMAN *et al.* (1959), and WALKER and WESTLAKE (1960) for the U.S.A., GRAHAM and ALLMARK (1958), and LU (1961) in Canada, the Toxicology Committee of the Medical Research Council (1957) in the U.K., VAN GENDEREN (1960) in Netherlands, EICHENBERGER (1960) in Switzerland, and MEDVED (1959) for the U.S.S.R.

III. United States

In the United States, under Public Law 518, tolerances for pesticides on or in raw agricultural commodities, including food and forage crops and animal products, are established by the U.S. Food and Drug Administration, after considering the usefulness of the material, its probable toxicity to human beings as judged by reports of laboratory tests on animals, and the amounts of residue likely to be found in or on raw agricultural commodities. If no harmful effect results from feeding at levels well in excess of anticipated residues, the pesticide can be safely used, and a tolerance may be granted. The tolerance level is determined on the basis of the following factors:

(a) Toxicological data translated into terms of use levels and margin of safety for humans.

(b) The amount of pesticide remaining in or on the treated commodity.

(c) The proportion of the usual diet composed of foods in which residues of the pesticide may appear.

(d) The extremes of probable intake of these foods.

(e) The extent of exposure of consumers to other pesticide chemicals or toxic compounds which would have a similar or additive toxic effect.

In no event will the tolerance be higher than necessary, as indicated by residues that result from proper use (WALKER and WESTLAKE, 1960).

A "zero tolerance" is set by the FDA if the toxicological data indicate that the compound cannot be present in some amount without endangering

public health. A "no residue" registration may be granted by the United States Department of Agriculture if the data submitted show that no residue will be present if the pesticide is used according to directions on the label. The case of the simultaneous presence on a food of several pesticide residues having related pharmacological effects has been provided for by a system of "mixed tolerances", which takes into account that, in the absence of evidence to the contrary, the various residues are considered as having an additive deleterious action (WALKER and WESTLAKE, 1960).

IV. Canada

The Canadian procedures and principles are similar to those applied in the United States. The main difference is that the Canadian Food and Drugs Act is restricted to food offered for sale for human consumption. Tolerances for some pesticide residues in some animal products have been established since 1960. The factors taken into consideration by the Food and Drug Directorate for the establishment of residue tolerances are those adopted by the Food and Drug Administration of the U.S.A. with the additional formal recognition of the importance of dietary habits. According to GRAHAM and ALLMARK (1958), and LU (1961), the permissible level of a pesticide on a foodstuff is calculated by means of the following equation:

$$PL = \frac{x \times 50}{S \times F}$$

PL = Permissible level of a pesticide in parts per million.
x = Maximum dose-level that causes no significant deleterious effect in the most sensitive species of animals. It is expressed in parts per million of the compound in the animal diet and converted into milligrams per kilogram of body weight.
50 = Average body-weight of man.
S = Safety factor. The size of this depends on the species of animals employed and the nature of the toxicity observed. It is often equal to 100, but may drop to 20 if cholinesterase inhibition is the only sign of toxicity observed at higher levels.
F = Total weight in kilograms of the average per-capita consumption of the foodstuffs in which the residue may occur.

The permissible level thus calculated is accepted as the tolerance if the figure obtained is close to those representing the range of actual residues resulting from all recommended uses of the pesticide under consideration. If the calculated permissible level is much higher, the tolerance is established at a level slightly higher than the one representing the actual residues.

"Mixed tolerances" for residues of pesticides having related pharmacological effects are calculated according to the same principle applied by the Food and Drug Administration of the United States (LU, 1961).

V. United Kingdom

In the United Kingdom, there is no prescribed pattern of toxicity tests to be applied routinely to every compound studied from the point of view of its toxic properties. The Advisory Committee on Poisonous Substances Used in Agriculture and Food Storage believes that the precise nature of

the tests to be applied after the preliminary results of examination of any compound become available must be determined by the investigator's knowledge and experience. Even with relatively extensive toxicity tests on animals, it is impossible to create in the laboratory conditions corresponding to the exposure of thousands or millions of the human race to a potentially toxic compound. Therefore, it is necessary to assume that man has a sensitivity to any chemical at least on a level for human exposure considerably below the threshold dose in the most sensitive animal, which could be regarded as providing an adequate margin of safety for human beings and which may be expressed by the term "safety factor". The magnitude of this "safety factor" must depend on the seriousness of the toxic reaction when it occurs. In general, a factor of 100 has come to be accepted as reasonable (Toxicology Committee 1957). A Scientific Subcommittee to the Advisory Committee reviews the toxicity and residue data, establishes "administrative tolerances", and makes recommendations with regard to the safe use of the pesticides including the period of time to be observed between last application and harvest of the crop. The Advisory Committee considers them and endorses or refers them back. If endorsed, they are forwarded to the government departments concerned, and if accepted by them, are passed to the Trade, the Official Agricultural Advisory Services, the National Farmer's Union, and other interested bodies.

The procedures for the control of sale and use of pesticides were reviewed by MILLER (1957) and a Research Study Group on Toxic Chemicals in Agriculture and Food Storage (1961).

