

Pesticide Residues and Exposure

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Jack R. Plimmer, EDITOR
USDA

Based on a symposium
sponsored by the Division of
Pesticide Chemistry at the
Second Chemical Congress of the
North American Continent
(180th ACS National Meeting),
Las Vegas, Nevada,
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FOREWORD

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PREFACE

The symposium upon which this book is based was organized by the Pesticide Chemistry Division to address the problem of exposure to pesticides. The choice of location of the symposium was the ACS National Meeting initially planned for San Francisco, California in August 1980, which was appropriate in view of the considerable agricultural interest within the state. The major concerns were the problems of measurement, monitoring, and safety in relation to the question of worker exposure and its many implications.

An undesirable consequence of increased use of synthetic organic pesticides is the increased potential for human exposure. Pesticide applicators and agricultural workers may be particularly at risk. The latter may be required to enter previously treated fields or orchards; in this case the problem is most acute when toxic organophosphorus compounds, such as parathion, have been applied. The definition of safe reentry intervals has been argued at many conferences and meetings. Many problems still remain: How does human poisoning correlate with exposure to pesticides and what can be done to predict when residue levels in a treated area will no longer be harmful? Although the acute toxicity of a pesticide may be low, will chronic exposure to low levels have adverse effects? Can workers be effectively protected from exposure to pesticides in the field?

The chapters in this volume are concerned with exposure to pesticides and address some of these topics. Within the United States, differences in climate and agricultural systems are responsible for substantial differences in the problems encountered. Rainfall, sunlight, and soil types have a considerable influence on the longevity and nature of pesticide residues.

California is a major agricultural state, and a number of chapters deal with its special problems. There is concern for establishing safe levels of foliar pesticide residues and in correlating these levels with effects in mammals. The use of animal models, such as the scaleless chicken, is suggested as a procedure for estimating potential exposure during agricultural operations, and the reentry problem and its implication for workers in different regions of the country are discussed. Another group of chapters deals with measurement of worker exposure to pesticides and its correlation with other variables. Dermal exposure is particularly important, and this volume includes studies of the usefulness of protective clothing in reducing worker exposure.

I would like to thank all the authors; M. L. Leng, W. Bontoyan, and R. Cannizzaro of the Pesticide Chemistry Division; and M. Inscoc and M. M. Scott of the Agricultural Research Service, USDA for their help and cooperation in preparing this volume.

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Trends in Chemical Residues Including Reentry Considerations

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Preface:

The development of synthetic organic pesticides passed through an accelerated phase during the decades following World War II. The discovery of the insecticidal activity of DDT, lindane and the organophosphates was followed rapidly by the introduction of carbamates and new organochlorine compounds. The 50's witnessed the introduction and widespread utilization of a variety of synthetic organic herbicides. The impact of these discoveries was dramatic. Human health benefited by the reduction of the incidence of malaria and other diseases carried by insect vectors. The World Health Organization proposed a campaign to control malaria in 1955 by using DDT and, by 1972, the disease had been eradicated in 36 countries (total population 710 million) (1). Selective herbicides eliminated much of the hand-weeding formerly needed in crop production and, by reducing the number of weeds that competed for water and nutrients, made possible substantial increases in crop yield. It also became possible to control undesirable vegetation in forests, on rights-of-way of highways or utilities, and in industrial areas without extensive use of hand-labor as in the past.

The production of synthetic organic pesticides increased from an estimated 464,000 pounds in 1951 to an estimated 1.4 billion pounds in 1977 (2). Increases in production were followed by the recognition that such increased use of synthetic chemicals would be accompanied by extensive human and environmental impact. Pesticide use was regulated by federal and state governments, but continued evolution of the regulatory position has been necessitated by increasing usage and changes in patterns of use. The U.S. Environmental Protection Agency (U.S.E.P.A.), is responsible for the registration of pest control chemicals, but many aspects of pesticide use and handling also fall under the responsibility of a variety of Federal and State agencies. With rapid increase in pesticide

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use, there has been a corresponding increase in public attention, and public concern has often expressed itself in legal and political action. However, in many cases there has been inadequate information on which to base definitive regulatory action. Data has accumulated slowly, but during the 1950's and 60's there was a major effort to develop sensitive and selective techniques of analysis and bioassay. Routes by which pesticides were transformed in man, plants and the environment were elucidated. The qualitative and quantitative aspects of dissipation of pesticides in the environment began to be understood. During the 1970's much greater attention was focussed on the implications of pesticide use for human health. Pesticide use patterns and the philosophy of pest control have undergone evolutionary changes in response to these developments.

Improvements in analytical techniques, particularly the gas chromatograph, and the intensification of monitoring programs revealed that organochlorine insecticides were common contaminants of environmental samples. The level of DDT in average U.S. inhabitants in 1973 was 2.3 - 4.0 ppm and this was accompanied by 4.3 - 8.0 ppm of DDE, a major degradation product. The corresponding figures for the inhabitants of India were 16 ppm of DDT and 10 ppm of DDE (1). Usage was higher in India than the U.S., but the presence of low levels in Eskimos, in an area where DDT was not used, points to world-wide distribution of residues (3). Levels of DDT residues stored in fat are proportional to intake, and the metabolism and excretion of DDT by mammals is slow.

Increased levels of DDT and other organochlorine insecticides residues in man and the environment and the increasing appearance of resistance among insects were among factors that contributed to change in use patterns. The organophosphate insecticides largely replaced organochlorines and were used on an increasing scale for control of insects in agriculture and public health. In 1972, 10 million pounds of parathion and 40 million pounds of methyl parathion were used for insect control. Despite the high mammalian acute toxicity of most organophosphates, they have been widely accepted, but stringent safeguards are essential to assure the safety of workers potentially exposed to these compounds.

Although organophosphates now predominate as high-use insecticides, a variety of chemicals of other functional types are used to control pests as herbicides, insecticides, fungicides, fumigants, defoliant etc. Several of these are the source of potential operational hazards that must be addressed in terms of worker protection and the necessity for analysis of exposure and assessment of its effects.

Pesticides may enter the body orally, through the skin (dermally), or through the respiratory tract. Some pesticides are so acutely toxic that their effects appear after brief exposure, and the most dangerous, in terms of acute toxicity, include several organophosphate insecticides such as parathion. The risk of poisoning by dermal exposure to parathion is great because it is effectively absorbed through the skin and its dermal toxicity approaches its oral toxicity. The dermal route of exposure is likely to be one of the most significant for field workers, and contributors to this symposium have described a number of techniques for direct and indirect analysis of worker exposure.

The primary biological effects of the organophosphates are well defined. They act generally by inhibition of cholinesterase, an enzyme that is involved in the nerve transmission process and is present in insects and mammals. Susceptibility to an organophosphate insecticide varies from species to species and from chemical to chemical. The symptoms of organophosphate poisoning are well characterized in humans. Biological effects that follow exposure include alteration of cholinesterase levels in plasma and cell, but the correlation of depressed cholinesterase levels with the appearance of clinical symptoms is poor, and normal levels of cholinesterase show great variability. However, correlation of mammalian cholinesterase levels with known doses of pesticides in statistically controlled experiments may provide useful guidelines when the information is combined with the corresponding residue dissipation data .

Although acute clinical symptoms may be rarely observed following exposure to pesticides of low mammalian toxicity, it is also important to monitor exposure levels. Such quantitative data may be especially important for epidemiological investigations in which a retrospective study of causative factors must be conducted. Without such data, conclusions can only be based on what may be unrepresentative sampling in addition to qualitative observations. Greater difficulties lie in assessing the effects of long-term exposure to low levels of pesticides. Occupational exposure may be quantitatively assessed, but the variations in individual susceptibility and the interaction of other factors that affect health must be considered in the interpretation of epidemiological studies. Most difficult are assessments of the effects of long-term, low-level exposures that may occur in the population at large as a result of dietary intake or environmental contamination. Nevertheless, it is important to monitor the levels of environmental pollutants in man and the food supply and to obtain baseline data that will indicate qualitative and quantitative fluctuations in the content of these pollutants.

We can expect that the controversy surrounding the long-term effects of pesticides on man will continue. The situation is increasingly complicated by the variety of man-made chemicals intentionally or unintentionally ingested during the human life span. Although resources available to science and medicine are adequate to evaluate only limited aspects of the problem, there is general agreement that measurements of human exposure represent an essential first step to understanding.

A priority for agriculture is to measure pesticide residue levels encountered by those most directly affected by reason of their occupation; the workers engaged in agriculture and pesticide manufacture. The latter present a somewhat different case because the factory environment can be more satisfactorily controlled than that of the field. Protective measures for field workers, such as prescription of reentry levels, or other regulatory action can only be intelligently applied if there are sufficient data available from the field to describe the duration and extent of hazard. Because the Second American Chemical Congress was scheduled to be held in California, the topic of worker-reentry was uppermost in the minds of many participants and their contributions reflect contrasting and controversial approaches to that problem. The majority of the symposium papers have addressed the problems of monitoring the exposure of field workers, although the scope of this volume also extends to problems of pesticides in the population at large and to considerations of industrial hygiene.

Several factors affect the extent of hazard associated with pesticide use. The compound used, the type of formulation, and the application equipment are important. Acute toxic hazards of active ingredients may be categorized but it must be recognized that the type of operation will also influence the hazard to the operator. For example, it has been stated that the application of parathion to fruit orchards by a power airblast sprayer is twice as hazardous to the operator as the application of dust to row crops with a boom duster (4).

The agricultural worker may be involved in one of many operations, each involving particular risk depending on the situation and the duration of exposure. For this reason, careful analysis of the operational site is essential and direct exposure measurements must be relevant to working conditions. Climatic conditions and other environmental factors will affect the dissipation of pesticide residues. Soil dust, residual particles of a formulation, or other pesticide-contaminated materials may easily be dislodged from foliage and the amount of dislodgeable material present when workers enter the field is an important guide to potential hazard.

Protection requires a combination of approaches. There are a number of factors that are intrinsic to the physiological and psychological makeup of the worker. Individual susceptibility and interaction with other biological stresses will vary from individual to individual. Personal hygiene and work habits also vary. It is important that the worker fully comprehends the nature of the hazards and the consequences of careless actions or failure to follow prescribed safe procedures. The attitudes of workers and managers are important in implementing working practices that will minimize risks.

The use of protective clothing, the observation of operational and regulatory guidelines and the observation of good work habits contribute to safe pesticide application. Access to regular trained medical advice and examination is also important.

If proper safeguards are to be maintained economically, it is essential to define the extent of the hazard and identify the problem areas. Research is needed to determine the sites and duration of exposure and to measure the amounts of residues and their rates of dissipation. Such measurements can be made with precision. The problem is to use knowledge gained in a particular situation to provide guidelines or models which can be applied more generally to field operations. Such extrapolations are controversial and they may also be dangerous if they are in error. The symposium includes descriptions of techniques for measurement of exposure, and some contributors indicate the controversial aspects of solutions that have been proposed.

The question of protective measures has been the subject of several studies and reports. In 1974 the Federal Working Group on Pest Management (F.W.G.P.M.) published the report of a task group on occupational exposure to pesticides (5). The task group recommended that registrants should "develop and submit data sufficient to enable the responsible federal agency to promulgate safe reentry levels for each crop for which any new organophosphorus pesticide is to be registered, if the responsible agency has reason to believe that exposure to foliar residues may pose a significant hazard to agricultural workers". Additional recommendations included: the consideration of significant geographical differences in the prevalence of the worker reentry problem; the necessity for health surveillance systems; research to clarify factors that influence reentry intervals, such as the effect of personal hygiene, work practices, degradation of foliar residues; and research to reduce reliance on the use of chemical pest control systems. Research to reduce reliance on the use of chemical pest control agents has received considerable support, and

systems of integrated pest management are evolving in which chemical pesticides may be more efficiently used by techniques such as improved prediction of pest population and combinations of chemical with nonchemical pest control methods.

The report also stated that in 1971 about 4,500,000 persons in the U.S. on an average were engaged in farm employment and that about 8,000,000 to 9,000,000 probably do some work in commercial agriculture. Although the annual average figure for farm employment had fallen to about 3,800,000 (6) in 1979, a considerable number of persons may be exposed to pesticides by reason of their employment or involvement in agriculture.

It is difficult to obtain figures that accurately reflect the incidence of pesticide poisoning, and the number of documented cases of direct human poisoning in the USA varies from source to source. It was estimated that there are 100,000 nonfatal cases of human poisoning each year from pesticide exposure (7). In 1973 there were 1,474 cases of occupational illness associated with pesticide exposure in California (8). Organophosphate insecticides are a major cause of occupational poisoning.

The F.W.G.P.M. Task Force addressed itself mainly to the problem of organophosphates, and its terms of reference were to identify areas in which information on occupational exposure to workers was unavailable, to make recommendations for the development of research protocols to determine safe reentry levels for the protection of agricultural and forest workers, and to suggest interim reentry standards based on existing knowledge. The report was controversial but drew attention to the lack of a substantial data base and to the urgent need for surveillance of pesticide-related morbidity and mortality and for research to identify factors influencing safe worker reentry levels.

The U.S.E.P.A. has concerned itself with the problem of reentry intervals, and the quantitative measure of human exposure has become part of the RPAR (rebuttable presumption against registration) process. U.S.E.P.A. requirements will be promulgated as Subpart K of the guidelines for pesticide registration under the title of "Reentry Data Requirements". Differing opinions concerning the value of guidelines have been expressed (WRCC-38) and a committee was constituted in 1979 to involve the medical as well as the agricultural community in pesticide residue research. One outcome of a seminar-workshop held by that committee was an emphasis on minimizing human occupational exposure. The seminar-workshop closely preceded the current symposium (9), and some of the contributions in this volume are focussed on the same areas.

The increasing amount of research data concerning pesticide exposure is to be welcomed: without this, it is unlikely that there can be rational correlations between exposure-related illness and use of pesticides. Minimization of occupational pesticide exposure can be attained by increased worker protection, changes in practice or by reductions in pesticide use. In view of the unknown total burden of synthetic chemicals among the population at large, it would seem prudent to minimize occupational exposure. Regulatory actions reinforce standards and prescribe safe operating conditions. However, by seeking to reduce potential exposure hazards to the worker, individual factors such as personal hygiene, education, etc. become less significant. Pesticide use throughout the world is increasing and protection from exposure is a world-wide problem. The problem is international in scale and must be faced at that level. The differences in pesticide use patterns, climatic conditions and attitudes to chemical pesticides among the nations of the world are so great that extreme differences are to be expected in the exposure status of their populations. Measures to safeguard the agricultural worker will depend on international cooperation because a considerable amount of information must be exchanged. Operational guidelines must be supplemented by field studies of worker exposure and measurements of residue dissipation in the zone where actual use will occur. Increased security for workers and for the population at large can only be achieved by adopting practices and procedures which seek to minimize exposure. This goal may be achievable by good planning and management of pest control practices. Its attainment also requires the availability of considerable data that must be provided by thorough analytical, biological, and epidemiological investigation. The symposium papers reflect progress and discuss issues in the United States in relation to exposure of agricultural workers and, to a lesser extent, of the community at large. However, the problem must ultimately be addressed on an international scale.

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Methodology for Estimating the Dietary Intake of Pesticide Residue

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The Environmental Protection Agency (EPA) is responsible for the registration of all pesticide products sold or distributed in the United States. The Agency will register a pesticide if--among other things--it performs its intended function without unreasonable adverse effects on the environment.

Before the Agency can register a pesticide for a use that could result in residues in food or feed, a tolerance must be established. EPA has the authority under the Federal Food, Drug, and Cosmetic Act, Sections 408 and 409 for setting tolerances. A tolerance is the legal maximum residue concentration of a specific pesticide chemical allowed in or on a specific food or feed item. If residues exceed the tolerance, the food or feed is considered adulterated and is subject to seizure as it travels in interstate commerce. Tolerances are set at a level that represents the maximum residue likely to occur if the pesticide is used in accordance with the registered directions for use.

In order to establish a tolerance, the Agency must be able to predict the level of residue that will occur. The data used for this purpose includes data on metabolism, analytical methodology, and the results of field trials conducted to determine the actual level of residue anticipated (1).

Metabolism data are needed to identify the nature of the terminal residue(s). These studies generally require the use of radiolabeled chemicals. Harvested portions of the crop are analyzed and as many metabolites or alteration products as possible are identified. The tolerance regulation includes identification of the chemical entities covered by the tolerance. The determination of which chemical entities are to be included in the tolerance will depend on their toxicological significance, their relative proportion of the total residue, and whether analytical methods are available to detect the entity.

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The tolerance level established is set high enough to cover residues of all the significant components of the residue.

In estimating exposure to pesticide residues, the level of residue used reflects both the parent compound and all significant metabolites or, in other words, the tolerance level. Although the toxicity of metabolites may be significantly higher or lower than the toxicity of the parent, toxicology data is generally not required on metabolites per se. In general, the Agency assumes that animal toxicology studies reflect exposure to a plant metabolite if the plant metabolite is also an animal metabolite. In cases where a plant metabolite is not also an animal metabolite, toxicology studies on the plant metabolite have been required by the Agency.

Analytical methodology used to determine the level of residue likely to occur must be suitable for enforcing the tolerances. That is, the methodology must: (1) be capable of determining all components of the residue as specified in the tolerance; 2) be of adequate specificity and sensitivity; (3) avoid the use of exotic reagents and equipment not available to the regulatory agencies; and, (4) be such that samples can be analyzed within a reasonable period of time.

Residue field trials are carried out to determine the level of residue that will occur as a result of the use of the pesticide. The pesticide is applied to the crop(s) in a manner consistent with the directions for use which will appear on the pesticide label. Frequently, samples will be collected that reflect exaggerated application rates and/or variable intervals between application and harvest to give an idea of the variation in residues with changes in how or when the pesticide is applied. The field trial studies are designed to determine the maximum residue likely to occur. The tolerance is set at a level such that registered use of the pesticide will not result in residues exceeding the tolerance.

If a pesticide is to be applied to livestock, or will result in residues in the feed of livestock, the possibility of residues in meat, milk, poultry, and eggs arises. Data on metabolism, analytical methods, and level of residue in animal food products are needed in those cases. The same considerations of identification of the terminal residue and developing analytical methods suitable for enforcement mentioned previously also apply to residues in animal products. The tolerances for animal products are based on the tolerances on the animal feed items, the significance of those feed items in the diet of livestock, and the potential

for transfer of residues to meat, milk, poultry, and eggs as reflected by the results of feeding studies.

The exposure of humans to pesticides from residues in food is dependent both on the quantity of a food consumed and the residue levels therein. The Agency has traditionally used a simplified method of estimating chronic exposure to pesticide residues that was originally developed by the Food and Drug Administration. This exposure method is based on the assumptions of tolerance level residues in food and national average food consumption per capita.

Tolerance levels in foods are estimated based on the parameters previously described. The average food consumption estimates are based on crop production data (2) or food consumption survey data (3) provided by the United States Department of Agriculture. The food consumption data used reflects per capita average consumption. The food consumption estimates currently used approximate total U.S. consumption of a food divided by total U.S. population.

The following theoretical example illustrates the procedure described above:

<u>Crop</u>	<u>Tolerance (ppm)</u>	<u>Food Consumption (grams/day)</u>	<u>Exposure (mg/day)</u>
Potatoes	1	80	0.08
Milk	0.01	430	0.004
Lettuce	10	20	0.2
			<u>0.284</u>

TMRC = 0.3 mg/day = 0.005 mg/kg bw/day, assuming a 60 kg person.

TMRC is an acronym for Theoretical Maximal Residue Contribution, and is an estimate of chronic dietary exposure which could result from the consumption of the foods on which tolerances for a specific pesticide are established.

When considering the establishment of the initial tolerance for a specific pesticide on a food item--and also when considering the establishment of each succeeding tolerance for that pesticide on subsequent food items--the Agency compares the TMRC with the Acceptable Daily Intake, the ADI, to determine whether granting the proposed tolerance(s) would result in an unsafe of residue in food.

The ADI is the daily exposure level of a pesticide residue which, during the entire lifetime of man, appears to be without appreciable risk based on all facts known at the time. The ADI is based on toxicological considerations only and is independent of level of exposure.

If the tolerances(s) under consideration will result in the TMRC exceeding the ADI, then the exposure is considered unacceptable and new tolerances(s) are denied unless data are available to show that actual exposure is less than the theoretical exposure(s) represented by the TMRC.

In the example illustrated, if the ADI were 0.002 mg/kg bw/day, which is equivalent to 0.12 mg/day for a 60 kg person, the potato and milk tolerances would be acceptable since their TMRC--either alone or in combination--would not exceed the ADI of 0.12 mg/day, but the lettuce tolerance would be denied--unless there were mitigating circumstances--since it would cause the TMRC to exceed the ADI. It should be noted that this procedure is not used by the Agency for suspected carcinogens for which alternative risk assessment methodology is used.

The exposure estimation procedure described above has been criticized on the grounds that it may either overestimate or underestimate the actual exposure. The assumption of tolerance level residues ignores the fact that tolerances are set at the maximum level anticipated and that 100% of a crop is not actually treated with the pesticide. Also, since tolerances are set on the basis of the raw agricultural commodity as it travels in interstate commerce, the assumption of tolerance level residues does not take into account any loss of residue that occurs as a result of processing, trimming, washing, etc. Thus, the assumption of tolerance level residues tends to overestimate the actual exposure. Conversely, the assumption of national average food consumption per capita leads to an underestimation of the exposure to pesticide residues on foods that are only eaten infrequently or are eaten primarily by a small subgroup of the U.S. population.

The criticisms of the procedure for estimating dietary exposure have led the Agency to review its present practice. The issues discussed above were referred to the Science Advisory Board of the EPA Environmental Health Advisory Committee. The Agency has received a draft response from the Science Advisory Board in which changes in the procedures used to set tolerances are recommended. The Agency is in the process of implementing appropriate changes.

Among the changes the Agency is considering implementing to allow a more accurate assessment of the dietary exposure are:

(1) The estimation of the Actual Daily Exposure assuming a person consumes a large portion of food. If this one-day exposure exceeds the Acceptable Daily Intake, then this use of the pesticide could be denied.

(2) Wider application of the use of a residue level estimate based on residues in food as actually consumed whenever tolerance level residues are considered unacceptable. Since tolerances are set on the raw agricultural commodity as it moves in interstate commerce, residue levels in food as consumed are frequently lower than the tolerance due to processing, trimming, cooking, etc.

(3) Use of estimates on the percentage of a crop that is treated with a pesticide. Although this would not influence the level of exposure to persons consuming treated commodities, it would influence the estimate of the number of persons exposed.

(4) The use of data on the frequency of consumption of foods. By combining data on the frequency with which various foods are eaten with data on the quantity of food consumed and residue levels, the variation in dietary exposure to pesticides in food can be estimated. This could be applied in estimating exposure for any given segment of the population; for example, infants, the elderly, women of child-bearing age, ethnic or regional groups, etc.

(5) The inclusion of an estimate of all potential sources of human exposure and not just pesticide residues in food. This would include an evaluation of the possible environmental contamination of air, water, and soil from a chemical's use as a pesticide and consideration of whether the chemical is also used as a food additive, drug, or industrial chemical.

These and other changes in the methodology used by the Agency in estimating dietary exposure to pesticides are currently under review.

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The Biophysiologic Analysis of Chemical Residues in Human Tissues

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The analysis of biological tissue to determine its chemical composition has been a recognized challenge for the biologist and chemist alike. It is felt by many that knowledge of the chemical composition of biological tissues may unlock the secret of the living process itself. Molecular structure is dependent upon chemical reactivity and other properties of the individual components which go together to form the living cell. Originally, chemical analysis allowed the chemist to define the components of different tissues in a chemical sense. As skill progressed and technology improved, the chemical pieces were put together in a way for us to identify molecular structures as well. Many challenges remain along these lines in order to uncover the chemical determinants upon which molecular structure is based.

Homeostasis

It is well recognized that living biological tissues are not in a state of chemical stagnation. In order to maintain the living state, a biological equilibrium exists which requires the input and utilization of an energy source, the utilization of raw materials, and the elimination of waste products. Thus, there is an attempt to maintain a state of homeostasis; homeostasis being a dynamic equilibrium.

Living organisms are indeed "chemical scavengers." The system is presented with many materials from which a selection is made. Some chemicals are useful as they are, and others can be made useful by some chemical alteration. Other materials are not useful and represent either waste, or potential harm or disruption to the system. There are certain basic biological

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processes necessary to understanding the interaction between the environment and living systems. These processes must be understood in order to fully appreciate the potential for reactions within the living system when it impacts with its environment. The processes referred to are absorption, distribution, metabolism, and excretion.

Chemical injury may occur when a living cell comes into contact with a chemical foreign to its system. Chemicals which enter the living organism which are foreign to the system are known as xenobiotics. Chemical injury may be external as a relatively non-selective process wherein an overall disruption takes place. An example of this would be an acid burn on the surface of the skin, wherein a general protein coagulation takes place with tissue and cellular disruption. The process of absorption is not specifically involved in this type of chemical injury.

Intoxication And Poisoning

Poisoning is usually defined as a harmful or fatal influence, and a poison is generally a chemical agent which is capable of producing such harmful or fatal effect on a living system. Intoxication generally implies absorption of a chemical which has a disruptive influence on normal biological processes into the living system. The most common intoxication we all are aware of is that with ethyl alcohol. The degree of influence of alcohol on the homeostatic state is related to the amount present in the system or the dose absorbed. Alterations in biologic function can be predicted on the basis of how much alcohol is present in the system. An individual who is intoxicated with ethyl alcohol may appear to behave normally; however, chemical analysis may reveal the presence of an amount of alcohol known to interfere with normal biological functioning.

Fate Of Chemicals Entering The Body

Most chemists are familiar with the routes of entry for chemicals into the body. They may enter either through the gastrointestinal tract, by direct absorption through the skin and mucous membranes, by the respiratory passages and lungs, and also by injection or parenteral administration. Once a chemical enters the body, biological interaction is dependent upon chemical reactivity. An agent may pass through the body without any interaction with the biological system, or the material may be selectively removed by certain organs or tissues. Whether

or not a chemical entering the living system will preferentially locate in one organ or another may relate to the biochemical reactivity of the individual organ. For instance, iodine is selectively removed from the bloodstream by the thyroid gland and is utilized by the cells of the thyroid gland in its manufacture of the thyroid hormones. Xenobiotics which have a chemical reactivity similar to iodine might therefore be expected to distribute preferentially to the thyroid. Chemicals which distribute preferentially to one organ or another in the body may have a chemical reactivity which may have no effect on normal chemical processes or could potentially interfere with organ functioning and, thereby, homeostasis. Some chemicals entering the system may become hung up within a tissue due to active cellular processes designed specifically to protect the homeostatic state by not allowing the foreign substance to become widely distributed within the organism. Thus, consideration should be given to analyzing why chemicals distribute to where they do and what this distribution may tell us about the body's living process.

One can think of metabolism similar to that of an automobile salvage operation. Useful parts are removed and used as replacement for worn out parts or put to an entirely different use, perhaps in a new configuration. Other parts may be stored for use at a later time and still others may be discarded. Thus, chemicals taken into the body are not necessarily used intact, but may be dismantled and reassembled within certain chemical limitations. An isolated molecule may become an integral part of the body in its original molecular form or it may be altered and incorporated in a new configuration. The body's system for doing this, however, is subject to error and potential breakdowns.

Sir Randolph Peters, in demonstrating the biochemical defect involved in the body's handling of sodium fluoroacetate, presents a good demonstration of how chemical reactivity and similarity may result in altered biological processes (1). For those who are unfamiliar with this classic story, sodium fluoroacetate, when absorbed into the mammalian biological system, behaves chemically much the same as sodium acetate without the fluorine atom. When fluoroacetate is present, it competes with normal acetate and enters the citric acid cycle producing a bogus molecule of fluoroacetate which then interacts with the enzyme aconitase and inhibits enzyme activity. The normal cycle is blocked and the body is no longer able to maintain homeostasis. If sodium fluoroacetate is in the system, various studies have shown that the presence of large amounts of normal acetate will antagonize fluoroacetate by limiting its utilization by the system. Knowing the chemical processes involved in homeostasis, one might be able to predict the effect of a foreign chemical in the system on the basis of its chemical reactivity. Knowing the enzyme

characteristics of the body and the chemical reactivity of specific molecules may provide us with clues to the possible influence individual chemicals may exert on normal functioning.

It is known that some chemicals tend to store in the body primarily due to solubility characteristics. The majority of chemicals entering the body which have no biological usefulness, however, will be excreted by the body. Products of excretion are generally thought of as waste. The body maintains specific capabilities to rid itself of unnecessary, unwanted, and harmful materials. Excretion is not necessarily a passive process, and chemical reactivity plays an important role in this process. Indeed, some metabolic processes seem specifically designed to facilitate excretion.

It is necessary to chemically understand the processes of absorption, distribution, metabolism, and excretion, the chemical reactions that are a part of these processes, and the homeostatic state in order to do a biophysiological analysis of chemical residues detected in human tissues. The mere presence of a xenobiotic does not indicate harm to the system. The finding represents a single observation along a continuous journey from absorption to excretion. Unless the processes are evaluated in their dynamic state, it is very difficult to determine the meaning of specific residue findings within the biological system. It is important to obtain a good history when checking biological tissues for "residue." When did it enter the system? Did absorption occur on a single exposure basis or was continuous or repeated absorption involved? What is the chemical reactivity of the material? What effects might be suggested on the basis of this chemical reactivity?

Our capability in measuring residues is becoming increasingly sensitive. The identification of a molecular structure from biological tissue does not, per se, establish prior exposure to the molecule in the form it is found. If a minute chemical residue is found in biological tissues for which there is no identifiable source of exposure, it's very logical to assume such exposure took place unobserved. It is also possible, however, that the individual molecule which was isolated from the system was somehow part of a larger structure and represents a waste product of some other molecule which has already been acted upon by the system. It is also possible that such a molecule in very minute amounts, was formed by the biological system, perhaps not by design, but due to circumstance.

Much toxicological investigation has occurred with respect to a series of pesticide chemicals known as Captan, Folpet, and Difolatan. The molecular structure of these pesticides is exceedingly similar to that of thalidomide. Thalidomide is the

drug which was found to cause abnormalities in developing human embryos. Because of the molecular similarity of these pesticide chemicals to that of thalidomide, many animal studies have been conducted specifically for the purpose of determining whether or not these pesticide chemicals will have the same effect on living systems as does the thalidomide molecule. Without going into detail, suffice it to say that the findings from these biological assays are not as evident or clearly distinctive as are the effects with thalidomide. As mentioned earlier, the inclusion of a fluorine atom onto an acetate molecular changes the chemical reactivity of this molecule. The influence of the different radicals contained within individual molecules may significantly alter the biological interactions which are possible. Structural similarities must be viewed with respect to the influence of molecular groups on chemical reactivity. Finding a molecular configuration suggestive of a particular chemical residue with no obvious source of contamination deserves careful interpretation.

Chemical molecules may be looked upon as if they are puzzles. Based on chemical reactivity, different molecules will become attached to one another. The individual pieces that went to making the puzzle lose their individual identity. When the puzzle is disassembled, however, new individual molecular configurations may result. The per cent recovery of material administered to an animal is frequently less than 100 percent. It's sort of like trying to follow an intact automobile as it proceeds through a dismantling operation. What was once a single automobile may now become parts of several different vehicles, each one going in a different direction, and the original parts perhaps losing their original characteristics.

A small amount of fluoroacetate in the total biological system may have a minimal influence on homeostasis. The material would eventually proceed through the system and lose its potential influence on that system. In order for homeostasis to be significantly altered, an adequate amount of material must be present relative to the biological substrate with which it may interact. There is a certain "critical mass" which must be present to overcome the normal homeostatic state of affairs. If the system becomes overloaded, there may be a breakdown. If, on the other hand, the system is functioning within its needed capacity, no abnormalities may be expressed.

In order to interpret biologically the chemical residues which one may find in the body, chemical processes of the body must be understood in much greater detail. The mere detection of a residue in tissue will only let us know whether or not a substance exists within the system, and does not address the potential such substance may have to disrupt that system. One must, therefore, search for alterations in the normal biological processes which might be associated with residue findings.

We should keep in mind that the term "pesticide" refers to a chemical use category and not a specific chemical characteristic. It does impart the knowledge that a substance has toxic properties which can be used to advantage. Only with proper chemical characterization of residues found in the body and knowledge of their potential influence on homeostasis will it be possible to dispel popular misconceptions to the effect that pesticide chemicals are handled differently from other chemicals which enter the body or are unique in the hazards they present to the system.

Conclusions

Xenobiotics, for the most part, have limited biological half-lives. The extent of their influence on the biological living system is limited on the basis of the amount absorbed and the duration of their presence within the system. Once exposure to a pesticide chemical occurs, individuals need not be unduly fearful of adverse health impacts slowly and relentlessly progressing regardless of dose and duration of exposure. There are effects such as cancer induction and genetic mutations which have yet to be characterized and fully understood from a chemical standpoint. Consideration of these special conditions has been the subject of entire seminars and obviously cannot be covered fully in a presentation such as this.

The body's chemical processes are the same for handling chemicals used as pesticides as they are for other xenobiotics. The body does not know the use category of a chemical entering its system. The study of chemical residues in the body and their effects on homeostatic processes offers some hope of a better biological, as well as chemical understanding, of the life process itself.

Abstract

The chemical analysis of biologic tissues is a reflection of biologic activity. The human body tries to maintain itself in a state of homeostasis. Homeostasis is a dynamic equilibrium and not a chemical stagnation. Homeostatic mechanisms tend to keep the chemical makeup of the body constant. Chemical analysis of various human tissues will, therefore, yield relatively consistent findings in healthy tissues.

Xenobiotics are chemicals foreign to the biologic system. When one speaks of chemical residues in human tissues, a xenobiotic is usually implied since a residue is what remains after a removal process. In order to interpret biologically the chemical residues found in the body, it is necessary to examine the chemical processes of the body and develop chemical road maps of xenobiotics from the moment they contact the biologic system to their eventual journey's end. Without such knowledge, mere detection of residue in tissue will only let us know whether or not a substance is present without addressing its potential effects on that system. Alterations in normal biological processes associated with residue findings offer some hope of mechanistic explanations.

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The Safe Level Concept and the Rapid Field Method

A New Approach to Solving the Reentry Problem

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The use of organophosphorus (OP) insecticides on tree and vine crops in California has resulted in blood cholinesterase depression among some field workers who have come in contact with toxic dislodgeable residues on the crop foliage (1) during harvesting, thinning and pruning operations. To prevent these illnesses among agricultural workers, the California Department of Food and Agriculture established reentry waiting intervals for 16 organophosphorus insecticides in 1971 and extended this list in the subsequent 9 years to include 21 insecticides (2). The reentry intervals were set to allow sufficient time for dislodgeable residues to dissipate to lower and thus safer levels when pesticides are used at the maximum rates allowed on their labels. This procedure consequently results in unnecessarily long safety intervals on crops when lower rates are used. To alleviate this problem, the California Legislature passed in 1979 a law (AB 1090) which permits growers by regulation to have their orchards or vineyards tested for toxic dislodgeable insecticide residues prior to allowing workers to reenter a treated field. The establishment of safe insecticide levels on foliage will be required prior to the implementation of the regulations. Knaak *et al.* (3) reported a procedure for establishing these levels on foliage using dermal dose-red cell cholinesterase (ChE) response curves and field reentry data. This procedure meets the needs of growers as well as regulatory and safety officials in the Department. The rapid field method (RFM) developed by Gunther *et al.* (4, 5) provides a sensitive and reliable procedure for estimating organophosphorus residues on crop foliage. The establishment of safe pesticide levels on foliage and the use of the rapid field method to test for insecticide residues provides a means for assuring safe working conditions for field workers. The rapid field method colorimetrically analyses for total OP residues on leaf surfaces. Total OP residues include both the thions and their toxic oxons. Safe levels for total OP residues are therefore needed for use in conjunction with the rapid field method.

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This paper establishes toxicologically-safe levels for total residues of parathion, azinphosmethyl, methidathion and their oxons on tree foliage and reports these levels in terms of absorbance units as determined by the rapid field method. Safe levels for a new insecticide, chlorthiophos, are also proposed based on preliminary residue data. Chemical structures of the four insecticides mentioned above are shown in figures 1, 2, 3 and 6.

Dissipation of Dislodgeable Residues

Figures 1A, 2A and 3A give representative dissipation curves for parathion, azinphosmethyl and methidathion on orange trees in California (6). Parathion dissipates with the formation of considerable amounts of paraoxon. Low volume application (100 gal/acre) of these insecticides results in high levels of OP residues and thus longer dissipation times to safe levels. Azinphosmethyl does not dissipate as rapidly as parathion under field conditions. Azinphosmethyl oxon is formed during the process and dissipates slowly with time. Azinphosmethyl oxon levels were determined only for azinphosmethyl at 6.0 lb AI per 100 gal/acre. Methidathion dissipates on citrus also with the formation of its oxon.

Figures 1B, 2B and 3B, drawn using the data from Figures 1A, 2A and 3A, give the dissipation curves for the total residues (thion + oxon) of parathion, azinphosmethyl and methidathion. These dissipation curves are similar to the curves obtained when OP residues are determined by the rapid field method as shown by the extensive studies conducted by Gunther et al. (5) which compare gas chromatographic values for thion + oxon with RFM values for total OP residues.

Safe Levels for Parathion, Azinphosmethyl, Methidathion and Their Oxons on Tree Foliage

Table I was constructed according to the procedure of Knaak et al. (3) using the dermal dose-ChE response curves in Figures 4 and 5. Paraoxon was used as the pesticide standard for methidathion and chlorthiophos, while azinphosmethyl oxon was used as a standard for chlorthiophos oxon sulfoxide and methidathion oxon. The groupings are based upon similar slope values for the dose-response curves. Parathion and azinphosmethyl acted as their own standard. A standard is a pesticide for which safety information is available. In this Table, safe levels are given for the thions and their respective oxons. The safe level for total residues (thion + oxon) lies between the safe levels for the thion and its oxon if the oxon level is at or below its safe level.

Table II gives a procedure for establishing safe levels for total dislodgeable thion + oxon residues on tree foliage.

Table I. Establishment of Safe Levels on Tree Foliage (in $\mu\text{g}/\text{cm}^2$) Using the Results of Dermal Dose-ChE Response Curves and Field Reentry Studies according to Knaak *et al.* (3)

Insecticide or Alteration Product ^{a/}	Slopes	ED ₅₀		Safe Level on ^{c/} Foliage in $\mu\text{g}/\text{cm}^2$
		in $\mu\text{g}/\text{cm}^2$ of Total Body Surface	Relative ^{b/} Toxicity	
Methidathion	2.9	10	30	0.6
Chlorthiophos	2.4	8.8	27	0.54
<u>Paraoxon</u>	2.3	0.33	1.0	0.02 ^{d/}
<u>Azinphosmethyl-oxon</u>	2.0	0.82	1.0	0.05 ^{e/}
Chlorthiophos-oxon sulfoxide	1.9	0.69	0.84	0.04
Methidathion-oxon	1.8	2.2	3.0	0.15
<u>Parathion</u>	1.3	2.4	-	0.09 ^{d/}
<u>Azinphosmethyl</u>	0.9	25	-	3.0 ^{f/}

a/ Pesticide standard is underlined; a standard is a compound for which safety information is currently available.

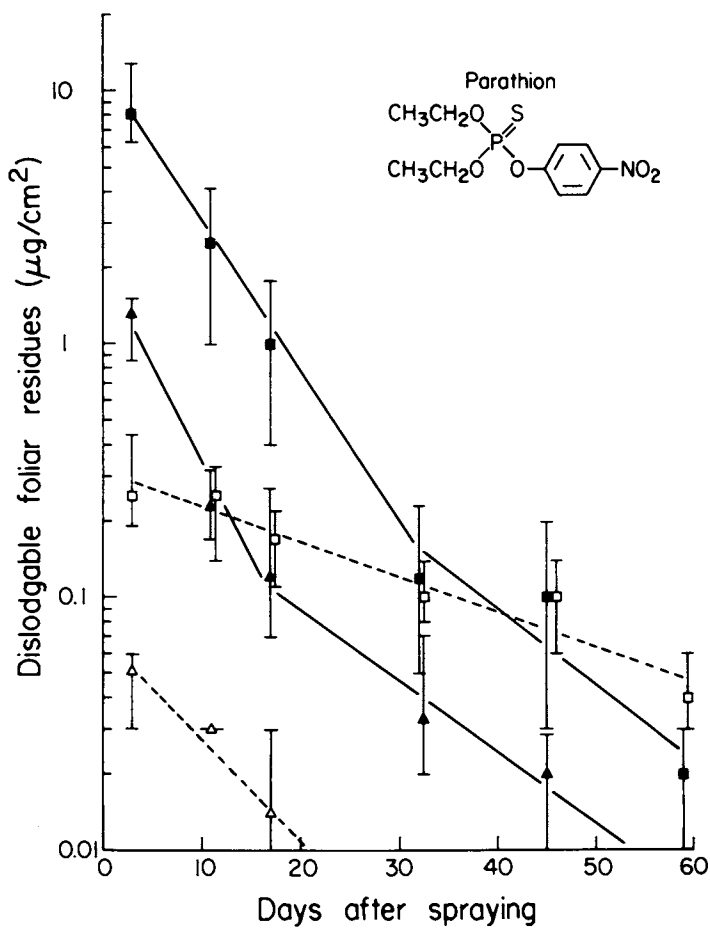
b/ ED₅₀ of pesticide under investigation divided by ED₅₀ of pesticide standard.

c/ Relative toxicity multiplied by established safe level of standard.

Safe levels for standards determined by reentry studies:

d/ Spear *et al.* (7); e/ estimated. f/ Richards *et al.* (8).

The procedure involves converting oxon to thion toxicity equivalents by multiplying the oxon value by its relative toxicity (ED_{50} of thion \div ED_{50} of oxon) in Table I. The ED₅₀ value is the dermal dose in $\mu\text{g}/\text{cm}^2$ of total body surface which produces 50% inhibition of red cell ChE activity 72 hours after application. The total thion and oxon level is then divided by the thion toxicity equivalents and the factor is multiplied by the safe level established for thion in Table I. This procedure was conducted for the dislodgeable residues of parathion-paraoxon, methidathion-methidathion oxon, and azinphosmethyl-azinphosmethyl oxon. The safe levels for the total dislodgeable residues were determined to be 0.06, 0.2 and 1.6 $\mu\text{g}/\text{cm}^2$, respectively, for



Residue Reviews

Figure 1A. Dissipation of parathion (closed symbols) and paraoxon (open symbols) on orange trees by GC method (6). Key: ■ and □, 10 lb AI parathion/100 gal/acre; and ▲ and △, 10 lb AI parathion/1,600 gal/acre; and ---, safe level for thion + oxon.

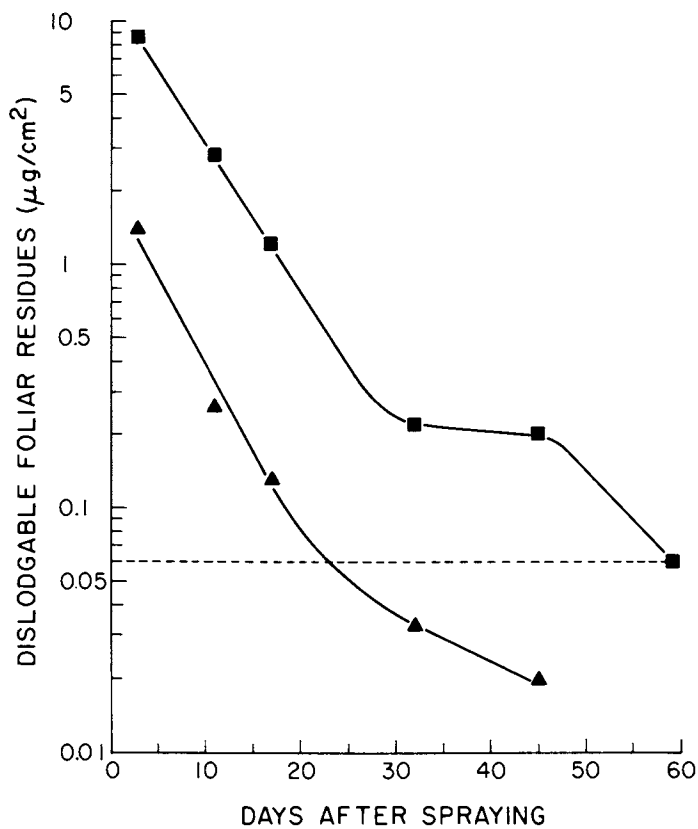
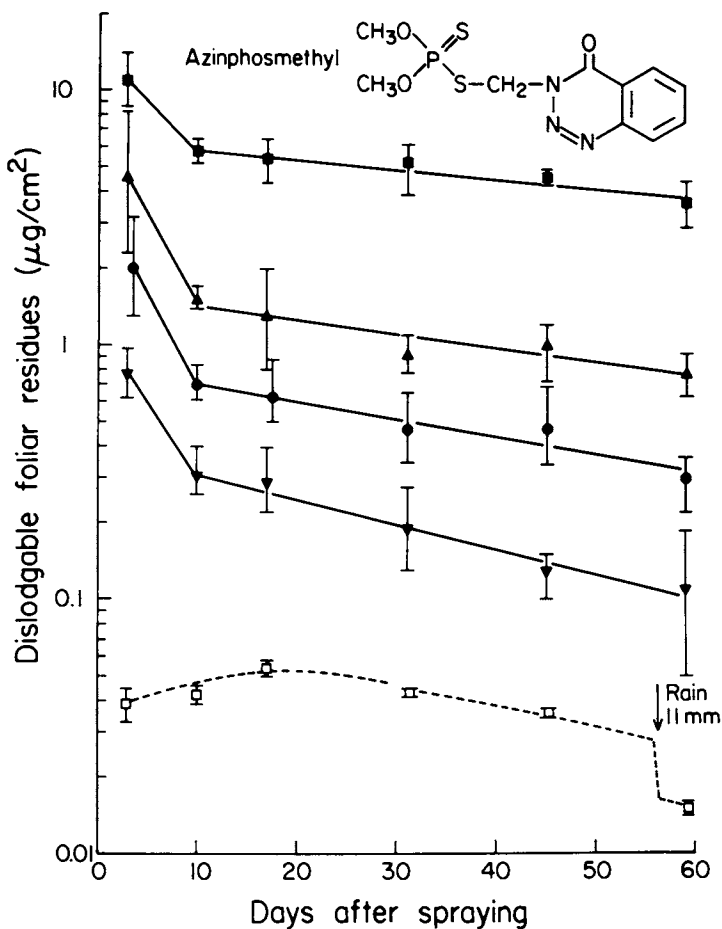


Figure 1B. Dissipation of parathion (closed symbols) and paraoxon (open symbols) on orange trees by total OP method. Curves are drawn from Figure 1A. Key: see Figure 1A.