VI. The Netherlands

In the Netherlands, an interdepartmental Commission on Pesticides establishes tolerances for pesticide residues on the basis of residue data resulting from agricultural practices in the Netherlands and from animal toxicology data. These tolerances, although not published, may be used officially to take punitive measures against those who produce food containing residues in amounts higher than permitted. Official recommendations are made on the interval to be observed between last application of the pesticide and harvest in order not to exceed the tolerances. The calculation of the permissible levels, developed by VAN GENDEREN (1960) as "corresponding experimental content in fruit and vegetables to maximum harmless dose in animal" is based on the following equation:

$$PL = \frac{x \times 3000}{S \times 3700 \times 0.4}$$

PL = The dose expressed in parts per million in fruit and vegetables that corresponds to the maximum harmless dose in animal.

x = Maximum dose-level that causes no significant deleterious effect in the most sensitive species of animals. It is expressed in parts per million of the compound in the animal diet.

$\frac{3000}{3700}$ = Ratio of calorie intake between man and animal, i.e. conversion factor for extrapolating x to a body weight of 70 kilograms.

S = Safety factor.

0.4 = Daily consumption of 400 grams of fruit and vegetables. This factor theoretically makes it possible to establish the same tolerance for a given pesticide on all the crops.

The permissible levels obtained by this calculation are used as tolerances only in the cases in which they are close to the figures representing the average amount of residues resulting from all recommended uses of pesticides. In most cases, however, the actual numerical values chosen as tolerances are much lower than those resulting from the calculation. The main differences between the Netherland and North American systems are:

(a) the process of extrapolation of toxicological data from animal to man, and

(b) the method of employment of the concept of the "food factor".

In North America it is considered necessary to have a range of tolerance levels for the same pesticide on different foodstuffs, each one being related to the rate of intake of the foodstuff concerned. In the Netherlands an attempt is made to simplify the system and to provide for the cases in which the use of a new pesticide increases with increased experience of its potentialities or in which treated food may be imported from foreign countries.

VII. Switzerland

In Switzerland the Intercantonal Commission on Poisonous Substances has established tolerances for some pesticide residues in some fruits and vegetables on the basis of the figures resulting from the correct use of the pesticides and from the examination of market samples in Switzerland. These tolerances are always lower than the permissible levels for human beings that are calculated from the toxicological data. The difference between the "use level" and the "permissible level" is considered as an additional safety factor that can be removed in case of necessity, such as the control of new pests with the same pesticide. Minimum intervals between the last application and harvest based on these tolerances are prescribed (EICHENBERGER, 1960).

VIII. U.S.S.R.

In the U.S.S.R. a National Commission made up of administrators and experts from the departments of health, agriculture, and chemical industry must reach unanimous agreement before new pesticides can be introduced to agriculture. The available information outlined by MEDVED (1959) suggests that the principles and procedures involved are similar in general to those of other countries, with particular emphasis on joint decision-making involving all the agencies concerned. Stricter approaches to the initial hurdles of arbitrary $LD_{50's}$ in acute toxicity studies and six-month long chronic toxicity studies, plus a requirement for no cumulative effects, are probably responsible for some residue tolerances approved in the U.S.S.R. being lower than in other countries.

IX. Discussion

For many pesticides, the residue tolerances that have been established in Europe are lower than those in North America, and there are differences between European countries. A few examples are provided in Table I.

Table I. *Tolerances in p.p.m. for some pesticides residues in various countries*

Pesticide	U.S.A.	Canada	Australia (Victoria)	New Zealand	U.S.S.R.	Netherlands	Switzerland
DDT	1.0 –7.0	7.0	7.0	5.0	0–1.0	5.0	2.0–4.0
Aldrin	0.0 –0.25	0.1 –0.25	0.1	0.25	0	0.1	0.1
Diazinon	0.75–1	0.25–0.75	0.15[b]	–	–	1	0.75
Parathion	1.0	1.0	0.15[b]	1.0	0–5.0[d]	0.5	0.75
Methyl Parathion	1.0	–	0.15[b]	–	–	0.5	0.75
Thiometon	–	–	0.15[b]	–	–	0.5	0.1–0.5
Arsenic	2.3[a]	1.0 –2.0[a]	–	1.0[a]	0	0.7[c]	1.0[a]

[a] Calculated as arsenic.
[b] Calculated as phosphorus.
[c] Allowed only for the residues of Tuzet [methylarsine bis-(dimethyldithiocarbamate)] and calculated as arsenic.
[d] "Pure thiophos" permitted to 5.0 p.p.m. on the grounds that it is selflimiting due to organoleptic properties. "Impure thiophos" restricted to 0.0 p.p.m.