Residue Reviews

Figure 2A. Dissipation of azinphosmethyl (closed symbols) and its oxon (open symbol) on orange trees by GC and LC method (6). Key: ■ and □, 6 lb AI azinphosmethyl/100 gal/acre; ▲, 6 lb AI azinphosmethyl/1,600 gal/acre, and at 2.0 (●) and 1.0 (▼) lb AI/500 gal/acre; and ---, safe level for thion + oxon.

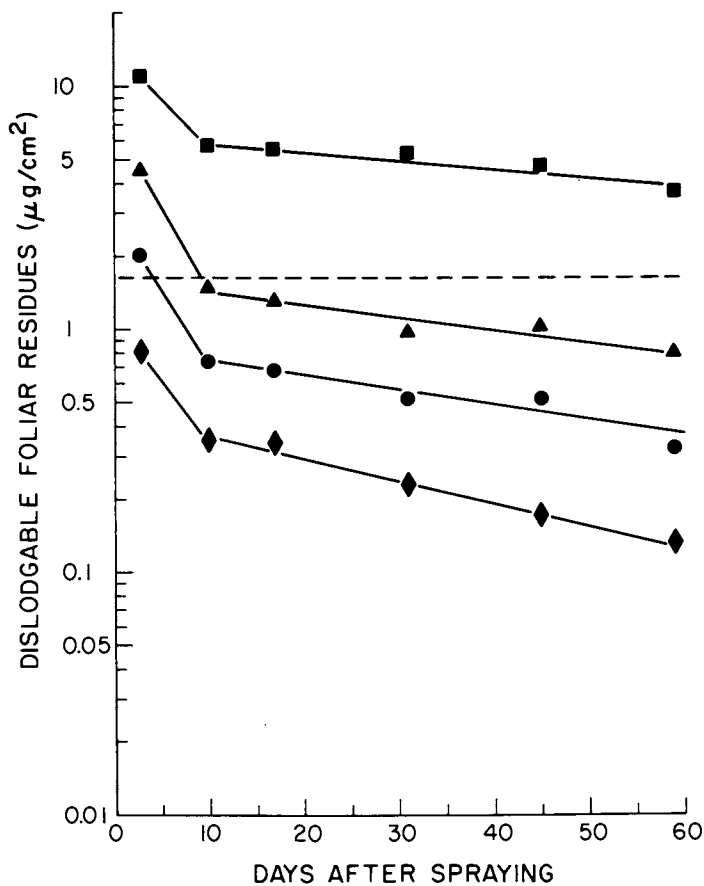


Figure 2B. Dissipation of azinphosmethyl (closed symbols) and its oxon (open symbol) on orange trees by total Op method. Curves are drawn from Figure 2A. Key: see Figure 2A.

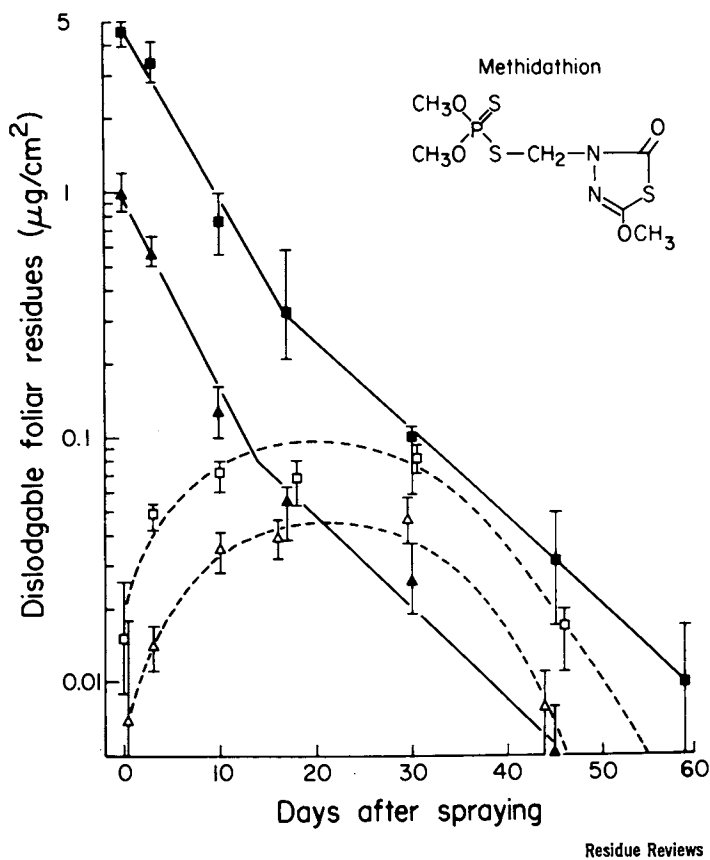


Figure 3A. Dissipation of methidathion (closed symbols) and its oxon (open symbols) on orange trees by GC method (6). Key: ■ and □, 5.6 lb AI methidathion/100 gal/acre; ▲ and △, 5.6 lb AI methidathion/2,250 gal acre; and (●) 11.3 lb AI methidathion/2,250 gal/acre; and ---, safe level for thion + oxon.

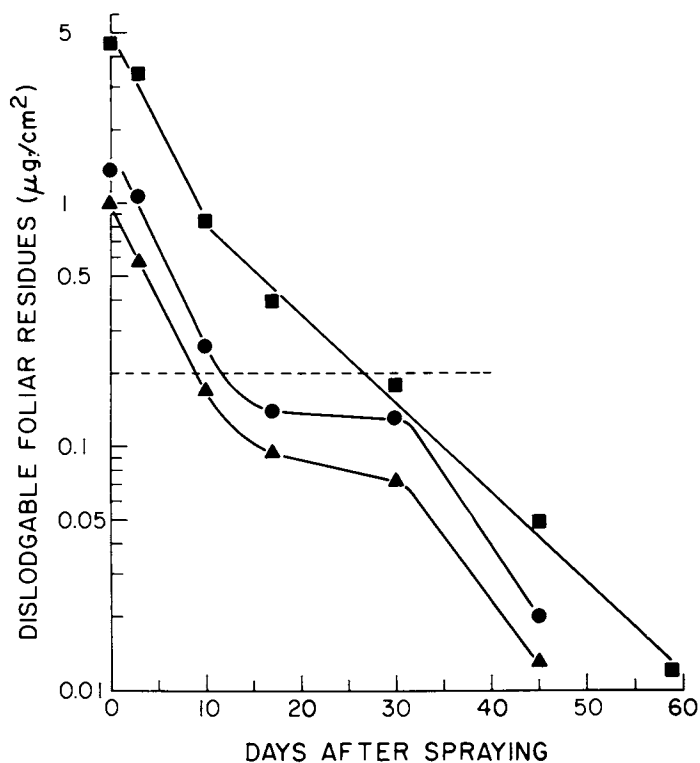


Figure 3B. Dissipation of methidathion (closed symbols) and its oxon (open symbols) on orange trees by total OP method. Curves are drawn from Figure 3A. Key: see Figure 3A.

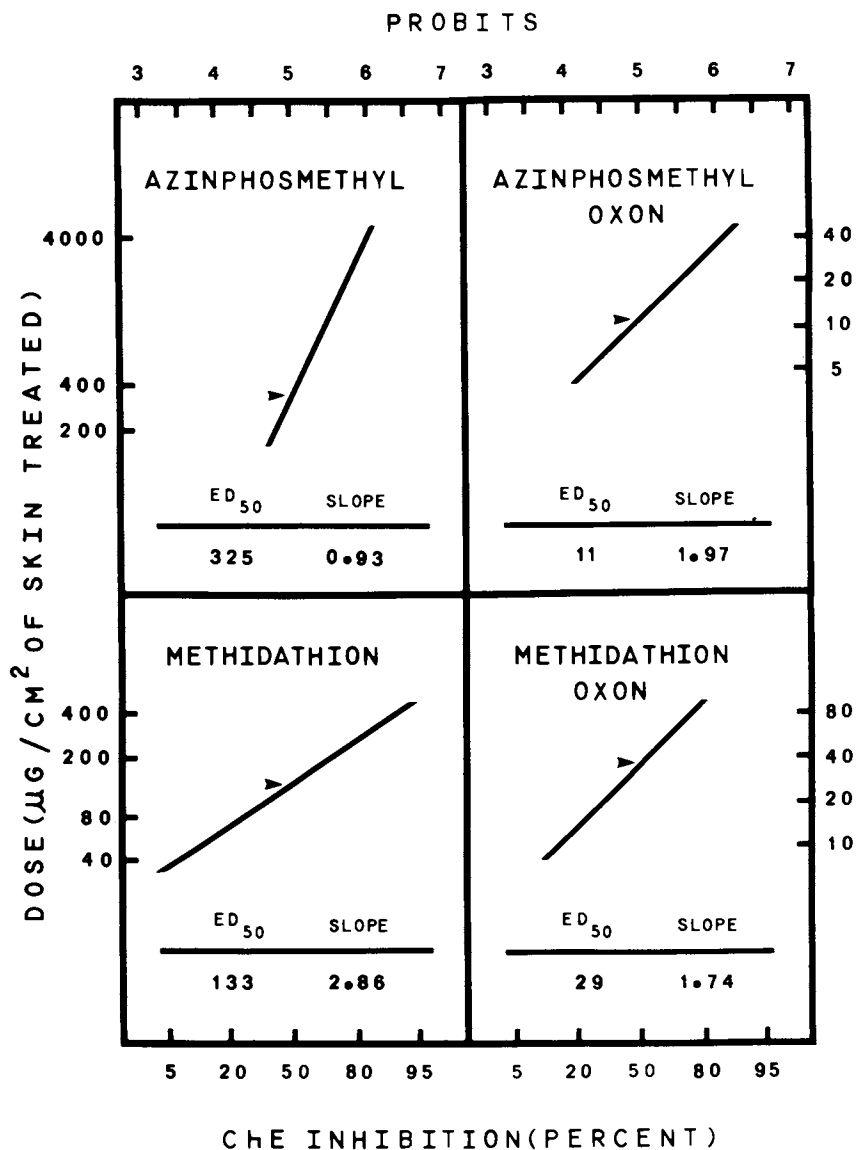


Figure 4. Dermal dose-ChE response curves according to Knaak et al. (3, 9). Male Sprague-Dawley rats weighing 220-240 g were used. A 25-cm² area of skin was treated. Red blood cell ChE was determined 72 h after the application of the OP compound in acetone.

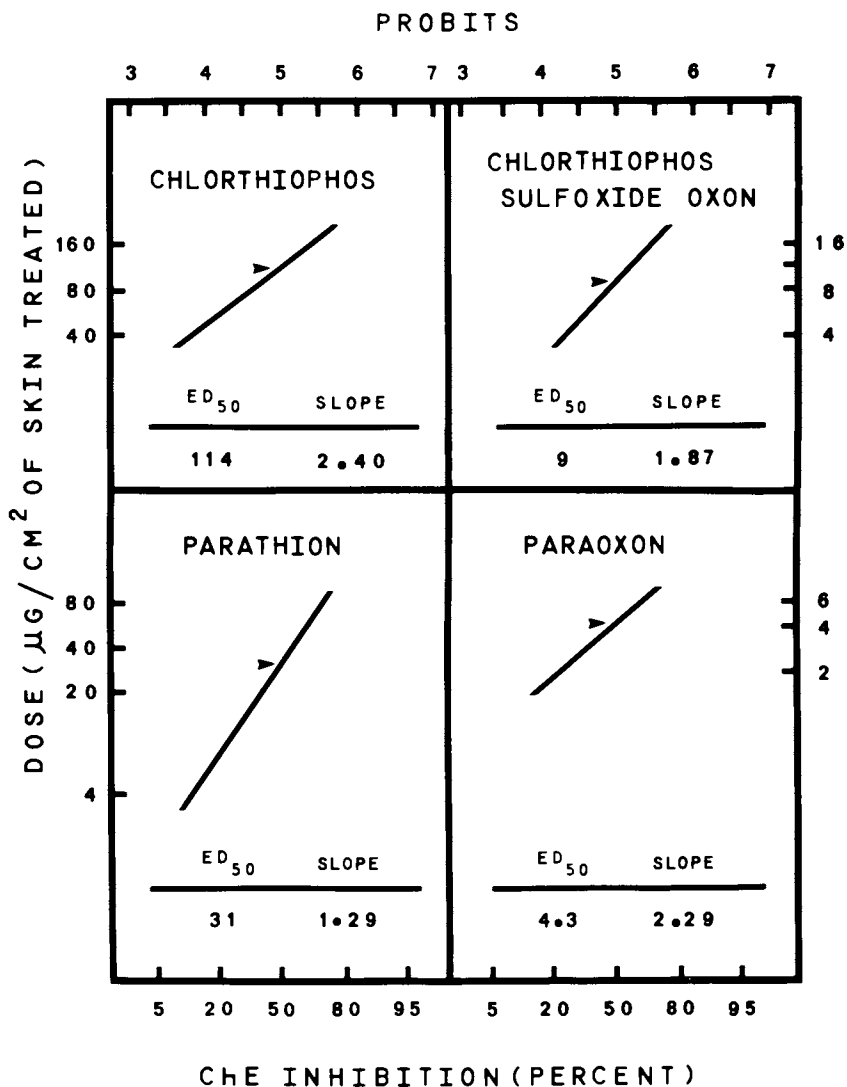


Figure 5. Dermal dose-ChE response curves according to Knaak et al. (3, 9). See Figure 4 for conditions.

Table II. Procedure for Establishing Safe Levels (in $\mu\text{g}/\text{cm}^2$) for Thions + Oxons on Tree Foliage

Application to Citrus ^{a/}	Days Elapsed ^{b/}	$\frac{\text{a/Thion}}{\text{Oxon}}$	$\frac{\text{a,b/Thion}}{\text{Oxon}}$	$\frac{\text{Thion + Oxon}}{\text{Oxon} \times \text{RT}^{\text{c/}}}$	$\frac{\text{Thion + Oxon}}{\text{Thion + Oxon} \times \text{RT}}$	$\times \text{SL}^{\text{d/}}$ for Thion
Parathion 10 lb AI per 1,600 gal/A	10	0.35	0.02	0.37	0.49	0.07 ^{e/}
	20	0.09	0.01	0.10	0.16	0.06 ^{e/}
Methidathion 5.6 lb AI per 100 gal/A	10	1.0	0.08	1.08	1.38	0.4
	20	0.25	0.1	0.35	0.73	0.3
	30	0.11	0.08	0.19	0.50	0.2 ^{e/}
Azinphosmethyl 6.0 lb AI per 1,200 gal/A	10	1.5	0.05	1.55	2.91	1.7 ^{e/}
	20	1.3	0.05	1.35	2.86	1.6 ^{e/}
	30	1.1	0.05	1.15	2.51	1.5

a/ Taken from Figures 1A, 2A and 3A.

b/ Oxons must be at safe level indicated in Table I. Method assumes oxons will be at a safe level when safe level for thion + oxon is reached.

c/ RT = Relative Toxicity from Table I (ED₅₀ of thion + ED₅₀ of oxon)

d/ SL = Safe levels for thions from Table I.

e/ Safe levels for thion + oxon.

parathion, methidathion and azinphosmethyl. This method assumes that the oxon level will be at or below the safe level by the time the safe level for the thion + oxon is reached. When this condition is not met (i.e. Figure 1A, parathion applied at 10 lb AI per acre in 100 gallons of water) the safe level value for the oxon would take precedence over the safe level value for the thion + oxon.

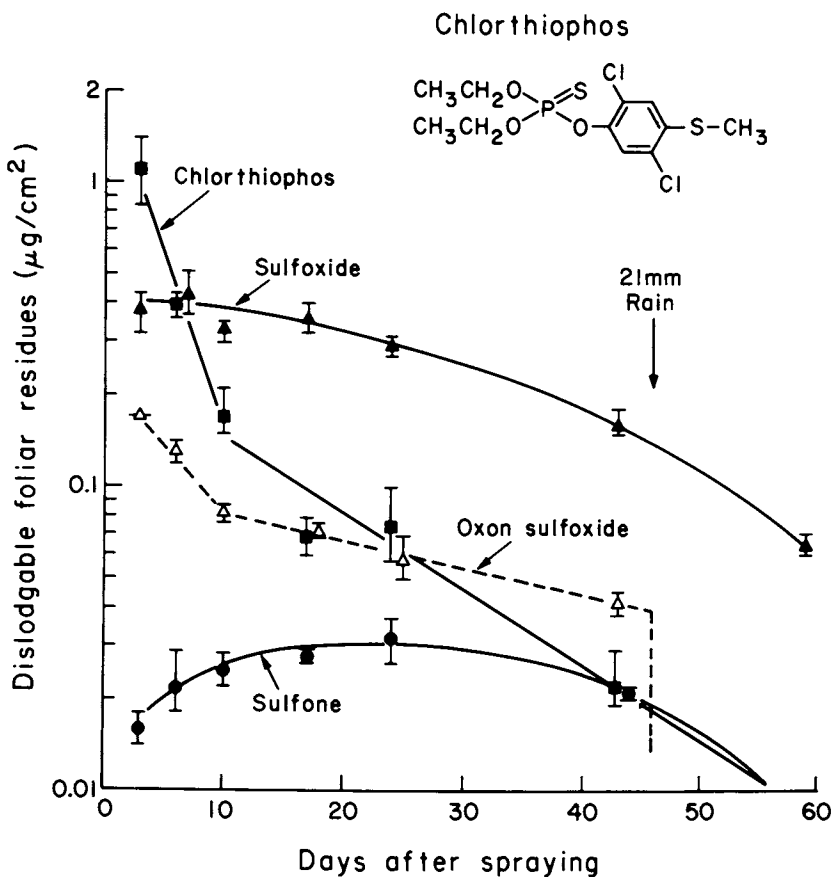
Safe Levels for a Currently Unregistered Insecticide, Chlorthiophos and its Oxon Sulfoxide

Figure 6 gives the dissipation curves for chlorthiophos and its alteration products on lemon foliage (6); these are data from preliminary experiments using higher application rates than would be expected to be normally used. The dermal dose-ChE response curves for chlorthiophos and chlorthiophos oxon sulfoxide were determined by the procedure of Knaak *et al.* (3) and presented in Figure 5. Table 1 gives the safe levels established for this insecticide and its oxon sulfoxide in terms of paraoxon and azinphosmethyl oxon as standards. Chlorthiophos and methidathion were found to have similar dermal toxicities, while chlorthiophos oxon sulfoxide is more toxic than azinphosmethyl oxon. According to the safe level for the oxon sulfoxide, the reentry interval for chlorthiophos would be approximately 45 days. Rain appears to remove the oxon sulfoxide from the leaves as indicated in Figure 6. Washing foliage with water is a feasible, although costly, way of reducing oxon sulfoxide levels.

Testing Foliage for Safe Insecticide Residue Levels Using the Rapid Field Method

The rapid field method is a kit for making analyses in the field. A leaf punch sampler is used to collect samples of known surface area. The surface residues are removed using 20% NaCl solution and then residues are partitioned into hexane. The hexane is boiled off and the insecticide residues are reacted with 4-(p-nitrobenzyl) pyridine for 3 minutes at 150°C. Addition of base solution produces a purple color whose intensity is measured using a portable mini-spectrophotometer. The absorbance value of the colored solution is used to estimate the residue level present in the collected sample.

The safe levels established for parathion + paraoxon, azinphosmethyl + azinphosmethyl oxon and methidathion + methidathion oxon on foliage have absorbance values determined by the rapid field method (4) equal to those given in Table III. Absorbance values greater than those listed in Table III signal an unsafe working condition. Field testing can also be conducted by standard gas chromatographic analysis of the leaf disk samples by state-approved laboratories.



Residue Reviews

Figure 6. Dissipation of chlorthiophos (■), its sulfoxide (▲), and its sulfone (●), and chlorthiophos oxon sulfoxide (△) after an application of 0.8 lb AI chlorthiophos/100 gal of spray on lemons (6).

A 2.54-cm diameter leaf punch sampler is normally used to collect leaf samples from citrus, while a 1.8-cm diameter punch sampler is more suitable for peaches with narrow leaves. A forty-1.8-cm diameter leaf disk sample represents a total surface area of 200 cm². An additional 40 leaf disks, therefore, must be collected to give a sample representing 400 cm² as required by the method.

Table III. Absorbance Values for Safe Levels of Total OP Residues on Foliage

Pesticide	(ug/cm ²) Safe Level	(mL) 20% NaCl ^{a/}	(mL) Hexane ^{b/}	Absorbance ^{c/}
Parathion + oxon	0.06	30/40	5/15	0.17
Azinphosmethyl + oxon	1.6	10/100	5/30	0.42
Methidathion + oxon	0.2	30/100	5/30	0.28

a/ mL decanted off leaf disks divided by total mL used to wash leaf disks.

b/ mL of hexane placed in reaction tube divided by mL of hexane used to extract decanted salt solution.

c/ Absorbance = Safe level (thion + oxon) x 400 cm² x NaCl fraction x Hexane fraction x Absorbance of 1.0 ug of thion x recovered fraction. Absorbance and recovery values from Gunther et al. (4).

Discussion

The toxicological safe levels established in this paper and the rapid field analytical method developed by Gunther et al. (4) meet the initial provisions of the regulations written for implementing California law AB 1090. During the writing of these regulations, Department of Food and Agriculture officials recognized the need for a provision in the regulations that would allow county agricultural commissioners to test foliage for growers and to certify that the OP residues on the foliage were at or below the safe levels established by the director. A bill (AB 2198) is presently being considered by the California Legislature which will allow county agricultural commissioners to charge fees for this service. The passage of the bill will make it possible for the Department of Food and Agriculture to fully implement the regulations required by AB 1090.

The success of these regulations will depend upon their acceptance by growers in California. If accepted, the testing program will provide safer working conditions for field workers

and in many cases it will shorten the waiting period between application and reentry. Safe levels will be established for all OP pesticides having reentry intervals longer than 3 days. Lower levels may be required for OP insecticides on grapes as suggested by the problems encountered with the use of dialifor in California (10). During field operations, OP insecticides and their alteration products are transferred via dust from foliage to the clothing and skin of workers. The safe levels established in this paper are appropriate when the level on skin ($\mu\text{g}/\text{cm}^2$) does not exceed the level on foliage. The amount of insecticide ($\mu\text{g}/\text{cm}^2$ of skin) available for absorption is dependent upon the transfer rate from foliage to skin and the rate the residue is removed by washing. Clothing serves as a barrier to the transfer of residues from foliage to skin, but may also function to supply residues when clothing is contaminated. Clean clothing must be worn each day and a shower must be taken at the end of the work day. The dermal exposure of workers to residues several fold higher than those present on foliage is believed to be the major reason for the reentry problems involving the use of dialifor on grapes in California (10).

The work on chlorthiophos, although incomplete, was included in this paper to provide data on an OP presently undergoing commercial development. The information should help scientists presently being faced with developing reentry data on new compounds.

Abstract

The California Department of Food and Agriculture is in the process of adopting Pesticide Worker Safety Regulations which will allow agricultural workers to enter a pesticide-treated area to engage in activities involving substantial contact with plant surfaces prior to the expiration of a safety interval, when the director has established a safe level ($\mu\text{g}/\text{cm}^2$) for the pesticide on foliage, and on-site tests have been performed for dislodgeable residues by methods approved by the director. The procedures published by Knaak et al., in the Bull. Environm. Contam. Toxicol. 24, 796 (1980) for establishing safe levels on foliage will be used to set safe levels on foliage; while the rapid field method published by Gunther et al., in the Bull. Environm. Contam. Toxicol. 24, 903 (1980) will be approved as a field residue method. The Rapid Field Method provides a colorimetric procedure for estimating organophosphorus insecticide residues on crop foliage in $\mu\text{g}/\text{cm}^2$. The success of this program will depend largely on its implementation and acceptance by agribusiness.

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Monitoring Pesticide Safety Programs by Measuring Blood Cholinesterase and Analyzing Blood and Urine for Pesticides and Their Metabolites

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Agricultural workers can be divided into two groups according to the degree of risk from pesticide poisoning; those who mix, load, and apply pesticides, and those who work in the fields after the pesticide has been applied. The group at highest risk (mixers, loaders, and applicators) is protected by regulations specifying medical supervision, protective clothing, and closed-transfer systems for mixing and loading pesticides. Field workers are at risk when exposed to dislodgeable residues of pesticides on foliage. These workers are protected by reentry times which allow pesticide residues to degrade to safe levels. Medical service, in the event of poisoning due to unsafe residues, is available to them, but they are not under continuous supervision. This report will discuss the results of studies designed to monitor the two types of workers; mixer-loaders, applicators, and field workers.

Monitoring Workers Using Organophosphate (OP) Pesticides

In California, mixer-loaders and spray applicators who work with toxicity category I and II organophosphates or N-methyl carbamates more than 30 hours per 30-day period are required to have medical supervision. Supervision consists of an interview and a medical examination to determine if a medical condition exists which would make the worker unusually susceptible to poisoning due to cholinesterase inhibition, and to caution the individual about the use of certain drugs such as the phenothiazine tranquilizers which potentiate the effects of cholinesterase (ChE) inhibition. Two blood samples, taken several days apart, are analyzed to determine the individual's preexposure plasma and red blood cell (RBC) ChE activity (baseline value). The physician arranges a routine ChE testing program and provides for extra ChE tests should the worker be accidentally exposed to OP's. If ChE activity is depressed to 50 percent of the baseline value, the physician may ask the employer to place the worker on

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another job until ChE activity returns to 70 percent of the individual's baseline value.

This program has worked well in reducing the incidence of OP poisoning in workers. However, physicians who use this program have questioned the need for repeated ChE determinations to detect the effect of multiple exposures which cause a gradual decrease in the ChE level. Some physicians claim they rarely see this effect; they usually see a precipitous drop associated with an accidental spill. Symptoms of poisoning are usually present. There is a reason for this. Small amounts of OP's absorbed each day inhibit ChE activity; the body replaces this enzyme, possibly at an accelerated rate, with the result that baseline levels are maintained. An individual must absorb sufficient OP over a relatively short time period to change ChE activity. This condition is met when spills occur. The implementation of control programs, including the closed-transfer of toxicity category I liquids, has reduced exposures and has contributed to the finding of fewer workers with low ChE levels.

Exposure to OP pesticides can also be detected by analyzing urine for alkyl phosphates (1, 3). The method identifies the alkyl phosphate present and assists in the identification of the particular pesticide involved. The method detects ug quantities per mL of urine. Alkyl phosphates, however, are only present in the urine for 24 to 48 hours after exposure, and the test is not useful in a poisoning case seen several days after pesticide exposure.

Monitoring Workers Exposed to N-methyl Carbamates

The monitoring of workers exposed to N-methyl carbamates presents problems not found with the OP pesticides due to the nature of the enzyme inhibitor complex. The OP compounds inactivate the ChE by phosphorylating serine, the esteratic site of the enzyme, thereby preventing the enzyme from catalyzing the hydrolysis of acetylcholine. The phosphate ester is stable, but it can be removed by oximes such as pyridine-2-aldoxime methiodide (2-PAM). If the use of this antidote is delayed, the inhibited enzyme becomes "aged" (a methyl or ethyl group is removed from the OP), and the oxime is not effective in removing phosphate. Carbamates bind to the esteratic site of the ChE, inactivating the enzymes. The carbamate rapidly dissociates from enzymes in the body and the symptoms of poisoning are of short duration. The 2-PAM has no effect on this reaction and is of no value in therapy. Experiments with dogs have shown that 2-PAM increases the toxicity of carbaryl. Physicians are cautioned about the use of 2-PAM for treating poisoning cases involving carbamates. However, OP's and carbamates are often applied by the same applicator on a single day. The use of 2-PAM in poisoning cases involving OP's and carbamates is clearly justified.

A satisfactory test for carbamate metabolites in the urine has not been worked out for clinical laboratory use.

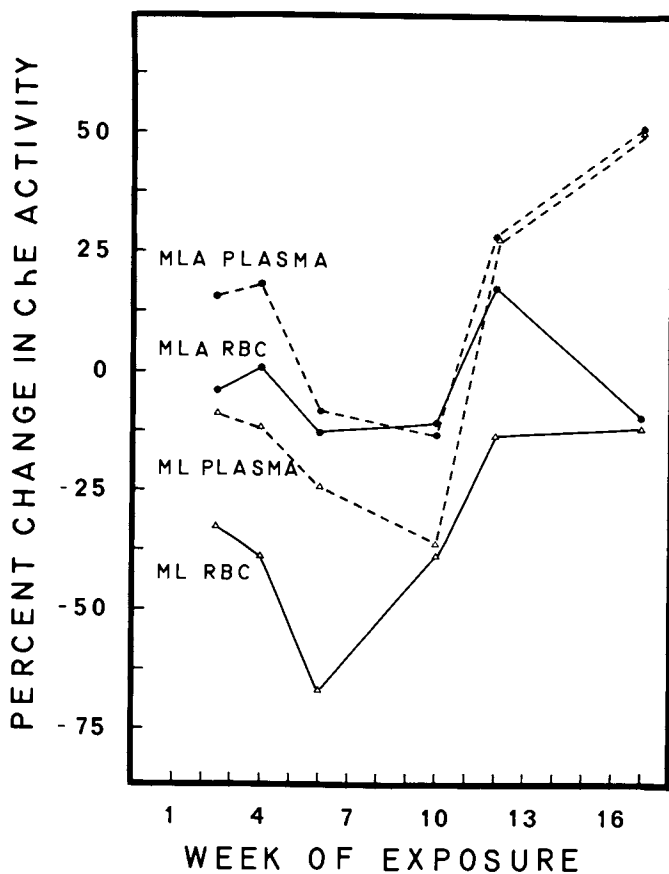
Studies on the Effectiveness of Closed-Transfer Systems for Mixing and Loading Toxicity Category I and II Products Containing Organophosphates and N-methyl Carbamates

The regulations adopted by the California Department of Food and Agriculture requiring the use of closed-transfer systems went into effect in April 1977. At that time, several types of equipment were put into use, and a series of studies were undertaken to evaluate their effectiveness in protecting workers.

In one study (2), plasma and RBC ChE activities were followed from May to September in two mixer-loaders (ML) who used a Swampmate closed-transfer system (Cherlor Manufacturing Co., Salinas, California), and in three mixer-loader applicators (MLA) who used a Model SS 12-4 closed-transfer system (Soil Serv, Salinas, California). The results shown in Fig. 1 indicate that the activities of plasma and RBC ChE were depressed during the application season, but returned to normal by the middle of September. The MLA showed less plasma and RBC ChE depression. It is interesting to note that the plasma ChE showed the "rebound effect," recording levels way above baseline.

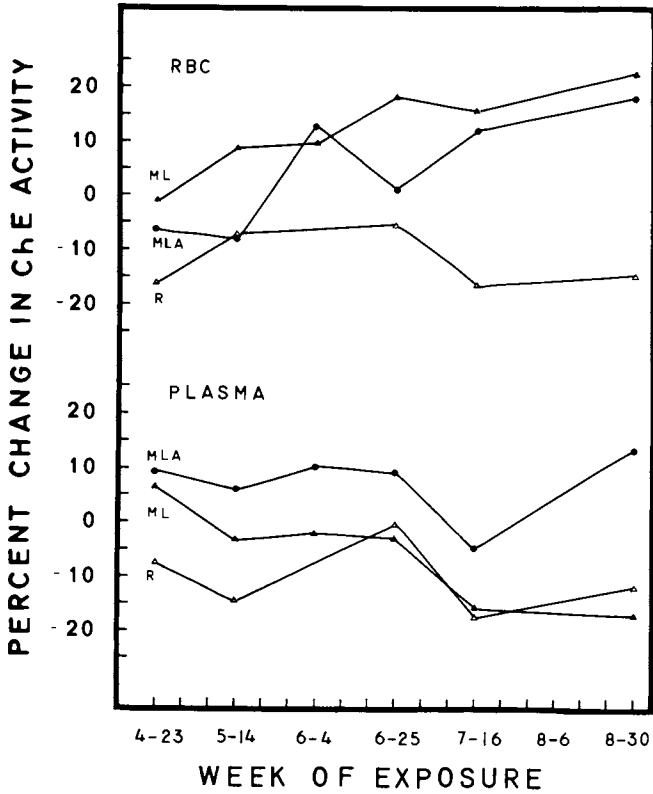
In another study involving the Model SS 12-4 closed-transfer system (3), blood ChE and urinary dialkylphosphate levels of five mixer-loaders and four mixer-loader applicators were followed over an 18-week period. During a four-day period, the concentration of airborne pesticide residues in the breathing zone was measured during mixing and transferring of concentrated liquid pesticides from their original containers to mix and spray tanks and during ground application. The fallout on the clothing was measured by analyzing gauze patches attached to various areas of the clothing. Blood ChE levels are shown in Fig. 2. The mean RBC ChE activity of three mixer loaders (ML) rose above the baseline value, while in two (R) individuals, it fell 42 percent below the baseline value. It was found that these workers (R) did not use the closed-transfer system. The mean plasma ChE activity in three mixer-loaders (ML) decreased moderately while the activity of the two mixer-loaders (R) varied. The mean RBC ChE activity of three mixer-loader applicators (MLA) rose 18 percent above the baseline value by the end of the monitoring period. The mean plasma value generally remained above the baseline value.

Since a variety of pesticides were used, the alkyl phosphates present in urine could not be related to a particular pesticide application. The method identified and quantitatively determined the alkyl phosphates derived from the organophosphates the men were applying. Values in unexposed workers varied from .02 to .24 ppm, and from .02 to 2.4 ppm in unexposed workers.



Archives of Environmental Contamination and Toxicology

Figure 1. Change in blood cholinesterase activities of two mixer-loaders (ML) and three mixer-loader applicators (MLA) from their April 4 baselines during the weeks between May 14 and September 13, 1976 (2). Mixer-loaders used the Swampmate (Cherlor Mfg. Co., Inc., Salinas, CA) while mixer-loader applicators used a Model SS 12-4 Closed System (SoilServ, Salinas, CA).



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Figure 2. Changes in mean blood cholinesterase activities of three mixer-loaders (ML), two mixer-loaders King City (R), and three mixer-loader applicators (MLA) from their April 2 baselines (3).

The concentration ($\mu\text{g}/\text{m}^3$) of mevinphos, methomyl, and acephate present in air during mixing, loading, transfer, and application are given in Table I. The high value for methomyl (1332 mg/m^3) was due to the breaking of a defective bag. The high acephate level was due to the dispersion of the fine powder in the air as the containers were emptied into the powder box. Residues found on the cloth patches worn by the workers are given in Table II.

The results indicate that the closed-transfer system reduced worker exposures below that of previously used methods. Powders are hard to handle, however, and workers must be trained to use the equipment effectively.

Monitoring the Exposure of Field Workers to Organophosphate Residues

The studies were of two types: (1) studies on the cholinesterase activities of workers during the spraying season compared to nonworkers, and (2) worker reentry studies relating the cholinesterase activities of workers to dislodgeable foliar residues.

In 1975, blood samples were obtained from workers via farm worker health clinics (1) during May, July, and September, and were tested for ChE activity. During the grape harvest season, 154 urine samples were collected and, of these, 25 contained detectable levels of various alkyl phosphates. The plasma and RBC ChE values of 24 of these workers is given in Table III which shows there was no significant difference in the ChE activities of exposed and unexposed workers. The urine analyses indicate that organophosphates were absorbed but were not sufficient to cause a reduction in blood ChE activity.

In a parallel study (4), blood samples were obtained through cooperating physicians in farm worker health clinics during the growing and harvesting season for lettuce, grapes, peaches, and citrus during January, May, July, and September, and were analyzed for blood ChE. The statistical results are given for plasma and RBC ChE in Figures 3, 4, 5, and 6, and in Tables IV and V. The mean plasma and RBC ChE activities of female and male workers were not significantly different from nonfield workers.

A study was carried out to determine a safe worker reentry period for phosalone on citrus (5). Fourteen male agricultural workers (volunteers), ranging in age from 18 to 72 years, were given physical examinations, including a blood ChE test, prior to three days of work in the citrus grove 14 or 21 days after it was sprayed with phosalone at six pounds A.I. per acre. Four men acted as controls.

Cloth patches were attached to the clothing in four areas to collect dislodgeable residues. Blood and urine samples were collected at the end of each work day. Leaf samples were taken daily to determine dislodgeable residues of phosalone and its

Table I. Monitoring of airborne residues during closed transfer, mixing-loading, (T, M-L) and application^b

Date ^c	Mevinphos			Methomyl			Acephate		
	Number of lbs. used	ug/m ³ during T, M-L application	Number of lbs. used	ug/m ³ during T, M-L Application	Number of lbs. used	ug/m ³ during T, M-L Application	Number of lbs. used	ug/m ³ during T, M-L Application	
7-18	9.3	0.3	55.7	nd(5) ^e	3.6	27.0	261.0		
	5.4	--				14.0	331.0		
	<u>14.7</u>	2.2				19.9	21.0		
						27.8	nd(1) ^e	2.6	
						<u>88.7</u>			
7-19	8.3	0.1	76.9	nd(5)	7.5	10.5	1.4		
	20.9	0.4				15.0	40.7		
	<u>29.2</u>	9.5				23.6			
						<u>49.1</u>		1.6	
7-20	16.6	28.0	18.9	8.1		23.2	118.0		
	11.7 ^d	2.0	2.3	1332.0		10.9	nd(1)		
			<u>39.6</u>	nd(3)		<u>34.1</u>		6.1	
			60.8		3.9				
	3.9	--							
7-21	57.5	9.4	31.4	nd(3)	0.0	7.5	174.0		
	7.3	0.3				22.5	292.0		
	<u>68.7</u>	3.7				<u>52.5</u>	nd(1)		
						<u>82.5</u>		2.3	

^aStaplex Air Sampler, Flow rate = 0.566 m³/min. held near breathing zone of workers.

^bGlass Impinger, Flow rate = 0.014 m³/min. tractor mounted near breathing zone of driver.

^cSamples collected from work zones of 4 mixer-loader applicators (F, G, H, & I).

^dMixer-loader.

^end Not detected, numbers in () represent the number of batches monitored. Knaak et al. (3).

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Table II. Mevinphos residues on cloth patches^a worn at various sites on the body of mixer-loader applicators

Residues recovered (ug/cm ²)								
Date								
7-18		7-19		7-20		7-21		
M/L/A ^b		M/L/A		M/L/A		M/L/A		
Body Site	G	G	I	I	G	H	I	
Shoulder	0.08	0.14	0.23	nd ^c	0.02	0.05	--	
Chest	0.04	0.016	0.23	0.03	nd	0.01	0.05	
Waist	0.14	0.016	0.53	0.05	nd	--	0.05	
Knee	0.11	0.052	0.58	0.016	0.023	0.02	0.1	

Values from patches were added to the values obtained for backing.

^aCotton

^bMixer-loader applicator

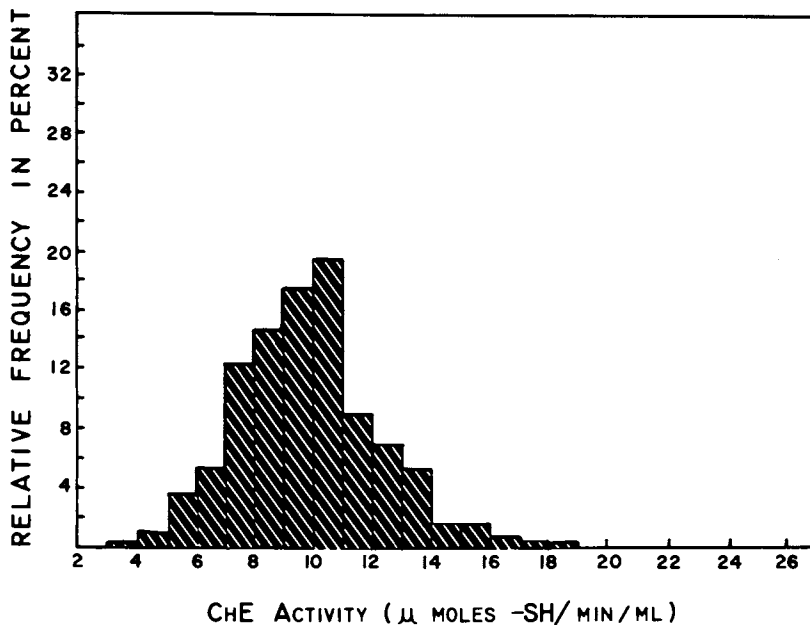
^cNot detected. Table from Knaak et al. (3).

Table III. Statistical Analysis of Cholinesterase Values
From Male Field Workers and Nonfield Workers

Statistic	ANALYSIS OF VARIANCE			
	Plasma		Red Blood Cells	
	Field Workers	Nonfield Workers	Field Workers	Nonfield Workers
Sample Size	24	95	24	95
Mean, \bar{x}	9.6	9.4	28.8	27.5
Std Deviation, s	1.41	1.62	4.3	4.1
F Ratio	0.25*		1.95*	

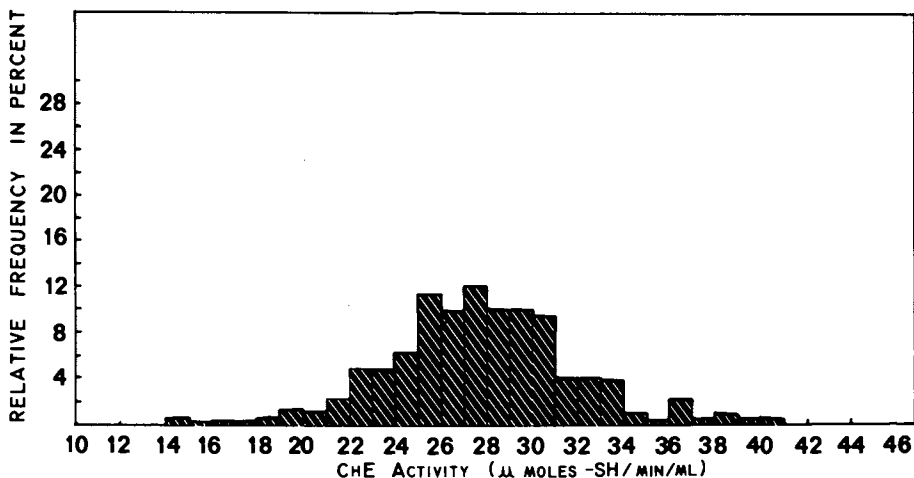
Statistic	FREQUENCY DISTRIBUTION STATISTICS			
	Plasma		Red Blood Cells	
	Field Workers	Nonfield Workers	Field Workers	Nonfield Workers
Median	9.6	9.4	29.0	27.5
Kurtosis, g_2	-0.67	0.61	-0.39	-0.57
Skewness, g_1	-0.09	-0.18	-0.19	-0.03
Minimum	6.8	3.8	21.0	18.2
Maximum	12.2	13.1	36.8	35.4

*Not significantly different at 5% level. ChE activity expressed in umoles of -SH released/min/mL of sample. Knaak *et al.* (1).



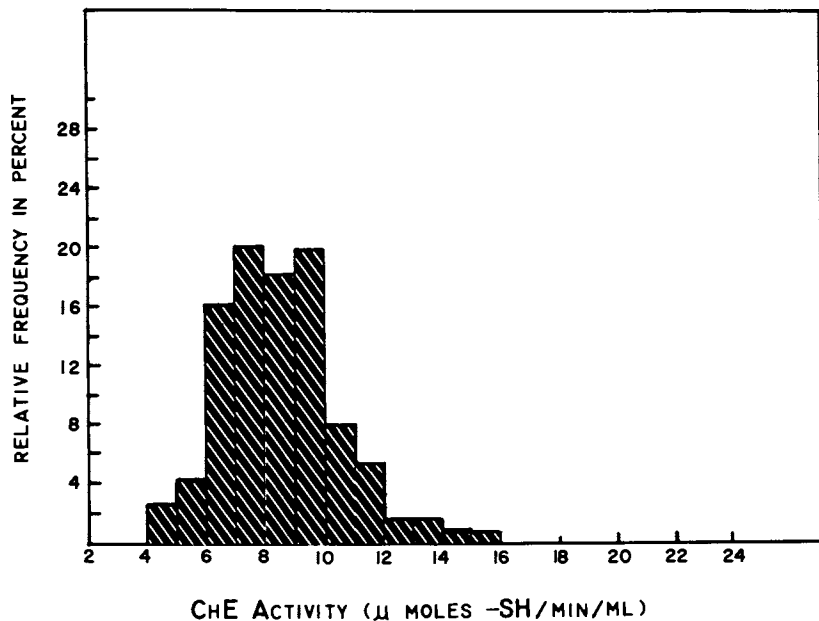
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Figure 3. Histogram of plasma cholinesterase values obtained from male field workers: $n = 459$, $\bar{x} = 9.7$, $s = 2.4$, median = 9.7, kurtosis (g_2) = 0.47, skewness (g_1) = 0.41, min = 3.2, and max = 18.4 (4).



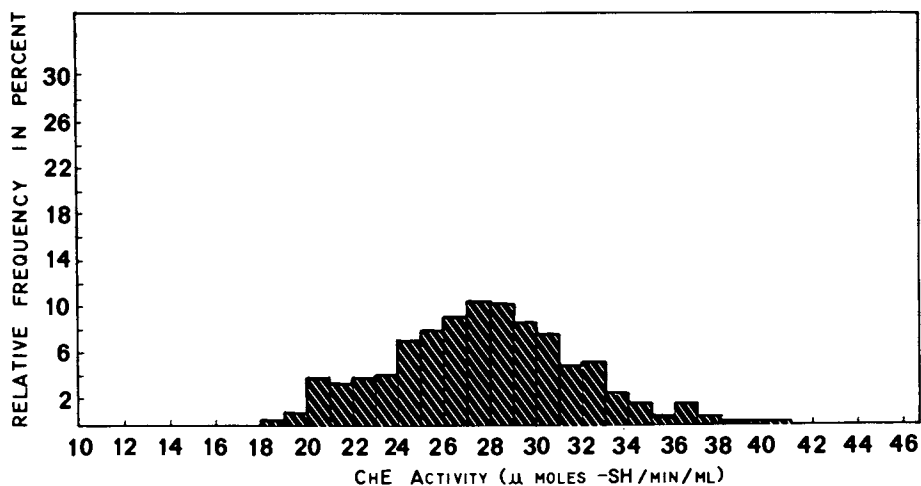
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Figure 4. Histogram of red cell cholinesterase values obtained from male field workers: $n = 459$, $\bar{x} = 27.7$, $s = 4.0$, median = 27.5, kurtosis (g_2) = 0.85, skewness (g_1) = 0.15, min = 14.3, and max = 40.9 (4).



Toxicology and Applied Pharmacology

Figure 5. Histogram of plasma cholinesterase values obtained from female field workers: $n = 258$, $\bar{x} = 8.4$, $s = 2.0$, median = 8.2, kurtosis (g_2) = 1.31, skewness (g_1) = 0.76, min = 4.2, and max = 15.9 (4).



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Figure 6. Histogram of red cell cholinesterase values obtained from female field workers: $n = 258$, $\bar{x} = 27.7$, $s = 4.1$, median = 27.6, kurtosis (g_2) = 0.28, min = 18.1, and max = 40.6 (4).

Table IV. Statistical Analysis of Cholinesterase Values From Females - Nonfield Workers and Field Workers^a

Analysis of variance									
Statistic	Plasma				Red blood cells				
	Nonfield Workers		Field Workers		Nonfield Workers		Field Workers		
	FWC ^b	SBB	NW	W	FWC	SBB	NW	W	
Sample Size	220	44	57	207	220	44	51	207	
Mean, \bar{x}	8.7	8.1	8.9	8.3	28.1	28.1	26.4	27.9	
SD	2.3	1.6	2.2	1.9	4.5	3.8	4.3	4.0	
F ratio (FWC, SBB, NW, W)			2.3 ^c				2.3 ^c		

Frequency distribution statistics									
Statistic	Plasma				Red blood cells				
	Nonfield Workers		Field Workers		Nonfield Workers		Field Workers		
	FWC	SBB	NW	W	FWC	SBB	NW	W	
Median	8.4	7.9	8.9	8.1	27.9	29.1	25.7	28.0	
Kurtosis g_2	0.54	0.04	1.10	1.22	0.21	0.22	1.3	0.03	
Skewness, g_1	0.67	0.0	0.73	0.73	-0.02	-0.55	0.9	0.15	
Minimum	3.8	3.8	4.3	4.2	13.5	17.0	19.6	18.1	
Maximum	18.4	11.6	15.9	15.9	40.7	36.4	40.6	39.4	

^aCholinesterase activity expressed in micromoles of -SH released per minute per milliliter of sample.

^bFWC, Farm Workers Clinics and Sacramento Blood Bank, 1975; SBB, Sacramento Blood Bank, 1976; NW, no field work past 30 days; and W, worked in fields past 30 days.

^cNot significantly different at 5% level. Knaak *et al.* (4).

Table V. Statistical Analysis of Cholinesterase Values From Males - Nonfield Workers and Field Workers^a

Analysis of variance								
Statistic	Plasma				Red blood cells			
	Nonfield Workers		Field Workers		Nonfield Workers		Field Workers	
	FWC ^b	SBB	NW	W	FWC	SBB	NW	W
Sample Size, n	147	95	62	397	147	95	62	397
Mean, \bar{x}	9.4	9.4	8.9	9.9	29.6	27.5	27.5	27.7
SD	2.6	1.6	1.9	2.4	4.6	4.1	4.1	4.0
F ratio (FWC, SBB, NW, W)			2.89 ^c				8.29 ^c	
Multiple range test, (means underscored are not significantly different)	8.9	<u>9.4</u>	<u>9.4</u>	<u>9.9</u>	<u>27.5</u>	<u>27.5</u>	<u>27.5</u>	29.6

Frequency distribution statistics

Statistic	Plasma				Red blood cells			
	Nonfield Workers		Field Workers		Nonfield Workers		Field Workers	
	FWC	SBB	NW	W	FWC	SBB	NW	W
Median	9.1	9.4	9.2	9.8	29.3	27.5	27.5	27.5
Kurtosis g_2	0.63	0.61	-0.63	0.43	0.54	-0.57	0.91	0.87
Skewness, g_1	0.53	-0.18	-0.12	0.41	0.43	-.03	0.47	0.10
Minimum	3.8	3.8	4.6	3.2	18.9	18.2	19.3	14.3
Maximum	18.2	13.1	13.2	18.4	45.7	35.4	40.9	40.7

^aCholinesterase activity expressed in micromoles of -SH released per minute per milliliter of sample.

^bFWC, Farm Workers Clinics and Sacramento Blood Bank, 1975; SBB, Sacramento Blood Bank, 1976; NW, no field work past 30 days; and W, worked in fields past 30 days.

^cSignificantly different at 5% level. Knaak *et al.* (4).

oxon. The statistical results shown in Table VI indicate there was a small but significant difference in plasma ChE between the exposed workers and their controls. Significant differences were not detected between exposed workers and controls for RBC ChE. The amount of dislodgeable residues transferred to clothing was measured by analyzing the residue on the cloth patches as shown in Table VII. The major metabolite of phosalone in animals, 2-amino-5-chlorophenol, was not detected in urine. Based on this data, the reentry time was set at 21 days.

Monitoring Workers Using Pesticides Which Are Not Cholinesterase Inhibitors

In the case of pesticides which are not ChE inhibitors, exposure is measured by the analysis of blood and/or urine for the active ingredient or its metabolites. Baseline levels of pesticides and/or metabolites are not usually determined, with the exception of methyl bromide. In this case, a blood sample is taken to check for bromide ion before fumigators use the pesticide. Blood and urine tests are run only in the case of spills or other accidents to assist in identifying the cause of poisoning or to monitor workers in a workplace. Paraquat, chlorinated hydrocarbons, mercury, p-nitrophenol, and dinitrophenol are examples of pesticides or metabolites of pesticides that have been found in the urine of exposed workers.

The proper protection of these workers usually depends on specifying protective clothing, closed-transfer systems for mixing and loading, and application equipment rather than specifying a medical monitoring program. The safety requirements for pesticides causing the greatest risk to health are based on field tests conducted by this Department and information supplied by manufacturers. Evaluation of medical reports of suspected poisonings assists in evaluating protection procedures.

Summary

The Department has developed methods for monitoring the exposure of workers exposed to organophosphate and carbamate pesticides. These methods utilize the determination of plasma and red blood cell cholinesterase activities and urinary alkyl phosphates. Studies are reported which show that these methods have proven useful in evaluating the safety effectiveness of closed-transfer systems and in determining reentry times for field workers.

Table VI.. Analysis of RBC and Plasma ChE Values of Exposed Workers and Controls

t	Test on deviations of red blood cholinesterase from baseline ($\bar{d}_{FW-d_C}^a$)													
	21-day reentry						14-day reentry							
	9-24		9-25		9-26		9-30		10-1		10-2 ^b		10-3	
	FW ^c	C ^c	FW	C	FW	C	FW	C	FW	C	FW	C	FW	C
Sd	7.2	2.2	14.5	5.2	-3.6	-0.5	9.7	6.4	-15.7	-3.1	-8.9	-0.3	-2.6	-2.0
\bar{d}	0.5	0.7	1.1	1.3	-0.3	-0.1	0.7	1.6	-1.4	-0.8	-0.9	-0.1	-0.3	-0.5
n	14.0	3.0	13.0	4.0	14.0	4.0	13.0	4.0	11.0	4.0	10.0	4.0	10.0	4.0
(Sq) ² /n	3.7	1.6	16.2	6.8	0.93	0.06	7.2	10.2	22.4	2.4	7.9	0.02	0.68	1.0
Sd ²	16.5	2.3	27.8	11.6	19.2	4.4	20.5	10.8	29.8	4.7	18.2	0.47	6.9	4.4
t	-0.36		-0.31		-0.20		-1.56		-1.30		-1.46		0.45	
t	Test on deviations of plasma cholinesterase from baseline ($\bar{d}_{FW-d_C}^a$)													
	21-day reentry						14-day reentry							
	9-24		9-25		9-26		9-30		10-1		10-2 ^b		10-3	
	FW ^c	C ^c	FW	C	FW	C	FW	C	FW	C	FW	C	FW	C
Sd	1.7	0.3	-2.4	0.0	-1.1	0.3	-3.7	-0.5	-2.5	-0.1	-1.1	0.4	0.9	0.6
\bar{d}	0.1	0.1	-0.2	0.0	-0.1	0.1	-0.3	-0.1	-0.2	-0.0	-0.1	0.1	0.1	0.1
n	14.0	3.0	13.0	4.0	14.0	4.0	13.0	4.0	11.0	4.0	10.0	4.0	10.0	4.0
(Sq) ² /n	0.21	0.03	0.44	0.0	0.09	0.02	1.05	0.06	0.57	0.0	0.12	0.04	0.08	0.09
Sd ²	0.47	0.11	0.74	0.12	0.67	0.13	1.33	0.11	0.81	0.19	0.85	0.18	0.91	0.20
t	0.22		-1.94 ^d		-1.30		-1.90 ^d		-1.91 ^d		-1.32		0.36	

^aSokal and Rohlf (1969).

^bWorkers did not work on this day because of rain the previous evening.

^cFW, field workers; and C, controls.

^dSignificantly different at the 5% level, but not at the 1% level; other values are not significantly different at the 5% level. Table from Knaak et al. (5).

Table VII. Phosalone and Phosalone-Oxon Residues on Patches Worn At Various Sites on the Body^a

		Residues recovered (ug/cm ²)					
		21-day study			14-day study		
Body site	Prewashing of fabrics	Chambray (1st day)		Jersey (2nd day)		Avril-polyester (1st day)	
		Phosalone	Oxon	Phosalone	Oxon	Phosalone	Oxon
Shoulder	No	0.92	0.09	0.62	ND ^b	0.51	0.11
	Yes	0.73	0.10	0.65	0.14	0.38	ND
Chest	No	0.67	ND	0.43	0.12	0.24	0.09
	Yes	0.43	ND	0.30	ND	0.34	0.11
Back	No	1.49	0.17	0.91	0.15	0.76	0.16
	Yes	0.80	0.13	0.60	ND	0.65	0.11
Thigh	No	2.55	0.13	1.71	0.16	1.24	0.19
	Yes	1.14	0.12	0.87	ND	0.94	0.14

^aValues were obtained by pooling the patches and backing (from six or seven workers) according to body site, extracting the pooled samples, and analyzing the extracts for residues of phosalone and phosalone-oxon by gas chromatography. Values from patches were added to the values obtained for backing.

^bND, not detectable. Table from Knaak *et al.* (5).

Abstract

Monitoring methods have been used by the Department to evaluate the safety measures taken to protect workers from exposure to pesticides. The most effective method for organophosphate and carbamate pesticides is the determination of plasma and red blood cell cholinesterase before the spraying season to get baseline levels, and repeating the tests during the work season. The determination of alkyl phosphates in urine is a sensitive method for detecting exposure, and aids in the identification of the OP used. The carbamates cause cholinesterase inhibition of short duration and, at present, there is no method for determining their urinary metabolites. Studies are reported which demonstrate the usefulness of these monitoring methods in evaluating the value of closed-transfer systems in protecting mixer-loaders and applicators and in determining reentry times for field workers.

The monitoring of pesticides other than cholinesterase inhibitors is done by measuring the concentrations of the compound or its metabolites in blood and urine.

The program for the medical supervision of mixer-loaders, applicators, and flaggers exposed more than 30 hours in 30 days to Category I and II OP's and carbamates has been successful but the details of the plan are under review.

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Regional Considerations in Worker Reentry

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Over 25 years ago Carman and coworkers recognized the adverse potential of fieldworker exposure to pesticide residues (1). Subsequent fieldworker acute organophosphate intoxications and resulting political pressure led to regulations by the Occupational Health and Safety Administration and the Environmental Protection Agency. These regulations and the worker reentry situation have been the subject of reviews (2, 3).

The Milby report listed 18 reentry cases from 1949 to 1971 in California citrus (2). Gunther and coworkers cited 47 reentry incidents from 1949 to 1976 in California citrus (3). Peoples *et al.* reported 38 cases of occupational illness in California due to aldicarb exposure from 1974 to 1976 (4). Approximately 14 of these cases were due to exposure to aldicarb residues.

Peoples *et al.* (5) reported that 351 cases of occupational illness caused by parathion exposure were seen by California physicians in 1975. Seventeen of these cases can be regarded as worker reentry incidents. Knaak *et al.* (6) reported a case of 118 worker illnesses from dialifor exposure in a California grape vineyard in 1976. Davies *et al.* (7) reported 2 cases (24 fieldworkers) involving parathion in Florida sweet corn. In 1975, one death was reported from North Carolina workers reentering a parathion-treated tobacco field (8).

A major summary of reentry incidents from 1966 to 1979 classified 86 of 25,500 pesticide incident reports as reentry incidents (9). These reported cases included all classes of reentry incidents from pest control operators to fieldworkers. Of these 86, 47 were fieldworker reentry incidents (Table I). Nine states reported incidents. California had the highest number with 34 incidents, followed by North Carolina with 3. Sulfur led with 9 cases and parathion was involved in 6. All the sulfur cases were from California, usually involving eye irritation. Twenty-five different pesticides, covering all pesticide classes, were implicated in these reported incidents (Table II). But this summary must be judged incomplete as the

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Table I. Summary of agricultural reentry incidents by state, 1966 to 1979 (from Ref. 8).

State	No. Incidents	No. Persons
California	34	62
Hawaii	1	1
Illinois	1	79
Indiana	2	77
Florida	1	17
Michigan	2	6
North Carolina	3	5
North Dakota	1	2
Washington	2	4
Total	47	253

Table II. Pesticides involved in agricultural reentry incidents (from Ref. 8).

Pesticide	No. Incidents
Aldicarb	2
Azinphosmethyl	2
Benomyl	1
Carbaryl	1
Carbofuran	1
Chlorothalonil	1
Copper sulfate	1
Demeton	1
Dichlorvos	1
Difolatan	2
Dimethoate	3
Disulfoton	1
Endosulfan	2
Lime	1
Malathion	2
Methidathion	1
Methomyl	2
NAA	1
Naled	2
Oryzalin	1
Oxydemetonmethyl	1
Parathion	6
Phosalone	1
Sulfur	9
Unknown	1
Total incidents	47
Total pesticides	25

1976 dialifor incident in California (118 persons) (6) and the cases reported by Peoples (4, 5) were not included.

The single most frustrating aspect of worker reentry episodes has been their unpredictability. This frustration was voiced by the Milby committee report (2) and in a major review of the California situation by Gunther *et al.* (3). Are the reported acute cases, in fact, the tip of an iceberg or do they fairly represent the actual frequency of such occurrences?

Reentry Classes

An examination of reported worker reentry incidents gives a clear picture of the immediate problem.

Sulfur. Symptoms of sulfur exposure were dermatitis and eye irritation. Incidents generally occurred within 1 to 4 days after application, but have occurred up to 3 weeks after application during mowing operations.

Carbamates (carbaryl, benomyl, carbofuran, aldicarb, methomyl). Symptoms of exposure were nausea, dizziness, blisters, dermatitis, malaise, sweating, tearing, breathing difficulty, and chest tightness. Usually, incidents have occurred within 1 to 2 days after application except for aldicarb (up to 4 days after application).

Chlorinated hydrocarbons (chlordane, endosulfan, and chlorothalonil). Symptoms of exposure were dermatitis, eye irritation, nausea, and dizziness. Entry into treated areas was from 0 to 1 day after application.

Organophosphates (parathion, naled, phosalone, dialifor, oxydemetonmethyl, dimethoate, azinphosmethyl, methidathion). Exposure to organophosphates resulted in headache, nausea, vomiting, dizziness, malaise, abdominal pain, and weakness. The organophosphates have been implicated in reentry incidents from 0 to 120 days after application.

Based on this record, worker reentry incidents can be placed in either of 2 classes. One class occurs shortly after application. Workers reenter a treated area by mistake or by design, and are exposed to toxic and fast-acting parent compounds. Additionally, these residues may be readily available to the worker because the field is still wet from the application itself or dew, or the residue is in a confined space such as a greenhouse or mushroom facility. Closer coordination between supervisor, the applicator, and fieldworkers; airing of greenhouses; and 1 or 2 day reentry times would easily preclude this class of reentry incident.

The second type of reentry incident involves entry into a treated area after a period of time usually considered safe: 5

to 120 days after application. This class of reentry incident has been limited primarily to California and to organophosphate insecticides, principally parathion and dialifor. This class has stimulated the research to date on worker reentry.

Reentry Research

The rarity of acute worker reentry illness has hampered research into this occupational illness. A North Carolina study of peach thinners exposed to foliar dislodgeable residues of 2.36 $\mu\text{g}/\text{cm}^2$ of parathion with 0.13 $\mu\text{g}/\text{cm}^2$ of paraoxon on fruit and 0.03 $\mu\text{g}/\text{cm}^2$ paraoxon on leaves showed no significant depression of either red blood cell (RBC) or serum cholinesterase (8). A California study of peach harvesters over 4 weeks, where both phosalone and azinphosmethyl had been applied, showed no depression of RBC cholinesterase (10). The dermal doses these subjects received ranged from 122 to 232 mg phosalone/week, 26 mg azinphosmethyl/first week only, and 2 to 3.4 mg phosalone oxon/week. Dislodgeable residues of azinphosmethyl on plant surfaces were 0.40 $\mu\text{g}/\text{cm}^2$ for the first week and not detected thereafter; phosalone ranged from 2 to 7 $\mu\text{g}/\text{cm}^2$ and phosalone oxon from 0.02 to 0.2 $\mu\text{g}/\text{cm}^2$. A similar experiment with apple thinners exposed to parathion (11) showed exposure ranging from 5 to 12 mg/hr depending on reentry time with dislodgeable residues of 1 to 5 $\mu\text{g}/\text{cm}^2$. No ill effects on the thinner were noted. A study of peach thinners in California showed no effect on RBC cholinesterase to thinners initially exposed to 2.6 $\mu\text{g}/\text{cm}^2$ azinphosmethyl on reentry. No residue was found in workers shirts, but urinary metabolites of azinphosmethyl were detected at levels up to 20 ppm by day 5 of exposure (12). Agricultural hand labor was studied in Washington using cholinesterase determinations and urinary metabolite analyses (13). No organophosphate residues were detected in urine and cholinesterase values were normal. In a study of peach thinners exposed to azinphosmethyl, the workers reentered the orchard only 12 to 18 hr after the application due to a 'misunderstanding' (14). Residues were about 2.6 $\mu\text{g}/\text{cm}^2$ of azinphosmethyl with very low azinphosmethyl oxon residues. Low urinary excretion of metabolites and a low (15% or less) cholinesterase depression were noted over 5 exposure days. In a reentry study of California citrus trimmers and pruners exposed to phosalone, results were unremarkable (15). No cholinesterase depression or urinary metabolites were noted. Reentry was at 14 and 21 days with exposure to 2.6 and 3.6 $\mu\text{g}/\text{cm}^2$, respectively, on each reentry day. In a major study of peach thinners (previously referenced, 12, 14, 16), lettuce harvesters, and artichoke harvesters, low or no urinary metabolites were observed and cholinesterase values were within the normal ranges. Phosdrin and methomyl had been applied to lettuce, and parathion and methyl parathion to artichokes. Dislodgeable residues were

less than $1 \mu\text{g}/\text{cm}^2$. A human subject experiment to determine tobacco reentry after a monocrotophos application showed no urinary metabolites and insignificant cholinesterase depression when reentry was 48 hr or greater (17). These controlled studies are reassuring in that under 'normal' conditions illness of farmworkers from exposure to residue is apparently not common.

However, an examination of published worker reentry cases presents a frustrating problem. A compilation of incidents from 1949 to 1955 described cases of organophosphate intoxication from workers in pears, apples, grapes, citrus, and hops (18). Workers were picking, thinning, cultivating, and irrigating. Most cases occurred 8 days after application; 1 case occurred 33 days after application. Although these were descriptive cases, there is no doubt that they were real organophosphate intoxications. The first inkling of why residues made workers ill 8 or more days after application came in 1964 (19). Ninety-four orchard workers became ill when the parathion residue present should not have made them ill. Paraoxon was postulated to be the culprit. One residue sample contained more paraoxon than parathion. Sweet corn workers in North Carolina showed significant depression of RBC and serum cholinesterase after parathion exposure. The residues were $0.26 \mu\text{g}/\text{cm}^2$ parathion, $0.35 \mu\text{g}/\text{cm}^2$ paraoxon (24 hr residue) and $0.16 \mu\text{g}/\text{cm}^2$ paraoxon, $0.23 \mu\text{g}/\text{cm}^2$ paraoxon (48 hr residue) (20). No clinical symptoms were observed. The simple expedient of wearing gloves appeared to reduce cholinesterase depression (20). A recent episode involved 118 workers harvesting grapes treated with dialifor and phosalone (6). Based on previous information and the residue data, phosalone was not implicated. However, dialifor was more persistent in the San Joaquin central valley than anticipated and vineyards varied considerably in their dialifor content. Vineyards had been sprayed 15 to 40 days earlier. Cholinesterase values were reduced 49 to 58% in workers who became ill. Analyses of trousers, patches, and skin swabs indicated a higher level of residue on the worker than on the foliage. The skin swabs had approximately 10 times the level of foliage. Unfortunately, analyses for dialifor oxon residues were not performed until later (21). Analyses for dialifor oxon on a row by row basis showed possible spot treatment of rows and dialifor oxon residues were as much as one-third of the dialifor residues. It was speculated that dialifor oxon contributed to this reentry incident (21). Cotton scouts in North Carolina showed occasional RBC and serum cholinesterase depressions of 20 to 50% over 8 growing seasons after exposure to toxaphene-methyl parathion, EPN-methyl parathion, monocrotophos or azinphosmethyl 1 to 7 days after application (20). These depressions were linked to early reentry of fields (prior to 48 hr), entering dew-wet fields and poor personal hygiene practices such as wearing contaminated clothing more than 1 day (20).

A reentry study 12 hr after application in Arizona cotton, exposing volunteers to methyl parathion, ethyl parathion, or monocrotophos for 5 hr produced no clinical signs of poisoning. However, cholinesterase depression averaged 14%, and both ethyl- and methyl-parathion were found in the blood as well as p-nitrophenol (PNP) in the urine. These workers were not judged in jeopardy as cholinesterase depression was less than 30% and the PNP excretion was less than 4 mg following exposure (22).

The reasons for negative and positive results seem evident. Negative results appear linked with legal or extended field reentry and low oxon metabolites. Positive results appear linked with early field reentry and/or the presence of unexpected levels of organophosphate oxon metabolites.

Regionality

A common conclusion of 2 worker reentry reviews is that the worker reentry 'problem' differs in various regions (2, 3). If organophosphate pesticides regularly caused fieldworker intoxication, reports should be increasing. Farm labor advocates are increasingly vocal about pesticide exposure. They use this issue as a bargaining tool, and the agricultural community is cognizant of this concern. Carbamate and organophosphate pesticide use doubled from 1964 to 1976 (23). By weight carbamates represented 19% and organophosphates 50% of insecticides in use in 1976, up from 10% and 25% in 1964. Of all pesticides in use worldwide, 21% were in cotton and 32% in fruits and vegetables, crops which employ scouts or hand labor. These increases should result in more hazard and thus more cases of fieldworker intoxication. But there have been no such increases.

The lack of an increase in reentry incidents can be partly attributed to harvest times: that time a grower must wait after a pesticide application before the crop can be harvested (Table III). The control of food quality through residue monitoring was established in 1947 and reenforced in 1954 (24). Harvest times are generally longer than reentry times and do not vary regionally (Table III). Harvest times have probably prevented harvester illnesses, but this is not necessarily so. For instance, parathion in citrus has a 30-day national harvest time and a 2-day national reentry time, but up to a 60-day reentry time in California. Azinphosmethyl treated fruit may be harvested in 7 days, groves may be reentered in 1 day nationally, but not for 30 days in California. These differences reflect the experience of agricultural labor with pesticide exposure in various regions. By examining the factors which have contributed to a worker reentry incident, one can understand why the California experience has not been shared by the rest of the United States. We provide as an example a comparison of California with Florida.

Table III. Comparison of California and Florida citrus harvest and reentry times in days (from Ref. 31 and 41).

Pesticide	Harvest		Reentry	
	Calif.	Fla.	Calif.	Fla.
1. Azinphosmethyl	7 (28) ^a	7 (28)	30	1
2. Carbophenothion	30	14 or 30	14	2
3. Demeton	21	21	5	2
4. Diazinon	21	21	5	0
5. Dimethoate	15	15	4	0
6. Dioxathion	0	0	30	0
7. Ethion	0 (21) ^b	0 (21)	30	1
8. Malathion	7	7	1	0
9. Methidathion	14	14	30	0
10. Sulfur	0	0	1	0
11. Parathion	30	R.N.R. ^c	30, 45 or 60	R.N.R.

^a28 days for two applications

^b21 days for lemons

^cR.N.R. = Registered but not recommended—2 day national reentry time; 14 or 30 day harvest depending on application rate.

^dDepending on application rate

Reentry Factors

Four primary factors apparently lead to an incident 5 or more days after application: a dusty work environment, use of a sufficiently toxic organophosphate, conversion of the parent compound to its oxon metabolite, and dry conditions. The dusty working environment has been recognized since 1952 as a key element for transferring pesticides from leaf, fruit, and soil surfaces to a field laborer (1). The type of dust, i.e. soil type, also influences the rate of conversion of an organophosphate to its more toxic oxon form (3). And dry conditions are necessary for the persistence of these oxon residues over long periods of time (3).

Dusty conditions. Florida citrus is grown in primarily sandy soils with clay content of 2% or less (25). Clay is defined as particles of $< 2 \mu$. Also, the Florida silt fraction contains less than 8% particulates 2 to 50 μ . California soils are reported as having over 10% particulates $< 2 \mu$ and 28 to 57% particulates from 2 to 50 μ (26, 27, 28). The limit for particulate matter swept up by grove operations and wind, i.e., airborne particulates which settle on foliage, is about 50 μ . Consequently, these data indicate a five-to-eightfold difference in the potential for dusty leaves and the transfer of soil surface residues to workers directly or via plant surfaces.

Pesticide use. California agriculture has a history of using ethyl parathion. This one compound has been implicated in almost every case of delayed reentry illness, with the recent exception of dialifor (2, 3, 6). Florida had difficulty with parathion applicator intoxication over 25 years ago which substantially reduced the use of ethyl parathion (29, 30). Also about 25 years ago, Florida citrus growers discovered that a decreased use of organophosphates assisted pest management by increasing the natural enemies of scale insects. Parathion is apparently not used in Florida citrus (30). Neither parathion nor dialifor is recommended for use in Florida citrus (31). Sulfur is recommended for one application per year in citrus and growers are clearly warned that sulfur is irritating to the eyes of applicators, loaders, and fruit harvesters (32); aldicarb may never be recommended for general use because of its toxicity (31). Similar events in California agriculture might have obviated their reentry problem.

Oxon metabolites. The organophosphate oxons have been a major factor in (0-120 days) worker reentry incidents (2, 3). This fact seems to have been lost in the search for a worker reentry 'solution'. Differences between California and Florida are again evident. In a study of ethion on grape foliage in the California central valley, ethion dioxon was above the level of

the parent compound in 7 days and the monoxon and dioxon combined were approximately 3 times the level of ethion after 28 days (33). A similar study in Florida citrus under various weather conditions found little ethion dioxon and less than 10% of the dislodgeable residue was the monoxon (34). Of 32 citrus groves in Tulare County, California, substantial paraoxon residues were found in 31 groves (35). In fact, on day 2 after application, one grove contained twice as much paraoxon as parathion, and paraoxon was about twice as long-lived as parathion in this study. Experiments in Florida citrus over a 2 year period under various weather conditions showed that dislodgeable paraoxon levels were always well below the level of parathion and disappeared at about the same rate (36, 37). Similar data have been reviewed (3). The point here is that oxon levels differ regionally in the United States and, in fact, regionally within California (3). This is reasonably well understood.

Environmental conditions—residue dissipation. It is well established that environmental conditions modify the behavior of pesticides (38). Long-term worker reentry (5 to 120 days) illnesses have been limited primarily to the hot and dusty central California valley. Since these incidents have been linked to the oxon metabolites and these metabolites reach higher levels in dry areas, rainfall and dew would appear to be additional major components for regionalizing the United States for worker reentry regulations. Davies *et al.* (7) proposed such a scheme in 1973.

- Arizona citrus belts— two wet seasons (July to September, December to February); mean annual precipitation 3" Yuma area, 7" Phoenix area, 11" Tucson area. Normal harvest seasons dry.
- California citrus belts—one wet season (November to March); mean annual precipitation 20" central district, 11" southern district. Except for navel oranges, normal harvest seasons dry.
- Florida citrus belts— no prolonged dry season; mean annual precipitation 52", higher south of Orlando, wet summers (8" in Orlando in July, 7" in Miami in June). Normal harvest seasons wet (7).

Beyond these differences in rainfall, most areas of the United States, excepting desert areas, have frequent dew condensation at night. In Florida, which is probably the extreme, dew presence ranges from 2 hr in the 'dry' winter to 12 hr in the 'wet' summer. In extensive and repeated studies of organophosphate insecticides and their oxon metabolites in Florida

citrus, the oxons have always been in low quantity (below the parent) and have dissipated rapidly (25, 34, 36, 37, 39). Rainfall has also been linked to oxon dissipation in California (3) and moisture to dissipation of oxons on soil surface particulates (40).

Reentry regions. Based on the factors contributing to a worker reentry illness, the United States could easily be regionalized using a combination of the following:

Areas of the United States with a history of incidents involving 5+ day reentry incidents should be regulated accordingly.

Those areas with very dry periods, particularly around harvest, may need special reentry regulations.

The soil type and its particulate profile determine the potential for transfer of pesticide to worker. This criterion is linked, however, to rainfall. Those areas with extended dry periods and a large quantity of particulate matter below 50 μ should receive closer attention.

Areas of the United States where the use of organophosphates (particularly parathion and dialifor) is low, have a low potential for acute fieldworker illness.

There are additional miscellaneous factors which impinge on the worker reentry process, the most important being: crop, pest management practices, work practices, and labor relations.

Most of the necessary information is currently available and could be woven into a flexible worker reentry regulatory model. Such a model would regionalize worker reentry regulations.

Regulatory Options and Research

There appear to be 3 viable regulatory options:

- (a) Continue the present system of 1 or 2 day reentry intervals for selected chemicals, the decision for longer intervals being left to the states.
- (b) Flexible reentry intervals based on toxicity, residue behavior, and exposure mechanisms.
- (c) Monitoring of pesticide levels prior to reentry.

Each option has problems. The present system, option (a), is limited to 14 organophosphates. This leaves unregulated many chemicals which have caused worker illnesses, so it would seem reasonable to extend a 1 or 2 day reentry time to all agricultural chemicals. This approach would provide a base for longer reentry times where necessary, such as in California. Another weakness of the present system, however, is the 24 and 48 hr times. The intent of these regulations might be better served by requiring full working days to elapse after the day of application prior to reentry. This would extend reentry times slightly, but would remove the guesswork as to when the

application was actually completed. This regulatory approach requires little, if any, additional research.

Flexible reentry intervals, option (b), would regionalize worker reentry regulations. Of necessity, this approach would involve a reentry model as a base. A model would set priorities, i.e., the relative importance of factors which lead to an acute reentry pesticide intoxication. Much of the necessary information is available for such a model: weather data, some information on oxon levels, some pesticide use data, actual worker reentry studies, and acute dermal toxicities. For the ultimate success of this approach, there are some important factors which need investigation. The tendency of pesticides to convert to a more toxic product and the parameters (environmental and otherwise) affecting the conversion rate, need further investigation. The dermal toxicity of pesticides and their more toxic environmental products would be required. The dermal dose a worker receives as related to work practices, soil type and foliage levels is a key factor. But the model could be constructed now with the available information and updated as data becomes available. The process is really not very complicated and reasonable priorities could be set on worker reentry 'factors'. This approach appears to be the most practical long-term solution because it would allow screening of compounds prior to use and would lead to an indepth understanding of fieldworker exposure, from work practices to human toxicology.

Monitoring of fields for residue levels is currently in use in California. This approach reflects the unpredictability of worker reentry incidents in California. Present scientific and empirical evidence, however, suggests that monitoring is not needed in other regions.

Reentry regulations should be promulgated only after carefully considering the accuracy of the applicable data. Many reported incidents are will-o-the-wisp cases. The physician has only the patients' word and a few symptoms for a diagnosis. Conversely, the worker and his foreman would probably never correlate increased 'sick days' with periods of pesticide application. Sometimes union activities and human suggestibility are involved. Sometimes the social aspects of big agriculture and exploited labor are involved. Agriculture has a diametrical anathema to any regulatory decision for which they see no immediate and personal justification. Reason dictates that all aspects of the reentry issue be considered.

The option suggested, the reentry model, can be constructed now. But the future requires enlightened, humane practices governing agricultural labor exposed to pesticides that continued careful investigation should help foster.

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Pesticide Safety Program of the California Department of Food and Agriculture Based upon Measurements of Potential Workplace Exposure and the Elimination of Excess Exposures

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Pesticides are selected because they have specific biological effects; usually they are toxic to some organism. These chemicals may not have an acute toxic effect on man, but often, with adequate dosage through time, adverse effects may occur. The pesticide safety program of the California Department of Food and Agriculture has included the taking of measurements of: (1) pesticide vapors and mists in the breathing zone of persons exposed; (2) pesticide residues on the skin and clothing of persons applying pesticides; and (3) pesticide residues, including more toxic breakdown products on foliage and soil in fields where work is to take place subsequent to application. In the past, little of this type of information was supplied by pesticide registrants. Studies to monitor exposure levels of pesticides in the workplace are being conducted by the Department for certain pesticides already registered; also, these kinds of data are now being requested from the registrants prior to registration of certain products. These measurements are of value in designing ways to keep exposures of users at low levels.

Discussion

It is considered desirable to keep workplace exposure to pesticides as low as practical regardless of the current knowledge of their acute and chronic toxicity. All too often pesticides which were considered to be of negligible toxicity are later found to have a potential for causing adverse health effects following a sufficient period of exposure to an adequate dose. DBCP and nitrophen are good examples of this type of problem. We also gather and analyze detailed information on more than 2,000 illness reports per year from physicians who describe possible occupational exposures to specific pesticides. We also obtain or make detailed workplace measurements of the levels of pesticides to which workers may be exposed.

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Inhalation Exposure Levels. For setting acceptable maximum inhalation exposure levels, we usually use the lowest threshold limit values of those set by Federal OSHA (Occupational Safety and Health Administration), California OSHA, or the ACGIH (American Conference of Governmental Industrial Hygienists). Sometimes, as with ethylene dibromide, we accept a NIOSH (National Institute of Occupational Safety and Health) recommended standard which is lower than the three standard-setting entities previously mentioned. In other cases, when a standard has not been set by any of these groups, we set one ourselves based on available inhalation toxicity information.

Dermal Exposure Levels. Setting acceptable maximum dermal exposure levels to specific pesticides has been difficult. This is primarily due to a lack of specific data on dermal transport rates for specific pesticides as related to adverse effect levels and presumed no-effect levels. We are now requiring such data from the registrants, and our Department has a suggested protocol (1) that is offered to registrants that will provide such information from animal exposure studies. This dermal transport rate information is important in setting minimum field reentry intervals for field workers as well as in evaluating exposure levels of mixers, loaders, and applicators.

If the products are cholinesterase inhibitors, a dermal dose-response rate protocol (2) is recommended.

Reentry-Type Data. Reentry-type data pertaining to entering areas where plants have been treated with pesticides is needed in certain situations to determine if a potential hazard exists for workers who might go back into a treated area. The following is a guideline suggested and used by the Department in deciding whether or not reentry data is needed:

The product is to be applied to a plant and, particularly, to its foliage, and

Cultural practices (such as pruning or harvesting) of that crop involve substantial (human) body contact with foliage or bark or exposure to pesticide residues shaken from the foliage or bark, and

The product contains:

- a.) a cholinesterase inhibitor, or
- b.) a significantly toxic principle that can cause a detrimental acute systemic toxic reaction or is suspected of causing a chronic effect, and may be readily absorbed through the skin or inhaled following exposure to pesticide residues contacted while conducting usual cultural practices, or
- c.) a chemical which causes a significant primary skin irritant reaction in appropriate test animals or man, or

d.) a chemical which is a significant skin sensitizer in appropriate test animals or man.

There are several suggested sources which are useful guidelines for determining residues of pesticides on soil and leaf surfaces (dislodgeable) and conducting field reentry studies involving human volunteers (3), (4), and (5). Human exposure studies may not be required if adequate animal dermal dose-response data, as well as other data, are available.

Mixer, Loader, Applicator Exposure Studies. A major difficulty in making hazard assessments for mixers, loaders, and applicators is the lack of information on how much of the pesticide may be inhaled or may reach the skin under a typical use situation.

The following is an example of a protocol used by the Department in conducting our studies on previously registered pesticides to measure exposure levels of mixers, loaders and applicators. This is the guideline that is suggested by the Department and is provided to registrants. From data collected in this manner, along with the dermal absorption or dermal dose response data, we can determine the adequacy of label instructions in minimizing exposure levels.

Substance to be tested: Testing should be performed using the formulated product to be marketed and in accordance with the proposed label.

Rate of application: At least some of the applications being studied should be at the maximum use rate specified on the label.

Exposure: The period of exposure studied should be at least one work day and often for longer periods. Sufficient work should be accomplished during the work day to allow investigators to collect meaningful and representative data.

Number of workers: Sufficient numbers of workers should be monitored during the study. Reported values should include data from at least four workers. Informed consent and appropriate human subjects review are required for studies done in California if the pesticide or the use being studied does not have full California registration.

Workers: The workers should be employees who are routinely engaged in the mixing-loading or application of pesticides.

Protective clothing: Protective clothing (usually including long-sleeved cotton coveralls) and protective equipment, such as approved respirators, rubber gloves, and rubber foot covering must be worn to prevent or reduce exposure if proposed for or already on the existing labeling. Toxicity category one liquid pesticides should be transferred from their original containers to mixing or application tanks through closed transfer systems.

Measuring clothing or dermal exposure: A cloth pad sampling technique may be used to determine the amount of pesticide

sprayed or spilled on clothing and skin. Hand exposure can be measured by hand-washing procedures. Other techniques such as those involving skin wiping procedures or those involving the use of fluorescent brighteners may be used to estimate the amount of pesticide deposited on clothing or skin.

Collecting air samples: An appropriate number of air samples should be taken during the work day to establish the amount of pesticide in the breathing zone of the worker during normal work operation. This would usually involve the worker's wearing battery powered air pumps pulling air through appropriate sampling media. Measurements should be taken from both inside and outside the worker's respirator.

Measuring dermal absorption: Urine, blood, and/or fecal samples should be collected at the end of each work day to determine the presence or absence of the parent chemical or metabolites. Samples may be collected at other times depending upon how the chemical is metabolized in the body. If the product is a cholinesterase (ChE) inhibitor, blood cholinesterase measurements should be made prior to and after exposure. It may be appropriate to examine the blood and urine for changes from the normal characteristics, or to make other medical tests appropriate to the pesticide's particular effects.

Suggested methods: The study should be started at the beginning of a representative work day. Attach cloth patches to the skin areas of the back and front of the neck, on each cheek, and to clothing (two per site) at the shoulder, lower sleeve, chest, waist, and knees. Wash the worker's hands with 200 mL of soapy water and/or ethyl alcohol at the end of the work period. Collect air samples in the breathing zone of the worker during the entire work day, usually inside and outside a respirator. Collect blood, urine, and/or fecal samples at the end of the work day and/or other times when appropriate. Analyze cloth patches and air samples for pesticides. Analyze urine samples for parent compound and its metabolites, and blood samples for ChE activity or other expected chemicals or changes in body chemistry. Repeat the process several times to obtain sufficient information for evaluation.

Evaluation: The work situation being studied should be carefully selected and described so that it can be determined if it is typical of current practices, of those on the label, or if different work practices were in effect.

At the completion of the study, the data obtained should be evaluated in relationship to exposure (dermal and inhalation), absorption (urinary metabolites), effects (ChE inhibited), acute, chronic, and metabolic studies in laboratory animals. These findings should then be evaluated and discussed in relation to existing or proposed labeling or regulations.

Protocols: A study by Durham and Wolfe (6) provides useful protocol guidance for mixer, loader, and applicator studies.

The Department considers the protocols used in the Durham and Wolfe (6) study as quite useful guides; however, the percentage of the body reported as exposed in that study is considered as underreported unless impervious clothing is worn. The Department usually considers the skin on the entire body to be subject to some exposure. We use data from two articles as references for determining total body surface area; one by Berkow and Amboy (7), and another by DuBois and Dubois (8).

Use of Data Obtained: When data is received, such as that provided by a study conducted as suggested above, an assessment is made as to the adequacy of label instructions in mitigating any perceived hazards of use.

The label may be accepted and the product may be registered without further concern. On the other hand, one or more of the following conditions may be required:

(1) The Environmental Protection Agency (EPA) may be advised of the desirability of requiring a label change, (2) a use regulation may be enacted which will have the same effect as a label change, (3) the use of the product may be restricted, (4) closed system transfer of liquid pesticides may be required, (5) changes in the product may be required to reduce excess dustiness (and thus the hazard), (6) water-soluble packaging of the more toxic powders may be required, (7) minimum field reentry intervals may be set by regulation, (8) medical supervision may be required, and/or (9) detailed safety training may be required for specific pesticides.

The use of closed systems has been required in California for transfer of toxicity category one liquid pesticides from the manufacturer's container into the mix tank and then into the application vehicle tank. It appears that this process has considerably reduced both dermal and inhalation exposure. The use of probes that are inserted and then removed from containers reduces dermal exposure up to ten-fold; it also appears that the use of built-in probes further reduces exposure to the mixer-loader by up to another ten-fold factor in some cases.

Conclusions

No one enjoys complying with detailed government regulatory requirements or label instructions. Efforts are made to avoid regulatory overkill to the point that it would appear that most pesticides in use are highly hazardous. Excessive warnings on every use of every product could lead to workers taking a casual attitude in their use of all products. On the other hand, there has been so much scrutiny and concern about the use of pesticides in recent years that it is important to base safe use of pesticides on the best possible technical information. This is of benefit to (1) the manufacturer who has spent millions of dollars in developing a product and wishes to continue to sell it, (2) the users who may collectively receive benefits in

the millions of dollars from the proper use of the product, as well as (3) the many members of the general public who have concerns that pesticides are not being used as carefully as considered desirable.

Abstract

Pesticides are selected because they have specific biological effects; usually they are toxic to some organism. These chemicals may not have an acute toxic effect on man, but often, with adequate dosage through time, adverse effects may occur. The pesticide safety program of the California Department of Food and Agriculture has included the taking of measurements of: (1) pesticide vapors and mists in the breathing zone of persons exposed; (2) pesticide residues on the skin and clothing of persons applying pesticides; and (3) pesticide residues, including more toxic breakdown-products on foliage and soil in fields where work is to take place subsequent to application. Once these measurements are made and their significance is assessed: (1) medical supervision may be required; (2) safe-use wording on the label may be recommended through the Environmental Protection Agency; (3) protective clothing and use procedures may be changed by regulation; (4) safety information leaflets may be issued to supplement safety training and to condition use permits; (5) closed-system transfer of liquid pesticides may be required; (6) water-soluble packaging or reduction of dustiness of the more toxic wetttable powders may be recommended; and (7) field reentry times may be set by regulation to minimize human exposure to pesticides. Most aspects of the safety program have focused on separating man from the pesticide and its residues.

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Workers in the Agricultural Environment

Derma! Exposure to Carbaryl¹

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Abstract

The hourly dermal exposure (HDE) of agricultural workers to carbaryl applied by air or ground equipment was highest for the aerial flagger (rarely used nowadays), next highest for the mixer-loader, followed by the applicator and the bystander. The exposure of the later three types of workers was limited mainly to the hands. The hand exposure of the mixer-loader was much greater when gloves were not worn; the hand exposure of the applicator occurred because it was necessary to adjust spray nozzles during the spray operation. The bystander received the hand exposure because of hand contact with the sprayed crop foliage. When an aerial flagger was used, the HDE values for the face were higher than for other parts of the body and were about half the total body HDE.

The HDE on hands of thinners working in an apple orchard treated with carbaryl correlated with total extractable residues from apple leaves ($r=0.99$). The loss of residues of carbaryl on apple leaves was found to occur by 2 first-order processes: 87% of the residue was lost with a half-life of 8 days, and the rest with a half-life of 45 days.

¹ This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under FIFRA as amended. Also, mention of a commercial product in this paper does not constitute a recommendation for use by the U. S. Department of Agriculture.

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Once DDT had proved effective as a means of controlling insect pests of several agriculture crops (1), organic insecticides became the major method of control. This stimulated extensive research on the synthesis of other potential organic pesticides to control economic pests on food and forage crops. Subsequently, research was directed toward the evaluation of residues of these pesticides in our food supply and their effects on man and animals. The knowledge gained from these studies made it possible to use pesticides effectively and in ways that were not harmful to consumers of the produce. However, in recent years it became apparent that there is insufficient information concerning the hazards to workers involved in the production of pesticides and to workers in the agricultural environment.

Comer et al. (2) therefore evaluated the effect of exposure to carbaryl (1-naphthyl methylcarbamate) on formulating plant workers and on workers applying carbaryl to fruit orchards with a tractor-drawn air-blast sprayer. However, information was still needed on the persistence of carbaryl in apple orchards and on the resultant exposure of workers who re-enter treated orchards. Further, no studies had been done on other types of crops. The present study was undertaken to determine the exposure of agricultural workers to carbaryl as a function of type of formulation, application equipment, application method, and type of work performed (i.e., mixer-loader, applicator, flagman, bystander, and apple thinners).

Methods and Materials

Table 1 shows the number and types of workers and the various situations in which these workers were monitored for dermal exposure to carbaryl. Formulations of carbaryl that were evaluated included 80S, a wettable powder containing 80% active carbaryl; 50W, a wettable powder containing 50% active carbaryl; Sevimol® 4, a liquid suspension of carbaryl in molasses containing 40% active ingredient; and XLR, a water based flowable containing 4 lbs active carbaryl per gallon.

Workers associated with the application of carbaryl (applicator, mixer-loader, bystander and aerial flagger) and re-entry workers (apple thinners) were monitored by a modification of the procedure of Durham and Wolfe (3). The workers were fitted with a disposable jacket (Safety and Supply Co., Seattle, WA) to which 10 x 10 cm pads were attached with masking tape. One pad was attached as close to the neck as possible on each shoulder and on the chest and the back of each subject. One pad was also placed on each forearm midway between the elbow and wrist. The pads were constructed by backing an 8 ply gauze compress with two pieces of heavy filter paper and attaching these together in the center with a staple.

Table I.--Operations in which workers were monitored for dermal exposure to carbaryl.