These growing differences are being regarded with increasing apprehension as a hinderance to the free international movement of food and are causing fear in some quarters that unscrupulously employed they may be used in place of tariff barriers to restrict trade. Most of the national and international organizations that have studied this subject, such as the Food and Agriculture Organization and the World Health Organization of the United Nations and the European and Mediterranean Plant Protection Organization, recognize that residue tolerances in some form are required to ensure that pesticides are used without hazard to the consumer. BERAN (1961 a and b) regards the problem of multiple tolerances and separate intervals between last application and harvest as too complex for clarity of regulation in practical agriculture. Instead he proposes a uniform classification of pesticides for Europe that would rely upon a single interval between last application and harvest, for each pesticide, sufficiently long to do away with the necessity to consider the crop or pest concerned. There are bound to be serious differences of opinion as to how practical such an inflexible system would be for the practical purposes of plant protection. VAN GENDEREN (1960) considers that the establishement of international residue tolerances would be incompatible with a method employing a "food factor" that takes into account the intake of each type of treated fruit and vegetable, as is done in North America. By contrast, he feels it would be feasible if the assumption is made that the entire daily intake of 400 grams of fruits and vegetables contains the maximum likely residues of every pesticide involved.

Although these views are logical, there is little likelihood that the international standardization of the numerical values for residue tolerances

per se can be broadly achieved. To do so would be unrealistic, since the regional requirements for pest control, the nature and habits of the pests concerned, climatic conditions, and economic motivation for pest control and food consumption are different between countries. The amount of residue resulting from "good agricultural practice" is intimately associated with these factors. Resistance to insecticides in insects is becoming an increasingly important problem in both agriculture and public health. In many circumstances in which alternative pesticides are not available and resistance is becoming a problem, heavier and more frequent applications may be required as an emergency measure to save food crops. Similarly, the control recommendations for grasshoppers in the Great Plains areas of North America were developed under "normal" non-outbreak years. In 1958 only one ounce of dieldrin per acre once in the crop year provided adequate control. By contrast, in the outbreak conditions of 1960—1961 applications of three ounces per acre, as many as five times during the year to one crop, were required to protect crops due to high grasshopper population pressures, prolonged drought, and mobility of the insect. All of these circumstances can be regarded as normal to "good agricultural practice" since plant protection requires a fluid approach to be effective. Furthermore, if the calculation of the "permissible levels" takes into account the total intake of all food on the assumption that the total of all foodstuffs bear the residues found in field trials, rather than the intake for the particular special group of foods concerned, further restrictive gross differences would appear from one country to another. This is illustrated by the examples in Table II taken from the Food Balance Sheets published by FAO (1958).

Table II. *Per caput consumption in grams per day (average 1954—1957)* [a]

Country	Cereals	Roots & Tubers	Vege-tables	Fruits (fresh & dried)	Oils & Fats	Meat	Milk & Cheese
Belgium	273.9	407.4	178.4	207.7	60.5	143.3	305.1
Canada	202.7	185.2	195.4	180.5	60.7	220.6	580.0
Germany	262.2	429.3	123.4	192.2	69.1	131.9	401.4
Israel	386.5	119.7	317.3	295.5	49.6	54.7	290.8
Italy	396.6	128.1	266.7	183.3	37.4	55.4	171.1
Japan	403.6	130.6	183.3	43.4	7.1	8.8	34.1
Netherlands	245.3	260.0	181.3	149.6	75.5	104.8	531.2
New Zealand	236.1	139.4	199.4	152.0	64.1	288.4	610.9
Portugal	342.0	309.6	300.2	165.1	43.7	45.5	46.5
Sweden	208.5	279.5	68.5	177.1	57.8	140.9	579.9
United Kingdom	242.8	270.5	100.8	125.5	60.6	174.9	433.5
U.S.A.	189.0	134.1	268.3	223.0	60.5	223.2	533.5

[a] From FAO food balance sheets (1958).

Recently a joint meeting of the FAO Panel of Experts on the Use of Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues was held in Rome to consider some of the difficulties that are being encountered. The Meeting Report (1961) recognized that different residue tolerances for any one food in different countries may be justified, but that international agreement on the basic principles governing their establishment could be reached. The basic premise universally acknowledged

is that *the amount of residue allowed to remain in food should be no higher than that which results from "good agricultural practice", provided that the final amount of residue in the daily food is no greater than the amount acceptable as safe for long-term consumption by man.*

It should first be demonstrated by well-designed experiments that the pesticide will give effective and economical pest control in plant or animal production, or in the processing, storage, and distribution of food. In order to obtain the range of actual residues resulting from "good agricultural practice", experimental programs to decide amount, formulation, frequency, and timing of the pesticide required should aim at providing an accurate forecast of the control obtained in commercial practice, the rate of pesticide disappearance, and the residue likely to occur on that part of food reaching the consumer. In the design of these experiments provision should be made for factors such as differences in agricultural practice, soil type, husbandry practice, climatic conditions, and severity of pest control problems in different areas. By-products obtained from processing raw agricultural products into human food are often used as farm animal feed. Data are required on the effect of processing on the initial and ultimate residues in such agricultural products that are used as human and animal food. This information is of particular importance for staple foods such as cereals, dairy products, and root crops. In reporting such experiments, detailed information on the variables involved should be made available. It should not be redundant to reiterate that the decision-making process that is ultimately required affects the welfare and economic structure of agriculture as well as health. Therefore, at the planning stages and in the evaluation of data, adequate consultation should take place between all the research and regulatory agencies concerned, since frequently regulatory officials are removed from the realistic aspects of pesticide use and behaviour.