Exp. no.	Formulation applied	Rate of application	No. of reps. ^{a/}	Crop treated	Workers monitored ^{b/}
<u>Ground applications^{c/}</u>					
1.	80S	3 lb AI/acre	1	peas	M,A,B
2.	80S	3 lb AI/acre	10	potatoes	M,A,B
3.	Sevimol-4	3 lb AI/acre	10	potatoes	M,A,B
<u>Aerial applications^{c/}</u>					
4.	XLR	2 lb AI/acre	1	corn	M,A,F
5.	XLR	1 lb AI/acre	1	corn	M,A,F
6.	Sevimol-4	2 lb AI/acre	4	corn	M,A,F
7.	80S	2 lb AI/acre	7	corn	M,A,F
<u>Handgun applications^{c/}</u>					
8.	50W	4 lb AI/acre	1	apples	M,A,T
9.	50W	2 lb AI/acre	1	apples	M,A,T

^{a/} The mixing, loading, and application of one batch of pesticide was considered a replication.

^{b/} The workers monitored were the mixer-loader (M); applicator (A); aerial flagger (F), bystander (B) i.e. a field worker near (within 100 feet) the tractor during applications; and re-entry thinners (T).

^{c/} The ground application was made with a tractor equipped with a rear mounted boom sprayer. The aerial applications were made with a helicopter equipped with a boom. The hand-gun applications were made with a commercial high pressure hose-nozzle sprayer.

Then each pad was pre-extracted with dichloromethane (DCM), dried, and fastened along two edges to a water repellent, 10 x 13 cm piece of cardboard with masking tape. The back of the cardboard was then marked with a 6.5 cm square to expedite trimming the pad after it had been exposed. After an exposure, the pads were removed from the jacket and trimmed to the premarked size (42.25 cm²). Then the cardboard backing was discarded, and the small square remaining was folded and placed in a small wide-mouth bottle containing 50 ml of DCM. The bottle was sealed with a Teflon®-lined cap and stored in a refrigerator until we were ready to proceed with extraction of the residues. A bottle size was chosen so that the folded pad would be completely covered with DCM.

In addition, hand rinse samples were taken from each subject by placing each hand in a plastic bag, adding 150 ml of 95% ethyl alcohol, securing the bag tightly around the wrist, and shaking vigorously for 1 min. When the hands were removed, the bag was sealed and placed in a wide-mouth half-pint jar for transporting to the laboratory. In the laboratory, the bags were opened and the alcohol solution was decanted into 200 ml bottles and stored in a refrigerator until partition extraction.

For the study of the degradation of carbaryl, 25 leaves were picked from each of the 3 apple trees in each plot as follows: starting near the crown of the tree, 8 leaves were picked randomly down to the drip line and this procedure was repeated at 2 other positions approximately 1/3 of the way around the tree. At the 3rd position, 9 leaves were picked. The leaves from each tree were then composited into one sample, so there were 3 replicate samples from each plot, and taken to the laboratory where they were immediately prepared for the extraction. Leaf samples were taken at intervals of 0, 1, 3, 7, 17, 24, 31, 38, and 52 days after the trees were sprayed with a carbaryl.

Extraction and Cleanup Procedures. Before the pads were extracted, the bottles containing the exposed pads in DCM were removed from the refrigerator and allowed to warm to room temperature. Then they were shaken for 1 min and the contents decanted into a 125 ml Erlenmyer flask. The pad was extracted 2 more times by shaking for 1 min with fresh 25 ml portions of DCM and the combined extracts refrigerated until analysis. For quantitative extraction, it was necessary that the pads be held in the DCM for at least one day before extraction.

Likewise, the hand rinse samples in ethyl alcohol were removed from the refrigerator and allowed to warm to room temperature. Then a 100 ml aliquot of the solution was transferred to a 500 ml separatory funnel, and 200 ml of water and 1.0 ml of concentrated phosphoric acid were added. The resultant solution was extracted 4 times with 50 ml each of

DCM. The DCM extracts were dried by filtering through a funnel plugged with a small amount of cotton overlaid with anhydrous sodium sulfate into a 250 ml Erlenmyer flask. After all 4 extractions had been filtered, the filtering funnel and its contents were rinsed with two 10 ml portions of DCM, and the resultant solution was placed in a refrigerator and held until analysis.

For extraction of the apple leaves, discs were punched from the 25 leaves with a cork borer to produce 190 cm² of leaf surface area per sample. Residues of carbaryl were then extracted by placing the 25 discs in a 250 ml Erlenmyer flask with 100 ml of DCM and shaking on a wrist-action shaker for 15 min. Next the solution was filtered through a glass fiber filter, and an 80 ml aliquot of extract was transferred to a 125 ml Erlenmyer flask and evaporated in a 40-45°C water bath with the aid of a gentle stream of dry filtered air. The dry residue in the flask was cleaned up by a coagulation procedure that involved adding 4.0 ml of methyl alcohol and 1.0 g of Hyflosuper-Cel® and warming the slurry in a water bath with intermittent stirring for 10 min. A 20 ml portion of coagulating solution (2.0 ml of phosphoric acid and 1.5 g of ammonium chloride in 1 liter of water) was added, and the sample was refrigerated for 30 min. Samples were removed from the refrigerator one at a time and filtered through a Whatman glass fiber filter paper into a 250 ml separatory funnel, and the sample flask was rinsed into the filtering funnel with three 10 ml portions of a mixture of 10% methyl alcohol in water (v/v). This procedure was followed by three 10 ml rinses of the filtering funnel and its contents with the methyl alcohol-water mixture. The solution in the separatory funnel was extracted with one 50 ml portion and two 25 ml portions of DCM by shaking for 30 sec and then filtering through a funnel plugged with a small amount of cotton overlaid with anhydrous sodium sulfate. After the extracts were filtered, the filtering funnel and its contents were rinsed with two 10-ml portions of DCM, and the solutions were stored in a refrigerator until analysis.

Gas chromatographic determination of carbaryl residues (as the mesylate derivative) from exposure pads, hand rinses, and apple leaf extracts showed that some samples required an additional cleanup to remove interfering compounds. This was accomplished by evaporating the solution to dryness in the 40-45°C water bath, adding 15 ml of hexane and 2.0 g of Florisil® (PR grade), and shaking on a wrist-action shaker for 5 min. The solution was decanted through a funnel plugged with cotton and overlaid with anhydrous sodium sulfate. The flask was inverted and suspended over the funnel until the entrapped material in the flask had dried. A sharp tap on the flask dislodged the remaining material in the flask into the funnel. The sample flask was rinsed 3 times with 10 ml each of hexane,

and the rinses were poured into the filtering funnel. When these solutions had passed through the filtering funnel, the funnel and its contents were rinsed 3 times with 10 ml each of hexane. The collection flask was then changed, and the carbaryl mesylate was eluted from the Florisil with four 10 ml portions of a mixture of hexane and DCM (1:1). The solution was evaporated to dryness in the water bath and made up to a volume sufficient for gas chromatographic analysis.

Analytical Procedures. The extracts from exposure pads, hand rinses, and apple leaves were evaporated to dryness in the 40-45°C water bath, and the carbaryl residues were determined by the procedure of Maitlen and McDonough (4). In this procedure, the residues were hydrolyzed with methanolic potassium hydroxide to 1-naphthol which was then converted to the mesylate derivative by reaction with methanesulfonyl chloride. The carbaryl mesylate was quantitated with a Hewlett Packard Model 5840A gas chromatograph (GLC) equipped with a flame photometric detector operated in the sulfur mode. The GLC column was a 122 cm x 4.0 mm I.D. glass column packed with Chromosorb G (HP) coated with 5% OV 101. The column was operated at a temperature of 205°C with a nitrogen flow rate of 60 ml/min.

The total hourly dermal exposures (HDE) were calculated from the residues on exposure pads by the equation of Durham and Wolfe (3) as follows:

$$\text{Total HDE (mg/h)} = \frac{150A + 110B + \frac{650(C)}{2} + \frac{1210(D)}{2} + E}{1000t}$$

where:

- A = Residues on chest pad ($\mu\text{g}/\text{cm}^2$)
- B = Residues on back pad ($\mu\text{g}/\text{cm}^2$)
- C = Sum of residues on shoulder pads ($\mu\text{g}/\text{cm}^2$)
- D = Sum of residues on forearm pads ($\mu\text{g}/\text{cm}^2$)
- E = Sum of residues from hand rinses (μg)
- t = The number of hours of exposure

The constants in the numerator of the equation approximate the surface area (in cm^2) of the front V of the neck, back of the neck, face, forearms and hands, respectively.

The efficiency of the extraction and cleanup procedures was determined by fortifying control samples of exposure pads, hand rinses, and apple leaves with known amounts of pure carbaryl before extraction and determining the percent recovered. Exposure pads fortified with carbaryl over a range of 50 to 400 $\mu\text{g}/\text{pad}$ had an average recovery of 88.6% (range 72 to 119%). Hand rinses prepared with carbaryl over a range of 100 to 400 $\mu\text{g}/\text{sample}$ had an average recovery of 90.6% (range 73

to 121%). Apple leaf samples fortified with carbaryl over a range of 50 to 200 µg/sample had an average recovery of 83.7% (range 68.0 to 104.0%).

Results and Discussion

Mixer-loaders. The mixer-loader operation was not inherently different for ground, aerial, or handgun applications. Several factors affected the hourly dermal exposure (HDE) to these workers (Table II). These included (1) the formulation used, (2) whether or not the worker wore gloves, and (3) the method of removing the powdered insecticide from its container prior to mixing.

The powdered formulations (80S and 50W) produced higher total HDE than did the liquid formulations (Sevimol-4 and XLR). When gloves were worn, mixer-loaders received a total HDE of 43.3 mg/h from powdered and 3.0 mg/h from the liquid formulations (average values of all 8 experiments). The higher HDE to the mixer-loader handling the powdered formulation versus the liquid formulation and wearing gloves was a result of the greater residues on the forearms (31 vs 1 mg). When gloves were not worn, the powdered formulations produced an average total HDE of 107 mg/h compared to 40 mg/h for liquid formulations. These results not only demonstrated that there was a difference in exposure rates due to formulations, but that exposures were greatly reduced by wearing gloves.

An average of 76% of the total HDE to all mixer-loaders was found on the hands, suggesting that gloves are the most important item of protective clothing. Experiments 2 and 3 were designed to determine the value of wearing gloves. Workers wearing gloves received total HDE's of 43.3 and 3.41 mg/h from 80S and Sevimol-4, respectively; when gloves were not worn the total HDEs were 247 and 40 mg/h, respectively.

The techniques the mixer-loader used to handle powdered formulations also affected the exposure level. In experiments 2 and 9 the worker (without gloves) scooped the formulation from the container, whereas in experiments 1, 7 and 8 the worker (without gloves) poured the formulation from the container. These procedures produced average total HDE's of 176 and 38 mg/h, respectively.

Applicators. The workers involved in the ground applications of carbaryl received considerably less exposure than the mixer-loaders. Most of the exposure was to the hands and was attributed to adjusting the nozzles on the spray equipment. Thus, for the ground application of the 80S formulation, the total HDE was 1.6 mg/h and the HDE to the hands was 1.5 mg/h. For a similar application of Sevimol-4, the total HDE was 2.8 mg/h and the HDE to the hands was 2.7

Table II.--Dermal exposure rate to workers involved in aerial and ground applications of carbaryl to crops of peas, potatoes, corn, and apples.

Exp. no.	Worker monitored	Rep. No.	time (min.)	Hourly dermal exposure (mg/h)				
				Exposure Front V of neck	Back of neck	Face	Forearms	Hands
1.	Mixer-loader	1	10	0.24	0.14	1.46	13.21	61.10
2.	Mixer-loader	1	18	0.46	0.07	9.30	21.52	153.10
		2	11	2.19	ND ^a	9.45	18.28	161.24
		3	9	0.91	0.10	3.84	26.02	318.19
		4	11	1.10	0.22	3.76	31.94	184.06
		5	8	1.05	0.11	9.63	33.17	319.76
		6 ^b	11	0.25	0.14	2.68	53.53	117.69
		7 ^b	10	0.36	0.13	3.49	38.12	2.81
		8 ^b	10	0.72	0.05	7.25	20.58	6.82
		9 ^b	9	0.45	ND	4.31	38.72	8.08
		10 ^b	11	0.30	ND	2.77	29.37	4.71
						Avg	0.78 ^c	5.65 ^c
				Avg	0.08 ^c			5.61 ^e
3.	Mixer-loader	1 ^b	13	0.29	0.10	0.84	6.31	6.89
		2 ^b	14	ND	ND	ND	0.88	68.95
		3 ^b	13	ND	ND	ND	0.25	0.59
		4 ^b	14	ND	ND	0.36	1.32	3.75
		5 ^b	9	ND	ND	ND	1.37	1.07
		6 ^b	13	ND	ND	ND	ND	0.38
		7 ^b	9	ND	0.71	ND	ND	0.23
		8	8	ND	ND	ND	ND	39.33

Table II.--Cont.

Exp. no.	Worker monitored	Rep. No.	Exposure time (min.)	Hourly dermal exposure (mg/h)					
				Front V of neck	Back of neck	Face	Forearms	Hands	
4.	Mixer-loader	1 ^{b/}	9	ND	ND	0.15	ND	24.97	
			10	ND	ND	ND	ND	21.25	
5.	Mixer-loader	1 ^{b/}	Avg	0.03 ^{c/}	0.08 ^{c/}	0.14 ^{c/}	1.01 ^{c/}	38.63 ^{d/}	
			Avg					2.15 ^{e/}	
6.	Mixer-loader	1 ^{b/}	19	ND	ND	ND	ND	0.47	
		2 ^{b/}	7	ND	ND	ND	ND	8.28	
		3 ^{b/}	20	ND	ND	ND	0.42	0.86	
		4 ^{b/}	24	ND	ND	ND	ND	2.77	
7.	Mixer-loader	1	7	ND	ND	ND	3.54	14.57	
		2	7	ND	ND	ND	0.99	6.62	
		3	9	2.29	0.40	7.27	3.94	2.19	
		4	9	2.75	0.21	6.83	25.45	3.21	
7.	Mixer-loader	5	7	1.66	0.54	2.87	11.82	5.38	
		6	8	7.37	1.42	16.33	55.90	8.80	
		7	8	1.98	1.02	7.61	23.28	8.70	
		8	7	1.30	0.26	6.77	25.37	11.27	
		Avg					28.51	6.62	
		Avg							

Table II.--Cont.

Exp. no.	Worker monitored	Rep. No.	time (min.)	Hourly dermal exposure (mg/h)				
				Exposure Front V of neck	Back of neck	Face	Forearms	Hands
8.	Mixer-loader	1	3	ND	ND	ND	ND	61.64
9.	Mixer-loader	1	3	SL _f /	0.35	ND	5.08	99.10
1.	Applicator	1	26	ND	ND	ND	ND	0.03
2.	Applicator	1	17	ND	ND	ND	ND	0.49
		2	19	ND	ND	ND	ND	0.94
		3	15	0.05	0.03	0.18	0.19	2.10
		4	12	ND	ND	ND	0.36	2.44
		5	20	ND	ND	0.08	ND	1.10
		6	17	ND	ND	ND	ND	0.83
		7	20	ND	ND	0.19	ND	1.74
		8	15	0.07	ND	ND	ND	0.52
		9	15	ND	ND	ND	ND	1.66
		10	15	ND	ND	ND	ND	3.42
				<u>Avg</u> 0.01	<u>ND</u>	<u>0.05</u>	<u>0.05</u>	<u>1.52</u>
3.	Applicator	1	19	ND	ND	ND	ND	4.58
		2	27	ND	ND	ND	ND	8.52
		3	15	ND	ND	ND	ND	0.34
		4	16	ND	ND	ND	0.50	6.33
		5	21	ND	ND	ND	0.22	2.03

Table II.--Cont.

Exp. no.	Worker monitored	Rep. No.	Exposure time (min.)	Hourly dermal exposure (mg/h)				
				Front V of neck	Back of neck	Face	Forearms	Hands
		6	15	ND	ND	ND	0.19	2.75
		7	15	ND	ND	ND	ND	1.01
		8	15	ND	ND	ND	ND	0.43
		9	17	ND	ND	ND	ND	0.31
		10	16	ND	ND	ND	ND	0.73
			<u>Avg</u>	<u>ND</u>	<u>ND</u>	<u>ND</u>	<u>0.09</u>	<u>2.70</u>
4.	Applicator	1 g/	2	ND	0.83	15.50	233.41	116.79
5.	Applicator	1	3	ND	ND	ND	ND	3.38
6.	Applicator	1	6	ND	ND	ND	1.39	18.16
		2	3	ND	ND	0.78	0.97	72.82
		3	3	ND	ND	ND	ND	6.84
		4	3	ND	ND	ND	ND	5.12
			<u>Avg</u>	<u>ND</u>	<u>ND</u>	<u>0.20</u>	<u>0.59</u>	<u>25.74</u>
7.	Applicator	1	2	ND	ND	ND	ND	7.14
		2	2	ND	ND	ND	ND	2.49
		3	2	ND	ND	ND	ND	7.02
		4	2	ND	ND	ND	ND	13.29
		5	2	0.02	ND	ND	ND	3.56
		6	2	ND	ND	ND	ND	15.90

Table II.--Cont.

Exp. no.	Worker monitored	Rep. No.	Exposure time (min.)	Hourly dermal exposure (mg/h)					
				Front V of neck	Back of neck	Face	Forearms	Hands	
		7	2	ND	ND	ND	ND	2.37	
8.	Applicator	1	4	ND	ND	ND	ND	7.32	
			<u>Avg</u>	0.29	1.82	4.39	6.90	2.90	
9.	Applicator	1	4	SL	0.73	9.80	8.17	4.14	
1.	Bystander	1	26	ND	ND	ND	ND	ND	
2.	Bystander	1	17	ND	ND	0.16	ND	0.19	
		2	18	ND	ND	ND	ND	0.08	
		3	15	ND	ND	ND	ND	0.53	
		4	12	ND	ND	0.26	ND	0.18	
		5	18	ND	ND	ND	ND	0.08	
		6	16	ND	ND	1.02	ND	0.80	
		7	15	ND	ND	ND	ND	0.41	
		8	15	ND	ND	ND	ND	0.32	
3.	Bystander	1	19	ND	ND	ND	ND	1.78	
		2	27	ND	ND	ND	ND	0.05	
		3	15	ND	ND	ND	ND	0.02	
		4	16	ND	ND	ND	ND	0.05	
		5	15	ND	ND	ND	ND	0.20	
		6	15	ND	ND	ND	ND	0.08	

Table II.--Cont.

Exp. no.	Worker monitored	Rep. No.	Exposure time (min.)	Hourly dermal exposure (mg/h)				
				Front V of neck	Back of neck	Face	Forearms	Hands
		7	17	ND	ND	ND	ND	0.11
		8	16	ND	ND	ND	ND	0.03
			<u>Avg</u>	<u>ND</u>	<u>ND</u>	<u>ND</u>	<u>ND</u>	<u>0.29</u>
4.	Aerial Flagger	1	2	2.97	69.10	380.84	94.38	58.41
5.	Aerial Flagger	1	3	1.80	27.52	184.60	152.70	41.30
		1	6	11.03	7.46	258.38	219.56	32.53
		2	3	1.23	32.34	208.10	126.75	38.74
		3	3	4.97	2.90	131.20	59.84	19.13
			<u>Avg</u>	<u>5.74</u>	<u>14.23</u>	<u>199.23</u>	<u>135.38</u>	<u>30.13</u>
		1	2	9.81	4.95	64.45	12.52	23.37
		2	2	0.27	4.03	81.02	33.02	22.08
		3	2	7.29	7.85	128.31	73.69	45.81
		4	2	3.87	2.18	112.13	10.71	38.22
		5	2	4.01	6.04	121.00	57.54	83.52
		6	2	1.58	12.71	55.38	20.51	36.93
		7	2	4.50	4.13	61.04	45.38	38.31
			<u>Avg</u>	<u>4.48</u>	<u>5.98</u>	<u>89.05</u>	<u>36.20</u>	<u>41.18</u>

Table II.--Cont.

Exp. no.	Worker monitored	Rep. No.	Exposure time (min.)	Hourly dermal exposure (mg/h)						
				Front V of neck	Back of neck	Face	Forearms	Hands		
8.	Thinner									
	No. of days after the spray									
	0		6	ND	ND	ND	ND	ND	ND	1.20
	1		8	ND	ND	ND	ND	1.04	ND	1.00
	3		10	ND	ND	ND	ND	ND	ND	0.81
	7		9	ND	ND	ND	ND	ND	ND	0.43
	17		7	ND	ND	ND	ND	ND	ND	0.19
	24		7	ND	ND	ND	ND	ND	ND	0.07
	31		9	ND	ND	ND	ND	ND	ND	0.06
	38		11	--	--	--	--	--	--	0.11
	52		9	--	--	--	--	--	--	ND
9.	Thinner									
	No. of days after the spray									
	0		8	SL	SL	ND	ND	ND	ND	0.45
	3		9	ND	ND	ND	ND	ND	ND	0.16
	7		8	ND	ND	ND	ND	ND	ND	0.24
	17		7	ND	ND	ND	ND	ND	ND	ND
	24		10	ND	ND	ND	ND	ND	ND	ND
	31		7	--	--	--	--	--	--	ND
	38		10	--	--	--	--	--	--	ND
	52		8	--	--	--	--	--	--	ND

Table II.--Cont.

- a/ None detected (ND); limit of detection was $0.07 \mu\text{g}/\text{cm}^2$ on exposure pads and $4.0 \mu\text{g}/\text{hand}$ rinse; thus the HDE limit of detection varied with exposure time.
- b/ These workers wore gloves.
- c/ Avg of all ten replicates.
- d/ Avg exposure when mixer-loader was not wearing gloves.
- e/ Avg exposure when mixer-loader was wearing gloves.
- f/ SL = samples lost before analysis.
- g/ Malfunction of spray equipment caused abnormally high HDE.

mg/h. The difference in HDE values between 80S and Sevimol-4 was related to the greater tendency of Sevimol-4 to plug the nozzles and the screens of the spray apparatus, requiring the applicator to make more frequent adjustments.

The aerial applicators also had considerably less exposure than the mixer-loaders. Again, most of this exposure was to the hands and was acquired from adjusting nozzles on the spray equipment. For the 80S and the XLR formulations, the total HDE's were 7.4 and 3.4 mg/h, respectively, and almost 100% of the exposure was to the hands in both cases. Here, as for the ground application, the highest HDE was obtained from Sevimol-4 because of the more frequent plugging of the spray nozzels. Thus the total HDE for Sevimol-4 was 26.5 mg/h, and the HDE on the hands was 25.7 mg/h.

During one aerial application with XLR (experiment 4) the spray equipment malfunctioned. The applicator, in an attempt to correct the problem, accidentally opened the dumping valve to the spray tank and the formulation splashed on him. The result was a total HDE of 367 mg/h, with almost half of it on the forearms. Since such an exposure would not be continuous, the calculation on an hourly basis is unrealistic. Therefore, the data were not used in determining the HDE to applicators.

Although handgun spraying is no longer a common method of applying carbaryl to large acreages, it is still used on small acreages and by commercial applicators treating city yards. We had an opportunity to monitor workers using such equipment in conjunction with the experiment to determine the degradation rate of carbaryl on apple leaves. This applicator had the highest exposure of any of the applicators monitored. The average total HDE was 19.6 mg/h, and the exposure was uniform over the body. There were several reasons for this. Since tree height ranged from 11 to 20 feet, nearly all of the spray was directed into the trees at shoulder height or above. To spray a tree uniformly, the worker occasionally had to stand or hold his arms under the drip line of the tree, resulting in direct contact with the spray. Also there was some misting from the spray gun and splash-back from the limbs of the tree.

Bystanders. The bystander had the lowest exposure to carbaryl of all the workers monitored. In keeping the bystander within 100 feet and downwind of the ground applicator, the bystander often had to walk into the field while it was being treated. This practice resulted in exposure when the hands of the bystander touched the crop foliage. Thus, with peas, there was no exposure because the plants were too small at the time of spraying for any inadvertent contact, but with relatively mature potatoes, measurable residues were deposited on the bystander. For example, when 80S was applied to this crop, the bystander had a total HDE of 0.5 mg/h

with 80% of the exposure on the hands. The other 20% was on the face as a result of the bystander putting his hand on these exposure pads. When Sevimol-4 was applied the total HDE was 0.3 mg/h and 100% of the exposure was to the hands.

The Aerial Flagger. The aerial flagger, who had the highest HDE of all workers monitored, is not now commonly used in the aerial applications of pesticides to crops. Still, a flagger is sometimes used in special situations, and was therefore monitored. The flagger was the only worker that had a discernibly different exposure for different application rates. For example, the total HDE for XLR was 606 mg/h for a rate of 2 lb AI/acre and 408 mg/h for a rate of 1 lb AI/acre.

The flagger was the only worker who did not have most of the residues on the hands. Instead, they were more evenly distributed over the body with the face having most of the exposure. For example, in experiment 4 (XLR) the HDE for the face was 381 mg/h when the total HDE was 606 mg/h and in experiment 5 (Sevimol-4) was 185 mg/h when the total HDE was 408 mg/h. For experiment 6 (Sevimol-4) the total HDE to this worker was 385 mg/h and the HDE for the face was 199 mg/h. These totals were higher than the 177 mg/hr total HDE for experiment 7 (80S). However, during experiments 4, 5, and 6 it was necessary for the flagger to be in the corn field, but during experiment 7 the flagger was on the perimeter of the field.

Thinners. Carbaryl is applied to apple trees as a thinning agent, but sometimes it is necessary to send workers into the treated orchard to finish the thinning by hand. For this situation, we studied the relationship between the persistence of carbaryl on apple leaves and the exposure to thinners working in this orchard.

The data for the persistence of carbaryl residues on apple leaves are presented in Table III and diagrammed in Figure 1. The residues were lost from the leaves in a two-step process. For each process the loss followed first-order kinetics (the rate of loss was proportional to the amount left). Most of the residue (87%) was lost relatively quickly with a half-life of about 8 days, and the remainder was lost more slowly with a half-life of about 45 days.

The HDE's to the hands of the thinners versus the total residue found on the leaves is shown in Figure 2. The regression equation is $Y = 690X - 45$ where Y is the HDE in $\mu\text{g}/\text{h}$ and X is the leaf residue in $\mu\text{g}/\text{cm}^2$, and the correlation coefficient was 0.99. Since the regression line does not go through zero, the value of the X intercept corresponds to non-dislodgeable residues which is only 4% when the initial value was $1.71 \mu\text{g}/\text{cm}^2$ and 9% when the initial value was $0.70 \mu\text{g}/\text{cm}^2$.

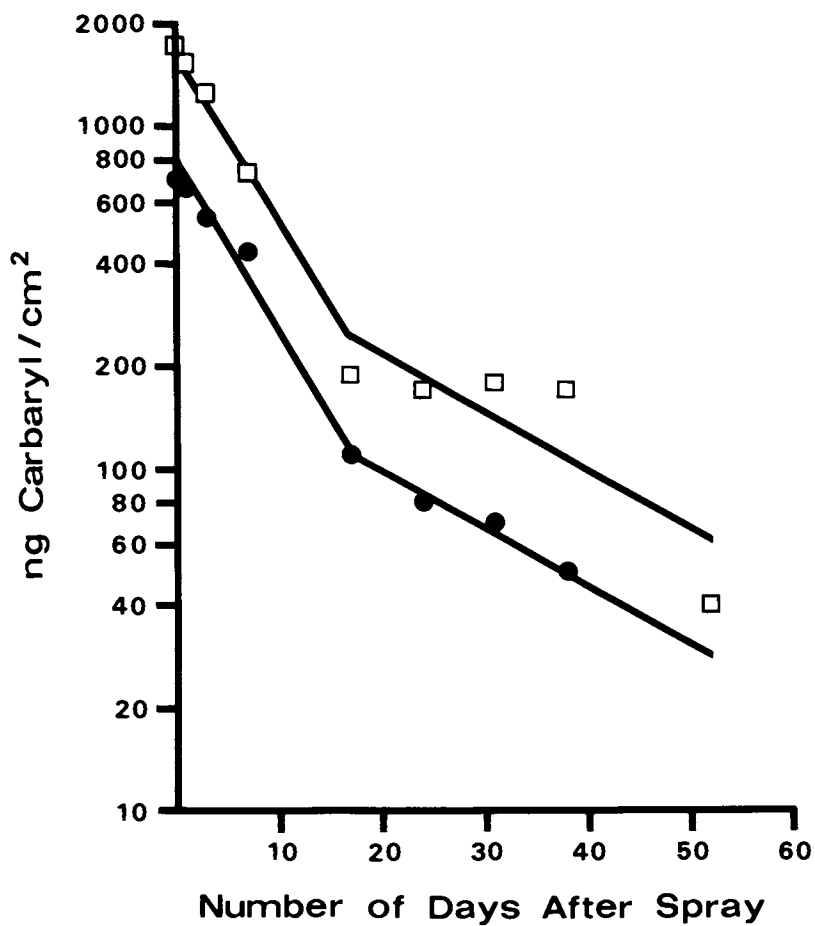


Figure 1. Degradation of carbaryl residues on apple leaves plotted on semi-log graph. Key: \circ , 0.5 lb/100 gal application rate and \square , 1.0 lb/100 gal application rate.

Table III.--Residues of carbaryl ($\mu\text{g}/\text{cm}^2$) found on apple leaves from two treated plots at various intervals after treatment.

Treatment rate AI/acre	Rep. no.	Residues found at sampling intervals (days) ^{a/}									
		0	1	3	7	17	24	31	38	52	
4.0	1	1.93	2.14	1.00	0.72	0.16	0.13	0.10	b/	ND ^{c/}	
	2	1.20	1.21	1.66	0.60	0.17	0.20	0.25	0.17	0.06	
	3	2.01	1.21	1.09	0.88	0.23	SL	0.18	0.16	0.07	
	<u>Avg</u>	<u>1.71</u>	<u>1.52</u>	<u>1.25</u>	<u>0.73</u>	<u>0.19</u>	<u>0.17</u>	<u>0.18</u>	<u>0.17</u>	<u>0.04</u>	
2.0	1	0.68	0.35	0.35	0.26	0.06	ND	0.04	ND	ND	
	2	0.94	1.10	0.58	0.63	0.21	0.15	0.14	0.06	ND	
	3	0.48	0.52	0.68	0.40	0.05	0.09	0.04	0.10	ND	
	<u>Avg</u>	<u>0.70</u>	<u>0.66</u>	<u>0.54</u>	<u>0.43</u>	<u>0.11</u>	<u>0.08</u>	<u>0.07</u>	<u>0.05</u>	<u>ND</u>	

^{a/} Values have been corrected to 100% based on recoveries found.

^{b/} SL indicates that the sample was lost.

^{c/} ND means that the residues were below the lower limit of detection for these samples ($0.03 \mu\text{g}/\text{cm}^2$).

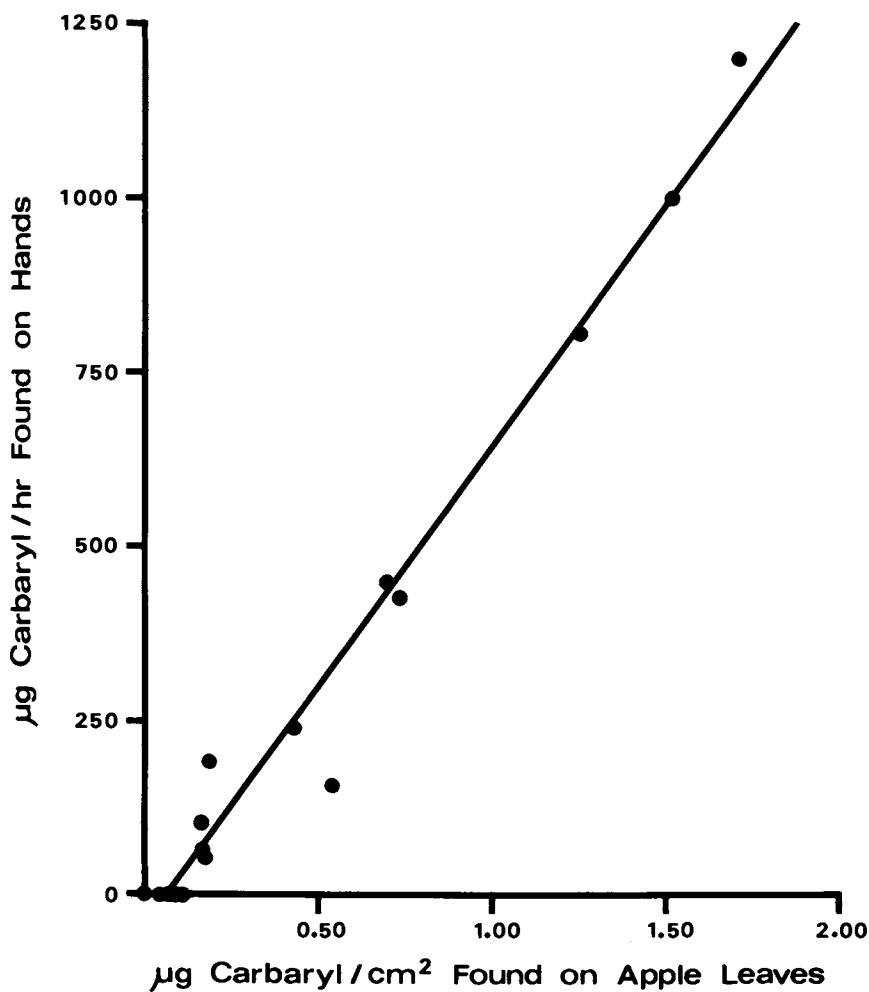


Figure 2. Residues of carbaryl found on thinners' hands (Y) as a function of residues found on leaves (X) for 0.5 and 1.0 lb/100 gal application rates (2.0 lb AI and 4.0 lb AI/acre) ($Y = 690X - 45$; correlation coefficient (r) = 0.99).

Iwata et al. (5) published a procedure for the extraction of dislodgeable residues from foliage. It involved the extraction of residues with a water-detergent solution and then partitioning the residues from this solution into dichloromethane. In our work, the total carbaryl residues were extracted directly from apple leaves with dichloromethane, and are not equivalent to dislodgeable residues. Nevertheless, because the total extractable residues correlate with HDE, they have the same value for predicting human exposure as dislodgeable residues. Since the total residue determination is less laborious, this method is preferred.

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Development of Methodology for Determining Human Exposure to Chlorobenzilate

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This paper discusses some of the investigative steps taken to develop an analytical procedure by which urinary levels of chlorobenzilate (CB), dicofol (DC) and suitable metabolites, especially p,p'-dichlorobenzophenone (DBP) can be determined. The approach is classical rat-dosing experimentation involving both oral and dermal administration of the two acaricides of interest.

Chemical and Physical Properties

Chlorobenzilate or ethyl 2-hydroxy-2,2-di-(p-chlorophenyl)-acetate is a chlorinated aromatic α -hydroxy ester. It has an empirical formula of $C_{16}H_{14}Cl_2O_3$ and a molecular weight of 325.18. It is insoluble in water but infinitely miscible in benzene, acetone, methanol and xylene. It is hydrolyzed by strong alkali and acid but is stable under normal storage conditions.

Chlorobenzilate is sold as emulsifiable concentrates and wettable powders under such formulation names as Akar^R, Folbex^R, Acaraben^R, Benzilan^R and Kop-Mite^R.

Dicofol or 1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol is a polychlorinated aromatic alcohol. It has an empirical formula of $C_{14}H_9Cl_5O$ and a molecular weight of 370.51. The technical material contains at least 70% of the p,p' isomer and about 18% of the o,p' isomer. A contaminant has been identified (1) in which the hydroxyl group has been replaced by a chlorine atom. Dicofol is insoluble in water but very soluble in aromatic and aliphatic solvents. It is incompatible with highly alkaline materials. On exposure to UV radiation at 2537 Å, it breaks down into the corresponding phenone, and it is also degraded by thermal sterilization.

Dicofol is formulated as an emulsifiable concentrate, wettable powder and dust sold under the name of Kelthane^R.

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Published acute oral LD₅₀ values (mg/kg) for chlorobenzilate administered to rats have included: 700, form unstated (2); 702 for technical material (3); 735 for xylene emulsion (3); 960-4850 for suspensions (4); 1500 for other emulsions (4); and 3100-4850, form unspecified (2). Acute dermal LD₅₀ values for rabbits include >2550 for 25WP wettable powder and >5000 for 4E emulsion (3).

Reported acute oral LD₅₀ values for dicofol have included 576 (2) and 600 (5) for unspecified forms. No dermal values have been reported for dicofol.

Bourke and coworkers (6) found that doses of 500 mg CB/kg/day administered to rats over a four-week period resulted in no toxic symptom. Horn and coworkers (7) reported that the maximum tolerated dose of CB for rats is about 500 ppm.

Metabolism

A review of chlorobenzilate has been published by Bartsch and coworkers (3), but no information on human metabolism of either chlorobenzilate or dicofol has been published. Possible pathways for human metabolism of the two compounds are:

Chlorobenzilate → p,p'-dichlorobenzilic acid (DBA) → p,p'-dichlorobenzhydrol (DBH) → p,p'-dichlorobenzophenone (DBP) → p-chlorobenzoic acid.

Dicofol → bis(p-chlorophenyl)methane (CPM) → p,p'-dichlorobenzhydrol (DBH) → p,p'-dichlorobenzophenone → p-chlorobenzoic acid,

Note that p,p'-dichlorobenzophenone (DBP) is a common intermediate in the two metabolic pathways.

Earlier Methodology

The earlier published analytical methods for chlorobenzilate and dicofol were colorimetric, usually employing the Schecter-Haller (8) procedure in the case of CB. Blinn and Gunther (9) developed an early CB method which relied on the hydrolysis of the ester linkage followed by oxidation of the DBA produced to DBP with final spectrophotometric determination at 265 mμ. The stability of the phenone allowed the strong oxidizing conditions employed. George and coworkers (10) degraded dicofol with KOH in pyridine and measured the absorption of the colored solution produced at 520-550 mμ. Other colorimetric methods based on the Fujiwara reaction have been published by Hughes (11), Eiduson (1), Gunderson (12) and Gordon and coworkers (13). All of the colorimetric methods were time consuming and relatively insensitive.

Most published methods are for analysis of crops and soil residues of the intact acaricides. Extraction has been done by stripping, blender or soxhlet. Extraction solvents have included petroleum ether, benzene, carbon tetrachloride, acetonitrile, diethyl ether, methanol and hexane/acetone. Clean-up steps have employed liquid/liquid partitioning and adsorption on activated charcoal, activated charcoal/Florisil, Florisil, alumina and silica gel. Burke (14) reported that CB is not completely recovered from Florisil. Horn and coworkers (7) found that no clean-up was necessary when analyzing dog urine for CB using a Schecter-Haller procedure. For detection of residues, the colorimetric and UV methods have been replaced by gas chromatographic methods employing microcoulometric or electron capture detectors.

Gas chromatographic analysis of dicofol is confounded in that the compound degrades in the instrument to produce several components including the corresponding phenones (15,16). Morgan (17, 18) found that the amount of breakdown varies with the type of carrier gas and the oven temperature. Westlake and coworkers (19) tried three different column packings and experienced compound breakdown on each of them. Ives (20) minimized decomposition by omitting the glass wool from the column inlet, using short (3') glass columns, and using a solid support material which had not been treated with alkali. Gunther and coworkers (21) found that degradation could be prevented by using firebrick as a solid support. Burke and Johnson (22) were exceptions in that they found only one dicofol component in their chromatograms. Gas chromatographic columns used with dicofol have included DC-11, DC-200, OV-17, OV-101, QF-1, XE-60, Carbowax 20M, SE-30, Polyethylenediol adipate, SF-96, Apiezon L, DEGS+H₃PO₄, QF-1+DC-200 and DC-710+SE-30.

Confirmation of the identity of the gas chromatographic components has been accomplished by thin layer chromatography, relative retention times on different gas chromatographic columns, "p" values, and most recently by mass spectrometry. Dicofol can be separated from its phenone by using a Florisil column (17) or TLC. Dehydrochlorination of dicofol to DBP can be used as a confirmatory test for the parent compound. Gajan and Lisk (23) used cathode ray polarography to analyze vegetables for dicofol residues.

Development of the Method Used

After trying several different derivatization procedures, it was decided that for use as a monitoring tool it would be more practical to analyze for only one urinary component. The most logical choice was DBP. Both acaricides, DBA from CB, and DBH could all be converted to DBP. This could be extracted from the urine, and final detection of DBP could be accomplished by ECD/GC. No hydrolysis step per se was to be included in the procedure although it was felt certain that the hot H₂SO₄ would liberate any metabolites from the corresponding conjugates.

The analytical method developed has been described elsewhere (24) and will be only briefly outlined here.

A 1-5 ml aliquot of urine, depending on the expected residue levels, is placed in a 60-ml separatory funnel, and 5 ml of oxidizing reagent (5% $K_2Cr_2O_7$ in 20% H_2SO_4) are added. The funnel is then placed in an oven at 90° for 1 hr. After cooling, the sample is diluted with distilled water, partitioned against hexane, and the aqueous layer is drained off. The hexane layer is washed with water again, and the combined aqueous layers are back washed with hexane. The combined hexane layers are washed once with water, dried with sodium sulfate, and injected into a gas chromatograph equipped with a ^{63}Ni electron capture detector and an analytical column of 1.5% OV-17 + 1.95% OV-210.

Following this procedure, recovery of greater than 80% was obtained from DBA-spiked urines at levels of from 0.05 ppm to 100 ppm. The electron capture detector was linear for chlorobenzilate from 0.1 $\mu g/ml$ to 2 $\mu g/ml$ and for DBP from 0.005 $\mu g/ml$ to 0.5 $\mu g/ml$. The lowest concentrations which produced reasonably good peak shapes (relative to noise) were 0.2 $\mu g/ml$ for CB and 0.02 $\mu g/ml$ for DBP. Background at the retention time of DBP averaged 0.014 ppm for control rat urines. Although this is a low value, it is highly recommended that further application of this method should include an adsorption column clean-up step such as the alumina column used by Bartsch and coworkers (3).

Injection of solutions of the parent acaricides resulted in components with the retention times of the phenones and no other components, so it was assumed that conversion within the instrument was complete. When a known amount of dicofol was taken through the entire method, better than 95% was recovered as DBP. DBH has been shown to be readily oxidized to DBP, so we assumed that any dicofol or DBH present in a sample was converted to DBP.

Rat-Feeding Studies

Seventy-two Sprague-Dawley male rats were placed in stainless steel cages equipped with a pan by which urine and feces were automatically separated. The rats were housed two to a cage in 32 cages and singularly in the 8 remaining cages. Water was supplied from bottles equipped with sipper tubes; standard Purina Rat Chow^R was supplied from feeding cups. Each was replenished each day. The rats were allowed to adapt to the cages for 3 days during which time no samples were collected, although, the pans were cleaned daily. On the morning of the 4th day, after the pans were cleaned, urine collection was started. This was considered time zero. Each morning the pans were cleaned and the collection bottles replaced. The first set of collection bottles was labeled day 1 and placed in the freezer pending analysis. The collection procedure was repeated each morning for 12 days. Days 1 and 2 were collected before dosing began to establish baseline values.

Preparation of Dosing Solutions

Samples of technical chlorobenzilate (97.0%) and dicofol (87.6%) were diluted to known concentrations with ethyl alcohol. From the stock solutions, dilutions were made in Mazola^R corn oil for oral dosing. Solutions for dermal application remained in ethanol. The concentrations on an active ingredient basis were:

	<u>Chlorobenzilate</u> (mg/ml)	<u>Dicofol</u> (mg/ml)
High dose	67.9	52.6
Middle dose	6.79	5.26
Low dose	0.679	0.526
Dermal application	388.0	175.2

Dosing solutions were mixed on a Fisher Rotorak^R for 45 min. Difficulty was encountered in weighing and preparing the technical grade dicofol. Whereas chlorobenzilate was a viscous material, dicofol was almost a wax. It was necessary to warm a small amount of the latter material in an aluminum weighing dish until it flowed. The liquid was taken up with a disposable pipet and quickly transferred to a tared volumetric flask on a balance. The effect of the heat (if any) on the material was not known.

Since less than 0.1 ml of ethanol per ml of solution was in the oral dosing solutions of chlorobenzilate, only plain Mazola^R corn oil was used for dosing the chlorobenzilate oral control rats. The dicofol dosing solution contained 0.3 ml of ethanol per ml of solution, so the dicofol oral control dose was composed of 3 ml ethanol and 7 ml of Mazola^R corn oil. Ethanol was used for both dermal controls.

Dosing the Rats

Dosing and application were done on the morning of the 3rd, 4th, 5th, and 6th days after the pans had been cleaned and the urine collection bottles changed. Dosing and changing of collection bottles was done every 24 hr \pm 30 min. Before the initial treatment the rats were individually weighed on a small scale. Oral dosing was done by delivering 0.2 ml of solution from disposable syringes through gavage needles. Dermal application was accomplished by delivering 0.5 ml of chlorobenzilate solution and 1.0 ml of dicofol solution dropwise over the surface of the abdomen of the animals which had been closely shaven. The solution was allowed to seep in or evaporate over a period of 2-3 min before the animal was placed back into its cage. Weights and dosing rates are given in Table I. Some of each dermal application ran into the animal's fur and quite possibly became ingested when it preened itself; therefore interpretation of the dermal application results may be somewhat confounded. By the 7th day (1st day after

Table I. Descriptive Data for Rat-Dosing Experiments

<u>Cage No.</u>	<u>Rats/Cage</u>	<u>Treatment^a</u>	<u>Day 3 Wt. (g)</u>	<u>Dose^a (mg/kg)</u>	<u>Final Wt. (g)</u>
1	2	CB oral control	255 255		250 260
2	2	CB oral control	260 245		250 200
3	2	DC oral control	275 300		290 290
4	2	DC oral control	250 260		260 270
5	2	CB derm control	265 235		290 240
6	2	CB derm control	275 250		280 250
7	2	DC derm control	265 240		280 250
8	2	DC derm control	255 255		240 270
9	2	High CB oral	260 280	52.23 48.50	290 300
10	1	High CB oral	290	46.83	300
11	2	High CB oral	235 250	57.79 54.32	260 290
12	2	Mid CB oral	210 270	6.47 5.03	220 290
13	2	Mid CB oral	260 280	5.22 4.85	280 290
14	1	Mid CB oral	200	6.79	250
15	2	Low CB oral	270 270	0.50 0.50	200 240
16	2	Low CB oral	280 290	0.49 0.47	260 290
17	2	CB oral control	160 240		134 ^b 270
18	2	CB oral control	280 265		260 260
19	2	CB derm control	260 200		300 200
20	2	CB derm control	250 275		230 280
21	2	DC oral control	260 290		270 300
22	2	DC oral control	250 280		250 300
23	2	DC derm control	270 250		290 270

Table I. (continued)

Cage No.	Rats/Cage	Treatment ^a	Day 3 Wt. (g)	Dose ^a (mg/kg)	Final Wt. (g)
24	2	DC derm control	265		270
			250		270
25	1	Low CB oral	240	0.57	280
26	2	High DC oral	250	42.05	250
			260	40.43	280
27	2	High DC oral	300	35.04	300
			250	42.05	280
28	1	High DC oral	230	45.70	280
29	2	Mid DC oral	260	4.04	280
			270	3.89	290
30	2	Mid DC oral	265	3.97	290
			270	3.89	300
31	1	Mid DC oral	255	4.12	270
32	2	Low DC oral	285	0.37	300
			285	0.37	280
33	2	DC dermal	230	761.74	183 ^c
			210	834.29	200
34	2	DC dermal	235	745.53	188 ^d
			265	661.13	195 ^e
35	1	DC dermal	240	730.00	188 ^f
36	2	CB dermal	270	718.52	270
			260	746.15	260
37	2	CB dermal	155	1251.61	160
			270	718.52	280
38	empty ^g	-	-	-	-
39	1	Low DC oral	220	0.48	250
40	2	Low DC oral	270	0.39	280
			250	0.42	280

^aThe oral dosing solutions were calculated to approximate 0.1, 0.01, and 0.001 of the 700 mg/kg acute oral LD₅₀ for technical chlorobenzilate and the 600 mg/kg acute oral LD₅₀ for technical dicofol.

^bDied on day 10; this is weight at death.

^cDied on day 8; this is weight at death.

^dDied on day 9; this is weight at death.

^eDied on day 9; this is weight at death.

^fDied on day 9; this is weight at death.

^gThis rat replaced the dead one in cage #37.

last treatment) the rats which had received the dicofol dermal application looked very unhealthy. They were suffering from diarrhea and their urine excretion and water consumption was greater than that of the other animals. By the 8th day one of these animals had died, two others could not stand up and the 4th was moving but moribund. By the end of the study the one remaining rat which had received dicofol dermal applications appeared to be recovering well. Throughout the study, all of the other animals (except for the deaths noted in Table I) remained in fair to excellent condition and exhibited only some slight diarrhea.

Results of Analyses of Rat Urines

The results of analysis of the urine samples from cages containing two treated rats are shown graphically in Figures 1-3. Data are plotted both in μg of residue/mg creatinine and ppm residue vs time in days. The results indicate that peak excretion of either chlorobenzilate or dicofol from dermal application occurs somewhat later than from oral dosing. The compounds given orally were rapidly eliminated after dosing was stopped but dermally applied material was still being excreted in appreciable amounts at the end of the study. No attempt was made to wash off the dermal applications after a specified timed period as is sometimes done in dermal toxicity determinations. This may explain why the cage 33 results differ from cage 34 results which seem to indicate more preening during the initial stages of the investigation. The dermal application rates for both chlorobenzilate and dicofol were less than twenty times the oral dosing levels, yet the average residue found from the chlorobenzilate dermal application was about seven times greater than that from the oral dose residues from the dicofol dermal application which approached 100 times the oral dose. It is not known if the much higher relative dermally derived residues from dicofol are due to increased absorption via the dermal route, as opposed to the oral route in the case of dicofol compared to chlorobenzilate, or due to some other factor such as increased adhesiveness of the dicofol.

When the residues found in the urine collected during the study were totaled by cage, divided by the total dose administered and averaged by treatment, the following recoveries were obtained:

	<u>Treatment</u>	<u>% Total Dose</u>
<u>Chlorobenzilate</u>	high	5.56
	middle	1.84
	low	11.96
	dermal	5.15
<u>Dicofol</u>	high	2.31
	middle	1.51
	low	1.82
	dermal	3.10

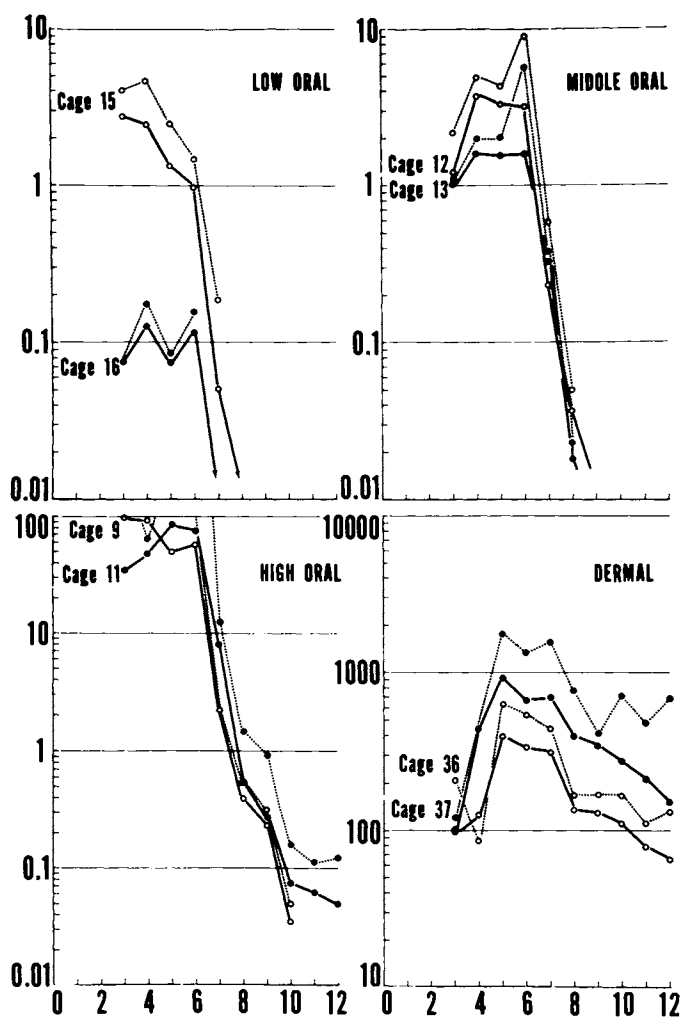


Figure 1. Semi-log plots of urinary chlorobenzilate residues vs. time in days. Specific animals have been cross-referenced to Table I by cage numbers. High-, middle-, and low-dosage results are shown as well as dermal values. Solid-line data are in ppm chlorobenzilate (as p,p'-dichlorobenzophenone) and broken-line data are in μg p,p'-dichlorobenzophenone/mg creatinine.

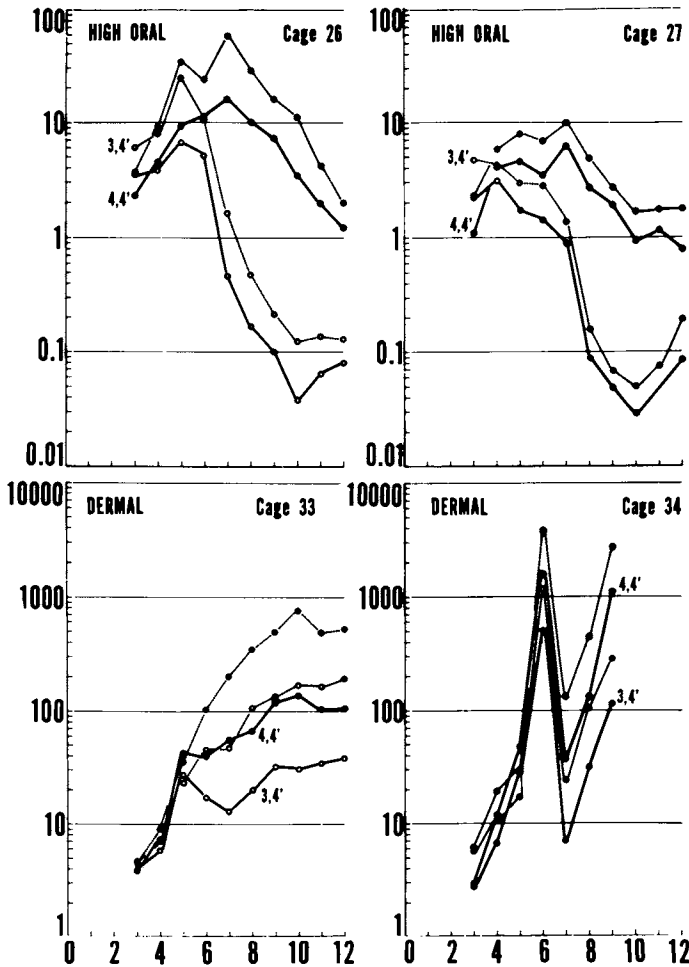


Figure 2. Semi-log plots of urinary dicofol residues vs. time.

Specific animals have been cross-referenced to Table 1 by cage numbers. Two sets of high-dosage results are given as well as two sets of dermal values which depict different results in the text. Data representation is as in Figure 1. The 4,4' refers to p,p'-dichlorobenzophenone; 3,4' (which should read 2,4') refers to o,p'-dichlorobenzophenone.

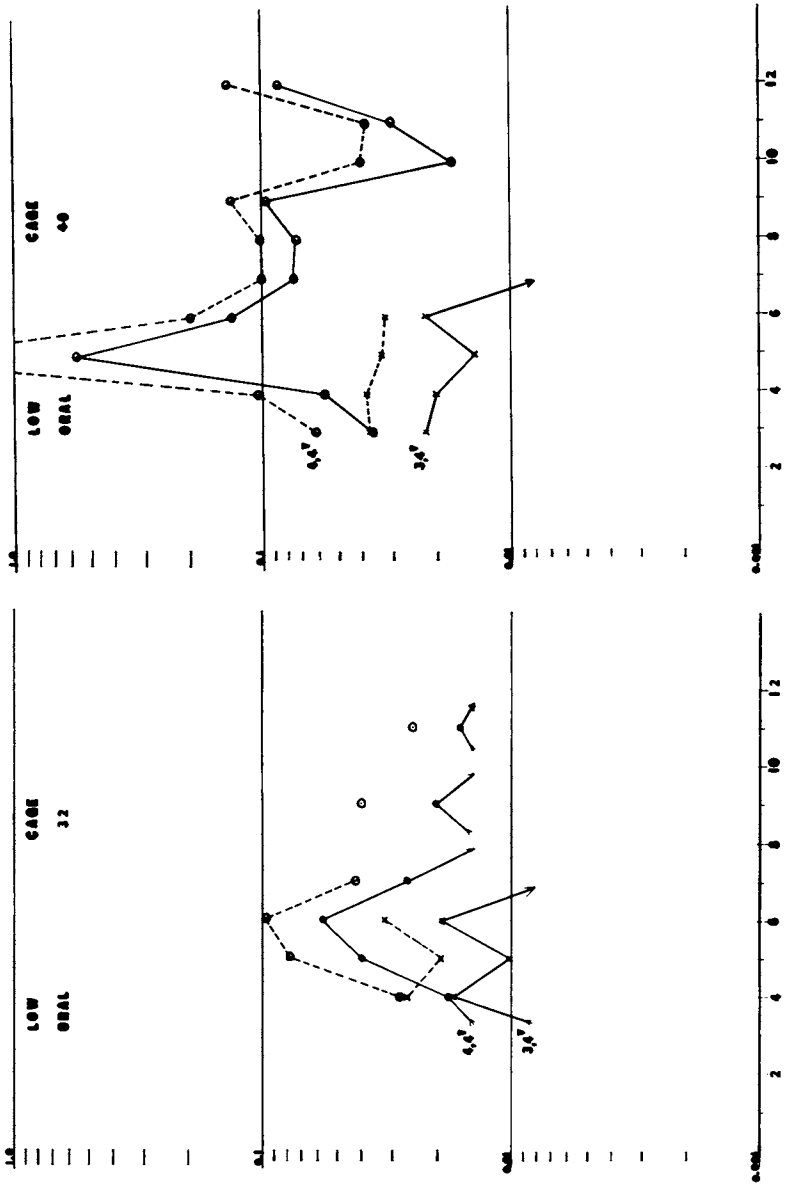
These recoveries do not correspond to the 25% recovery of ^{14}C chlorobenzilate found by Bourke and coworkers (6). However, for all treatments except the middle and low chlorobenzilate doses, residues were still detectable at the end of the study period. In most cases the level of residues from the dicofol oral doses began to increase again on days 11 and 12. This was possibly due to mobilization of dicofol stored in fat. We have no explanation for the low recovery data. As mentioned above, it is believed that the sulfuric acid used in the oxidation step was sufficient to cleave any conjugates present. The metabolism literature suggested that the parent compounds would be fully metabolized under our experimental conditions. We analyzed for intact residues in the first few samples and, finding none, we did not continue this practice. Our recoveries were based on spiked samples. Bowman and coworkers (25) have shown that this is risky in the case of crop extractions. Perhaps some mechanisms other than conjugation caused the biologically produced residues to be more difficult to extract than were the fortified samples.

Summary

A method has been developed for analyzing urine for residues of chlorobenzilate, dicofol, and the corresponding metabolites: p,p'-dichlorobenzilic acid, p,p'-dichlorobenzhydrol and p,p'-dichlorobenzophenone. This method has been applied for the analysis of urines from rats dosed by gavage at rates approximating 0.1, 0.01 and 0.001 of the acute oral LD_{50} for both acaricides, or treated dermally at rates of about 1g/kg. The method may be used as a monitoring tool to assess exposure to chlorobenzilate or dicofol; and urine of exposed citrus-grove workers is currently being analyzed. Graphic plots of the urinary residues vs time suggest rapid elimination of both acaricides from the body following removal of the exposure source.

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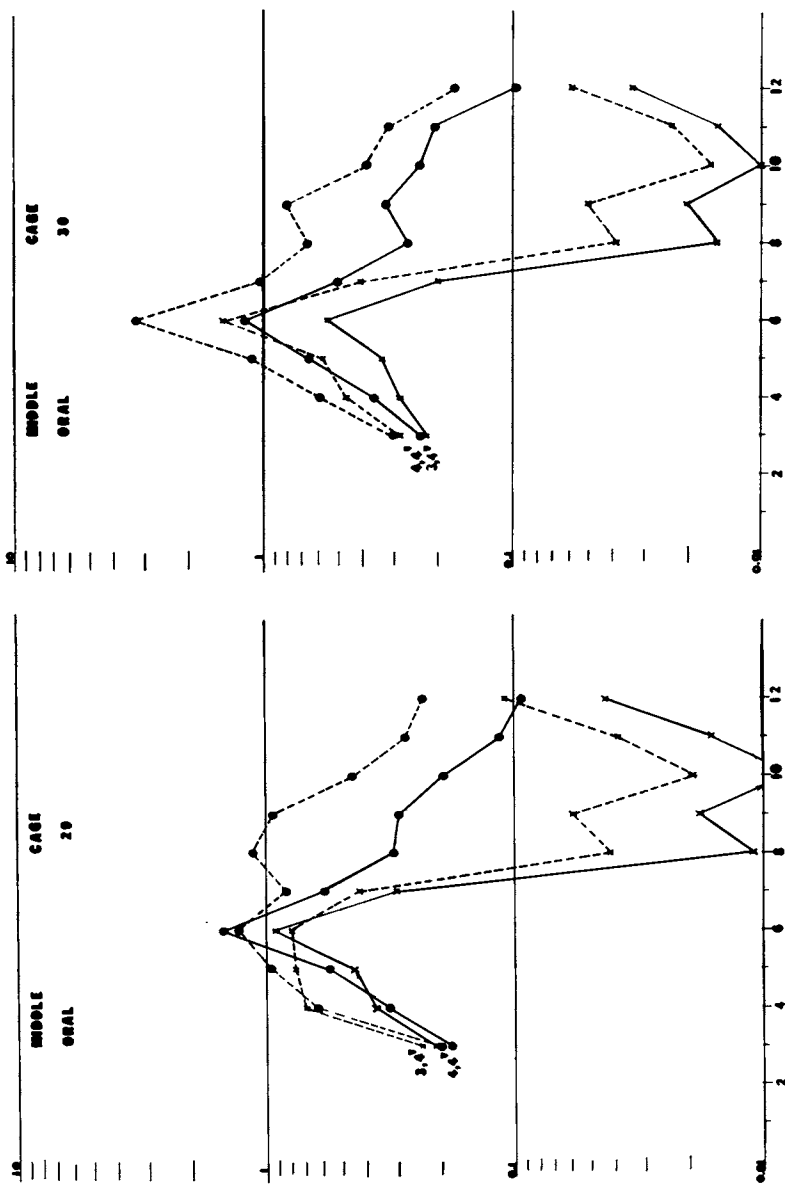


Figure 3. Plots similar to those discussed in Figures 1 and 2. Two sets of low- and middle-dosage results for both isomers of dicofol are included.

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Agricultural Applicators Exposure to 2,4-Dichlorophenoxyacetic Acid

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Abstract

Exposure of workers (applicators and mixer-loaders) to 2,4-D [(2,4-dichlorophenoxy)acetic acid], when applied to wheat under normal use, was determined by measuring urinary excretion of 2,4-D. The participants included 26 ground applicators in North Dakota after a single exposure and 17 aerial applicators in Washington during intermittent exposure. The objective was to ascertain worker exposure base-levels of 2,4-D under normal use conditions. Mean daily urinary excretion of 2,4-D by workers involved in aerial applications was 0.006 mg/kg body weight for pilots and 0.02 mg/kg for mixer/loaders from intermittent exposure. Workers involved in ground applications had maximum mean one-day 2,4-D urinary excretion of 0.002, 0.003, and 0.004 mg/kg, respectively, for applicators, mixer/loaders, and mixer/loader/applicators from a one-time exposure. The $E_{1/2}$ (half-elimination time for total 2,4-D amount excreted) values ranged from 35 to 48 h for the one-time exposed workers making ground applications. A correlation existed between 2,4-D excreted in the urine vs. worker duty for personnel involved in both the aerial and ground applications and 2,4-D excreted in urine from workers of ground application only vs. hours of exposure and vs. amount of 2,4-D applied. There was no apparent correlation between age (except where worker duty and age were correlated) weight, clothing, or 2,4-D formulation.

Exposure data are important in any assessment of pesticide safety. 2,4-D is a widely used herbicide so that opportunities exist for obtaining reliable quantitative data on exposure levels in several occupational situations. One method of measuring human exposure is by measurement of 2,4-D levels in urine, because most 2,4-D absorbed is excreted in the urine and because dermal exposure is considered the most likely exposure route (1,2,3). Excretion studies on phenoxy herbicides in man (3-6) show that 90% of the 2,4,5-T and 75% to 95% of the 2,4-D was excreted unchanged

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or as a conjugate within 96 to 144 hours, when each was administered orally at 5 mg per kg body weight. In rats and dogs, 2,4,5-T administered by intubation resulted in rapid unchanged 2,4,5-T urinary excretion by rats, but much slower excretion in dogs with some metabolism occurring. Excretion occurred by first order processes with half elimination values ($E_{1/2}$) for man, rats, and dogs of 23, 14, and 87 h, respectively. In man, Sauerhoff *et al.* (2) found $E_{1/2}$ values of 18 h for 2,4-D. Kohli *et al.* (5,6) and Sauerhoff *et al.* (2) found $E_{1/2}$ plasma values of 33, 19 and 12 h for 2,4-D, 2,4,5-T, 2,4-D respectively. Sauerhoff *et al.* (7) observed that urinary excretion was even more rapid ($E_{1/2}$ - 11 h) when 2,4,5-T was administered intravenously to rats. Distribution and elimination of 2,4,5-T was markedly altered when larger doses were administered.

Exposure studies by Lavy *et al.* (1) on forest applicators showed a 6-day mean excretion of 0.033 mg/kg body weight from single day exposures. A considerable range of 2,4,5-T was measured in the urine for different crew members, with a mixer excreting the highest level (0.096 mg/kg) and a flagman the lowest level (0.001 mg/kg) per exposure. In a subsequent 2,4-D study on forestry workers, T. L. Lavy, J. D. Walstad, R. R. Flynn, and J. D. Mattice (1980, *unpub*), found mean values of less than <0.02, 0.02, 0.006, 0.003, and 0.001 mg/kg body weight, respectively, for batchmen, pilots, mechanics, supervisors, and observers.

The objective of the current study was to obtain measurements of 2,4-D levels in urine from workers involved in the application of the herbicide under normal use conditions. Specifically, 2,4-D levels were measured in urine samples collected prior to, during, and after actual spray operations from workers involved in ground and aerial applications of amine and ester formulations of 2,4-D to wheat. The resultant data were used to calculate exposure doses and urinary excretion relationships.

Materials and Methods

Protocol. Volunteers for the 2,4-D exposure study consisted of 26 workers involved in the ground application in Eastern North Dakota and 17 workers involved in the aerial application in Eastern Washington during the spring of 1980. No suggestion or attempt was made to alter work habits or clothing worn by the workers. Instructions were given for the taking, handling, and storing urine samples and recording dates of 2,4-D application and urine collection, equipment used, hours and acres sprayed, formulation, wind condition, and job. A summary of the experimental conditions and parameters is given in Table I. Prior to the experiments most participants completed an information sheet which listed age, sex, weight, job, years of experience with 2,4-D, and applicator equipment type. During the experiments most participants listed type of clothing and weather conditions.

Sampling. Individual workers from aerial applications provided 24-hour urine samples on approximate alternate days for the continuous 2,4-D application. This represented what may be considered a typical situation during the height of 2,4-D applications to wheat in Eastern Washington. Individual workers from ground applications provided six consecutive day 24-hour urine samples for a one-week period following a single 2,4-D application. Each participant in both groups were requested to provide a one-time single voided urine sample prior to experimental initiation.

Table I. Summary of Conditions and Parameters of 2,4-D Exposure by Agricultural Applicators.

Item	Application Method and State			
	Aerial (Washington)		Ground (North Dakota)	
Equipment	4 Thrush Commanders		4 Pull type	
	4 Grumman Ag-Cats		21 Self-propelled	
	4 Pipers		10 Cab	
	1 Snow		16 No cab	
	1 Cessna			
Occupation	14 Pilots		9 Sprayers	
	5 Mixer/loaders		14 Mixer/loaders	
Clothing	Various		Various	
Rates lb/A	--		0.25 - 0.50	
kg/ha	--		0.28 - 0.56	
Formulation	--		24 Amine, 4 ester	
Sex	18 Males, 1 Female		25 Males, 1 Female	
	<u>Geometric mean</u>	<u>Range</u>	<u>Geometric mean</u>	<u>Range</u>
Age	33	21-64	39	18-63
Work experience	7	1-20	10	1-31
Sprayed acres	9000	2500-40,000	500	25-8000
ha	3650	1000-16,200	200	10-3200
Hours per day	5 ^a	3-16	--	--
Days per year	45	14-60	3	1-14

^aPilots, 4.2 h and mixer/loaders, 5.6 h.

Sample handling. All urine samples were stored frozen by each subject until collected and transported to a laboratory. Upon receipt of the frozen urine samples, they were weighed, thawed (in the case of the North Dakota workers they were subsampled at the Plant Metabolism Laboratory, Fargo, North Dakota, and refrozen prior to shipping to Beltsville, Maryland), assigned a code, and returned to a refrigerator (5°C) pending analysis.

Analysis. The Washington State urine samples were analyzed at the ARS Yakima Agricultural Research Laboratory and the North Dakota samples at the ARS Pesticide Degradation Laboratory at Beltsville, Maryland. The method used by both laboratories was a similar procedure based on a method developed at Yakima (Maitlen and Sell, unpub.). Several randomly selected urine samples were

exchanged between the two laboratories for comparative analysis. (Deviations from this method used at Beltsville are shown in the following parenthesis.) Briefly the method involved placing 15 mL urine (25 mL), 2 mL 37% KOH (5 mL 1 N KOH), 5 mL methanol, and glass beads (sand) in a boiling flask and refluxing the contents for 1 h. After cooling, the contents were transferred to a 250 mL separatory funnel, the flask rinsed with 25 mL water (50 mL sat. NaCl) and 25 mL dichloromethane (DCM) added. The funnel contents were shaken, allowed to separate, and the bottom DCM layer discarded. The upper layer was acidified with 2 mL H_3PO_4 (10 mL 20% H_3PO_4) and 20 mL DCM (15 mL) was added to the funnel. The funnel contents were shaken and allowed to separate. The bottom DCM layer was collected in a boiling flask. Two additional extractions with DCM were collected and the funnel contents discarded.

The DCM solution in the boiling flask was transferred to a separatory funnel the boiling flask was rinsed with 60 mL 3% $NaHCO_3$ (twice with 25 mL 3.6% $NaHCO_3$), and the rinses transferred to the funnel. The funnel contents were shaken vigorously for 1 min and allowed to separate and the bottom layer was discarded. Three mL H_3PO_4 (15 mL 20% H_3PO_4) was carefully added to the separatory funnel and gently swirled until effervescence diminished. The acidified solutions were extracted three times with 20 mL DCM (15 mL) and each DCM extract dried through anhydrous Na_2SO_4 in a 125 mL Erlenmeyer flask (100 mL beaker). The DCM solution was carefully evaporated to dryness.

Samples were methylated by one of two procedures. Ten milliliters methanol and 1 mL of 14% BF_3 in methanol were added to the residue in the flask and the contents boiled on a hot plate for 1.5 min. Excess methanol was evaporated with dry air. About 0.5 mL of a brown oily substance remained. Two mL of 95% hexane plus 5% toluene were added to the oil residue, mixed and placed into the refrigerator until gas-liquid chromatographic (GLC) analysis. An alternate methylation procedure involved transferring the residue to a 15 mL pointed tube with four 0.5 mL increments of $BF_3 \cdot CH_3OH$ in methanol (1:2 v/v). The pointed tube was stoppered and placed in an 80°C tube heater. After 1 h the tube was removed and allowed to cool. Five milliliters hexane and 2 mL saturated NaCl were added to the tube and the contents mixed thoroughly on a vortex mixer. Four mL of the hexane layer were pipeted from the tube and placed into a vial. The contents of the vial were evaporated just to dryness with dry N_2 at room temperature. Four milliliters of hexane were added to the vial and the contents shaken. Aliquots from the vial were taken for GLC assay.

GLC conditions. Dilutions for GLC assays were made when necessary. The GLC parameters were: ^{63}Ni electron-capture detector; glass columns were filled with 3% OV 101 on Gas Chrom Q (4 mm i.d. by 1.2 m), 11% (QF-1 + OV-17) on Gas Chrom Q (4 mm i.d. by 1.8 m), and 5% OV 101 on Chromsorb W (4 mm i.d. by 1.8 m) all

80/100 mesh; 5/95 CH₄/Ar gas at 60, 30, or 60 mL/min and 160, 200 and 180° column temperatures, respectively. The data reported represents the absolute values measured by the Yakima Laboratory, whereas the Beltsville laboratory subtracted from all field samples a value 2.5 times the recorder peak height of control urine samples.

Results and Discussion

Recoveries of 2,4-D (80%) and 2,4,5-T (92%) added to urine are given in Table II and comparative 2,4-D analysis in several laboratory exchanged samples is given in Table III. The 2,4,5-T was used as an internal standard at the Beltsville laboratory. The Beltsville laboratory found higher 2,4-D amounts in many urine

Table II. Recoveries of 2,4-D (propylene glycol butyl ether ester) and 2,4,5-T (isooctyl ester) Added to 25 mL urine.

2,4-D		2,4,5-T	
Added ng	Recovered %	Added ng	Recovered %
48	73 + 14 ^a	27	98 + 10
120	75 + 9	68	68 + 7
240	83 + 6	135	96 + 4
480	72 + 5	270	93 + 6
960	84 + 3	540	100 + 4
2,400	83 + 5	1,350	95 + 4
4,800	87 + 4	2,700	97 + 6
Average	80 + 7		92 + 6

^aStandard error of mean from 4 to 12 trials.

Table III. Comparative 2,4-D (ppm) Analysis of Urine Samples between the Yakima and Beltsville Laboratories.

Sample number	laboratory		Sample number	Laboratory	
	Yakima	Beltsville		Yakima	Beltsville
-----North Dakota Samples-----			-----Washington Samples-----		
1	ND ^a	ND	13	0.04	0.05
2	0.17	0.16	14	0.06	0.05
3	0.01	0.003	15	0.19	0.25
4	ND	ND	16	0.32	0.51
5	0.61	0.66	17	1.50	2.00
6	0.24	0.29	18	1.05	1.43
7	0.18	0.10	19	1.21	2.02
8	ND	ND	20	1.08	1.17
9	0.03	0.01	21	1.25	1.53
10	0.44	0.38	22	1.88	2.24
11	2.63	2.94	23	0.83	0.93
12	0.31	.033	24	0.20	0.23

^aNone detected.

samples, especially the high 2,4-D urine content Washington samples. The procedure measures conjugated 2,4-D, also (4).

Aerial application workers. The total amount of 2,4-D applied to wheat on a weight basis, exposure period, and mean daily excretion rate expressed on a body weight basis for the aerial application workers is shown in Table IV. A preexperimental one-time void sample was collected from most participants. These one-time samples contained from 0.1 to 1.6 ppm 2,4-D, with an average of 0.4 ppm. The mean daily excretion rate for all workers was 0.012 mg/kg body weight. From an occupational standpoint, the per day excretion rate was substantially higher for the mixer/loader (mean 0.02 mg/kg body weight) as compared to pilots (mean 0.006 mg/kg), and probably associated with their handling the 2,4-D concentrate. The low number of participants reporting exposure information relative to 2,4-D amount handled and hours of exposure negated (Table 1) any valid statistical analysis of the data when worker number 2 was not included.

From the information provided by the participants, we were unable to find any relationship between clothing worn and 2,4-D excretion levels. The mixer/loader excretion 2,4-D levels (Table IV) correspond to the 0.02 mg/kg 2,4-D excreted by batchmen of T. L. Lavy, J. D. Walstad, R. R. Flynn, and J. D. Mattice, (1980, unpub), but considerably lower (0.06 mg/kg) than the 2,4,5-T mixers of Lavy's study and the 0.077 mg/kg in pilots to a low of 0.01 mg/kg in supervisors of forestry workers in Smith's et al. (8) study. Likewise, the wheat 2,4-D pilots excreted 0.006 mg/kg compared to the 0.02 mg/kg forestry pilots of Lavy et al. (unpub).

Ground application workers. Urinary excretion samples from ground applicators and mixer/loaders from North Dakota were taken on 6 consecutive days after a single 2,4-D exposure. Most participants provided one preexperimental urine sample, which contained from nondetectable quantities to 0.4 ppm 2,4-D.

The total amount of 2,4-D applied, hours of exposure, and daily and total excretion on a body weight basis is shown in Table V. Three parameters—occupation, amount mixed or applied, and exposure period were correlated with the amount of 2,4-D excreted in urine. When the occupational series is compared to the total amount (mg/kg) of 2,4-D excreted, three subgroups are identifiable, (a) applicators, 0.005; (b) mixer/loaders, 0.007; and (c) mixer/loader/applicators, 0.018; i.e., the excretion levels increased a<b<c. This reflects, in part the longer average exposure time (8 h) and the handling of 2,4-D concentrate for the mixer/loader/applicator group when compared to the mixer/loader (2.4 h) and applicator (3.5 h) groups (Figure 1). For example, the calculated 2,4-D excretion rate for the mixer/loaders would be 0.002 mg/kg/hour of 2,4-D exposure.

Total 2,4-D excretion was also correlated with the amount of 2,4-D mixed or applied (Figure 2). Assuming a one day exposure to

Table IV. 1980 2,4-D 12-Day Intermittent to Continuous Exposure to Aerial Applicators in Washington.

Worker	Job	12-Day application			Mean daily excretion of 2,4-D (mg/kg X 10 ⁻³)
		2,4-D a.i. amount		Time hour	
		lb	kg		
1	M/L <u>a/</u>	500	225	6	6.5 <u>b/</u>
2	M/L	2,200	1,000	24	54.5 <u>b/</u>
3	M/L	1,600	730	28	4.9
4	M/L	---	---	--	0.8
5	M/L	---	---	--	34.0
6	M/L	---	---	--	18.5
7	M/L/P	860	390	22	18.0
8	P	1,050	475	8	3.3
9	P	1,120	510	14	9.7
10	P	950	430	20	7.7
11	P	---	---	18	3.3 <u>b/</u>
12	P	100	415	4	1.4
13	P	830	375	23	1.3
14	P	---	---	15	0.6 <u>b/</u>
15	P	740	335	10	10.1
16	P	---	---	--	2.5
17	P	---	---	--	20.2
Mean	overall	995	450	16	11.6
Mean	M/L <u>c/</u>	1,290	585	20	19.6
Mean	P	800	360	14	6.0
Ratio	M/L ÷ P	1.6	1.6	1.4	3.3

a/ Mixer/Loader/Pilot.b/ For 80 kg person.c/ Including No. 7.

Table V. 1980 2,4-D 1-Day Exposure to Ground Applicators in North Dakota.

Worker	Job	1-Day application		Daily excretion of 2,4-D, mg/kg X 10 ⁻³						Total exposure mg/kg X 10 ⁻³	
		2,4-D a.i. amount lb	Time hour	0	1	2	3	4	5		6
1	A	23	1	ND ^c	0.05	0.10	0.10	0.10	ND	0.05	0.30
2	A	--	1.5	0.09	ND	ND	ND	ND	ND	ND	0.09
3	A	40	3	ND	0.07	0.07	0.07	0.07	ND	ND	0.21
4	A	100	7	ND	ND	ND	ND	ND	ND	ND	0.0
5	A	120	54	ND	0.48	0.19	0.07	0.14	0.34	0.34	1.56
6	A	100	45	ND	1.03	2.12	1.71	0.68	0.27	0.14	5.95
7	A	--	--	ND	10.41	5.21	1.30	0.55	0.41	ND	17.9
8	A	43	20	ND	0.06	0.35	0.52	0.29	0.06	0.17	1.45
9	A	23	10	0.16	4.00	19.4	24.0	15.2	6.37	6.89	76.0
10	A	107	49	0.07	4.13	4.40	4.73	1.40	1.27	1.00	17.0
11	M/L	20	9	ND	0.93	0.83	0.50	0.07	0.07	0.14	2.54
12	M/L	23	10	ND	0.88	0.13	0.44	ND	0.20	ND	1.65
13	M/L	23	10	ND	0.33	1.13	1.13	0.47	0.33	0.20	3.59
14	M/L	50	23	ND	1.74	1.67	1.52	0.53	0.98	0.68	7.12
15	M/L	35	16	ND	3.96	5.19	4.22	1.17	0.91	0.97	16.4
16	M/L	58	26	0.13	0.75	0.84	2.16	5.64	4.26	0.54	14.3
17	M/L	42	19	ND	0.28	0.84	0.62	0.34	ND	ND	2.08
18	M/L/A	--	--	0.21	3.36	8.71	8.43	5.79	5.57	2.64	34.7
19	M/L/A	112	51	ND	5.60	1.60	2.33	2.00	0.27	1.20	13.0
20	M/L/A	200	91	0.38	1.29	2.80	6.36	10.4	2.50	20.5	44.2
21	M/L/A	14	6	ND	2.36	4.79	6.07	5.64	1.26	0.93	21.0
22	M/L/A	--	--	ND	1.35	4.38	4.16	4.78	2.92	2.53	20.1
23	M/L/A	30	14	0.12	1.07	0.73	0.33	1.13	0.93	--	4.31
24	M/L/A	40	18	0.93	2.07	3.50	2.93	1.86	2.79	1.07	15.2
25	M/L/A	83	38	ND	0.91	1.04	0.73	0.37	0.37	0.18	3.66
26	M/L/A	102	46	0.27	3.74	2.79	5.68	5.84	3.37	1.89	23.6
Mean overall		63	(29)	0.09	2.80	3.08	3.08	2.47	1.36	0.90 ^d	12.7 ^d
Mean e/	A	70	(32)	0.03	3.18	3.25	3.25	1.83	0.87	0.86	12.0
Mean	M/L	39	(34)	0.02	1.80	1.94	0.94	0.34	0.26	0.19	4.93
Mean	M/L/A	83	(18)	0.02	1.27	1.51	1.51	1.17	0.96	0.36	6.8
		83	(38)	0.21	2.42	3.37	4.11	4.02	2.22	1.49 ^d	17.8 ^d

a/ Day 2,4-D was applied. b/ Mixer/Loader/Applicator. c/ None detected. d/ Without worker 20. e/ Without worker 9.

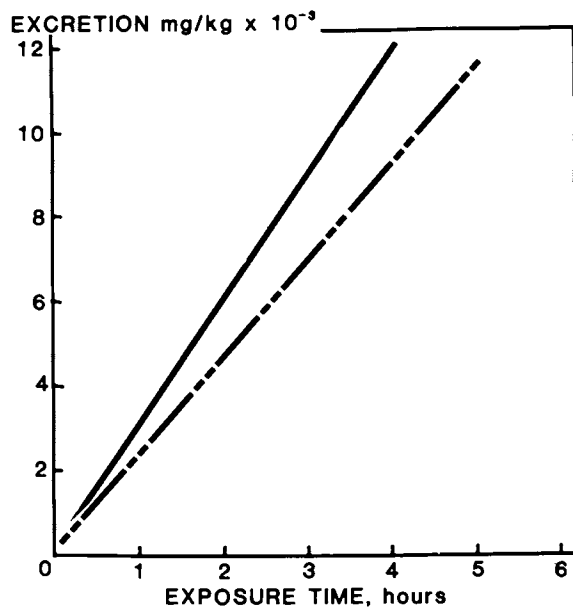


Figure 1. Relationship between hours of 2,4-D exposure and excretion rate. Key: —, mixer/loaders, excretion: $-1.8 + 3.8 h$ ($r: 0.78'$); - - - -, ground applicators, excretion: $0.55 + 2.2 h$ ($r: 0.68''$)

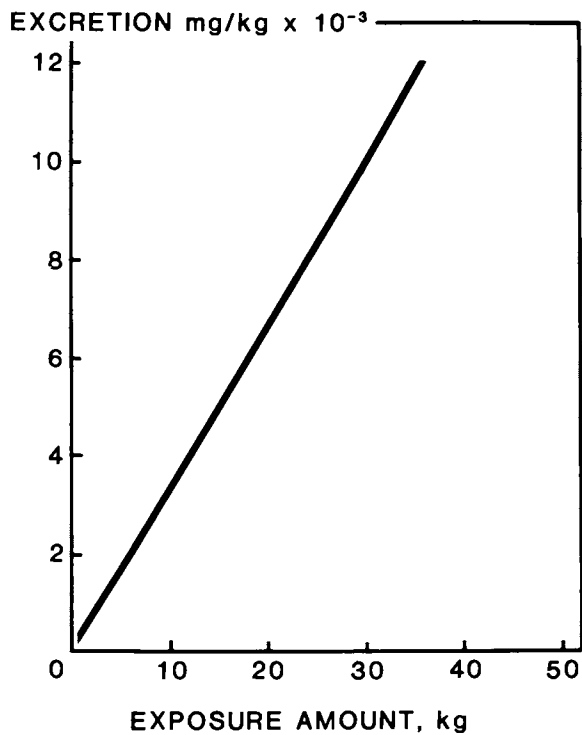


Figure 2. Relationship between amount of 2,4-D applied and excretion rate ($excretion = 1.15 + 0.31 \text{ mixed or applied}$ ($r: 0.61''$)).

45 kg (100 lb) of 2,4-D, the total amount excreted over 6 days would be 0.015 mg/kg body weight. It should be pointed out that the amount of 2,4-D mixed or applied or the exposure period may not be the most important relationship to urine excretion levels. The care or carelessness with which the worker performs his duty and the type of protective clothing worn may provide a more meaningful relationship. In this study, because the type of clothing worn was not specified in the protocol, the resultant clothing worn was too variable to make any statistical correlation with amounts of 2,4-D excreted.

One-half of all ground applicators exposed to 2,4-D had maximum daily excretions of <0.002 mg/kg (Table VI), which is probably more indicative of the exposure level for an average worker rather than the >0.003 mg/kg maximum mean given in Table V for all ground workers.

Table VI. Individual Frequencies for Maximum 2,4-D Daily Urinary Excretions of North Dakota Ground Applications.

2,4-D Excretion mg x 10 ⁻³ /kg body weight	Frequency
0 - 1.9	13
2 - 3.9	3
4 - 5.9	5
6 - 7.9	1
8 - 9.9	1
10 - 11.9	2
24 - 25.9	1

Comparisons with other studies. The urinary excretion level of 2,4-D by aerial and ground workers was not associated with age, weight, or type of clothing. However, 2,4-D excretion was associated with job for both aerial and ground workers and with amount of 2,4-D applied and with hours of exposure for ground workers. Presumably this reflects the amount of 2,4-D taken into the body by absorption through the skin, inhalation, and possibly some ingestion. Gehring *et al.* (4) Kohli *et al.* (6), and Sauerhoff *et al.* (7) observed that all ingested or bodily absorbed 2,4,5-T or 2,4-D by man was absorbed into the plasma and excreted in the urine either unchanged or as a conjugate in the case of 2,4-D. Kohli *et al.* (5), indicated 2,4-D would follow a similar pattern because they recovered 77% of the ingested 2,4-D and found no metabolites after 96 h.

Gehring *et al.* (4), and Kohli *et al.* (5,6), and Sauerhoff *et al.* (2) found urinary excretion and plasma clearances for man with $E_{1/2}$ times of 23 and 19 h, respectively, for 2,4,5-T, and 33 and 18 h, respectively, for 2,4-D. The $E_{1/2}$ times for applicators, mixer/loaders, and mixer/loader/applicators, respectively, were 35, 39, and 48 h. An $E_{1/2}$ time for forest 2,4,5-T applicators, calculated from Lavy's *et al.* (1) data was found to be 52 h. The longer $E_{1/2}$ times associated with the 2,4-D (this study) and

2,4,5-T (1) workers compared to 2,4-D or 2,4,5-T ingestion studies (2,4,5,6) probably reflects the slower rate of plasma absorption of the herbicides, compared to ingestion. The data suggests the workers did a poor job of skin protection and subsequent washing.

In the Gehring *et al.* (4) study, the one-time ingestion was 5 mg 2,4,5-T/kg body weight. The per day excretion was 1.9, 1.4, 0.71, and 0.33 mg/kg for days 1 to 4, respectively. The Kohli *et al.* (5,6), studies for 2,4-D and 2,4,5-T showed the same order of magnitude for 5 mg/kg dosages, but proportionately less for 2 and 3 mg/kg dosages. In this experiment, the per day excretion for the mixer/loader ground workers (Table V) on days 1 to 5, respectively, were 1.2, 1.4, 1.5, 1.4, and 1.1 mg $\times 10^{-3}$ /kg, about one-thousandth as much as the former.

Gehring *et al.* (4) also calculated the concentration of 2,4,5-T in plasma from repeated doses. They found that a maximum concentration was reached after 3 days when the rate of 2,4,5-T clearance from plasma equaled the amount ingested. The analogy can be applied to the urinary excretion amounts, also, because plasma clearance rate was exactly the same as the urinary excretion rate. Therefore, it would appear that the aerial workers (Table IV, Figure 3) had essentially reached the maximum 2,4-D excretion plateau.

Concluding Remarks. Based on the results presented here, some estimate of 2,4-D exposure can be calculated. For example, the ground application workers exposure to 2,4-D can be expressed as percent daily excretion (Figure 4). By knowing the total urinary excretion for any given day after exposure, the total exposure level can be calculated. In another example, the all-jobs per day urinary excretion of the aerial workers (Table IV) was 0.012 mg/kg. A 50 or 60 year-old 80 kg worker with 30 years experience of 30 days exposure per year could have absorbed and excreted as much as 0.9 g 2,4-D in his lifetime, which is about double the single injection dosages reported by Gehring *et al.* (4), Kohli *et al.* (5,6), and Sauerhoff *et al.* (2) without any known ill effects.

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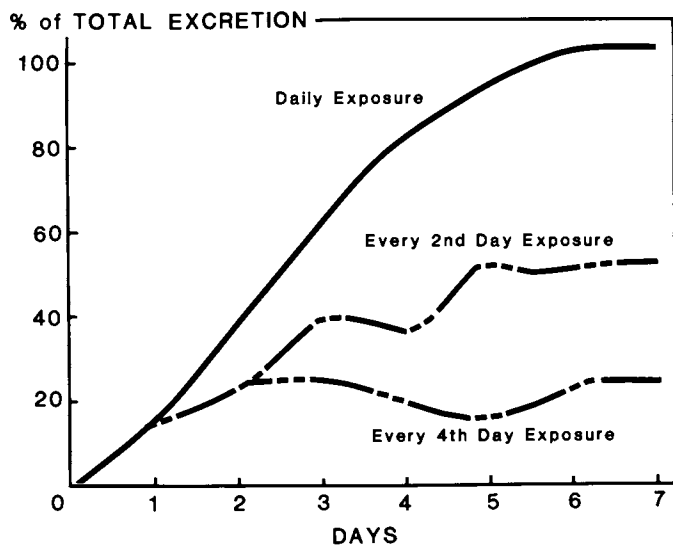


Figure 3. Percent of daily 2,4-D excretion levels from repeated exposures.

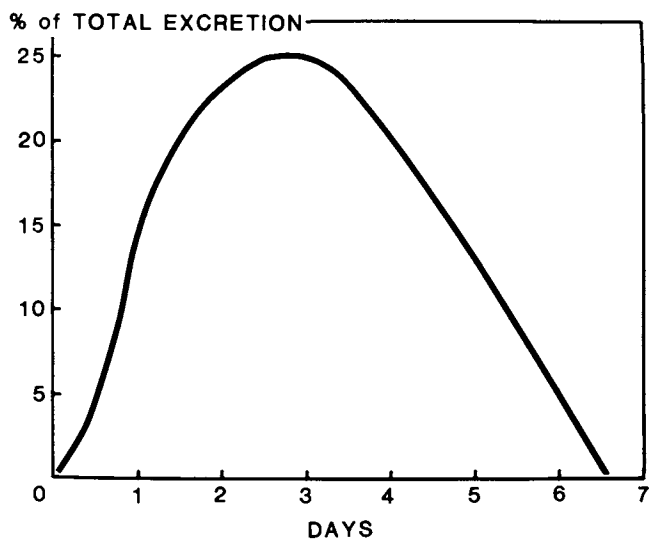


Figure 4. Percent of daily 2,4-D excretion from a single exposure for the first 6 days ($\%: 0.04 + 15d - 1.1d^3 + 0.12d^4$ ($r: 0.99''$) based on ground applications).