The amount of pesticide residue that may be accepted as safe for long-term consumption by man, i. e. the *permissible level*, expressed in parts per million of the fresh weight of the food, may be calculated from the *acceptable daily intake* and the *food factor*. These terms are employed as defined in the joint FAO/WHO Report (1961).

The *acceptable daily intake* is the daily dosage of a chemical which, during an entire lifetime, appears to be without appreciable risk on the basis of all the facts known at that time. It is expressed in milligrams of the chemical, as it appears in food, per kilogram of body weight (mg./kg./day) and implies for most chemicals an extrapolation from data obtained on the most sensitive animal species to man by means of a suitable safety factor.

The *food factor* has been defined as the average fraction of the total diet made up by the food or class of foods under discussion. It implies an exact knowledge of the national diet. Due to the emphasis that has been placed on this term by some authors, misunderstandings have been associated with assigning mathematical values to the term itself. This can possibly be avoided if the calculation of *permissible level* is related only to the amount consumed of the particular group of foods that will be involved, since only these contain the stated amount of residue.

When all these data are available, the *tolerance,* i. e. the permitted concentration of a residue in or on food, may be derived by taking into account both the range of residues actually remaining when food is first offered for consumption and the *permissible level.* The *tolerance* is expressed in p.p.m. of the fresh weight of the food. It is never greater than the *permissible level* for the food in question and is usually smaller.

Regardless of variations in food consumption habits, the nature of the pests, and agricultural practices the safety of the consumer can be assured with the same degree of confidence in different countries by means of different tolerances for the same pesticide if so required. The existence of different tolerances need not necessarily impede the free movement of a food in international trade. When systems of inspection and associated analytical services are sufficiently developed, a country can solve problems on an individual basis so that a particular shipment of food containing a residue level higher than that accepted in the importing country will not, in fact, create a hazard to the consumer (FAO/WHO 1961). The total amount of residue-bearing food that is imported would have to be known and the resulting incidence in the daily diet of the extra amount of residues introduced by the imported food calculated. If the potential intake of residue is still less than the *acceptable daily intake,* the importation can be safely justified.

Summary

The adoption of the use of published or unpublished numerical values for pesticide residue tolerances by some countries, and the use of fixed intervals between the last application of pesticide and harvest by others has caused concern that differences in these numerical values will interfere with the international movement and distribution of food. As long as the principles involved in establishing tolerances can be agreed upon, the published residue tolerances need not necessarily remain as a barrier to trade in food. The basic principle involved is that the presence of some pesticide residues in selected foodstuffs may be allowed in amounts demonstrated to be no higher than those resulting from "good agricultural practice", provided that the final amount of residue in the food is no greater than the amount accepted as safe for long term consumption by man. As long as agreement can be reached on the acceptable daily intake and permissible levels for each pesticide regardless of the food involved, their numerical values become the universally important parameters in calculating the specific tolerances required by an individual country, depending upon its eating habits. Requirements for pest control, amount, frequency and timing of pesticide applications vary from country to country and in regions within a country. As long as the total load of pesticide residues that result in the diet of the consuming country is below the defined permissible level, the safety of the presence of these residues in imports can be defined. The scope of investigations required to adequately define residues resulting from "good agricultural practice" is outlined. Examples of systems now employed in selected countries are used as background for the thesis developed.

Résumé*

La fixation, dans certains pays, de limites de tolérances pour les résidus de pesticides et, dans d'autres, d'intervalles entre la dernière application du pesticide et la récolte s'est traduite par la crainte que les différences observées dans les valeurs imposées n'entraînent un préjudice au commerce international et à la libre distribution des denrées alimentaires.

La limitation de la quantité de résidus de pesticides dans les aliments ne constitue pas nécessairement un obstacle aux échanges internationaux s'il y a accord sur les pricipes de base inhérents à la fixation des tolérances. En effet, le principe fondamental généralement reconnu est l'admission, dans certains denrées, de résidus de certains pesticides en quantités ne dépassant pas celles qui résultent d'une « pratique agricole rationelle », pour autant que la dose présente dans le régime alimentaire soit prouvée comme étant inoffensive pour une consommation à long terme chez l'homme. Dès lors, il suffit qu'il y ait accord sur les valeurs des doses quotidiennes maxima admissibles et des taux acceptables pour chaque pesticide, quelle que soit la denrée incriminée.

Ces valeurs étant admises comme paramètres fondamentaux, le calcul des tolérances tiendra compte des facteurs inhérents au régime alimentaire particulier à chaque pays ou région et aux conditions exigées par la lutte antiparasitaire qui correspond à ses besoins. Les chiffres qui en résulteront varieront nécessairement d'un pays à l'autre. Ils ne devraient néanmoins être rejetés lors d'echanges internationaux que si la quantité totale de résidus qui pourrait en résulter dans le régime alimentaire du consommateur du pays importateur dépassait le taux défini comme acceptable sur la base des résultats de l'expérimentation toxicologique.