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Review of Studies with 2,4,5-Trichlorophenoxyacetic Acid in Humans Including Applicators Under Field Conditions

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Studies in animals and humans have shown that oral doses of phenoxy herbicides are rapidly absorbed and are excreted virtually completely as phenoxy acids in urine with a half-life of less than 1 day. The rate of absorption of 2,4,5-T into the body appeared to be slower after external exposure than after oral administration in humans. Pharmacokinetic modeling indicated 97% of the 2,4,5-T absorbed through the skin would be cleared within 1 week. Measurement of 2,4,5-T excreted in urine of spray crews demonstrated that the maximum absorbed dose is not likely to exceed 0.1 mg per kg of body weight per work day. Urinary excretion provided a more reliable measure of dose than analysis of patches worn by the workers. Exposure was highest in mixers who handled the spray concentrate and in sprayers using backpack equipment. Some absorption of 2,4,5-T was apparently due to wearing contaminated clothing by field workers.

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5,T) has received considerable public attention in recent years. Concern that it might cause birth defects, or miscarriages, or even cancer, resulted in numerous investigations during the past decade (Table I). In April 1978 the U.S. Environmental Protection Agency (EPA) issued a Rebuttable Presumption Against Registration (RPAR) for all uses of 2,4,5-T (1). In February 1979 EPA issued a temporary suspension of uses in forestry, rights-of-way and pastures (2). Uses in rice and rangeland were not affected. Hearings began in March 1980 to evaluate all available data on potential risks, and to balance these risks against benefits from each use. No decision had been reached when this review was completed in August 1981.

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Table I

Chronology of Major Events in the 2,4,5-T Issue

-
- 1970 - USDA cancels or suspends uses around homes, in recreation areas, in aquatic sites, and in food crops.
- 1970 - Dow appeals cancellation of its use in rice.
- 1974 - EPA withdraws from cancellation hearings pending completion of additional studies.
- 1978 - EPA issues Rebuttable Presumption Against Registration for all uses of 2,4,5-T.
- 1979 - EPA temporarily suspends uses in forestry, rights-of-way, and pastures.
- 1980 - Cancellation hearings begin in March, to review all available evidence on risk, then to weigh benefits vs. risks for each use of 2,4,5-T.
- 1981 - Decision?
-

It should be noted that hazard or risk is a function of exposure as well as of toxicity. Thus, an important aspect of this continued debate on 2,4,5-T is the need for representative data on real exposure in humans, not only for applicators of the herbicide but for the populace at large. EPA's RPAR Position Document 1 (1) included estimates of exposure to 2,4,5-T based on direct measurements of external contamination by other pesticides applied with equipment similar to that used for 2,4,5-T. However, in most cases, application conditions were quite different from those used for 2,4,5-T.

The response submitted by The Dow Chemical Company included a more realistic evaluation of human exposure to 2,4,5-T (3). This evaluation relied on data from several studies in humans which showed that 2,4,5-T was rapidly excreted in the urine with nearly complete clearance from the body within less than a week after exposure. Based on these data, the dose received by a person using a backpack sprayer was estimated to be less than 0.1 milligram per kilogram of body weight per day of exposure spraying brush. This estimate was subsequently confirmed by an extensive study conducted in forest workers applying 2,4,5-T under field conditions in Arkansas.

The following review summarizes the findings in all reported studies on excretion of 2,4,5-T by humans given oral doses, or exposed to the herbicide under normal field conditions, including careless work habits.

Oral Administration Studies in Humans

Numerous studies on the metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D) and related herbicides in animals have shown that these chemicals are absorbed and distributed rapidly in the body, and are excreted, undegraded, relatively quantitatively in the urine within a week after administration (4). Pharmacokinetic studies with 2,4,5-T in rats and dogs (5) and in humans (6) supported these findings, and demonstrated that rates of clearance from plasma and elimination in urine depend on dosage level, animal species, and chemical structure of the phenoxy acid being studied (4). Corresponding chlorinated phenol metabolites were detected only in ruminants (4) or in trace amounts in urine of rats fed very high doses of phenoxy herbicides (7).

In a study by Gehring *et al.* with 2,4,5-T in humans (6), five male volunteers received a single oral dose of 5 mg/kg body weight. Plasma levels attained a peak of about 60 µg/ml within about 2 hours, and decreased rapidly with a half-life of about 23 hours. Urinary excretion was rapid with a diurnal fluctuation, and a total of about 90% of the dose was recovered, largely as free 2,4,5-T, within 4 days after administration. About 70% of each day's excretion was accounted for in the 12-hour daytime sample (Table II).

Similar results were obtained in two studies reported by Sauerhoff *et al.* in which 2,4-D was given at 5 mg/kg as a single oral dose in five males (8), and silvex [2-(2,4,5-trichlorophenoxy)propionic acid] was given at 1 mg/kg as a single oral dose in seven males and one female (9).

In a study by Matsumura with 2,4,5-T in Japanese volunteers (10), a peak plasma level of 21.1 µg/ml was reached at 4 hours after ingestion of a single dose of 150 mg by a male weighing 68 kg (i.e. 2.2 mg/kg). More than 80% of the administered 2,4,5-T was recovered in the urine within 3 days after a dose of 100 mg in two volunteers weighing 68 and 53 kg (1.5 and 1.9 mg/kg, respectively). About 45% of the administered 2,4,5-T was recovered in the first 24-hour urine collection compared to the average 38% recovered in the Gehring study in humans given 2,4,5-T at 5 mg/kg (6).

The fate of phenoxy herbicides in humans has also been studied by Kohli in India. When 2,4-D was given as a single oral dose at 5 mg/kg in six male volunteers, 75% of the administered dose was recovered unchanged in the urine within 96 hours after administration (11). When 2,4,5-T was given to a total of eight male volunteers as a single oral dose at 2, 3, or 5 mg/kg of body weight, the half-life for clearance of 2,4,5-T from the plasma was about 19 hours (12) compared to 23 hours in the Gehring study (6). Of the total 2,4,5-T recovered in 96 hours, nearly 80% was excreted in the first 48 hours, as in the Gehring study.

Table II

Excretion of 2,4,5-T in Urine after Single Oral Dose of 5 mg/kg Body Weight in Five Male Volunteers (6).

Interval After Ingestion		Incremental % of Dose Excreted	Cumulative % of Dose Excreted	Fraction in Daytime Collection ²
(hr)	(day)			
0-12	1	26.8	38.1 ¹	0.70
12-24		14.1		
24-36	2	20.7	67.5	0.70
36-48		8.7		
48-60	3	9.9	81.8	0.69
60-72		4.4		
72-84	4	4.8	88.4	0.72
84-96		1.8		
				av. 0.70

¹One subject pooled the 0-12 and 12-24 specimens so the mean excretion for day 1 is not the sum of the mean excretions for 0-12 and 12-24 hours.

²Calculated from $26.8/38.1 = 0.70$; $20.7/29.4 = 0.70$; $9.9/14.3 = 0.69$; $4.8/6.6 = 0.72$.

Although considerable variation was noted among individuals in each study, particularly in those conducted by Kohli, the overall agreement is very good. As summarized in Table III, 26 to 57% of the 2,4,5-T was recovered in urine collected the first day after administration of a single oral dose, compared to the 50% predicted by pharmacokinetic modeling (6).

Dermal Application Studies

In a study by Feldmann and Maibach (13), only about 6% of the 2,4-D applied to the forearm of human volunteers was recovered in urine collected during a week after exposure. Also, the rate of excretion was slower after dermal exposure than after intravenous injection (13), or after oral administration (8) of 2,4-D in humans.

Table III

Summary of Excretion of 2,4,5-T by Humans
After Administration of Single Oral Dose

Reported by	Dosage mg/kg	Number of Subjects	Mean % of Dose in Urine			
			Day 1	Day 2	Day 3	Day 4
Gehring (6)	5	5	38	29	14	7
Matsumura (10)	1.5	1	45	30	5	
	1.9	1	45	30	5	
Kohli (12)	2	1	26	22	18	7
	3	1	57	16	3	3
	5	6	27	23	10	4
Calculated ¹			50	25	13	6

¹Approximate values assuming instant absorption of the total dose and a half-life of 1 day.

Similarly, a slower rate of excretion was noted after application of 2,4,5-T esters to the shaved skin of rats than after oral administration in this species (Ramsey, unpublished data). The rate controlling step was shown to be absorption of the ester through the skin. Once inside the body, the esters were rapidly hydrolyzed to the acid, and the acid was excreted at the same rate as following oral administration of 2,4,5-T in rats. (29)

A controlled dermal exposure study was conducted recently at Oregon State University in which 2,4,5-T ester formulation was applied to the skin of humans (14). Four concentrations of 2,4,5-T ester emulsion were applied to the point of runoff on bleached denim patches, 900 square centimeters in area. The patches were then held in close contact with the skin on the upper thigh of four volunteers, including one woman. The patches were removed after 2 hours and total urine was collected for 5 days. As shown in Table IV, less than 0.5% of the applied 2,4,5-T was absorbed from the cloth, even when soaked with concentrated spray solutions.

Studies in 2,4,5-T Applicators

All available studies on exposure to 2,4,5-T in applicators are reviewed herein, with special emphasis on the "state of the art" studies by Lavy in forest workers under normal work conditions in Arkansas.

Table IV

Controlled Exposure Study in Human Volunteers
Wearing 900 cm² patch for 2 Hours (adapted
from Newton and Norris, 14).

Pounds 2,4,5-T per 100 gal ¹	Total mg 2,4,5-T Excreted in 5 Days ²	Estimated Total mg 2,4,5-T Absorbed ³	mg 2,4,5-T Absorbed, per kg bw ⁴ per cm ² per hr
2	0.38	0.44	0.000003
4	0.72	0.84	0.000007
16	0.66	1.14	0.000009
32	1.38	2.34	0.000011

¹Patches soaked with 40 ml of solution containing 2.4, 4.8, 19.2, and 38.4 grams of 2,4,5-T acid equivalent per liter, amounting to about 100, 200, 800 and 1600 mg per patch.

²Urine collection began 24 hours before application of treated patch and continued for a total of 5 days.

³Corrected for incomplete excretion in 4 days after exposure.

⁴For 70 kg person.

Dow Study. In a review of direct and indirect measurements of exposure in applicators, Leng (3) reported an unpublished early study by Dow in which 2,4,5-T exposure was measured in eight field personnel and two observers. Direct measurements of exposure were based on analyses of air samples and of patches simulating skin exposure. Indirect measurements of actual dose received were based on analyses of blood samples and 24-hour urine collections from these individuals.

The workers were applying a low-volatile 2,4,5-T butoxy-ethanol ester formulation diluted with fuel oil at 3 gal/100 gal (2% 2,4,5-T acid equivalent). The spray was applied selectively to the lower 3 to 4 feet of trees and brush in a utility right-of-way using hand-pressurized backpack sprayers (2.5 gal capacity). The applicators wore short-sleeved shirts open at the neck, long pants, no gloves and no hat. One of the backpacks leaked as evidenced by soaking of the applicator's clothing at the lower back.

Table V summarizes the results of analyses for 2,4,5-T in the 24-hour urine samples. Urinary levels ranged from <0.01 ppm for an observer to 17 ppm in urine collected from applicator 4 who was wearing the leaking backpack sprayer. Proper maintenance of equipment and better personal hygiene probably could have prevented the relatively high exposure experienced by this individual, estimated as a dose of about 0.2 mg/kg of body weight.

Table V

2,4,5-T in Urine of Spray Applicators:
Backpack Crew, 24 Hour Collection (3).

Subject	Occupation	kg bw ¹	2,4,5-T in Urine		mg 2,4,5-T per kg bw ²
			ppm	mg/24 hr	
1	Applicator	73	0.85	1.45	0.02
2	Applicator	73	4.30	7.06	0.10
3	Applicator	59	3.00	3.58	0.06
4	Applicator	68	17.00	13.18	0.20 ³
5	Foreman	77	0.75	1.25	0.02
6	Foreman	87	3.80	4.79	0.06
7	Foreman	70	0.07	0.09	0.001
8	Gen. Foreman	78	0.03	0.08	0.001
9	Ind. Hygienist	75	0.02	0.03	0.0004
10	Clin. Chemist	73	<0.01	<0.03	<0.0004

¹Kilograms of body weight for each subject.

²Calculated from the urinary levels using the pharmacokinetic model developed from an oral study in humans (6), assuming that repeated daily exposure had resulted in a steady state body burden of 2,4,5-T.

³Subject 4 wore a leaking backpack sprayer which soaked his shirt and the small of his back.

EPA Study. The first published report of studies on 2,4,5-T applicators was by Shafik *et al.* of EPA in 1971 (7). They analyzed urine samples collected from people occupationally exposed to 2,4-D and 2,4,5-T, and reported higher exposure in spray operators than in those who had less direct contact with the herbicides.

According to later recollection by observers in the EPA study as reported by Leng (3), the study with 2,4,5-T had been conducted in Arkansas in 1970 on workers applying a low volatile 2,4,5-T propylene glycol butyl ether ester formulation diluted with diesel oil at 2 gal/40 gal (about 3% 2,4,5-T acid equivalent). The spray crew consisted of a foreman and three applicators who used knapsack equipment to spray around the base of individual trees and up to 2 feet on the trunks. According to an observer, they wore jeans and gloves but little care was taken to avoid skin contact.

A total of eight urine samples were collected, one from each individual on the afternoon after spraying since 7 a.m. that morning, and one in the early morning the following day. The 2,4,5-T found in the urine samples ranged from 0.5 ppm to 3.6 ppm. Assuming a steady state body burden and an average 2.0 ppm 2,4,5-T in 1500 ml of urine per day, the estimated daily excretion in these applicators was 3 mg of 2,4,5-T. For workers weighing 75 kg, this corresponds to an effective dose of about 0.04 mg/kg which is similar to the doses observed in the Dow study.

Lavy Studies. The first study was in Arkansas in 1978 using 2,4,5-T under field conditions for forestry applications in that area. Complete details of the study were reported to EPA in February 1979 (15) along with kinetic analyses of the data by Ramsey *et al.* (16).

The study involved monitoring a total of 21 crew members applying 2,4,5-T with four types of equipment (Table VI). Two applications were made 1 or 2 weeks apart, and exposures were reported separately. One backpack sprayer did not take part in the second phase, making a total of 41 exposures.

Table VI

2,4,5-T Study in Forest Workers, 1978:
Total Urine Collection for One Week (Lavy, 15, 19).

<u>Type of Application</u>	<u>No. of Subjects</u>
Backpack spraying	7
Tractor driven mistblower	4
Helicopter using	
- microfoil boom	5
- raindrop nozzles	5
<u>Total:</u> 21 subjects, 41 exposures ¹ .	

¹One member of the backpack crew did not take part during the second spray period.

Monitoring during the application included measuring the 2,4,5-T level in air and on patches fastened to the clothing of the workers. Total urine collections were made over 12-hour periods before, during, and for up to 5 days after each exposure, and each sample was analyzed separately for 2,4,5-T.

The product used was a low-volatile 2,4,5-T propylene glycol butyl ether ester formulation containing 0.04 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It was applied as an emulsion in water at a rate of 1.6 pounds of 2,4,5-T acid equivalent per acre (lb/A) in the backpack study, and 2 lb/A in the other studies. These were low-volume applications at 10 gal/A in the ground studies and 5 gal/A by air. Thus, the workers in the aerial studies were exposed to about 5% 2,4,5-T spray solutions compared to the 2 or 3% used in the earlier studies by Dow and EPA described above (3, 7).

The four crews were selected from a group who normally did this type of work in that area of Arkansas. They were instructed to perform their routine duties in their usual manner with as little influence as possible from the test situations. Prior to the spray program each worker filled out a form which provided personal information regarding his or her vital statistics and history of any previous involvements with 2,4,5-T. Workers were selected who indicated they had not worked with 2,4,5-T for 2 weeks prior to the study (15).

For the most part, the workers did not wear gloves or special protective clothing. Typical attire for members of the spray crews included long trousers, long or short sleeved shirt, and cloth sneakers, leather shoes or field boots. All wore hats except four members of the backpack crew.

Each crew member wore six patches consisting of 10 cm x 10 cm squares of gauze, backed by filter paper, fastened to clothing on the chest, back, upper arms, and upper thighs. In addition, each worker wore a portable air pump which drew a known volume of air through a column of XAD-2 resin which trapped the 2,4,5-T from the air.

Total urine collection began the day prior to spraying (day 0), and was continued the day of spraying (day 1), and for at least 4 or 5 days following each of two spray operations 1 or 2 weeks apart. The total urine excreted by each worker throughout the entire test period was collected for 12-hour periods and kept refrigerated until analyzed. Analyses were conducted at Northrop Services, Inc. in Little Rock, Arkansas, using a modification of the gas chromatographic method of Nony *et al.* (17). The urine samples were hydrolyzed to release possible conjugates although 2,4,5-T has been shown to occur as the free acid in human urine (6, 17).

For most workers, the highest concentration of 2,4,5-T was in urine collected the first day after spraying. By the end of the second day, all but two workers had excreted more than 50% of the total amount they excreted in the 4 to 5 day post-spray

period. A diurnal variation was noted with more 2,4,5-T being excreted during daytime hours than at night. This was similar to the pattern noted following oral administration of 2,4,5-T in humans (6). A more regular pattern was obtained by adding the amounts excreted during each consecutive 24-hour period to give total 2,4,5-T excreted each day.

The apparent rate of excretion was slower after dermal exposure than after oral administration, probably due to slower absorption of the 2,4,5-T ester from the skin than 2,4,5-T acid from the gut. This is in agreement with observations made by Feldmann and Maibach for 2,4-D and other pesticides applied to the forearm of human volunteers (13). Calculations by Ramsey et al. using three methods showed that 97% of the 2,4,5-T absorbed by forest workers would be excreted in urine within 7 days following dermal exposure under typical field conditions (16).

The study demonstrated the variability in exposure of the 21 crew members depending on the job being performed and on the care taken by some individuals to avoid exposure. Tables VII through X report the apparent total dose absorbed by each test subject, based on the total 2,4,5-T recovered in urine during each test period divided by the person's body weight. Figures 1 through 4 illustrate the excretion pattern for those with the highest apparent exposure.

The backpack crew was made up of seven members, including two couples working as pairs in which the female sprayers weighed about 50 kg (110 lb). The total absorbed dose of 2,4,5-T ranged from 0.01 mg/kg in the mixer-supervisor to around 0.09 mg/kg for the first exposure in each of three sprayers (Table VII).

Table VII

Total 2,4,5-T Excreted in Urine per Exposure
in a Backpack Crew (adapted from Lavy, 15, 19).¹

Subject	Sex	kg bw	Job	mg 2,4,5-T per kg bw ¹
1	M	72.6	Mixer/Supervisor	0.011, 0.012
2	M	68.1	Sprayer	0.086, 0.074
3	F	49.9	Sprayer	0.087, 0.053
4	M	95.3	Sprayer	0.030, 0.033
5	F	52.2	Sprayer	0.031, 0.017
6	M	65.8	Sprayer	0.038, -----
7	M	74.9	Sprayer	0.093, 0.037

¹Based on excretion on day 1 of exposure and 5 days after the first exposure and for 4 days after the second exposure, excluding excretion on day 0 before each exposure.

The patterns of excretion were similar for the two people in each couple (Figure 1), with the lower dose in the female, even on a body weight basis. The second pair received somewhat less than the first pair, possibly due to more careful work habits. Wearing of contaminated clothing between sprayings might account for the increased excretion observed in these two male workers (Nos. 2 and 4) at the end of the first spray period.

Figure 2 gives the excretion pattern for the other two male backpack sprayers, one of whom did not take part in the study during the second test period.

Exposure in the mistblower study was somewhat less than in the backpack study. The highest apparent dose was about 0.08 mg/kg in the mixer (No. 11) during the first test period (Table VIII). The values shown in the last column of Table VIII were calculated by Ramsey *et al.* (16), and represent the maximum dose absorbed by each worker in the two sprayings 2 weeks apart. Figure 3 reveals that the mistblower driver (No. 10) was being exposed at a constant rate throughout the test period, possibly from wearing contaminated clothing.

Table VIII

Total 2,4,5-T Excreted in Urine, and Calculated Dose per Exposure in a Crew Using a Tractor Mounted Mistblower (adapted from Lavy 15, 19, and Ramsey *et al.* 16).

Subject	Sex	kg bw	Job	mg 2,4,5-T per kg bw	
				Measured	Calculated ¹
8	M	95.3	Supervisor	0.029	0.032
				0.008	0.012
9	M	84.0	Driver	0.042	0.046
				0.032	0.037
10	M	106.7	Driver	0.033	0.041
				0.040	0.054
11	M	79.5	Mixer	0.078	0.086
				0.043	0.053

¹Maximum value for total absorbed dose for each individual calculated by three methods based on the pharmacokinetic model for clearance after dermal exposure to 2,4,5-T (16).

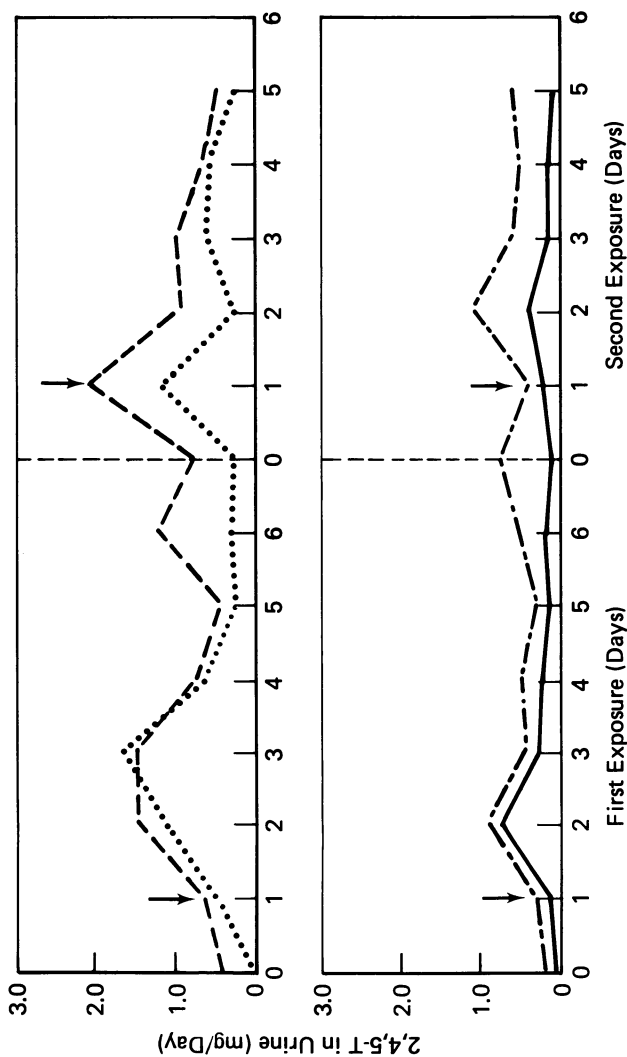


Figure 1. Excretion of 2,4,5-T in two couples applying the herbicide with backpack sprayers (arrows indicate application dates). Key: Couple 1: ---, male (2); ···, female (3); Couple 2: —, male (4); —, female (5).

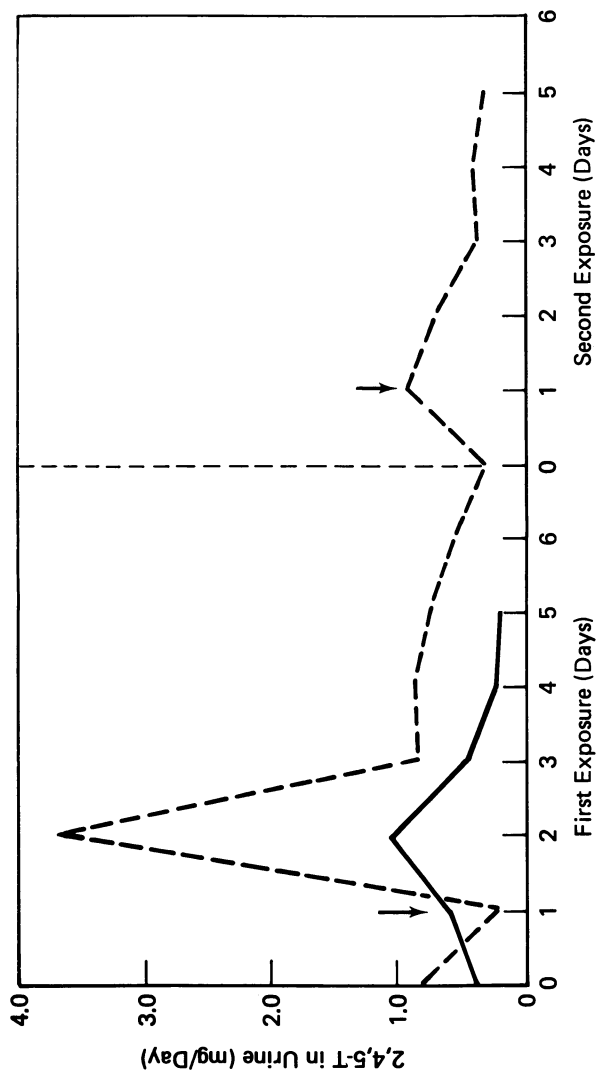


Figure 2. Excretion of 2,4,5-T in two men applying the herbicide with backpack sprayers (arrows indicate application dates). Key: —, male (6); - - -, male (7).

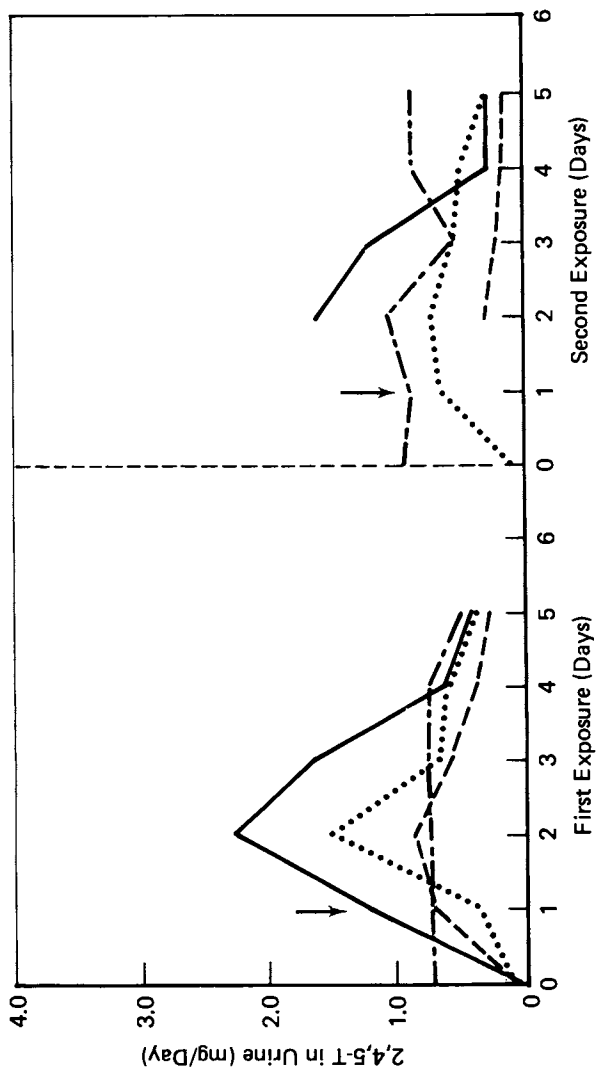


Figure 3. Excretion of 2,4,5-T in four men applying the herbicide with a tractor drawn mistblower (second application was 2 weeks later). Key: —, supervisor (8); ···, driver (9); ---, driver (10); — · —, mixer (11).

Values obtained for helicopter crews are presented in Tables IX and X for applications with a microfoil boom and raindrop nozzles, respectively. Flagmen in both studies excreted only 0.001-0.003 mg/kg indicating they received very little exposure even though they were standing under the spray path during repeated passes by the helicopter. The pilot in the raindrop nozzle study (No. 17, Table X) received a higher dose than the pilot in the microfoil study (No. 12, Table IX), possibly because he routinely checked and unplugged nozzles with his fingers at each fill-up time, and also helped to change the spray boom on the helicopter before and after each spray period.

Table IX

Total 2,4,5-T Excreted in Weekly Urine per Exposure
in a Helicopter Crew Using a Microfoil Boom
(adapted from Lavy, 15, 19).

<u>Subject</u>	<u>Sex</u>	<u>kg bw</u>	<u>Job</u>	<u>mg 2,4,5-T per kg bw</u>
12	M	95.3	Pilot	0.001, 0.011
13	M	109.0	Mixer	0.075, 0.061
14	M	84.0	Supervisor	0.004, 0.004
15	M	61.3	Flagman	0.001, 0.001
16	M	74.9	Flagman	0.002, 0.001

Table X

Total 2,4,5-T Excreted in Weekly Urine per Exposure
in Helicopter Crew Using Raindrop Nozzles
(adapted from Lavy, 15, 19).

<u>Subject</u>	<u>Sex</u>	<u>kg bw</u>	<u>Job</u>	<u>mg 2,4,5-T per kg bw</u>
17	M	72.6	Pilot	0.034, 0.039
18	M	86.3	Mixer	0.068, 0.127
19	M	81.7	Supervisor	0.007, 0.001
20	M	86.3	Flagman	0.003, 0.001
21	M	95.3	Flagman	0.001, 0.001

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Figure 4 illustrates the discrepancy in doses received by mixers. Mixer No. 1 (backpack crew) had very low levels of 2,4,5-T in his urine, possibly due to the fact that he wore gloves and was cautious in his work habits. Mixer No. 13 (microfoil study) had relatively high levels of 2,4,5-T in his urine on day 0 before each spraying, indicating he might have been wearing contaminated clothing. Mixer No. 18 (raindrop nozzle study) exhibited a much higher peak urinary level in the second test, and also received almost twice the dose (0.127 mg/kg) than he did in the first test (0.68 mg/kg), or than mixer No. 13 in both microfoil boom tests (0.075 and 0.061 mg/kg). These data support an observation made years ago by Wolfe (18) that the likelihood of serious contamination is greatest during handling of the pesticide concentrate and could be largely avoided by wearing impermeable gloves during mixing.

A summary is presented in Table XI showing the total 2,4,5-T excreted by the 21 forest workers in two exposures under typical field conditions. The highest dose was received by mixers who handled the herbicide concentrate, followed by applicators using backpack sprayers, spray tractor drivers, helicopter

Table XI

Summary of Total 2,4,5-T Measured in Urine of Spray Crews per Exposure Under Field Conditions in Forests in Arkansas (adapted from Lavy, 15, 19).

<u>Job Description</u>	<u>Number of Subjects</u>	<u>Range of mg 2,4,5-T per kg bw¹</u>
Mixers	4	0.012 - 0.138
Backpack Sprayers	6	0.019 - 0.104
Spray Tractor Drivers	2	0.033 - 0.049
Helicopter Pilots	2	<0.001 - 0.044
Supervisors	3	0.002 - 0.030
Flagmen	4	<0.001 - 0.003

¹Includes 2,4,5-T excreted on day 0 before each spraying due to wearing contaminated clothing or exposure of fingers during mixing of spray, and 2,4,5-T excreted on day 6 after first spraying (i.e. on day 0 of second spraying).

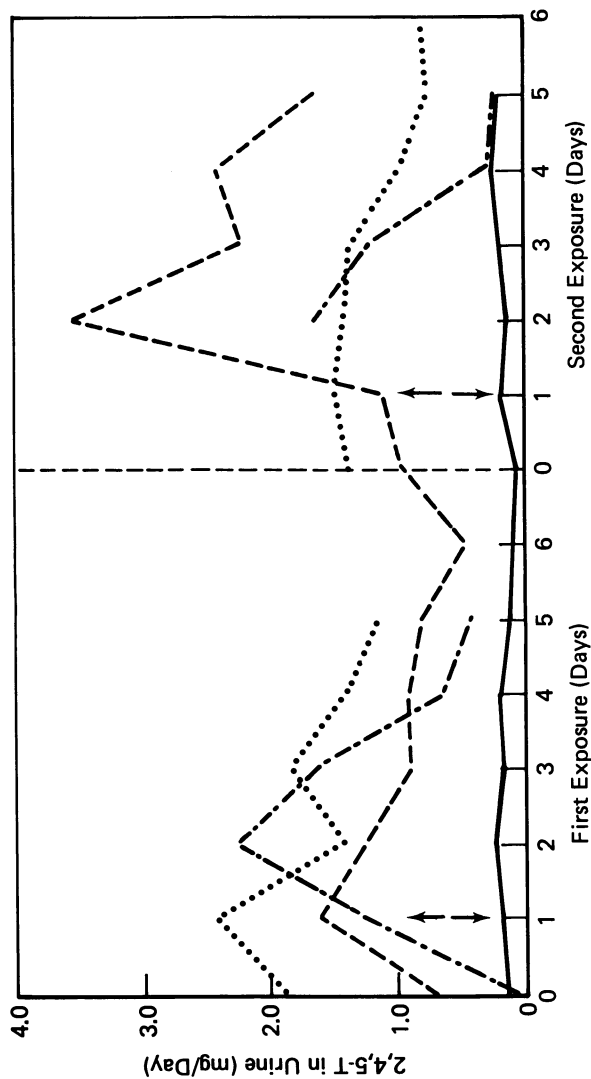


Figure 4. Excretion of 2,4,5-T by mixers in four crews applying the herbicide with different equipment. Mixer No. 1 (—) wore gloves and was cautious in his work habits. Key: ····, raindrop nozzle (18); ····, microfoil boom (13); - - - -, mist blower (11); ———, back-packer (1).

pilots, supervisors, and flagmen. The pattern of excretion for some of the workers indicated they wore contaminated clothing or were otherwise exposed prior to and between the two spray periods. Exposure would undoubtedly have been less if the workers had worn protective clothing such as better footwear, long sleeves, and gloves, and had practiced good work habits like mixer No. 1.

A subsequent study by Lavy *et al.* (20) demonstrated the effectiveness of wearing protective clothing in decreasing exposure to 2,4-D in spray crews during aerial applications in forests in the Pacific Northwest. Each of three crews, made up of six members per crew, were engaged in two helicopter applications of low volatile ester formulations. During one treatment the workers wore normal clothing and followed their own individual work habits and precautions during the spray operation. In the second treatment the same crews wore protective clothing and were supervised in their work to maintain more stringent protection from exposure to the herbicide. Comparisons of the two treatments indicated that, although low levels of exposure did occur, less exposure was evident when precautions were taken.

Data from analyses of air samples in Lavy's 2,4,5-T study (15, 19) and 2,4-D study (20) indicate that exposure via inhalation was insignificant during application of low volatile ester formulations of these herbicides.

Data obtained by direct measurements of contamination of patches worn by workers have been used for estimating exposure to pesticide sprays (1). However, such data are of limited value for predicting exposure to phenoxy herbicides applied as emulsions (3). For example, in Lavy's study on 2,4,5-T in forest workers (15, 19), the total exposed skin area was estimated for each worker according to the method of Wolfe (21), using photographs taken at the spray site. Total dermal exposure was based on the average amount of 2,4,5-T detected on the six patches worn by each crew member multiplied by the total estimated exposed skin area for that worker. As shown in Table XII, the observed dose (i.e. amount excreted in urine) was generally much lower than the estimated external dose. Also, patch data would not predict exposure from contamination of fingers while handling the concentrate during mixing of sprays.

The potential for exposure in bystanders has also been investigated. In a study by Lavy *et al.* (22) an individual wearing gauze patches stood directly under the spray path of a helicopter during application of 2,4,5-T at a rate of 2 lb/A. Based on finding an average 2 mg per 100 square centimeters of patch area, the external exposure calculated by Wolfe's method was 0.86 mg/kg for a person weighing 76 kg who was wearing shoes, short sleeves, long pants, and no hat. Although the urinary excretion of 2,4,5-T was not measured in this individual, flagmen exposed repeatedly during field applications of 2,4,5-T

Table XII

Apparent External Exposure Compared to
Actual Dose of 2,4,5-T Absorbed by Forest Workers
(adapted from Lavy *et al.* 19, 22).

<u>Job Description</u>	<u>Number of Exposures</u>	<u>mg 2,4,5-T per kg body weight</u>	
		<u>gauze patches¹</u>	<u>excreted in urine²</u>
Mixers	7	0.04 - 1.62	0.011 - 0.127
Backpack sprayers	11	0.20 - 2.54	0.017 - 0.093
Mistblower crew	5 ³	0.20 - 1.58	0.029 - 0.042
Helicopter crews	12 ⁴	nd - 0.02	<0.001 - 0.004
Pilots	4	nd - 0.11	<0.001 - 0.039

¹Based on average 2,4,5-T found on six patches per subject for each exposure, and estimated total skin area exposed on each subject as calculated by method of Wolfe *et al.* (21).

²Excluding amount found in urine on day 0 before spraying, possibly due to wearing contaminated clothing.

³Excluding mixer listed separately.

⁴Excluding mixers and pilots, listed separately.

excreted only <0.001 to 0.003 mg/kg (Table XI). This is a fraction of a percent of the exposure predicted by patch data, despite the fact that the flagmen were under the spray path for several passes of the helicopter.

Lavy also reported that no 2,4,5-T was detected on patches worn by an individual who walked through an area sprayed 2 hours earlier (22). Also, 84% of the 2,4,5-T applied by helicopter was found within 80 feet of the spray path and 99% of the spray was delivered within 160 feet of the spray path (22). Thus persons near or entering sprayed areas shortly after application are not likely to receive measurable exposure to 2,4,5-T.

Swedish Study. Occupational exposure to 2,4-D and 2,4,5-T was also studied in a forest situation in Sweden (23). The materials used were butoxy ethyl ester formulations of 2,4-D or of a 2:1 2,4-D/2,4,5-T mixture applied as a 2% emulsion in water using tractor drawn equipment. The spray was applied at a rate of 2 to 3 kilograms total active ingredient per hectare (1.8 to 2.7 lb/A) to a distance of about 20 meters from both sides of the tractor. Two areas of about 70 to 80 hectares were sprayed during the test week. The temperature was about 20°C from Monday through Thursday with little wind, and was 10-15°C on Friday with sporadic wind.

Four sprayers took part in the study, working in pairs of two, one driving the tractor and the other walking ahead to mark the areas to be sprayed. One tractor had a cab with a windshield but was open on the left side. The other tractor was completely open and both men got sprayed when the tractor turned.

Three of the four men took part in preparing the sprays from the herbicide concentrates. They wore jeans and shirts, with rubber boots or leather shoes and leggings. However, they had bad work habits, contrary to advice and recommendations outlined in the publication "Pesticides" published by the Council of Europe (24). They wore the same clothes all week as they had worn the week before doing the same work. Two of them lived in a house trailer in the spray area and took their rest periods in the trailer where the odor of the herbicide was noted. All were smokers and smoked during the day without washing their hands.

Monitoring consisted of measuring the 2,4-D and 2,4,5-T in the air during spraying, and in blood and urine of the workers during the 5-day work week and for 36 hours afterwards. The levels found in urine are shown in Table XIII, compiled from data given in the Swedish paper. In the first pair, urinary levels ranged from 1.0 to 6.3 ppm 2,4-D, and from 0.4 to 1.2 ppm 2,4,5-T during the whole week, with little difference between the two workers. Generally higher levels were found in the second team, peaking at 11.4 ppm 2,4,5-T in the urine of one worker on Tuesday morning, and 14.0 ppm 2,4-D in the urine of the other worker on Saturday evening.

The estimated mean 24-hour excretion in the Swedish study was 12 mg 2,4-D and 7 mg 2,4,5-T, amounting to 0.16 and 0.09 mg/kg in workers weighing 75 kg. In a later experiment, the authors calculated that exposure ranged from 0.10 to 0.60 mg/hr for 2,4-D and 0.03 to 0.07 mg/hr for 2,4,5-T in the four workers, based on 3 to 4 hours spent spraying each day during the week. The highest levels found in urine were lower than the 17.0 ppm found in the early Dow study in which one of the workers had a leaking backpack sprayer and whose estimated exposure was only about 0.2 mg/kg of body weight (Table V). Thus, despite poor work habits, the doses of 2,4-D and 2,4,5-T received in the Swedish study (23) were comparable to those received by the backpack sprayers in the studies done by Dow (3) and by Lavy in Arkansas (15, 19).

Table XIII

Levels of 2,4-D and 2,4,5-T Found in Urine
of Swedish Forest Workers
(adapted from Kolmodin-Hedman et al. 23).

Worker Herbicide ¹	Test 1				Test 2			
	KK		LJ		JG		LEO	
	D	T	D	T	D	T	D	T
Mon. pm	1.0	0.5	1.9	0.5	4.5	3.1	1.0	1.3
Tues. am					3.4	11.4	3.0	4.9
pm	2.4	1.0	1.6	0.4				
Thurs. pm					3.4	6.5	1.9	3.7
Fri. am					4.1	4.2	2.2	2.3
pm	6.3	1.2	3.0	1.2	5.6	3.0	4.0	3.3
Sat. pm	3.6	0.9	3.1	0.9	6.4	2.7	14.0	4.3
Sun. am	2.1	0.4	3.1	1.0	3.4	2.2	5.3	3.5
pm	1.5	0.7	1.4	0.7	1.6	2.1	4.2	2.5

¹D = 2,4-D; T = 2,4,5-T, as ppm found in urine.

Toxicological Significance of Exposure to 2,4,5-T Sprays

Sporadic exposure resulting in doses of less than 0.1 mg/kg of body weight per day spent spraying 2,4,5-T would not appear to pose a significant risk to applicators of the herbicide, even if the 2,4,5-T contains trace amounts of the impurity 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Perspective on the potential risk can be gained by comparison of this estimated maximum dose (0.1 mg/kg) with doses which caused no effect in animals treated daily for extended periods.

Numerous studies have demonstrated that the no-effect-level for 2,4,5-T in pregnant rats and mice is about 20 mg/kg of body weight when given daily for 10 days during the critical stage of development of the fetuses (1). In Dow studies with specially purified 2,4,5-T, no effect was observed in rats fed 3 mg/kg of body weight per day continuously for 2 years (25), or over three generations of reproduction (26). In studies in Germany with

technical 2,4,5-T containing 0.05 ppm TCDD, the no-observed-effect level was 30 mg/kg/day in rats which received this dosage prior to conception, through pregnancy, and for 30 months after birth (27), or during three generations of reproduction (28). Thus, all no-effect dosage levels are considerably higher than the estimated maximum dose from exposure to 2,4,5-T in humans, including applicators and mixers.

Conclusions

1. The maximum exposure expected under relatively careless field conditions for use of 2,4,5-T would be less than 0.1 mg/kg of body weight per day spent spraying.
2. In the study in forest workers, the highest exposure was in mixers who handled the herbicide concentrate, followed by applicators using backpack sprayers, spray tractor drivers, helicopter pilots, and flagmen. (See Table XI.)
3. Exposure would be considerably reduced if the workers wore gloves, particularly for the mixers during preparation of the spray. (See Figure 4, mixer No. 1, solid line.)
4. Measurement of contamination of patches worn by field workers is of limited use for estimating actual exposure to herbicides such as 2,4,5-T. (See Table XII.)
5. Bystanders or persons entering treated areas shortly after spraying are not likely to receive measurable exposure; thus exposure in the general population is extremely unlikely.

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The Assessment of Potential Health Hazards to Orchardists Spraying Pesticides

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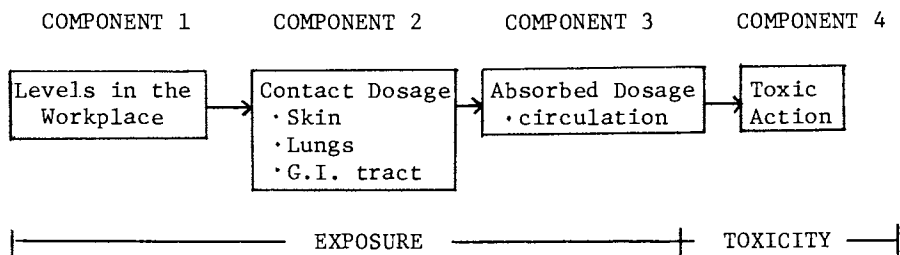
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The assessment of potential health hazards to workers resulting from the use of pesticides requires a knowledge of both the amount of pesticide to which a worker is exposed and its toxicity.

There are numerous variables which may affect the actual levels of pesticide in the workplace. One way to minimize their effect on the estimate of exposure would be to group the variables into subgroups or scenarios which describe a set of operational circumstances common to a specific use.

If field studies are conducted in which the variables are clearly delineated, it is possible that a model could be developed that would predict the maximum exposure under registered use conditions, and such a model would be of great value in the assessment of hazard to workers. This approach may be feasible for the orchard scenario because of the large number of studies that have been carried out. Some of the factors which should be taken into consideration in developing an exposure model and areas where there are insufficient data to make an accurate estimate will be discussed.

Hazard assessment can be subdivided into four major components whose interrelationships may be defined by the following diagram.



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For an orchard scenario, the three components contributing to exposure are:

Component 1: Levels in the Workplace

There are numerous variables that will have an effect on the actual amount of pesticide available for contact by the worker. Some of the variables such as type of equipment (high pressure, low pressure, tank size, nozzles, height of boom, spray volume), mode of application (aerial or ground application), kind of formulation (wetttable powder, granular, dust, emulsifiable concentrate, solvent soluble, etc.), volatility, type of activity (mixer, loader, flagger, etc.), duration of spraying and rate of application can be readily identified. There are many other variables such as ambient temperature, wind conditions and spills, which are more difficult to define and which may actually change during application. However, care should be taken when extrapolating data from exposure studies since some of these variables can have a profound effect on actual exposure.

In attempting to estimate the exposure of orchard workers, it could be presumed that, of the total amount sprayed, the amount that did not impinge upon the target or drift off would be available for contact on the worker. In a preliminary trial using air blast equipment, Chiba (1) found that 53% of the spray cloud hit the target, 13.5% fell to the ground and 33.5% remained in the air.

Using these preliminary data for an application rate of 454 g per 4047 m², the maximum potential amount available for contact would be:

$$0.335 \times \frac{454 \times 10^3}{4047} \text{ mg} \cdot \text{m}^{-2} = 37.6 \text{ mg} \cdot \text{m}^{-2} \quad (1)$$

The use of air sampling equipment would lead to a more realistic estimate of air concentration by taking into account the effects of multiple row treatment and the effect of drift. The use of the estimated value given above does not allow for these variables.

Component 2: Contact Dosage

All the pesticide that is in the workplace environment is potentially available for contact with the worker. The three major routes of exposure are dermal (percutaneous), inhalation and, to a much lesser extent, oral. The relative importance of each route will differ under various conditions, but the total body contact will be the sum of the amount of pesticide at each barrier (skin, lungs and G.I. tract).

Dermal Route. In orchard applications the dermal route of exposure has been established as the major one (2, 3). Durham

et al. (4) compared the dermal and respiratory routes and found that the former accounted for 87% of the total exposure.

The dermal exposure can be estimated by presuming that all of the pesticide in the air is available for contact. The total body surface has been estimated to be 2 m^2 (5), and under normal circumstances, approximately 15% of the body is uncovered (V of chest, back of neck, face, forearms, and hands).

Using the estimate of the amount of pesticide in the air (equation 1) and the total body surface area available for contact, the maximum potential contact for a 70 kg body weight (BW) would be:

$$2 \text{ m}^2 \times 0.15 \times 37.6 \text{ mg} \cdot \text{m}^{-2} \times \frac{1}{70} \text{ kg}^{-1} = 0.16 \text{ mg} \cdot \text{kg}^{-1} \text{ BW} \quad (2)$$

The total contact may be calculated by multiplying the value in equation 2 by the total kg of active ingredient applied.

Numerous methods have been utilized to actually measure the amount of pesticide which impinges on the skin. One of the more widely used ones involves attaching absorbent patches to various areas of the body and analyzing the patches for pesticide on termination of the spray operation. The concentration on the patch can then be extrapolated to the surface area of the body from which it was removed. The assumptions are that the pesticide will adhere to the patch in the same manner as it would to skin, that the distribution of the pesticide will be uniform over the area represented by the patch and that dermal contact occurs only on exposed skin surfaces and not on skin covered by clothing (6).

Wolfe et al. (2) tabulated the results of many different exposure studies and in those in which orchard air blast equipment had been used; the dermal contact exposure extrapolated from the patch data ranged from 0.66 to $2.08 \text{ mg} \cdot \text{kg}^{-1} \text{ body weight} \cdot \text{day}^{-1}$. In these studies the contact exposure was related to the time it took to apply the pesticide. However, there may be merit in relating the dermal contact to the amount of pesticide sprayed rather than the time to spray. The time required to spray one acre of orchard is influenced by the driving speed of the tractor, the row width and the row length of the trees. Given a driving speed of 1.5 mph and a row length of 200 feet, a decrease in the row width from 20 feet to 10 feet almost doubles the application time per acre (8). A priori, it seems reasonable to presume that the absolute maximum amount of pesticide that could impinge on the worker would be equivalent to the total amount that is applied. Then a worker who applies 454 g in one hour will be potentially exposed to the same amount of pesticide as a worker who applies 454 g in two hours.

In a study conducted in the Okanagan Valley in British Columbia (7) there were wide fluctuations in the number of kg of active ingredient applied in 1 hour by different orchardists as shown in the following table.

Table I. Variation in the amount of Guthion sprayed

Amount Sprayed (kg ai)	Duration (hrs)	Potential Exposure Rate (kg·hr ⁻¹)
1.1	2.5	0.44
1.1	3.0	0.47
1.4	3.5	0.40
1.4	4.8	0.29
1.4	5.0	0.28
1.7	4.5	0.38
1.7	5.0	0.34
1.7	6.0	0.28
2.0	5.0	0.40
2.3	2.8	0.82
2.6	8.0	0.31
2.7	3.5	0.77
2.8	4.5	0.62
3.4	3.5	0.97
3.4	7.0	0.49
4.5	4.0	1.12
4.5	9.0	0.50
4.8	6.5	0.74
Range 1.1-4.8	2.5-9.0	0.28-1.12

These differences in the amount of pesticide sprayed in a given time may be due to the roughness of terrain which would influence the tractor speed and other characteristics of the orchard.

Some of the assumptions that are made when using patches as indicators of dermal contact exposure may not be completely valid. The same study indicated that there were detectable levels of pesticide on skin that was covered by cotton clothing and also with rubberized protective clothing. Although some penetration may have occurred through the protective clothing, a more likely explanation is that the pesticide entered through gaps or openings in the clothing. Similar findings for workers exposed to foliar residues of organophosphorus compounds have been reported by Spear *et al.* (9), and Pependorf *et al.* (10), in which 25-47% of the pesticide on the outside of clothing reached the skin surface. When working with extremely toxic pesticides, even this small amount of material, which reaches the skin surface despite the protective clothing, may be toxicologically significant.

The reliability of patches as accurate indicators of dermal contact needs to be carefully studied. As with any method in which data have to be extrapolated, small deviations may result

in large errors. Thus, a splash of concentrate on a patch would lead to an erroneously high value when that patch data is adjusted to reflect the regional surface area. The opposite may be true as well, that a spill resulting in considerable dermal exposure might miss the patch and an unrealistically low exposure could be calculated. These types of possible errors may in fact be reflected in the wide variations seen in data from exposure studies. It would thus appear that more work should be done in developing new approaches, which would give more reliable estimates of dermal contact exposure.

Preliminary results using a fluorescent tracer, which was added to the spray tank at the same time as the pesticide (Guthion WP), indicated that the distribution of the tracer, and presumably the pesticide, was not uniform, emphasizing the difficulty in the placement of the patches (7). This tracer technique is currently being evaluated as a tool for quantitative exposure estimation. This could result in a more realistic measurement of pesticide contact on the skin and minimize the reliance on extrapolation from the patch data.

Inhalation Route - Estimation of Aerosol Contact. The inhalation route has been shown to be considerably less important than the dermal route in the exposure of orchard workers (4). This is somewhat surprising when one considers that air blast equipment, utilized in orchards, delivers a relatively small droplet spray cloud which one would expect to be readily respirable. The total mass of these droplets is small, however.

To estimate inhalation contact exposure, some assumptions must be made which err on the side of conservatism and which should be modified as more complete data become available. It is necessary to know the droplet size spectrum of the spray because the diameter of the droplet influences its movement down the respiratory system (11). The functional unit of the lung is the alveolus, which is the terminal branch in the system. It is presumed that pesticide particles which are soluble in respiratory tract fluid and are 5 μ or less in diameter will reach the alveolus where they will be readily absorbed through the cells of the alveolar membrane into the pulmonary capillary beds and hence into the circulatory system. A recent review by Lippmann *et al.* (12) discusses in depth the deposition, retention and clearance of inhaled particles.

There is information available on the droplet size generated from 3 of the more common nozzle types used on air blast equipment (13). The physical/chemical properties of the spray formulation and its end use dilution can have a marked influence on the droplet as it travels from the nozzle, resulting in a large decrease in diameter. There is presently inadequate information to enable accurate estimates of droplet size distribution at varying distances from the spray nozzle. These changes may not be important in estimating direct contact to the workers but become

so when estimating drift exposure. It should be borne in mind, however, that the total mass of these potentially respirable droplets is generally small relative to the total number.

A relatively accurate estimate can be made of inhalation contact exposure using the information on lung capacity and breathing rates (14). If it is assumed that an applicator occupies a space 2 metres square by 3 metres high, the immediate volume surrounding him would be 6000 litres. The concentration of pesticide that would be contained in this 6000 litres would be that which would impinge on the 2 square metres. For an application rate of 454 g.4047 m⁻² the concentration in the 6000 litres would be:

$$\frac{454 \times 10^3}{4047} \text{ mg} \cdot \text{m}^{-2} \times 2 \text{ m}^2 = 224 \text{ mg} \quad (3)$$

It has been shown that for air blast equipment using 3 types of nozzles, no droplets exist below 5 microns (13). If however we arbitrarily assume 0.5% for purposes of calculation, the respirable concentration would be:

$$224 \text{ mg} \times 0.005 = 1.12 \text{ mg} \quad (4)$$

Assuming 33.5% of the total spray cloud is in the air (1), the maximum amount available for inhalation becomes:

$$1.12 \text{ mg} \times 0.335 \text{ mg} = 0.38 \text{ mg} \quad (5)$$

Assuming a respiration rate of 29 L·min⁻¹, the inhalation exposure becomes:

$$29 \text{ L} \cdot \text{m}^{-1} \times \frac{0.38}{6000} \text{ mg} \cdot \text{L}^{-1} \times 60 \text{ min.} = 0.11 \text{ mg} \cdot \text{hr}^{-1} \quad (6)$$

Correcting for body weight, the inhalation exposure becomes:

$$0.11 \text{ mg} \cdot \text{hr}^{-1} \times \frac{1}{70} \text{ kg}^{-1} = 0.0016 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1} \quad (7)$$

Consideration should be given to the settling velocity of the droplets which is a combination of terminal velocity and transport velocity due to atmospheric turbulence. There are data to suggest that droplets 10 μ settle before the applicator comes into contact with any drift from the previous row sprayed (15).

Estimates of inhalation contact exposure can be made by sampling air in the breathing zone of the applicator. Filter pads attached to respirators can be presumed to trap the pesticide which would have otherwise been inhaled and they can be removed and analyzed. A second method is to use personal air sampling pumps in which air is drawn over a suitable absorbent material which can subsequently be analyzed for pesticide.

Using the air sampling pumps, the flow rate of air through the cartridge and the duration of the sample period are both related to the concentration in the cartridge as follows:

$$\frac{\text{weight of sample on cartridge (mg)}}{\text{flow rate (L}\cdot\text{min}^{-1}) \times \text{sampling time (min)}} = \text{mg}\cdot\text{L}^{-1} \quad (8)$$

The air concentration is frequently expressed in terms of $\text{mg}\cdot\text{m}^{-3}$ ($\text{m}^3 = 1000 \text{ L}$). The inhalation exposure is calculated by:

$$\text{respiration rate (L}\cdot\text{min}^{-1}) \times \text{air concentration (mg}\cdot\text{L}^{-1}) = \text{mg}\cdot\text{min}^{-1} \quad (9)$$

This value is dependent on both the total amount of pesticide applied and the spray time, as can be seen in the derivation of the equations. It does not differentiate between droplets that would be deeply inspired ($< 5\mu$) or those which would merely impinge upon the nasal pharyngeal passage and then be swallowed ($< 50\mu$ but $> 5\mu$).

In a study conducted on orchardists who applied Guthion at the rate of $568 \text{ g ai}\cdot 4047 \text{ m}^{-2}$, the mean air concentration based on seven samples was $0.05 \text{ mg}\cdot\text{m}^{-3}$ (range 0.02 to $0.11 \text{ mg}\cdot\text{m}^{-3}$) (7). This value falls in the middle of the range of 0.01 to $0.15 \text{ mg}\cdot\text{m}^{-3}$ reported by Wolfe *et al.* (3) for eleven different pesticides.

The inhalation exposure using the mean value of $0.05 \text{ mg}\cdot\text{m}^{-3}$ is:

$$\begin{aligned} 29 \text{ L}\cdot\text{min}^{-1} \times \frac{0.05}{1000} \text{ mg}\cdot\text{L}^{-1} &= 0.0014 \text{ mg}\cdot\text{min}^{-1} \\ &= 0.09 \text{ mg}\cdot\text{hr}^{-1} \end{aligned} \quad (10)$$

This estimate of inhalation exposure compares with the values reported by Wolfe *et al.* (3) which ranged from 0.02 to $0.26 \text{ mg}\cdot\text{hr}^{-1}$. This method does not differentiate with regards to droplet size. A proportion of pesticide droplets that are trapped on the cartridge are too large to be inhaled. However, any vapour that might be present would be absorbed on the cartridge, and an estimate based on droplet size includes the vapour component.

Inhalation Route - Estimation of Vapour Exposure. In a study of drift exposure following aerial application of an organophosphorus pesticide, Crabbe *et al.* (16) found that the vapour concentration in areas remote from the spray line increased gradually up to 10 hours after the spraying. Increasing temperature was undoubtedly the major explanation for this. Other factors such as volatility of the pesticide, windspeed and sorption properties of the target would also influence the actual vapour concentration on the target.

If the spraying occurs in the early morning or late afternoon when the air and surface temperatures are cooler, the vapour exposure would be less, and it is perceived that the vapour con-

tribution to the worker would be small relative to the aerosol concentration. The vapour portion becomes more important when assessing bystander exposure since there may be little or no aerosol involved depending on the distance from the spray line.

Oral Route. Detailed data are difficult to obtain to enable an accurate estimate of the potential oral exposure from inhalation of large droplets ($<50\mu$ but $>5\mu$) which are moved from the trachea to the hypopharynx and subsequently swallowed. It is also possible that poor hygiene may result in oral exposure, but under normal circumstances and with available information, this route would not be considered to make a significant contribution to the total contact.

Component 3: Absorbed Dosage

The three main barriers upon which pesticide will impinge are the skin, the lungs and the gastrointestinal tract. Each of these barriers possesses a unique structure and function which will influence the absorption of pesticide into the bloodstream. Although some product may exert local contact effects, it is usually only after they have been absorbed that they exert their toxic effects; therefore, it is important to understand the extent of the absorption.

Percutaneous Absorption. The skin is a complex organ consisting of a number of layers which are functionally unique. The stratum corneum consists of cells which have lost their nuclei and are continuously sloughed off. The outer surface of the stratum corneum is covered by sebum, a complex lipid substance with a high affinity for lipophilic substances. The thickness of this layer is quite variable depending on the particular area of the body, and it appears to be very important in the penetration of chemicals. The layer of cells beneath the stratum corneum is the Malpighian layer resting on the basal cell layer, which in turn borders the dermal-epidermal junction. The skin appendages such as the hair follicles, sebaceous glands and sweat glands are located in the dermal layer. The capillary beds also are found in the dermal layer, and it is here that the pesticide molecule would enter the bloodstream for distribution throughout the body. The appendages appear to have an important effect on percutaneous absorption, with the follicle rich areas such as the forehead exhibiting much greater penetration than the forearm (17).

Maibach and co-workers (18) have reported the percutaneous absorption of a number of pesticides using a technique they developed, in which ^{14}C labelled pesticide is applied to the skin, and the total urine output is collected until all the radioactivity has been excreted. These data are corrected for incomplete excretion by using the excretion data obtained following intravenous or intramuscular injection.

Once the percentage of pesticide that is absorbed and the dermal contact are known, the absorbed dosage can be calculated.

Using Guthion as the example, since it has been shown to be approximately 15% absorbed (18), the absorbed dermal dosage can be calculated using the estimate of dermal contact from equation 2 :

$$0.16 \text{ mg}\cdot\text{kg}^{-1} \times 0.15 = 0.024 \text{ mg}\cdot\text{kg}^{-1} \quad (11)$$

A better estimate might be achievable using dermal contact data actually measured using patches.

In addition to these calculated estimates of absorption, a specific estimate of absorbed dose can be made by measuring the metabolites of the pesticide in urine. For pesticides on which good data exist on metabolic excretion, it appears that this method is very sensitive. In a study conducted on orchardists (7), metabolites were detected in the urine samples of all workers, and a statistically significant correlation was found between the total 48 hour metabolite output and the total amount of pesticide sprayed. In contrast the same study indicated that the correlation between urinary output and the total spray time was not significant. This supports the point mentioned earlier that it seems reasonable to presume that exposure is related to the total amount available for contact, and that correlating exposure with the spray time may be misleading.

Alveolar Absorption. For droplets and particles which are soluble in respiratory tract fluid and are of an aerodynamic diameter that enables penetration to the alveolus, it is not unreasonable to presume that absorption may be relatively complete (100%) (12). Insoluble particles are handled in a very different manner and may be cleared as free particles or by transport within alveolar macrophages. Actual absorption measurements for specific products would of course enable a more accurate percentage to be applied.

Gastrointestinal Tract Absorption. The structure and function of this tract is varied and complex. The structure of the pesticide may be altered within the G.I. tract due to changes in pH in the stomach and intestine, or due to enzymatic action within the gut before it is absorbed into the lacteals and eventually into the hepatic portal system or lymphatic system.

Since the amount of exposure via this route is perceived to be small under normal circumstances it is probably not unrealistic to simply presume 100% absorption. This is not always the case and frequently there is information from metabolism studies which would indicate the actual percentage absorption.

Component 4: Toxicity

One principle that is central to the understanding of toxicology is the dose-response relationship, which implies that there is a threshold level below which no toxic effects are observed. This level can be approximated in studies in which animals are dosed with the pesticide; the maximum dose tested at which there are no detectable differences between treated and untreated control animals is called the no observed effect level (NOEL). The dosage slightly in excess of the NOEL at which toxic effects are observed is referred to as the lowest observed effect level (LOEL). These two dosages should be relatively close together in order to clearly define the threshold level.

There are numerous toxicity tests which must be conducted on a variety of animal species to establish the NOEL and to characterize the type of toxic effects exerted by the pesticide in question. The duration of exposure as well as the routes of exposure are important in assessing the effects, and therefore acute, short term and long term studies are conducted using oral, dermal and inhalation routes of exposure. However, a large volume of the toxicity testing on pesticides has been conducted using the oral route of exposure. One of the reasons for this may be that much of the emphasis in the past has been directed towards estimating the hazard resulting from residues of pesticides on food. Another reason is that it is generally held that the mechanism of the toxic response does not usually depend on the route of exposure. However, the dosage required to elicit a specific response may differ due to the variation in amount of the material which is absorbed. Since most of the predictive animal testing is generated using the oral route of exposure and most of the worker exposure is via the dermal route, care must be taken in extrapolating the data and correction for absorbed dosage becomes important.

Once the NOEL and the exposure to workers are known, the margin of safety (MOS) can be calculated. It then remains to be determined whether the MOS is adequate. Obviously the acceptability of a margin will depend upon the severity and reversibility of the toxic effect. Historically, a margin of 100-fold has been accepted for many toxic effects (19). This allows for a factor of 10 for extrapolation from animals to man, and a factor of 10 to allow for differences in sensitivity from one person to another. However much larger factors (up to 5000) have been used when the effects are more severe. A more complicated procedure is utilized when the product is a proven animal carcinogen. However, any method of risk analysis requires reliable assessment of both NOEL and exposure.

Summary

The importance of having good data on both exposure and toxicity in order to assess worker hazard has been discussed. It appears that the state of the art is not yet well enough defined to enable a definitive model to be constructed. However, this approach is useful in pointing to areas where more information is necessary to enable viable models to be developed.

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Protective Clothing Studies in the Field

An Alternative to Reentry

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Handling and application of pesticides in other than closed systems, results in exposure of the worker (1, 2, 3). Attendant with exposure, is the possibility of an intoxication resulting in morbidity or mortality.

A number of studies beginning shortly after 1945, addressed the route of exposure experienced by pesticide workers, particularly with organic pesticides (2-6). While both dermal and respiratory routes are possible it has been found that the principal route of exposure is dermal with the respiratory route generally many fold less. Dermal exposure cannot be equated with intoxication since the amount of chemical absorbed varies with the chemical, the carrier, and the proportion of the body surface exposed (7). However, prudence would indicate that appropriate protection against this exposure is highly desirable. Accordingly, attention has been given in many of the studies on worker exposure to the role of clothing that might afford such protection (8, 9, 3).

Although normal work clothing can provide some protection, assuming it covers a major portion of the body, several workers have found that the clothing may itself become contaminated and afford a continuing exposure (8, 10, 11). Rubberized or plastic clothing gives significantly more protection than usual textiles and has been used extensively for protective clothing (12, 5). However, even in this case, some permeation may occur (12).

Rubberized or plastic clothing though substantially better in reducing dermal exposure, may in hot climates, be quite uncomfortable. As a consequence, workers object to wearing such clothing, thus suffering a higher dermal exposure. Recently light weight disposable plastic suits reported to be much more comfortable, have been developed (5).

The work to be reported on here involves an approach of treating normal clothing in order to achieve a level of protection through repellency. It was reasoned that resin treated textile might be repellent to sprays, hence reducing dermal exposure. At the same time, it was hope that the woven textile might retain

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enough air exchange characteristics to be comfortable to wear in hot weather and particularly in subtropical and tropical regions, where both temperature and humidity may be high. Laboratory trials were undertaken to assess the capability of different textiles and weaves to afford protection and to determine whether some improvement in this protection, was possible through treatment with appropriate resin. The initial laboratory studies were sufficiently encouraging, that the Ministries of Health and Agriculture in El Salvador sponsored a field trial using cotton pickers as the subjects. Field studies gave very promising results indicating that even clean clothing afforded protection to these workers and that the treated clothing gave a very high degree of repellency.

Since earlier field studies in El Salvador had suggested significant worker protection against pesticide exposure by wearing fluoroaliphatic treated clothing, studies of repellency and penetrability of different fabrics and Scotchgard were measured in the laboratory. Additionally, field studies of pesticide penetrability were measured in ethion exposed workers in two orange groves in Central Florida.

Materials and Methods

Laboratory Studies. The objective of this portion of the study was to ascertain the repellency or penetrability of textiles untreated, and those treated with fluoroaliphatic resins. The cloth for the most part was that commonly used for clothing, particularly "work" clothes. Both cotton and a blend of polyester cotton (65/35) cloth of various weights and weaves were used. The 100% cotton cloths were denim, a coarse black cotton cloth from Pakistan, and single knit light weight cloth. The polyester cotton blend cloth used, was a light weight gingham and a denim.

Though several treatments to enhance repellency of cloth were evaluated, primary attention was given to treatment with fluoroaliphatic resins. These were applied from an aerosol can, spraying the cloth until wet as per directions and then allowing the solvent to dry off before using. Patches of approximately 10 by 20 cm were cut from the cloth to use for study.

Repellency Tests. To determine the repellency of treated cloth, equal size patches of treated and untreated cloth were suspended in a laboratory hood and given equally timed sprays of emulsified chemical. This treatment was reproducible to plus or minus 10% using the time application at a given pressure.

After the spray deposit had dried, cloth was extracted with solvent and after adjusting the volume, the chemical deposit on the cloth determined either by gas-liquid chromatography or spectrophotometric methods. The repellency of the treated cloth was calculated as the percent of the deposit found on the untreated cloth.

Tests of Penetrability. Treated and untreated cloth as described under Repellency, were used for these tests. In this case however, the test patch was backed by an alpha-cellulose pad (2-4, 6, 7, 13). The cloth with its alpha-cellulose pad was either suspended in the hood, or placed at a 30° working angle and sprayed with the appropriate chemical, in a timed spray application or treated with droplets applied by a micro syringe. After the cloth had dried, the cellulose patch was removed and extracted with solvent for determination of the chemical. Results were calculated, as percent of chemical penetrating the treated cloth compared to the amount penetrating the untreated cloth.

Chemicals Used. Chemicals used in this study were technical grade products or better, dissolved in xylene with 5% emulsifying agent. The chemicals used included an oil soluble red dye, Dinoseb (2-sec-butyl-4,6 dinitrophenol), pentachlorophenol (PCP), Isopropyl N-(3-chlorophenyl) carbamate (CIPC or Chlorpropham), Iso-octyl 2,4,5- trichlorophenoxy acetate (2,4,5-T), chlorpyrifos (0, 0-diethyl 0-(3,4,6-trichloro 2-pyridyl) - phosphorothioate), and Ronnel (0-dimethyl 0-2, 4,5-trichlorophenyl) phosphorothioate) for application as a spray. The emulsifiable concentrate was diluted in water comparable to 50 gallons of spray to contain the following amounts respectively: 2 lbs. for chlorpyrifos; 4 lbs. for 2,4,5-T; 3 lbs. CIPC and 2 lbs. for PCP.

Methods of Extraction and Analyses. Chlorpyrifos, Ronnel, chlorpropham and 2,4,5-T were extracted from the cloth or the cellulose pad with hexane. Pentachlorophenol residues were extracted with benzene. Pentachlorophenol was methylated with diazomethane prior to determination by gas chromatography while the other compounds were determined directly. Dinoseb, on the other hand was extracted with chloroform and partitioned into 2% Na₂ CO₃ solution. The yellow extract was analyzed colorimetrically using the method of Potter (14).

Determination by gas-liquid chromatography utilized the gas-chromatograph with Ni⁶³ electron capture detector. Column packing consisted of carbo-wax, 20m on 100-120 mesh chromosorb WHP. The gas flow rate was 20 ml per minute. Operating conditions for the gas liquid analyses, for the different compounds was as follows:

Table I

Operating Conditions for GLC Analyses of Different Compounds

Compound	Temperature	Column Dimensions
1. 2,4,5-T isooctyl ester	180° C	1½ ft' 1/8 inch
2. pentachlorophenol	140° C	1½ ft' 1/8 inch
3. chlorpropham	130° C	1½ 45' 1/8 inch
4. dursban	145° C	3 ft' 1/8 inch

Field Studies

Exposure assessment using alpha cellulose pads and DEP urine excretion were made on 8 workers applying ethion on a daily basis in two citrus groves in Orange County, Florida. The study format was divided into three phases:

Phase 1 - During this period the subjects were asked to wear their normal working clothes. Alpha cellulose patches were attached to the interior and exterior on opposing sides of the shirt. Timed urine voids were collected in the field.

Phase 2 - The same subjects were to repeat the first phase format in addition were requested to wear OSHA/NIOSH approved respirators for pesticide application during the mixing periods.

Phase 3 - The subjects were issued 100% cotton denim coveralls of uniform design and weight. These subjects were divided into two groups, one wearing Scotchgard treated uniforms and one wearing non-treated coveralls. During this study phase, respirators were not worn. As in the first and second phase, pads and urines were collected daily.

In the laboratory the alpha-cellulose pads were received in labelled 60 ml hexane washed jars containing 10 cc of methylene chloride. The outside or exterior patches were identified by the letter O after the pad number and the inside or interior pad by an I. These pads were stored at -4° until they were analyzed.

In order to compensate for varying work loads specific to each individual, ethion concentrations were expressed as μg ethion per 25 cm^2 per hour of exposure.

In order to maintain a constant error factor inside and outside pad pairs were analyzed on the same day. Blank analyses were run on each lot of solvent and pads to insure there were no interfering peaks. Each blank was concentrated from 50 ml to 1 ml prior to injection, thus insuring that the field samples which needed concentrating would be free of interfering peaks in the ethion position. No such peaks were found in any of the blanks.

Instrument and column conditions. A Tracor #222 gas chromatograph equipped with a flame photometric detector (FPD) operating in the phosphorus mode (526 mu) filter was used. The operating conditions of the flame photometric gas chromatographic analyses were as follows:

Column 6" x 1/4 glass, carbowax 4% OV-210 on Gas Chrom Q 100/120 mesh 190°C , nitrogen flow rate of 32 cc/min. detector temperature 200°C , inlet 230° hydrogen 60 ml/min, air 60 ml/min attenuator 103 x 8, recorder speed 1/4 inch/min.

An injection of 0.535 of ethion produced a peak of 115 mm.

Detector Sensitivity (ng) = 0.08_2

Limits of detectability $\mu\text{g}/25\text{ cm}^2 = 01016$

*Detector sensitivity was based on 15% scale deflection.

Timed urine voidings were collected and analyzed by the Sha-fik and Peoples modified method (15). Differences in urine were standardized to creatinine levels on the basis of the recommenda-

tions of Metzger et al. (12). The Rapid-Stat Kit was used for the quantitation using the colorimetric determination of creatinine in urine (16). During the third week the 100% cotton denim coveralls provided by the project were washed commercially six times to remove sizing.

Results

1. Laboratory Studies. - The initial experiments to determine the repellency of treated and untreated cloth involved the use of an emulsifiable concentrate of oil soluble red dye in xylene. A timed application of an emulsion was made to the cloth and following drying, the amount of dye deposited was determined by extraction and determination in a spectrophotometer. Portions of the patches of cloth were sprayed a second time, to determine whether or not there would be a build up of chemical. The results are presented in Table II.

Table II
Comparison of Deposition of Oil Soluble Dye Emulsion of Treated and Untreated Cloth
mg/cm² Deposited on Cloth After Both:

Cloth	One Spray		Two Sprays	
	T ^a	U ^o	T ^a	U ^o
Denim 100% Cotton	0.11	0.44	0.29	0.86
Coarse White 100% Cotton	0.28	0.52	0.40	0.76
Coarse Black 100% Cotton	0.25	0.64	0.57	0.80
Single Knit 100% Cotton	0.36	0.51	0.87	1.17
50/50 Polyester/Cotton, Gingham	0.14	0.19	0.22	0.42
50/50 Polyester/Cotton, Denim	0.20	0.45	0.43	0.77

T^a = treated with Scotchgard
U^b = untreated

It did indicate that the fluoroaliphatic resin treatment imparted considerable repellency to all of the cloths with the possible exception of the cotton polyester gingham. It would be presumed that if the treated cloth repelled sprays, as suggested by the foregoing data, clothing treated similarly would afford protection to the worker exposed to chemicals.

A wide array of chemical types are used as pesticides so that it is entirely conceivable that while the treated cloth may be repellent to one chemical, another may be deposited and penetrate with facility. Several types of materials representing different

compositions and weaves, to ascertain whether different chemicals had varying abilities to deposit and penetrate were tested. The results of this study is presented in Table III.

Table III

Percentage Repellency Imparted by Scotchgard^R Treatment
To Different Chemicals

Cloth Type and Treatment Given to Cloth	<u>Concentration of Chemical</u>		
	Chemical Emulsion	Mean ₂ (ug/cm ²)	% Repellency Afforded by Scotchgard
65/35 polyester/ cotton Scotchgard treated	Dinoseb	0.81	69.3
65/35 polyester/ cotton untreated	Dinoseb	2.63	
65/35 polyester/ cotton Scotchgard treated	Chlorpyrifos	3.28	62.8
65/35 polyester/ cotton untreated	Chlorpyrifos	8.82	
65/35 polyester/ cotton Scotchgard treated	Chlorpro pham	112.5	4.74
65/35 polyester/ cotton untreated	Chlorpro pham	118.2	
100% cotton Scotchgard treated	Chlorpro pham	113.6	1.45
100% cotton untreated	Chlorpro pham	135.5	
65/35 polyester/ cotton Scotchgard treated	PCP	60.9	45.5
65/35 polyester/ cotton untreated	PCP	111.7	
100% cotton Scotchgard treated	PCP	87.8	43.3
100% cotton untreated	PCP	154.8	

It will be noted that while the treatment imparted repellency to Dinoseb, chlorpyrifos and pentachlorophenol, it was relatively ineffective in the case of chlorpropham. However there is no indication from these data whether this means a chemical like chlorpropham, may penetrate the cloth, or whether what was being determined was merely a surface deposit.

In Table IV, data are presented where the penetration of chemicals through treated and untreated cloth was determined. In this case, the emulsion was applied as a spray to treated and untreated cloth backed by an alpha-cellulose pad. After drying, the pad was taken, extracted with the appropriate solvent, and the amount of chemical penetrating the cloth to the pad, determined.

Table IV
Penetration of Chemicals Through Scotchgard^R and Untreated Cloth to Alpha-Cellulose Pads

Cloth Type and Treatment ^a	Surface Deposit of Chemical		Penetration to A-cellulose Pad (ug/cm ²)
	Chemical Emulsion	ug/cm ² ug/cm ²	
65/35 polyester/cotton (T)	Chloropyrifos	57.83	0.88
65/35 polyester/cotton (U)	Chloropyrifos	49.63	1.2
100% cotton (T)	PCP	53.34	0.01
100% cotton (U)	PCP	51.95	0.54
65/35 polyester/cotton (T)	PCP	45.35	0.0008
65/35 polyester/cotton (U)	PCP	39.91	10.9

^aT=treated with Scotchgard^R
U=untreated

Clearly the amount of chemical penetrating the cloth to the pad, is far smaller than the surface deposit, despite the direct spraying of the cloth. This would suggest that clean, relatively heavy weight clothing in and of itself, serves as a partial barrier to the chemicals.

In order to insure a heavy surface deposit with ample opportunity to penetrate to the alpha-cellulose pad, patches of cloth were fixed at 30° angle backed with the alpha-cellulose pad and then the chemical emulsion applied dropwise with the microsyringe. The droplet was allowed to dry and only then was the pad removed for analyses. This, it was felt might stimulate the exposure that could occur if clothing received a spill and were not removed immediately. Table V summarizes the results obtained.

Table V

Penetration of Chemically Treated and Untreated Cloth to Alpha-Cellulose Pads

Cloth Type and Treatment	Chemical Emulsion	Concentration of chemical on cloth (ug/cm ²)	Penetration to a-cellulose (ug/cm ²)
100% cotton (T)	2,4,5-T isooctyl ester	489.6	0.015
100% cotton (U)	2,4,5-T isooctyl ester	507.4	0.118
65/35 polyester/cotton (T)	2,4,5-T isooctyl ester	473.4	0.016
65/35 polyester/cotton (U)	2,4,5-T isooctyl ester	203.8	218.1
100% cotton (T)	PCP	9.7	N.D.
100% cotton (U)	PCP	5.96	0.002
65/35 polyester/cotton (T)	PCP	7.76	0.002
65/35 polyester/cotton (U)	PCP	3.73	0.968
100% cotton (T)	Chlorpropham	43.85	0.068
100% cotton (U)	Chlorpropham	43.66	0.204
65/35 polyester/cotton (T)	Chlorpropham	50.67	0.219
65/35 polyester/cotton (U)	Chlorpropham	42.88	2.31

^a(T) = treated with Scotchgard^R
(U) = untreated
N.D. - Not detectable

In the repellency test (Table III), chlorpropham showed a much higher deposition on the treated cloth than the other chemicals tested. However, in this particular test of penetration while it is higher than other chemicals the percent penetrating, in comparison with the surface deposit, is small. The treatments seemed to be effective for both PCP and 2,4,5-T isooctyl ester.

As a final evaluation, it was felt necessary to assess the efficacy of the cloth in preventing dermal absorption. In this instance, small pieces of cloth were sewn into a sleeve that would just fit over the tail of a rat. Treated and untreated sleeves were fitted to animals and a time spray application of

appropriate emulsion applied. The animals were then held in metabolism cages for 48 hours, during which time the urine was collected. The urine extracted and analyzed for the appropriate chemical or its metabolite, to determine the amount adsorbed. In this test, it was possible only to compare between treatments i.e., treated versus untreated. Table VI presents the data for three different chemicals.

Table VI

Chemical Penetration Through Cloth Excretion Following Dermal Absorption

Cloth Type and Treatment ^a	Chemical Emulsion	Total Amount (Mg/rat/48 hrs.)
65/35 Polyester/ Cotton (T)	PCP	0.28
65/35 Polyester/ Cotton (U)	PCP	1.11
65/35 Polyester/ Cotton (T)	2,4,5-T isooctyl ester	1.53
65/35 Polyester/ Cotton (U)	2,4,5-T isooctyl ester	4.65
65/35 Polyester/ Cotton (T)	Ronnel*	1.02
65/35 Polyester/ Cotton (U)	Ronnel*	28.8

^a(T) = Treated with Scotchgard^R

(U) = Untreated

*Analyzed as 2,4,5-trichlorophenol, the major metabolite.

Quite large differences in the amount of the chemical excreted by animals in treated tail covers as compared to untreated covers is noted. Even allowing for some variation in spray deposition, the differences of 3 to 28 fold, probably indicates significant protection in the case of the treated cloth.

Field Studies

The individual personal clothing worn by the 8 mixers are shown in Table VII.

We measured the occupational exposure of the 8 mixers to ethion during the three phases of the study. Their pesticide exposure with the different clothing modalities was determined on the basis of (1) the daily percentage penetration of ethion

TABLE VII

"Own" Clothing Characteristics of Pesticide Citrus Grove Mixers,
Orange County, Florida, 1978

Subject Number	Type of Clothing Worn
1.	Synthetic, short sleeve shirts (thin) frequently open. Work pants cotton and/or synthetic
2.	Cotton Shirts, sweat shirts and "T" shirts occasionally. Work pants. Low shoes.
3.	Primarily a thin synthetic shirt and trousers. Occasionally synthetic/cotton shirts. Rubber boots.
4.	Combinations: "T" shirts and short sleeve shirts. Variety of work pants, light weight.
5.	Variety of short sleeve shirts, "T" shirts and sweat shirts. Work pants varied from cotton twill to cotton synthetic.
6.	A varied assortment of shirts, sweat shirts (long and short sleeved) and athletic jerseys. Trousers varied including shorts and sandals.
7.	Wore heavy army fatigue coveralls of a heavier twill finish than the University of Miami protective clothing. These military green fatigue were from a surplus store (had no labelling to determine type and weight of fabric-long sleeve).
8.	Same as #7

in each worker (15), and (2) the daily creatinine corrected urinary excretion of DEP in each worker (16, 12). There were multiple observations for each worker. Results from any wet patch or patches which had been dropped were excluded in the compilation of the data. In essence, each subject acted as his own control.

For statistical evaluation of the different clothing modalities, "the unweighted means analysis of variance for repeated measures" was used.

The average percent penetration of ethion in each of the pesticide workers during phases 1 and 2 combined and phase 3 (untreated and treated clothing) are shown in Table VIII. The mean percent penetration of ethion from phases 1 and 2 combined was 27.6 compared to 4 (untreated) and 3.6 (treated) in phase 3 when protective clothing was worn.

The protective potential of these clothing modalities was also tested by urinary alkylphosphate excretions (15). The mean DEP concentrations during the several different clothing modalities worn by the two groups are shown in Table IX.

Average corrected DEP concentrations for the mixers was 1.05 ug/ml when wearing their own clothing; 0.89 ug/ml during phase 2 when they wore their own clothing and a respirator, and 0.68 ug/ml and 0.69 ug/ml in phase 3 when these same workers wore new 100% cotton denim coveralls, SCOTCHGARD treated and untreated.

DISCUSSION

A significant degree of protection was apparent when the penetrability of clothing was assessed on the basis of percentage penetration. The differences between percentage ethion penetration and workers wearing their own clothing when compared to the penetration observed with the new clothing (treated or untreated) was significant at the $p < .001$ level. There was no statistical difference between treated and untreated new uniforms.

Similarly when the corrected urinary DEP concentrations were used as the exposure instrument, the analysis of variances showed that there were differences among the modalities of protection which were significant at the $p < .01$ level. Urinary DEP concentrations for workers wearing their own clothing with or without mask were significantly different from those observed when new uniforms were worn whether treated or untreated; the mask did not significantly lower average DEP concentration values. Penetration being significantly lower when the new uniforms were worn. The wearing of the mask did not significantly lower DEP concentrations.

Worker pesticide exposure which is primarily dermal can be acquired either as a result of accidental spillage or as a result of saturation of the clothing and penetration of the chemical through the fabric. Both laboratory and field data from these studies suggested that significant worker protection against

Table VIII

Percent Penetration of Ethion in Each of the Pesticide Workers Wearing Different Clothing Modalities in Orange County, Florida 1978

Subject	Job	Phases 1 & 2	Phase 3	
		Own Clothing & Mask	Untreated Uniform	Treated Uniform
R.S.	Mixer	47.3	8.1	5.9
D.O.	Mixer	20.8	4.9	0.3
R.S.	Mixer	16.1	0.4	0.6
P.M.	Mixer	21.0	0.3	0.8
J.N.	Mixer	40.6	9.0	4.7
G.C.	Mixer	42.6	0.3	0.8
J.C.	Mixer	20.0	2.4	0.0
W.B.	Mixer	12.4	7.7	16.0
Mean of Group =		27.6		
*S.D.+		13.59		

*S.D.-Standard Deviation

Table IX

Mean Urinary DEP Concentration (Creatinine Corrected mg/ml) Observed with the Different Clothing Modalities Worn by Citrus Grove Workers Occupationally Exposed to Ethion, Orange County, Florida, 1978

Subject	Job	Phase 1	Phase 2	Phase 3	
		Own Clothing	Own Clothing and Mask	100% Cotton Denim Coveralls Untreated	Treated
R.S.	Mixer	1.82	0.91	0.87	0.93
D.O.	Mixer	1.92	0.98	0.76	0.44
R.S.	Mixer	0.65	0.59	0.49	0.55
P.M.	Mixer	1.16	0.80	0.52	0.79
J.N.	Mixer	0.70	0.77	0.93	0.78
G.C.	Mixer	0.91	1.20	0.91	1.13
J.C.	Mixer	0.89	1.64	0.80	0.61
W.B.	Mixer	0.32	0.21	0.17	0.25
Mean of Group =		1.05	0.89	0.68	0.69
S.D. +		0.563	0.421	.266	.288

penetration was afforded by 100% cotton-denim coveralls used in this study bearing in mind that clean coveralls were provided daily. The laboratory studies suggested that the addition of SCOTCHGARD to the clothing was additionally protective because of the ability of this treatment to increase the repellency of the fiber and thereby obviate the exposure potential of an accidental spillage. Understandably this eventually was neither tested nor encountered in the field studies. These findings suggest to us that 100% cotton-denim coveralls, adequately laundered, should be a requirement for all occupational pesticide workers and that standards should be promulgated to require the frequency and efficacy of laundering. The further addition of fluoroaliphatic carbon resin treatment suggested additional protection against a spillage and clothing so treated produced no heat exchange problems in these studies. The apparent protection afforded by these clothing modalities suggested that satisfactory worker protection against both acute and chronic pesticide penetration should be considered as an alternative to the establishment of the field re-entry time.

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RECEIVED June 18, 1981.

Reentry: An Industrial Viewpoint

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This presentation deals with a limited aspect of the subject of this meeting, namely the exposure of agricultural workers to pesticide residues. This is commonly referred to as "reentry." In this paper, I am expressing a personal point of view and my opinions should not be construed as reflecting the position of the agricultural chemical industry on the question of reentry. However, I feel that many of my viewpoints are shared with a number of my colleagues in the industry. With this consideration in mind, some basic principles may be stated.

It is the right of everyone to work in an environment which poses no unreasonable risk to their health. It is the responsibility of industry to develop the information which provides the basis for deciding what these conditions should be to meet this basic standard. It is the responsibility of government to provide the legislative and regulatory framework to achieve this common objective which is shared by workers, industry and government. The basic principles to develop the necessary information to make decisions are simple. All one needs to do is to determine the exposure and relate this to effects. Armed with this information, one can then determine the level which would be "safe" and the level which would be "unsafe". Like most basic principles, they can be simply stated, but it is often difficult to determine how to figure out the answers. Figuring out the answers with respect to exposure of agricultural workers to pesticide residues has been a complex and difficult problem. The basic strategy, which has been adopted by regulatory officials to deal with this issue, has been to determine the time one must wait before entering pesticide treated fields. This time lapse is referred to as the reentry interval. However, the exposure variables one encounters in a field situation are obvious. These include climatic factors (temperature, moisture, sunlight and atmospheric conditions), pesticide formulation (wetable powder, emulsifiable concentrate, granules), rate of application and the crop itself. These variables complicate decision making to a much greater extent than in a manufacturing plant. Nevertheless,

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the issue of overexposure of agricultural workers to certain pesticides during harvesting and other field activities is real, especially in California. Specifically, illnesses have occurred as a result of exposure to certain organophosphorus insecticides. This has led to a rather extensive research effort, primarily on the part of chemists, which has brought the issue, and the means to quantitate it, into sharper focus.

Reentry Research Results and Conclusions

Some research conclusions may be enumerated:

1. Methods for Determining Residues

Due to the pioneering efforts of Gunther, et al, (1) we now have a standard procedure to determine dislodgeable residues. This is the component of the residue which is considered available for exposure during harvesting and other cultural activities that involve intimate contact with treated foliage. The technique has been widely used and allows the comparison of residue decay dynamics for different compounds under different situations. The data gathered in this way have relevance to potential exposure.

2. Regional Difference in Residue Decay Dynamics

When the issue of harvest worker illness resulting from exposure to organophosphorus insecticides began to surface as a national issue 10 to 12 years ago, it seemed to many, including myself, that there was a different phenomena occurring in California which seemed to be absent in other parts of the country. There was the ever present question - why were such incidents essentially restricted to California? This was a highly debated question which is probably still not resolved in the minds of some. However, there are now data which speak to this question. Takade (2) reported on the study of regional comparison of insecticide decay in 1976 at a Symposium on Pesticide Residue Hazards to Farm Workers in Salt Lake City. Most of his work was with Parathion, the insecticide which is associated with the vast majority of reentry illness reports. He reported that in the dislodgeable residue on citrus leaves the ratio of Paraoxon to Parathion in California and Arizona to be significantly greater than the ratio observed in Florida. In California the ratio was greater than in Arizona.

It was also reported by Spear (3), at that same conference, that the dislodgeable residue level of Paraoxon on leaves and soil in a California citrus grove in which a residue poisoning occurred the day before, were unusually high.

Spencer, et al, (4) reported that they found residue concentrations were much higher on loose dust particles than in bulk soil in certain California citrus groves. It was established that the residue containing dust can be transferred to the foliage with which workers may later come in contact. Also, workers entering the treated fields can be contaminated by direct contact with the soil surface or by stirring up the dust as they move through the fields.

These data suggest that arid, dusty conditions result in residue exposures different than areas where conditions are just the opposite; for example, Florida. As a matter of fact, it has been suggested that if Parathion was never used in California that the reentry issue would not be a matter of concern today.

3. Routes of Exposure

It is now understood and accepted that the dermal route is the route of exposure in field situations. There is ample research data to back this up. Also, this fact is reflected in the California regulations on reentry. This point will be discussed later.

4. Coupling Exposure with Effects

Relating dislodgeable residues to absorbed dose and absorbed dose to effects still remains a complex and difficult problem. We will probably never be able to determine exposure to the nth degree or down to the last gnat's eye. There simply are not enough resources available to measure absorbed dose for every formulation, at each rate, by each method of application, on every crop at different times after treatment. This means that we are going to have to rely on models. It may be safe to say that many of us will find such models to be imperfect. However, we are close to having models that allow an approach to the problem. For example, the procedure recently published by Knaak (5) on a method for establishing safe pesticide residue levels on foliage, is a significant advance and should be considered as one of the options available to establish reentry intervals for organophosphate insecticides. This technique involves the utilization of dermal dose red cell cholinesterase response curves for animals and existing field reentry data with bench mark pesticides.

Another interesting model, which is under development, is relating dislodgeable residue data to dermal dose (6). This would be the amount which would reach the skin of an individual engaged in various cultural activities that

involve intimate contact with the foliage. It would seem to be a relatively simple matter to then relate this to basic toxicological effects data along with dermal penetration data from animal experiments. Again, as with any model, it will not be perfect. There will be situations where the answer may be unwelcome. In such circumstances this would mean that more detailed experiments with a particular product would be in order.

Reentry Regulations and Guidelines

The EPA is in the process of developing reentry guidelines. As I mentioned previously, the basic thrust is to require data to establish reentry intervals. There are two points to be made concerning this endeavor:

1. The issue of when special reentry studies are required.
2. The issue of what constitutes chronic effects.

When Required

It should be obvious to anyone who is familiar with the reentry issue that concern should be restricted to situations where work practices involve substantial and prolonged contact with foliage which has been treated with a foliar spray or dust. To put it in another way, special reentry studies should not be required for soil applied pesticides, crops which are mechanically harvested, or in other situations where it is obvious that dermal exposure to foliar residues is not a factor. The other factor which should be taken into consideration is the properties of the pesticide itself. The current California regulations speak to this point. The criteria for the pesticide, the use of which results in substantial and prolonged contact with the treated foliage in these regulations are - if (a) any active ingredient or alteration product has an acute dermal toxicity (LD50) of 2000 or less mg/kg, (b) is highly irritating to the skin, (c) is a sensitizer, or (d) involves a potential risk of a chronic health effect. I suggest that the Federal Guidelines use the same criteria. However, there is a need to sharpen up what is meant by chronic health effects.