Les auteurs soulignent l'importance et décrivent la nature des recherches nécessaires à la connaissance d'une « pratique agricole rationnelle ». Le développement de leur exposé est illustré d'exemples de systèmes actuellement en application dans différents pays.

Zusammenfassung**

Die Angabe von Toleranzen für Schädlingsbekämpfungsmittel-Rückstände in einigen Ländern und die Vorschriften in anderen, bestimmte Zeiträume zwischen der letzten Anwendung der Schädlingsbekämpfungsmittel und der Ernte einzuhalten, legen die Befürchtung nahe, daß unterschiedliche Zahlenwerte im internationalen Lebensmittelhandel auftreten. Wenn die Prinzipien, die bei der Festlegung von Toleranzen zugrundegelegt werden, in Übereinstimmung gebracht werden können, müssen diese Toleranzen aber nicht unbedingt ein Hindernis für den Lebensmittelhandel bleiben. Das Grundprinzip besteht darin, daß Rückstände von Pesticiden in ausgewählten Lebensmitteln nur in Mengen zugelassen werden, die die Rückstände, die bei „good agricultural practice" verbleiben, nicht übersteigen, und daß der Endgehalt im Lebensmittel auch bei fortwährendem Genuß eine für unbedenklich erachtete Grenze nicht überschreitet. Wenn eine Übereinstimmung zwischen

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** Übersetzt von S. W. Souci.

der täglichen Aufnahme und der zulässigen Grenze für jedes Schädlingsbekämpfungsmittel — unabhängig davon, in welchem Lebensmittel es enthalten ist — erreicht werden kann, stellen diese Zahlenwerte einen allgemein gültigen Maßstab für die Berechnung spezifischer Toleranzen für ein bestimmtes Land je nach dessen Ernährungsgewohnheiten dar. Die Erfordernisse für die Schädlingsbekämpfung, Menge, Häufigkeit und Zeitraum der Pesticid-Anwendung, sind von Land zu Land und sogar innerhalb einzelner Landstriche unterschiedlich. Solange die Gesamtmenge der mit der Nahrung aufgenommenen Schädlingsbekämpfungsmittel-Rückstände unter der festgelegten zulässigen Menge bleibt, kann die Unbedenklichkeit dieser Rückstände in Importen als sicher angenommen werden. Die Untersuchungen werden umrissen, die für eine ausreichende Bestimmung der Rückstände notwendig sind. Einige Anwendungsbeispiele, die in bestimmten Ländern üblich sind, wurden den aufgestellten Leitsätzen zugrunde gelegt.

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Pesticide residue analysis by oxygen flask combustion

By
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With 1 figure

Contents

I. Introduction

SCHÖNIGER (1955, 1956) burned organic compounds in an oxygen-filled Erlenmeyer flask prior to analysis of sulfur and halogens. Combustion gases were absorbed by shaking an alkaline solution contained in the flask and analysis of the resulting solution followed. This procedure has been modified by LISK (1960) and ST. JOHN and LISK (1961) for direct combustion of evaporated crop extracts containing chlorinated pesticides.

II. Chlorine

The combustion apparatus as modified for residue analysis is shown in Figure 1 (A and B). It consists of a 1-liter borosilicate glass round-bottomed flask (A) with a 34/28 standard-taper outer ground joint sealed on the neck. A platinum sample holder (B) is constructed by sealing a 12-cm. length of "B and S" 16-gage platinum wire[1] onto a 34/28 standard-taper inner ground joint which has been drawn out about 1.5 cm. below the ground portion. At the lower end, 2 cm. of wire are bent to form a right angle. A piece of Style 4 perforated platinum sheet[2], 2 cm. wide and 4 cm. long and bent as shown is electrically welded to the wire at the lower end. A side arm (1 to 1.5 cm. in outside diameter, 7 cm. long) is sealed to the flask (A) at the position shown. An ordinary rubber balloon about 7 cm. long is secured to the side arm with a rubber band. The flask also contains a

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[1] Engelhard Industries, Inc., Baker Platinum Div., 113 Astor St., Newark 2, New Jersey.

[2] J. Bishop and Co., Platinum Works, Malvern, Pennsylvania.

1⁵/₈-inch long, Teflon-sealed, egg-shaped, magnetic stirring bar for mixing the solution to hasten gas absorption. This apparatus is available commercially[3].

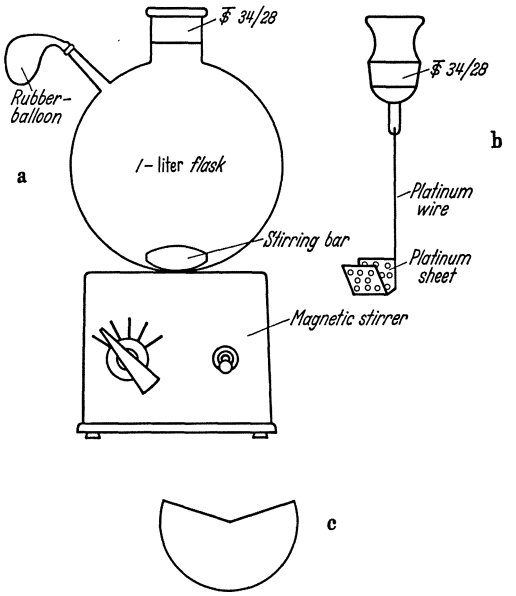

Figure 1. Combustion apparatus: A. Combustion flask, B. Platinum sample holder, C. Cardboard template for sample holder

The sample holder (Fig. 1, C) consists of a cone made from S-600, 1-mil. cellulose acetate film[4]. The cone is prepared as follows:

Wearing cloth gloves, cut the plastic using a cardboard template having the shape (4-cm. radius) shown in Fig. 1, C. Manually join the two edges to form a cone. Heat-seal[5] the joined edges to form a seam about 5 mm. wide. Immerse the seam for 10 seconds to about one-half its width in acetone contained in a watch glass. Remove and immediately dry it in the warm air stream of a hair dryer. Using forceps, wash each cone by dipping for 10 seconds in 6 *N* sodium hydroxide solution warmed to about 60° C. in a 1-liter beaker. Rinse each cone thoroughly in running distilled water and allow it to drain dry on a piece of aluminum foil. Then place each cone in a clean 65-mm., 60° funnel.

The procedure for combustion and determination of chloride is as follows:

Pipet 5 to 10 ml. of the solvent extract of the sample into the cone. Allow the solvent to evaporate. Wearing rubber gloves, remove the cone and its dry contents from the funnel. Fold the cone over and up to form a small packet about 1 cm.² in area. Compress and place the packet in the center of the platinum holder which is supported from a ring stand. Insert a fuse (3 mm. wide and 5 cm. long, cut from Whatman No. 42 filter paper) into the top of the holder between the folds of the

[3] Arthur H. Thomas Co., Philadelphia, Pennsylvania.
[4] Celanese Corp. of America, 290 Ferry St., Newark, New Jersey.
[5] Dobeckman Company Heat Sealer, Model No. DS-150-W, Cleveland, Ohio.

packet. Pipet exactly 30 ml. of distilled water into the combustion flask. Place the magnetic stirring bar in the flask and clamp the flask in place on a magnetic stirring motor. Throughly purge the flask with oxygen. Light the fuse and place the holder in the flask when about one-half of the fuse has burned away. Hold or clamp the top on until combustion is complete. Turn on the stirring motor and spin the bar rapidly for 10 minutes to splash the water and hasten absorption of combustion gases. An automatic shut-off timer on the stirrer is advantageous here. Pick up the flask and roll it manually so that the solution will wash all inside glass surfaces. Also, invert the flask several times so that the balloon is rinsed through the side arm by the water.

Pipet exactly 15 ml. of the resulting solution into a 50-ml. Erlenmeyer flask. Add 1 ml. of 0.25 *M* ferric ammonium sulfate solution in 9 *M* nitric acid, followed by heating, with agitation, an excess of powdered mercuric thiocyanate in Mix the resulting solution and allow it to stand for 10 minutes. Using a Beckman DU or equivalent spectrophotometer, measure the absorbance of this solution at 460 mμ in a 2-cm. cell with distilled water in the reference cell. To minimize chloride absorption from air make the measurement promptly (BERGMANN and SANIK, 1957).

The calibration curve (0 to 50 μg. of chloride per ml. of solution) is prepared as follows:

Pipet 0, 2, 4, 6, 8 and 10 ml. of a standard sodium chloride solution (5 μg. of chloride per ml.) into a series of 50-ml. Erlenmeyer flasks. Make each up to 15 ml. with distilled water and add 1 and 3 ml., respectively, of the above reagents. Determine the absorbance as described above.

The recovery of the organohalogen pesticides dalapon, DDT, dieldrin, endrin, heptachlor, lindane, methoxychlor, pentachloronitrobenzene, and thiodan from forage and fruit at levels of 1 to 14 p.p.m. ranged from 80 to 109 percent. The sensitivity of the method using the cellulose acetate sample holder is about 0.25 p.p.m. of chloride based on a 50-gram sample. The cellulose acetate cone as prepared contains about 10 μg. of chloride. PHILLIPS *et al.* (1961) have reported sensitivities of 0.025 p.p.m. of chloride when using a simple cotton plug as a sample holder.

The cone weighs about 85 mg. A total weight of 200 mg. for cone-plus-contents can be burned in the 1-liter flask with no carbon formation. Carbon forms if the flame touches glass, however. If carbon forms as an indication of incomplete combustion, the absorbing solution will be colored and the sample should be discarded. Repeating the combustion using a smaller sample will usually correct this trouble. Cellulose acetate burns more vigorously than Schöniger's original paper sample holders, and the balloon allows for the consequent more rapid expansion of gases. About a thousand combustions have been safely conducted in this type of flask in the author's laboratory.