Chronic Effects

The attention of those concerned with occupational exposure to pesticides has turned from immediate acute effects to chronic effects. The California regulations lists chronic health effects as a criteria for special reentry studies. The EPA, in their first public draft of reentry guidelines, also lists chronic effects as a criteria to be utilized in setting reentry intervals. One problem with this is determining what constitutes a chronic health effect and what information should be used? It is clear that animal studies which unequivocally demonstrate onco-

genic, teratogenic, mutagenic and delayed neurotoxic effects, need to be taken into consideration in reentry exposure situations. However, what about substances which have none of these properties? In these situations, I suggest that the no effect level obtained from 90 day feeding studies can be used in reentry calculations, for situations where the acute dermal toxicity trigger stated above, has been tripped.

Summary

A significant amount of knowledge has been gained over the last ten years which has resulted in a better understanding of the nature of the reentry problem and means to deal with it. In the development of regulations and guidelines on reentry for outdoor uses of pesticides, the EPA should focus on work practices where substantial dermal exposure can occur and couple this with the basic toxicological properties of a particular chemical. A systematic appraisal of these factors, taking into consideration the current knowledge, leads to the conclusion that the need for special reentry studies as part of the registration process should be the exception rather than the rule.

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An Animal Model for Testing Organophosphates in the Field

S,S,S-Tributyl Phosphorotrithioate and the Scaleless Chicken

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The General Problem of Risk Assessment

Although there has been much research on agricultural chemicals and their toxic effects, and although each agent must be subjected to a battery of short and long-term tests before it is registered, there is still no agreement on what is required to establish safe levels of exposure of many widely used insecticides such as the organophosphates. One problem is the kinds of data available. There are dose-response data for laboratory animals, residue analyses for plants and soils under several environmental conditions and information on the effects of low levels of chemicals on field workers (Figure 1). However, there is little known about the extent of absorption of organophosphates through the skin even though most of them enter the body by this route, and there is no universally accepted way to compare data on laboratory animals to the information available for humans. Figure 2 diagrammatically illustrates an integrated approach to establishing safe levels of exposure. The major differences between it and today's practices are the use of skin absorption data on experimental animals to develop effective dose-response curves for the agents under study, and the exposure of experimental animals to conditions encountered by humans in the field.

A strong point of the scheme is that it bridges the gap between lab data on experimental animals and field data on humans by exposing experimental animals in the field to levels and lengths of exposure not permissible with human subjects. One problem with the approach is inherent in the use of experimental animals, the possibility that the toxicity of the agents will be considerably different from one animal to another.

Whether or not all the steps shown in Figure 2 should be performed for a given chemical depends on how similar its properties are to the standards used in the program. In the

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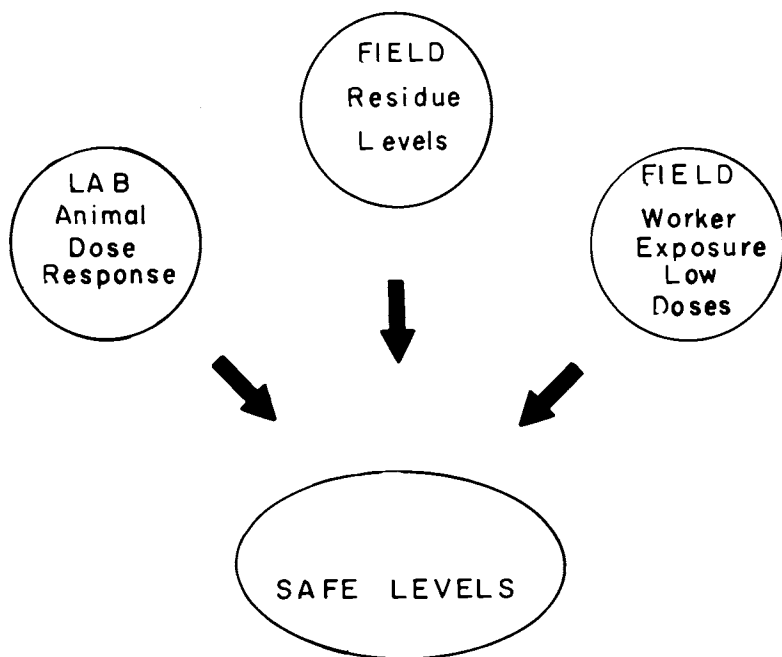


Figure 1. Current information for setting safe levels of agricultural chemicals.

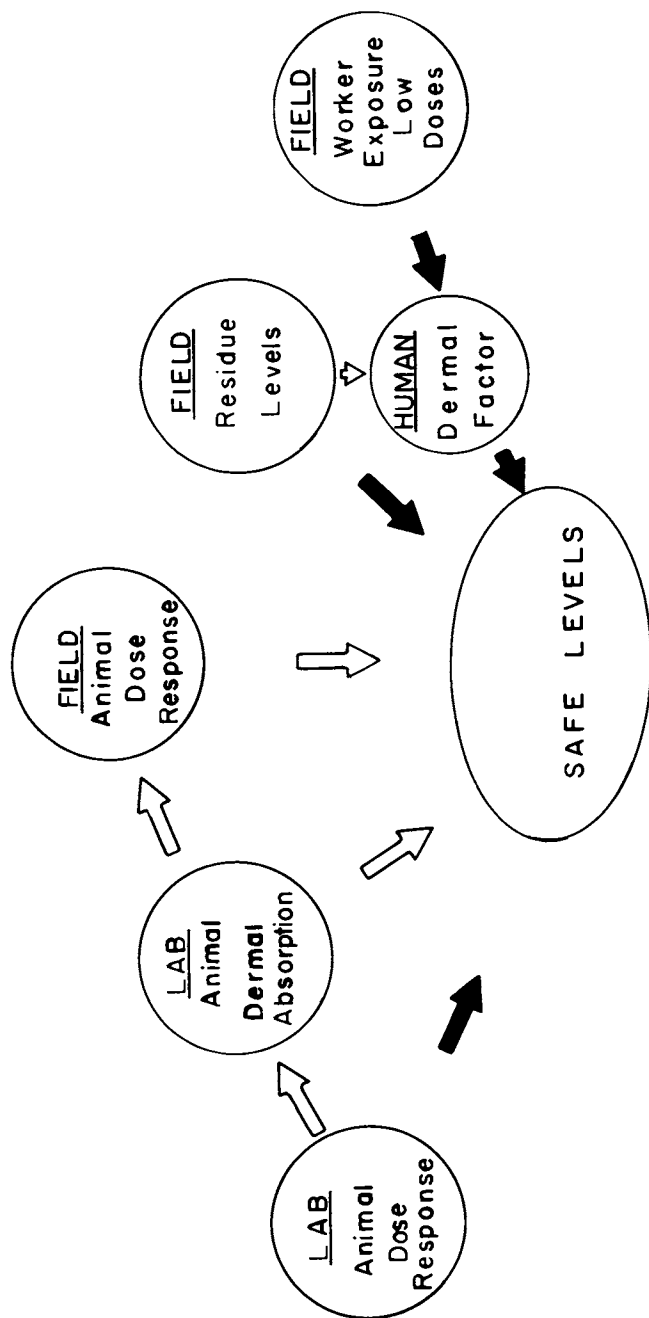


Figure 2. Proposed kinds of information for setting safe levels of agricultural chemicals.

case of the organophosphates, rates of dermal absorption may be needed for several agents and chronic as well as simulated field studies may be necessary for those suspected of causing delayed neurotoxicity. Indeed, data could be obtained and stored in a manner suitable for computer simulations.

Elsewhere in the Symposium (1), Dr. Knaak and colleagues examine the dermal absorption of a series of organophosphates in the rat using cholinesterase inhibition as a parameter. Here we describe a field test with a special experimental animal, the scaleless chicken.

The Scaleless Chicken

The ideal animal for studying organophosphates would be one without hair, fur or feathers capable of contracting delayed neurotoxicity. Such a creature is the scaleless chicken, a mutant with a defect in feather and scale development (2,3), developed and maintained by Dr. Ursula K. Abbott of the Department of Avian Sciences, University of California, Davis. (Figure 3). Its absence of feathers makes it an excellent animal for studies of dermally applied toxicants. Renden and Abbott found that the scaleless mutant was more sensitive to applications of trithion in mineral oil than were either normal chickens or another mutant "ichthyotic", in a study of mineral oil-induced dermatitis (4).

Studies on the scaleless chicken are underway examining its suitability as a model for assessing toxicity of organophosphates. The first compound selected for field trials was the defoliant DEF (S,S,S-tributylphosphorotrithioate) used during the harvesting of cotton in California and Arizona in the fall (October–November) when air movements are frequently restricted by inversions. DEF has been the subject of sufficient complaints to place it on the pre-RPAR list, although there are no reports of acute or delayed neurotoxicity in humans when it and related chemicals are used according to recommendations. It both inhibits cholinesterases and causes delayed neurotoxicity in hens (5,6).

Field Test of DEF

Twenty-seven two-year-old scaleless hens were transported to Visalia, CA on October 1, 1979, housed in cages overnight with food and water provided *ad lib*, and exposed to field applications of DEF on October 2. Some birds were repeatedly treated over the next five days. The mature cotton fields (Diversified Farming Inc.) were sprayed from a ground vehicle that treated 8 rows simultaneously with DEF-6 (0.72 kg/l, 6 lb/gal; Mobay Chemical Co.) at 0.37 gal/acre and Accelerate (amine salt of endothall (7-oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid, 0.06 kg/l, 0.5 lbs/gal) at 0.19 gal/acre in 25 gal water/acre.

There were 3 hens to an experimental group. One control was approximately 400 m away from the site (OFF); another was between the two fields (ON). Two groups were sprayed directly in the rows of cotton (ROW) approximately 4 meters from the edge of the field. Another was in unsprayed cotton, 8 rows away from the path of the applicator (ADJ). One set was in a specially designed cage on top of the spray rig (RIG), immediately behind and slightly above the operator (Figure 4). Water and shade were provided.

Mylar sheets (1858 cm²) were at the OFF and ON locations, and on the spray rig (464 cm²) for the 7 hour duration of the test. Duplicate sets of petri dishes (67.5 cm) were placed at the ROW and ADJ locations. Samples were collected and extracted with acetone, taken to the laboratory in dry ice and stored frozen until analyzed. High and low volume air collectors were set up at the OFF, ON and RIG locations. DEF and Accelerate were analyzed by gas chromatography (7,8). The tank mix was diluted 1/10000 for DEF analysis and 1/2 for Accelerate. Minimum detectability was <0.2 ng DEF and <0.1 ug Accelerate. The ratio of DEF to Accelerate in the tank mix (approximately 10.4 mg/ml of DEF and 0.5 mg/ml of Accelerate) was similar to the ratios found in the samples collected.

Repeated exposures were done by setting one group in the path of the sprayer once a day (ROW), and another adjacent to it (ADJ) as described above. A control set (CON) was left in the automobile.

Birds were weighed and blood samples taken the day before they left Davis; The birds on the acute test were returned to Davis on the same day; those that received repeated exposures were returned one week later, two days after their last exposure in the field. In both cases, blood samples were taken the day after their arrival in Davis. The birds were weighed and blood samples taken 2, 8, 15, 22 and 29 days after their first exposure and observed daily for signs of ataxia and other evidence of neuropathy or illness.

Plasma CHE was determined by a radiometric assay (9) using acetylcholine and BW 284c51. Plasma creatine kinase (CK) was determined by a spectrophotometric method (10).

Results

The recently reported results of the study (8) demonstrated the feasibility of using the scaleless chicken for field research.

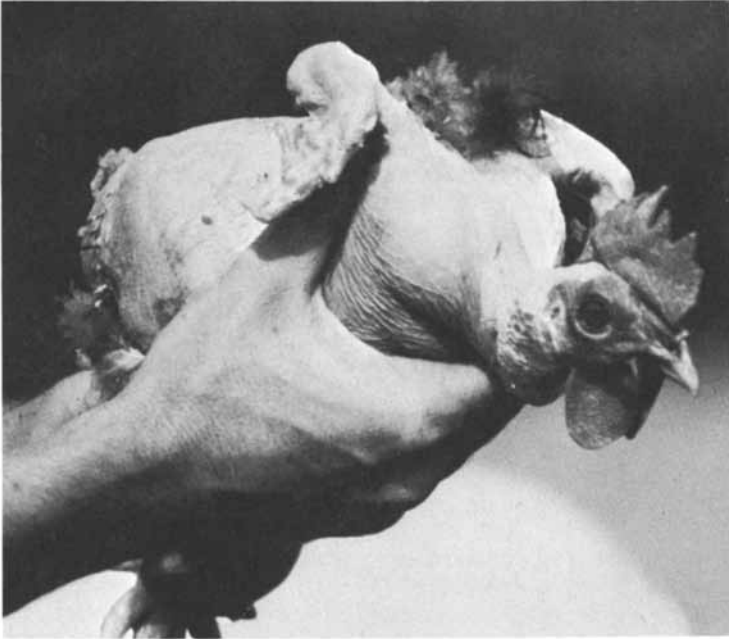


Figure 3. The scaleless chicken.



Figure 4. Three scaleless chickens in a specially designed cage atop the spray rig.

TABLE I
Plasma CHE Levels

Group Site	DEF $\mu\text{g}/\text{cm}^2$	Days After Exposure				
		0	2	8	15	22
Single Exposure						
OFF	0.0017	379	413	389	294	361
ADJ	0.0092	380	360	385	300	333
ON	0.04	292	300	314	294	329
ROW	4.4	354	251	374	291	319
ROW	17.7	301	190*	252	290	309
RIG	47.8	379	299	332	320	408
Repeated Exposure						
ROW	108	429	—	223*	323	352
ADJ	3.9	388	—	323*	349	337
CON	-	401	—	368	294	384
Grand Mean		367	NA	NA	306	348
Mean		± 45			± 20	± 32

DEF: means of 2-4 samples. CHE: means of blood samples from 3 birds/ group in nmoles ACh hydrolyzed /min/ml. Grand Means + S.D. Standard deviations of groups and values for day 29 omitted for brevity. *Statistically different from day 0, $P < 0.05$, t-test paired variates calculated for days 2 and 8. (Modified from Wilson *et al.*, 8.)

DEF levels ranged from negligible to a total of more than $100 \mu\text{g}/\text{cm}^2$ (Table I). Plasma CHE decreased with increasing single exposures of DEF [except for the birds riding on the rig (RIG)] to approximately 50% the CHE of off-site birds. Both ROW and ADJ birds showed decreased CHE levels with repeated exposures. CHE activity took 2-3 weeks to recover.

There was a log-linear dose-response relationship for DEF and CHE, particularly for birds in the acute, one day study (Figure 5).

Plasma CK rose (one group increases more than 17-fold) but the increases did not correlate with the exposures of the birds to DEF (Table II). CK values had returned to normal within 2 weeks.

TABLE II
Creatine Kinase Activity
of Scaleless Chickens
(Milliunits/ml plasma)

Group	0	Days 2	after 8	exposure 15	22	29
Single Exposure						
OFF	121	823	87	153	107	187
ADJ	192	687	260	360	233	287
ON*	154	2710	350	80	70	40
ROW 1	160	373	253	187	133	133
ROW 2	199	753	323	187	127	260
RIG	192	660	267	213	167	240
Repeated Exposures						
ROW	173	-	247	167	113	140
ADJ	205	-	167	267	87	127
CON	192	-	233	220	213	130
Grand Mean	176	1000	243	204	139	172
	± 27	± 851	± 78	± 78	± 55	± 79

*3 birds to a group, except for ON with 2 after day 8.

The DEF levels to which the applicator was exposed during the study were measured from 4 x 4 cm gauze patches inside and outside a coverall worn during the 7 hour duration of the field test (Table III). DEF levels outside the coveralls were as high as 250-260 $\mu\text{g}/\text{cm}^2$, and ranged from above 1 to almost 7 $\mu\text{g}/\text{cm}^2$ inside of the garment, comparable to levels to which the chickens were exposed.

DEF levels measured in the air and on the surfaces of mylar and petri dishes during the 7 hour interval exhibited similar trends. For example, the concentration of DEF in the air increased from 0.111 $\mu\text{g}/\text{m}^3$ at the OFF site to 6.2 $\mu\text{g}/\text{m}^3$ at the ON site while the fallout increased from 0.0017 $\mu\text{g}/\text{cm}^2$ to 0.040 $\mu\text{g}/\text{cm}^2$ at the same locations. Calculations based on an approximate respiration rate of 1 l/min for the birds suggest that the levels of DEF in the air were too low for inhalation to be a major route of exposure (8).

TABLE III
Exposure of Applicator to DEF
($\mu\text{g}/\text{cm}^2$)

Coverall	Outside	Inside
Sleeve	194 (260,254,68)	—
Shoulder	38 (24, 50)	1.52 (1.37,1.66)
Chest	47 (24,68,48)	2.88 (2.66,2.84,3.13)
Thigh	82 (102,85,59)	5.92 (6.76,4.55,6.44)
Leg	37.0 (44.0,50,18)	—

Values determined from 4 x 4 cm gauze patches worn for 7 hours by the applicator. Means of values in parentheses. (Modified from Wilson *et al.*, 8.)

Birds did not exhibit ataxia or other behavioral signs of delayed neurotoxicity during the more than 30 days they were observed after they returned to Davis. One died of causes not related to the study.

Featherless chickens treated with DEF under laboratory conditions developed delayed neurotoxicity. Three birds were pretreated with atropine sulfate (20 mg/kg s.c.) 30 minutes before they were injected with DEF (800 mg/kg s.c. technical grade, 94% purity, Mobay Chemical Co.). (A second injection of atropine sulfate was administered 2 hours after treatment with DEF.) Control hens received two injections of atropine sulfate as above. The birds were observed daily for changes in behavior, weighed and blood samples taken 2, 6, 13, and 20 days after treatment. Ataxia developed 12 days after treatment with DEF. CHE decreased to low levels as expected, in both scaleless (Figure 6) and normal birds (not shown), and, in this study, did not return to normal levels by 20 days. CK levels rose after treatment with DEF, but did not rise in the controls. Plasma CK activity also rose in birds treated with TOCP and parathion (11).

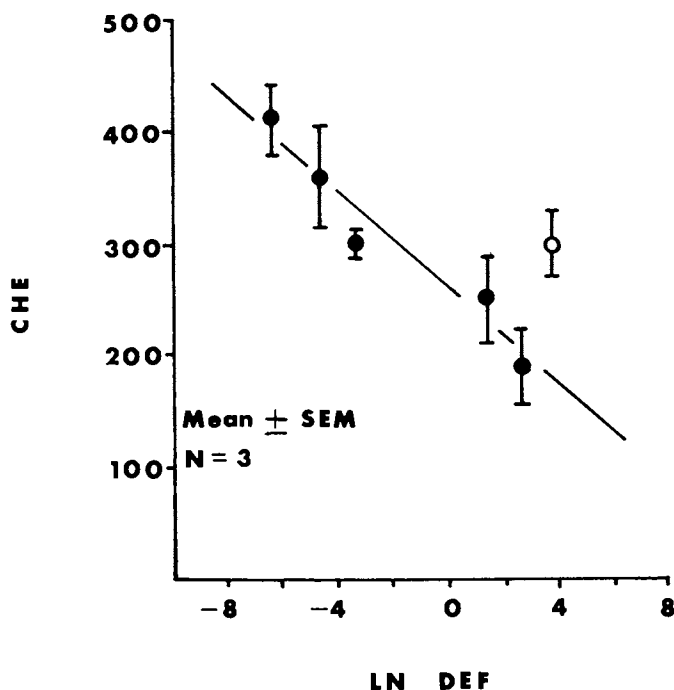


Figure 5. Plasma CHE in scaleless chickens after field applications of DEF (CHE in nmol/min/mL; DEF in ln $\mu\text{g}/\text{cm}^2$). Single-day exposures of groups are in Table 1, omitting birds on the sprayer (RIG). Modified from Ref. 8.

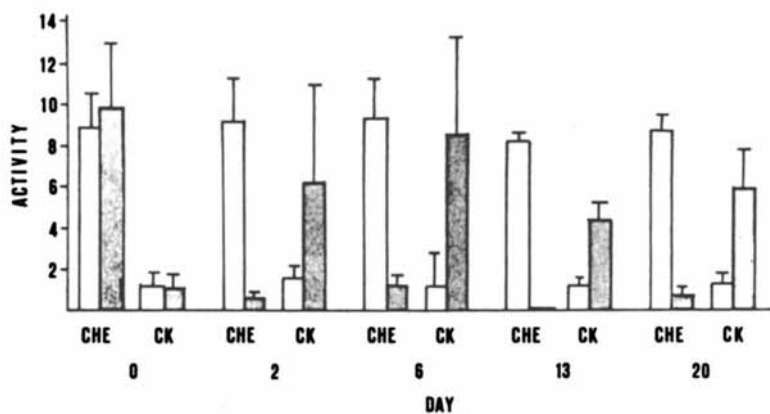


Figure 6. Plasma levels of CHE and CK in scaleless chickens given 800 mg/kg DEF and 40 mg/kg atropine, or atropine alone, and sampled on the days indicated (8). Three birds per group; dark bars are for DEF-treated birds.

Bulletin of Environmental Contamination and Toxicology

Discussion

The dose-response relationship between DEF fallout and plasma CHE levels was remarkably consistent, extending over 5 orders of magnitude. The lack of a large decrease of CHE in the birds that rode on the applicator may have been due to impeding the fallout of DEF in the cage by a thick rubberized screen with which we lined the cage to protect the birds (unnecessarily as it turned out) from jostling.

The fact that the birds developed delayed neurotoxicity under laboratory conditions indicates that the lack of it in the field study was not due to an inability of this mutant to develop the neuropathy. "Early warning" tests for delayed neurotoxicity are lacking. The laboratory data suggest that serum enzymes like CK may be useful markers for organophosphate exposure. However the conditions of this test must be controlled. The increase of CK in all birds taken to Visalia, regardless of their exposure to DEF in the field, suggests that the rigor of the trip may have stressed the birds and increased CK levels. Plasma CK activity in humans is known to increase under stress, such as after heavy exercise (12).

The Canary in the Mine

Most tests involving experimental animals are carried out in the laboratory, reducing the direct applicability of the results to the field. For example, none of the published laboratory studies on DEF have involved mixtures of agents even though the formulation used to spray DEF includes other chemicals such as the desiccant and RNA inhibitor, Endothall. In addition, conditions in the field may cause reactions not seen in the laboratory, particularly those catalyzed by sunlight. The experiments reviewed here established the feasibility of using the scaleless chicken in field tests to establish safe levels for use of organophosphates, particularly those causing delayed neurotoxicity. The use of such "sentinel" birds may be a model for future ways to bridge the gap between laboratory findings and their application.

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Appendix

List of Common Names Mentioned in Text and Chemical Names

<u>Chemical or Pesticide Designation</u>	<u>Chemical Name</u>
acephate	<u>O,S</u> -dimethyl acetylphosphoramidothioate
aldicarb	2-methyl-2-(methylthio)propionaldehyde <u>O</u> - (methylcarbamoyl)oxime
azinphosmethyl	<u>O,O</u> -dimethyl <u>S</u> -[(4-oxo-1,2,3-benzotriazin- 3(4H)-yl)methyl] phosphorodithioate
azinphosmethyl oxon	dimethyl [4-oxo-1,2,3-benzotriazin-3(4H)- yl)methyl] phosphate
benomyl	methyl 1-(butylcarbamoyl)-2-benzimidazole= carbamate
captafol	<u>cis-N</u> -[(1,1,2,2-tetrachloroethyl)= thio]-4-cyclohexene-1,2-dicarbox= imide
captan	<u>N</u> -[(trichloromethyl)thio]-4-cyclohexene- 1,2-dicarboximide
carbaryl	1-naphthyl methylcarbamate
carbofuran	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
carbophenothion	<u>S</u> -[(<u>p</u> -chlorophenyl)thio]methyl] <u>O,O</u> - diethyl phosphorodithioate
chlordane	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a- tetrahydro-4,7-methanoindan (60% minimum and not over 40% of related compounds)
chlorobenzilate	ethyl 4,4'-dichlorobenzilate
chlorothalonil	tetrachloroisophthalonitrile
chlorthiophos	<u>O</u> -[2,5-dichloro-4-(methylthio)phenyl] <u>O,O</u> - diethyl phosphorothioate and the 2,4,5 and 4,5,2 isomers (73:14:13)
chlorthiophos oxon sulfoxide	[2,5-dichloro-4-(methylsulfinyl)phenyl] diethyl phosphate with its isomers

2,4-D	(2,4-dichlorophenoxy)acetic acid
DBCP	1,2-dibromo-3-chloropropane
DDE	1,1-dichloro-2,2-bis(<u>p</u> -chlorophenyl)= ethylene
DDT	1,1,1-trichloro-2,2-bis(<u>p</u> -chlorophenyl)= ethane
DEF ^R	<u>S,S,S</u> -tributyl phosphorotrithioate
demeton	<u>O,O</u> -diethyl <u>O</u> (and <u>S</u>)-[2-(ethylthio)ethyl] phosphorothioates
dialifor	<u>O,O</u> -diethyl phosphorodithioate <u>S</u> -ester with <u>N</u> -(2-chloro-1-mercaptoethyl)= phthalimide
dialifor oxon	<u>O,O</u> -diethyl phosphorothioate <u>S</u> -ester with <u>N</u> -(2-chloro-1-mercaptoethyl)phthalimide
diazinon	<u>O,O</u> -diethyl <u>O</u> -(2-isopropyl-6-methyl-4- pyrimidinyl) phosphorothioate
dicofol	4,4'-dichloro- -(trichloromethyl)= benzhydrol
Difolatan ^R	(see captafol)
dimethoate	<u>O,O</u> -dimethyl <u>S</u> -(<u>N</u> -methylcarbamoylmethyl) phosphorodithioate
dioxathion	2,3- <u>p</u> -dioxanedithiol <u>S,S</u> -bis(<u>O,O</u> -diethyl phosphorodithioate)
endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a- hexahydro-6,9-methano-2,4,3-benzodiox= athiepin 3-oxide
endothall	7-oxabicyclo[2.2.1]heptane-2,3- dicarboxylic acid
EPN	<u>O</u> -ethyl <u>O</u> -(<u>p</u> -nitrophenyl) phenylphosphonothioate
ethion	<u>O,O,O',O'</u> -tetraethyl <u>S,S'</u> - methylene bis(phosphorodithioate)

ethion dioxon	<u>0,0,0'0'</u> -tetraethyl <u>S,S'</u> , -methylene bis(phosphorothioate)
folpet	<u>N</u> -[(trichloromethyl)thio]= phthalimide
Guthion ^R	(see azinphosmethyl)
malathion	<u>0,0</u> -dimethyl phosphorodithioate of diethyl mercaptosuccinate
methidathion	<u>0,0</u> -dimethyl phosphorodithioate <u>S</u> - ester with 4-(mercaptomethyl)-2-methoxy- 2-1,3,4-thiadiazolin-5-one
methidathion oxon	<u>0,0</u> -dimethyl phosphorothioate <u>S</u> -ester with 4-(mercaptomethyl)-2-methoxy- 2-1,3,4-thiadiazolin-5-one
methomyl	<u>S</u> -methyl <u>N</u> -[(methylcarbonyl)oxy]= thioacetimidate
methyl parathion	<u>0,0</u> -dimethyl <u>0</u> -(<u>p</u> -nitrophenyl) phosphorothioate
mevinphos	methyl (<u>E</u>)-3-hydroxycrotonate dimethyl phosphate
monocrotophos	dimethyl phosphate ester with (<u>E</u>)-3- hydroxy- <u>N</u> -methylcrotonamide
naled	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
nitrofen	2,4-dichlorophenyl <u>p</u> -nitrophenyl ether
oxydemeton-methyl	<u>S</u> -[2-(ethylsulfinyl)ethyl] <u>0,0</u> -dimethyl phosphorothioate
paraoxon	diethyl <u>p</u> -nitrophenyl phosphate
paraquat	1,1'-dimethyl-4,4'-bipyridinium ion
parathion	<u>0,0</u> -diethyl <u>0</u> -(<u>p</u> -nitrophenyl) phosphorothiate
parathion-methyl	(see methyl parathion)
phosdrin	(see mevinphos)

phosalone	<u>0,0</u> -diethyl <u>S</u> -[(6-chloro-2-oxobenzoxazol- in-3-yl)methyl] phosphorodithioate
2,4,5-T	(2,4,5-trichlorophenoxy)acetic acid
TCDD	2,3,7,8-tetrachlorodibenzo- <u>p</u> -dioxin
toxaphene	chlorinated camphene containing 67 to 69% chlorine

INDEX

- A**
- Absorption of pesticides
 alveolar 165
 gastrointestinal tract 165
 percutaneous 164-165
- Absorption studies vs. 2,4-D
 ingestion 129-130
- Absorption studies vs. 2,4,5-T
 ingestion 129-130
- Acaricides 107
- Acceptable daily intake (ADI) 11
- Acephate 46
- Acetate, sodium 17
- Agricultural
 chemicals, setting safe levels 190f, 191f
 reentry incidents, pesticides
 involved 61t
 workers using pesticides,
 protection 5
 workers using pesticides, risks 4
- Alcohol, polychlorinated aromatic 105
- Aldicarb exposure 59
- Alkyl phosphates, analyzing urine 42
- Alveolar absorption of pesticides 165
- Alveolus 161
- 2-Amino-5-chlorophenol 54
- Analytical methodology to determine
 pesticide residue levels 10
- Animal food products, level of
 pesticide residue 10
- Animal toxicology studies 10
- Apple leaves, degradation of
 carbaryl residues 99, 100f
- Applicator(s)
 2,4-D, agricultural
 conditions and parameters 121
 carbaryl exposure 89
 DEF, exposure 197t
- Applicators, studies in 2,4,5-T 137-153
- Arizona citrus belts 68
- Azinphosmethyl-azinphosmethyl oxon
 dislodgeable residues 25
 dissipation, on orange trees 24, 28f-29f
 safe levels 35
 on tree foliage 24-35
- B**
- 1,1-Bis(*p*-chlorophenyl)-2,2,2-
 trichloroethanol 105
- Blood**
- cells, red, cholinesterase activity 41
 cholinesterase levels 43
 cholinesterase depression 23
 and urine, analyzing for pesticides
 and their metabolites 41-57
- Bromide, methyl 54
- C**
- California
 agriculture 67
 citrus belts 68
 citrus harvest and reentry times,
 comparison with Florida 66t
 Department of Food and Agricul-
 ture, pesticide safety program 75-80
 use of dialifor on grapes 38
- Captan 18
- Carbamate exposure, symptoms 62
- Carbamates, *N*-methyl 41
 monitoring workers exposed to 42
 toxicity category I and II products
 containing 43-46
- Carbaryl (1-naphthyl methylcar-
 bamate) 84
 exposure
 applicators 89
 dermal 83-103
 rate to workers involved in
 aerial and ground
 applications 90t-97t
 mixer-loaders 89
 thinners 99
 residues on apple leaves,
 degradation 99, 100f
- ChE (*see* Cholinesterase)
- Chemical
 injury 16
 residues in foods and human
 tissues 1-7
 fate in humans 16-20
 routes of entry into humans 16
- Chicken, scaleless 192, 194f
 creatine kinase activity 196t
- Chlorinated hydrocarbon exposure,
 symptoms 62
- Chlorobenzilate [ethyl 2-hydroxy-
 2,2-bis(4-chlorophenyl)acetate] .. 105
 colorimetric analysis 106
 determining human exposure 105-117

Chlorobenzilate (*continued*)

human metabolism	106
method for analyzing urine for residues	105-117
rat feeding studies	108-115
residues, urinary, plots vs. time	113f
Chlorpropham	174
Chlorpyrifos	174
Chlorthiophos	
dissipation	35, 36f
oxon sulfoxide, dissipation	35, 36f
oxon sulfoxide, safe levels	35
Cholinesterase (ChE)	3
activities of workers	46
activity, red blood cell	41
depression, blood	23
determinations	63
enzymes, esteratic site	42
inhibition	41
inhibitors	76
levels, blood	43
monitoring pesticide safety programs by measuring blood	41-57
response curves, dermal dose-red cell	23
Citrus	
belts	68
Florida	67
harvest and reentry times, comparison of California and Florida	66t
safe worker reentry period for phosalone	46
Closed-transfer system(s)	
mixing and loading toxicity category I and II products, effectiveness	43-46
Model SS 12-4	43
Swampmate	43
Cloth	
chemical penetration following dermal absorption of pesticides	175t-177t
repellency imparted by Scotchgard treatment to different chemicals	174t
repellency of treated	170
tests of penetrability	171
Clothing	
or dermal exposure to pesticides, measuring	77-78
fluoroaliphatic-treated	170
protective	
effectiveness	150
studies in the field	169-181
Colorimetric analysis of chlorobenzilate and dicofol	106
Crop foliage, estimating organophosphorus residues	23

D

DBP (<i>see p,p'</i> -Dichlorobenzophenone)	
DCM (dichloromethane)	86
DDE in humans	2
DDT in humans	2
DEF (<i>see S,S,S</i> -Tributyl phosphorotrithioate)	
Dermal	
absorption of pesticides, chemical penetration through cloth following	175t-177t
absorption of pesticides, measuring	78
application studies of 2,4,5-T in humans	136-137
dose-red cell cholinesterase response curves	23
dose, relating dislodgeable pesticide residue data	185
exposure	
carbaryl	83-103
rate to workers involved in aerial and ground applications	90t-97t
levels, maximum pesticide	76
pesticides	158-161, 169
measuring clothing	77-78
Dialifor	
exposure	59
on grapes in California	38
oxon	64
Dialkylphosphate levels, urinary	43
<i>p,p'</i> -Dichlorobenzilic acid, method for analyzing urine for residues	105-117
<i>p,p'</i> -Dichlorobenzophenone (DBP) method of analyzing urine for residues	105-117
Dichloromethane (DCM)	86
2,4-Dichlorophenoxyacetic acid (2,4-D)	135
aerial and ground workers, urinary excretion level	119-131
agricultural applicators exposure	119-131
applied and excretion rate, relationship between amount	128f
exposure by agricultural applicators, conditions and parameters	121
exposure and excretion rate, relationship between hours	127f
ingestion vs. absorption studies	129-130
levels in urine	119-131
in Sweden, occupational exposure	152
Dicofol (1,1-bis(<i>p</i> -chlorophenyl)-2,2,2-trichloroethanol)	105
colorimetric analysis	106
gas chromatographic analysis	107
human metabolism	106

- Dicofol (*continued*)
 method for analyzing urine for
 residues 105-117
 rat feeding studies 108-115
 residues vs. time, plots of urinary .. 114f
- Dietary exposure of pesticide, method-
 ology for estimating 9-13
- Difolatan 18
- Dioxon, ethion 67
- Dinoseb 174
- E**
- Entry for chemicals into humans,
 routes 16
- Environmental Protection Agency
 (EPA) 1, 9, 59, 133
- Enzymes, esteratic site of the ChE 42
- Ester, α -hydroxy, chlorinated
 aromatic 105
- Ethion 172
- Ethion dioxon 67
- Ethyl 2-hydroxy-2,2-bis(4-chloro-
 phenyl)acetate (*see* Chloro-
 benzilate)
- Ethyl parathion 67
- Excretion studies on phenoxy herbi-
 cides in humans 119
- Exposure
 applicator to DEF 197t
 carbaryl
 applicators 89
 dermal 83-103
 rate to workers involved in
 aerial and ground
 applications 90t-97t
 thinners 99
 chlorobenzilate, determining in
 human 105-117
 2,4-D, by agricultural applicators,
 conditions and param-
 eters 119-131, 121
 2,4-D in Sweden, occupational 152
 dermal, levels, maximum pesticide 76
 with effects, coupling pesticide 185
 field workers to organophosphate
 residues, monitoring 46-54
 humans to pesticides from
 residues in food 11
 inhalation, levels, maximum
 pesticide 76
 minimization of occupational
 pesticide 7
 mixer-loader, carbaryl 89
 organophosphorus pesticides,
 detecting 41-42
 pesticides
 dermal 158-161, 169
 clothing or dermal, measuring 77-78
- Exposure (*continued*)
 pesticide (*continued*)
 occupational 5
 oral 164
 procedure to monitor worker 84-89
 pesticide residue
 agricultural workers 183
 estimating 10
 methodology for estimating
 dietary 9-13
 routes of pesticide 185
 studies, pesticide mixer, loader,
 applicator 77-79
 2,4,5-T sprays, toxicological
 significance 153-154
 2,4,5-T in Sweden, occupational 152
- F**
- Federal Food, Drug, and Cosmetic
 Act 9
- Federal Working Group on Pest
 Management 5
- Florida citrus 67
 belts 68
 harvest and reentry times, com-
 parison with California 66t
- Fluoroacetate, sodium, in the human
 body 17
- Fluoroaliphatic-treated clothing 170
- Foliage
 absorbance values for safe levels of
 total organophosphorus
 residues 35, 37t
 estimating organophosphorus
 residues on crop 23
 pesticide residues on soil 77
 safe levels
 azinphosmethyl-azinphosmethyl
 oxon 35
 insecticide residue using the
 rapid field method, testing .. 35
 methidathion-methidathion oxon 35
 parathion-paraoxon 35
 skin, transfer of pesticide residues .. 38
 tree
 establishment of safe levels of
 pesticides 25t
 oxons 24-35
 procedure for estblishing safe
 levels for total dislodgeable
 thion-oxon residue 24, 34t
- Folpet 18
- Food(s)
 chemical residues in human tissues 1-7
 exposure of humans to pesticides
 from residues 11
 pesticide tolerance levels 11
 products, animal, level of
 pesticide residue 10

G		L	
Gas chromatographic analysis of dicofol	107	Livestock, pesticide residues	10
Gastrointestinal tract absorption of pesticides	165	M	
Grapes in California, use of dialifor ..	38	Methidathion-methidathion oxon	
Guthion	165	dislodgeable	25
H		dissipation on orange trees ...	24, 30f-31f
Harvest times	65	safe levels	35
and reentry times, comparison of California and Florida citrus ..	66t	on tree foliage	24-35
Herbicides, phenoxy, in humans, excretion studies	119, 135	Methomyl	46
Homeostasis	15-16	Methyl bromide	54
Human		N-Methyl carbamates	41
DDE	2	effectiveness of closed-transfer systems for mixing and loading	
DDT	2	toxicity category I and II products	43-46
dermal application studies of 2,4,5-T	136-137	monitoring workers exposed	42
excretion studies on phenoxy herbicides	119	Mevinphos	46
exposure to chlorobenzilate, determining	105-117	Mixer-loaders, carbaryl exposure	89
fate of chemicals entering body	16-20	Monocrotophos	64
fate of phenoxy herbicides	135	N	
long-term effects of pesticides	4	1-Naphthyl methylcarbamate (see Carbaryl)	
oral administration studies of 2,4,5-T	135-136	Neurotoxicity	192
pesticide entry	3	p-Nitrophenol (PNP)	65
pesticides from residues in food, exposure	11	O	
routes of entry for chemicals into ..	16	Occupational exposure to pesticides ..	5
sodium fluoroacetate in body	17	Occupational Health and Safety Administration	59
2,4,5-T	133-154	Occupational pesticide exposure, minimization	7
tissues, biophysiological analysis of chemical residues	15-21	Oral exposure to pesticides	164
tissues, chemical residues	1-7	Orchardists spraying pesticides, assessment of potential health hazards	157-167
Humans excretion of 2,4,5-T after oral dose	137t	Organic pesticides, synthetic	1
Hydrocarbon, chlorinate, exposure, symptoms	62	Organochlorine insecticides	2
α -Hydroxy ester, chlorinated aromatic	105	Organophosphate(s)	
I		assessing toxicity	192
Ingestion vs. absorption studies, 2,4-D and 2,4,5-T	129-130	biological effects	3
Inhalation of pesticides	76, 161-164	effectiveness of closed-transfer systems for mixing and loading	
Insecticide(s)		toxicity category I and II products containing	43-46
organic	84	exposure, symptoms	62
organochlorine	2	in the field, animal model for testing	189-199
organophosphate	2	intoxication	64
organophosphorus (OP)	23	insecticides	2
residues, tests for toxic dislodgeable	23	oxons	67
testing foliage for safe residue levels using rapid field method	35	pesticides, monitoring workers using	41-42
Intoxication	16	residues, monitoring the exposure of field workers	46-54

- Organophosphorus (OP)
insecticides 23
pesticides, detecting exposure 41-42
residues on crop foliage, estimating 23
residues on foliage, absorbance
values for safe levels 35, 37t
- Oxon(s)
dialifor 64
organophosphate 67
residues on tree foliage, procedure
for establishing safe levels for
total dislodgeable thion 24, 34t
sulfoxide, safe levels for
chlorthiophos 35
tree foliage 24-35
- Oximes 42
- P**
- 2-PAM (pyridine-2-aldoxime
methiodide) 42
- Paraoxon 63, 64, 184
on orange trees, dissipation 24, 26f-27f
- Parathion 63, 64, 184
ethyl 67
exposure 59
on orange trees, dissipation 24, 26f-27f
-paraoxon
dislodgeable residues 25
safe levels 35
on tree foliage, safe levels 24-35
toxicity 3
- Pentachlorophenol 171, 174
- Percutaneous absorption of
pesticides 164-165
- Pest management, federal working
group 5
- Pesticide(s)
alveolar absorption 165
assessment of potential health
hazards to orchardists
spraying 157-167
chemical penetration through
cloth, following dermal
absorption 175t-177t
contact dosage 158
dermal exposure 158-161, 169
maximum levels 76
detecting exposure to
organophosphorus 41-42
dose-response relationship 166
entry into humans 3
exposure
with coupling effects 185
dermal 158-161, 169
minimization of occupational 7
occupational 5
oral 164
routes 185
- Pesticide(s) (*continued*)
gastrointestinal tract absorption 165
on humans, long-term effects 4
impinging on skin, measuring
amount 159
inhalation 161-164
maximum exposure levels 76
in agricultural reentry incidents 61t
levels in workplace 158
measuring clothing or dermal
exposure 77-78
measuring dermal absorption 78
and metabolites, analyzing blood
and urine 41-57
mixer, loader, applicator exposure
studies 77-79
monitoring workers using
organophosphate 41-42
not cholinesterase inhibitors,
monitoring workers using 54
percutaneous absorption 164-165
poisoning 6
procedure to monitor worker
exposure 84-89
protection of agricultural workers
using 5
registration, guidelines 6
residue(s)
animal food products, level 10
data to dermal dose, relating
dislodgeable 185
decay dynamics, regional
difference 184-185
estimating exposure 10
exposure of agricultural workers 183
field trials 10
food, exposure of humans 11
levels, analytical methodology
to determine 10
livestock 10
methods for determining 184
methodology for estimating the
dietary exposure 9-13
soil and foliage 77
transfer from foliage to skin 38
risks to agricultural workers using .. 4
safety program of the California
Department of Food and
Agriculture 75-80
safety programs by measuring
blood cholinesterase,
monitoring 41-57
synthetic organic 1
tolerance levels 9
in foods 11
tree foliage, establishment of safe
levels 25t
- Phenoxy herbicides in humans,
excretion studies 119

- Thion-oxon residues on tree foliage,
 procedure for establishing safe
 levels for total dislodgeable 24, 34*t*
- Tissues, chemical residues in human .. 1-7
 biophysiological analysis 15-21
- Tolerance levels, pesticide 9
 foods 11
- Toxic dislodgeable insecticide
 residues, tests 23
- Toxicity 166
 category I and II products con-
 taining organophosphates and
N-methyl carbamates 43-46
 organophosphates, assessing 192
- Toxicology studies, animal 10
- Tree foliage
 establishment of safe levels
 of pesticides 25*t*
 oxons 24-35
 procedure for establishing safe
 levels for total dislodgeable
 thion-oxon residues 24, 34*t*
- Trees, orange, dissipation
 azinphosmethyl-azinphosmethyl
 oxon 24, 28*f*-29*f*
 methidathion-methidathion
 oxon 24, 30*f*-31*f*
 paraoxon 24, 26*f*-27*f*
 parathion 24, 26*f*-27*f*
- S,S,S*-Tributyl phosphorotrithioate
 (DEF) 189
 exposure of applicator 197*t*
 field test 192-193
- 2,4,5-Trichlorophenoxyacetic acid
 (2,4,5-T) 133
 applicators, studies 137-153
 humans 133-154
 dermal application studies 136-137
 excretion after oral dose 137*t*
 oral administration studies 135-136
 ingestion vs. absorption studies 129-130
 pharmacokinetic studies 135
 sprays, toxicological significance
 of exposure 153-154
 in Sweden, occupational exposure .. 152
 in urine 120
 excretion after oral dose 136*t*
 spray applicators 139*t*, 148*t*
- U**
- Urinary
 chlorobenzilate residues vs. time,
 plots 113*f*
 DEP concentrations 179
 dialkylphosphate levels 43
 dicofol residues vs. time, plots 114*f*
 excretion level of 2,4-D by aerial
 and ground workers 119-131
 excretion of 2,4,5-T after oral dose 136*t*
- Urine, analysis
 alkyl phosphates 42
 chlorobenzilate 105-117
 2,4-D levels 119-131
 dicofol 105-117
p,p'-dichlorobenzhydrol 105-117
p,p'-dichlorobenzillic acid 105-117
p,p'-dichlorobenzophenone 105-117
 metabolites 63
 pesticides and metabolites 41-57
 spray applicators, 2,4,5-T 139*t*
 spray crews, 2,4,5-T 148*t*
 2,4,5-(trichlorophenoxy)acetic
 acid 120
- W**
- Worker(s)
 carbaryl-dermal exposure rate, in
 aerial and ground
 applications 90*t*-97*t*
 cholinesterase activities 46
 field, monitoring exposure to
 organophosphate residues 41-54
 monitoring exposure to pesticides,
 procedure 84-89
 monitoring pesticides which are not
 cholinesterase inhibitors 54
 pesticide residues, exposure,
 agricultural 183
 reentry
 illness, acute 63
 period for phosalone on citrus 46
 regional considerations 59-70
 urinary excretion level of 2,4-D
 by aerial and ground 129
- X**
- Xenobiotics 16