Plant waxes burn satisfactorily but oils may drip out of the platinum holder before combustion is complete; addition of a small amount of filter paper pulp to the cone to absorb the oil will reduce this effect. Too vigorous burning with resultant carbon formation may also be reduced by addition of sodium borate powder to the residue in the cone. Large amounts of oils or fats in sample extracts often can be conveniently reduced by freezing out of solution before transfer of the extract to the cone. At least 30 ml. of water must be used in the 1-liter flask in order that the liquid will

be effectively splashed by the magnetic stirrer for gas absorption. Flammable solvents should never be burned as samples in the flask, or used in cleaning the apparatus. Residues are never burned until all of solvent has evaporated as judged by sight and odor.

The standard curve for chloride follows Beer's law so long as the mercuric thiocyanate solution remains saturated. This solution is best prepared by heating, with agitation, an excess of powdered mercuric thiocyanate in absolute ethyl alcohol and allowing it to settle for 48 hours before use. The resulting clear solution and additional solid mercuric thiocyanate should be mixed, heated, and allowed to settle again once every two weeks during use. Amperometric (PHILLIPS and DE BENEDICTIS, 1954) or potentiometric (HELMKAMP *et al.*, 1954) detection methods are also suitable for the chloride determination.

III. Other elements

The use of oxygen flask combustion employing the same design of flask but of 5-liter capacity has been extended to other elements in pesticides.

Mercury (GUTENMANN, 1960), selenium (GUTENMANN, 1961), arsenic, bromine, chlorine, manganese, and nickel (GUTENMANN *et al.*, 1961) have been determined in apples, potatoes, cherries, onions, cabbage, and oats. In these procedures 10 grams of whole tissue is weighed on a square of cellophane and dried. It is then burned in a 5-liter oxygen-filled flask. Dry samples such as oats are ground, pelleted, and burned. This procedure is especially advantageous for mercury and selenium because they are almost impossible to ash in open systems without loss. Good recoveries of these elements are obtained. Mercury is extracted directly from its absorbing solution (0.1 *N* sulfuric acid) with dithizone and determined colorimetrically. Selenium is absorbed in distilled water and determined directly as piazselenol using the method of CHENG (1956), but fluorometric methods (WATKINSON, 1960) are presently being investigated for greater sensitivity in this determination (ALLAWAY, 1961). TAUSSKY (1961) has also used a 1-liter Schöniger flask for combustion of small tissue samples for selenium determination.

Arsenic is absorbed in 1 *N* hydrochloric acid, extracted into butanol as molybdoarsenic acid, and determined after reduction to the heteropoly-blue complex. Bromide is absorbed in water, separated from chloride by ion exchange, and determined by the ferric thiocyanate method of BERGMANN and SANIK (1957). Manganese is absorbed in 6 *N* hydrochloric acid and determined colorimetrically after oxidation to permanganate. Nickel is determined colorimetrically as the diethyldithiocarbamate complex after absorption in 0.1 *N* hydrochloric acid.

Dried tissue samples usually contain about 5 percent moisture when burned. For analysis of mercury, selenium, bromide, and chloride the tissue is dried at room temperature in an evacuated desiccator containing sulfuric acid. For analysis of arsenic, manganese, and nickel, oven drying at 100° C. suffices. The volume of absorbing solution for the 5-liter flask is 100 ml. This is sufficient to absorb the gases during 10 minutes of stirring.

A smaller volume of solution would probably suffice if stirring time was increased.

The entire absorbing solution is used for analysis with mercury, selenium, and bromide in the above determinations. If necessary, absorbing solutions can be concentrated by boiling with arsenic, manganese, and nickel analyses without loss of these elements. The solution for chloride analysis can be made alkaline and evaporated by boiling. Ion exchange might also be employed for concentrating the various elements.

An excellent review of other elements (not necessarily concerning pesticides) to which the Schöniger method has been applied was made by SCHÖNIGER (1960).

IV. Miscellaneous

Infrared and electrical igniters are available for Schöniger flasks. For analysis of carbon where a positive blank from the paper fuse is to be avoided, electrical ignition is advantageous. If the sample is not dark in color an infrared igniter will not initiate combustion; a black strip of paper must then be used as a fuse and a carbon blank is again obtained. Samples so very combustible as to require the operator to use infrared or electrical ignition from behind a shield should not be burned in the present flask. The introduction of impurities by match-produced combustion has never been mentioned in the literature nor experienced by the writer. Fuses of the length described allow ample time for introduction of the sample when using a match for ignition. The latter method certainly involves a smaller investment in equipment and is simple and rapid for most samples.

Summary

Residues of pesticides may be determined in crops by combustion in an oxygen-filled (Schöniger) flask. Absorption of combustion gases by an appropriate solution contained in the flask is followed by spectrophotometric analysis of the desired element. For residues of chlorinated pesticides a solvent extract of the plant sample is evaporated in a cone of cellulose acetate. Combustion of the cone and contents in a 1-liter flask produces hydrogen chloride which is absorbed in water and determined spectrophotometrically by the ferric thiocyanate color reaction. A balloon attached to the flask for pressure control assures safe combustions. The recovery of dalapon, DDT, dieldrin, endrin, heptachlor, lindane, methoxychlor, pentachloronitrobenzene, and thiodan from forage and fruit at levels of 1 to 14 p.p.m. ranged from 80 to 109 percent. The sensitivity of the method is about 0.25 p.p.m. of chloride based on a 50-gram sample.

Mercury, selenium, arsenic, bromide, chloride, manganese, and nickel have been determined in apples, potatoes, cherries, onions, cabbage, and oats by combustion of whole dried tissue in a similarly constructed 5-liter oxygen-filled flask. These elements are also determined colorimetrically in appropriate absorbing solutions contained in the flask. This method is particularly advantageous for elements such as mercury and selenium which may volatilize when ashing samples in open systems. The practical points concerning sample handling and analysis are discussed.

Résumé*

Les résidus de pesticides peuvent être analysés dans les récoltes par la méthode de combustion dans un flacon rempli d'oxygène (Schöniger). L'absorption des gaz de combustion par une solution appropriée introduite dans le flacon est suivie d'une analyse spectrophotométrique de l'élément désiré. Pour les résidus de pesticides chlorés, après extraction de l'échantillon végétal par un solvant organique, ce dernier est évaporé dans un cône d'acétate de cellulose. La combustion du cône et de son contenu dans un flacon de un litre produit de l'acide chlorhydrique qui est absorbé dans de l'eau et dosé spectrophotométriquement par la réaction colorée du thiocyanate ferrique. Un ballon adapté au flacon assure une combustion sans danger en régularisant la pression. De 80 à 109 pour cent de dalapon, DDT, dieldrin, endrin, heptachlor, lindane, methoxychlor, pentachloronitrobenzène et thiodan ont été de recouvrés de fourrages et de fruits à des teneurs de 1 à 14 ppm. A partir de 50 grammes d'échantillon, la sensibilité de la méthode est d'environ 0,25 ppm de chlore (Cl).

Les éléments suivants: mercure, sélénium, arsenic, brome, chlore, manganèse et nickel ont été dosés dans des pommes, des pommes de terre, des cerises, des oignons, des choux et de l'avoine par combustion du tissu entier desséché, dans un flacon analogue de 5 litres rempli d'oxygène. Ces éléments sont également dosés colorimétriquement dans les solutions absorbantes particulières introduites dans le flacon. Cette méthode est particulièrement avantageuse pour les éléments tels que le mercure et le sélénium qui peuvent se volatiliser lorsqu'on incinère les échantillons en enceinte non fermée. On discute les aspects pratiques de la manipulation des échantillons et de l'analyse.

Zusammenfassung**

Rückstände von Schädlingsbekämpfungsmitteln in Ernteprodukten können durch Verbrennung in einer mit Sauerstoff gefüllten (Schöniger-)Flasche bestimmt werden. Das Verbrennungsgas wird von einer geeigneten in der Flasche befindlichen Lösung absorbiert und das entsprechende Element spektralphotometrisch bestimmt. Zur Rückstandsbestimmung chlorierter Schädlingsbekämpfungsmittel wird die Pflanzenprobe mit Lösungsmittel extrahiert und der Extrakt in einem aus Celluloseacetat bestehenden Kegel verdampft. Bei der Verbrennung des Kegels einschließlich Inhalt in einer 1-Liter-Flasche wird Chlorwasserstoff erhalten, welcher von Wasser absorbiert und spektralphotometrisch mittels der Eisen(III)-thiocyanat-Farbreaktion bestimmt wird. Durch Kontrolle des Sauerstoff-Druckes in der Flasche wird eine vollständige Verbrennung gewährleistet. Bei der Bestimmung von Dalapon, DDT, Dieldrin, Endrin, Heptachlor, Lindan, Methoxychlor, Pentachlornitrobenzol und Thiodan in Pflanzen und Früchten, die in Mengen von 1—14 mg/kg enthalten waren, wurden 80—109% wiedergefunden. Die Empfindlichkeit liegt ungefähr bei 0,25 mg/kg (ber. als Chlorid), bezogen auf eine Probe von 50 g.

* Traduit par R. Mestres.

** Übersetzt von S. W. Souci.

In Äpfeln, Kartoffeln, Kirschen, Zwiebeln, Kohl und Hafer wurden Quecksilber, Selen, Arsen, Bromid, Chlorid, Mangan und Nickel durch Verbrennung des gesamten getrockneten Gewebes in einer ähnlich konstruierten 5-Liter-Flasche, die mit Sauerstoff gefüllt war, bestimmt. Die genannten Elemente wurden ebenfalls colorimetrisch in geeigneten in der Flasche befindlichen Absorptionslösungen bestimmt. Diese Methode ist besonders für die Bestimmung von Elementen, wie Quecksilber und Selen, vorteilhaft, da sich letztere bei Veraschung in offenen Gefäßen verflüchtigen können. Es werden ferner praktische Hinweise hinsichtlich der Behandlung und Untersuchung der Proben diskutiert.

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