AGRICULTURAL CONTROL CHEMICALS

Collected Papers from the Symposia on Economic Poisons presented before the Division of Agricultural and Food Chemistry of the American Chemical Society at the 115th national meeting in San Francisco, March 28 to April 1, 1949, and the 116th national meeting in Atlantic City, September 18 to 23, 1949

AMERICAN CHEMICAL SOCIETY
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INTRODUCTION

The growth in the number of symposia presented at meetings of the AMERICAN CHEMICAL SOCIETY has been rapid in the last several years. The importance of these symposia is widely recognized. They provide opportunities to review and evaluate scientific and technological advancements at periodic intervals. In an era when research is expanding at a rapid rate and scientists and technologists are finding it increasingly difficult to keep abreast with even a small portion of the published literature, periodic symposia perform a most useful purpose.

Occasionally, however, symposia are of such highly specialized nature that only a relatively small percentage of the readers of any one of the established A.C.S. journals would be served by journal publication. The problems of publishing such symposia in their entirety in the regular journals are increasing, particularly so when the demand continues unabated for editorial pages to report the results of original research.

Not infrequently, too, symposia contain a large number of papers that do not fall within the editorial scope of the established A.C.S. publications. In these instances many papers are released for publication elsewhere, and many may not appear in print in any established journal.

To satisfy the obvious need of providing a medium for the publication of highly specialized symposia in their entirety, the editors of *Industrial and Engineering Chemistry*, *Analytical Chemistry*, and *Chemical and Engineering News* proposed to the Board of Directors of the AMERICAN CHEMICAL SOCIETY the establishment of an ADVANCES IN CHEMISTRY SERIES. Permission was readily received from the Board of Directors on June 4, 1949.

The volumes of ADVANCES IN CHEMISTRY SERIES will be numbered consecutively. It is expected that the ADVANCES IN CHEMISTRY SERIES will be recognized as an integral part of the permanent literature, and will be covered by the abstract journals.

Number one of ADVANCES IN CHEMISTRY SERIES, "Agricultural Control Chemicals," is a collection of papers from the Symposia on Economic Poisons, organized under the chairmanship of J. L. St.

John and presented before the Division of Agricultural and Food Chemistry of the AMERICAN CHEMICAL SOCIETY at the spring 1949 meeting of the AMERICAN CHEMICAL SOCIETY in San Francisco and the fall meeting in Atlantic City.

This first number is a perfect illustration of the desirability of establishing a special medium for the publication of certain symposia presented at AMERICAN CHEMICAL SOCIETY meetings. Only a relatively small percentage of the papers can be said to be strictly chemical in nature. Many deal with such subjects as the toxicological effects of economic poisons, public health aspects, etc. Only through the establishment of the ADVANCES IN CHEMISTRY SERIES was it possible to present the symposia in their entirety.

In introducing this series we are highly optimistic in our belief that it will help to solve at least some of the present publication problems facing the AMERICAN CHEMICAL SOCIETY.

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Objectives and Scope

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Further information is needed on the fundamental chemistry, mechanism of action, and toxicity of the newer economic poisons. An outline is presented of a research program that emphasizes the breadth and scope of the problem and the variety of research and educational needs.

During the organization of these symposia much interest in the field of agricultural control chemicals was evident. It is difficult adequately to express appreciation to those who have so generously responded with both suggestions and papers. A broad field of topics was included in the suggestions received. It became evident in attempting to organize the suggested topics into a program of titles that it would be imperative to limit the field to be covered. The symposia were thus confined to certain phases of the field of insecticides.

Many other topics in addition to those covered by the titles in these symposia were proposed, and may form the basis for future symposia. Other suggestions included the broad and important topic of formulation, which may many times have an important relation to the effectiveness of the economic poison for the purpose for which it is designed, may be modified to prepare a given pesticide for different uses, and may also influence its toxicity to warm-blooded animals. A report of work on methods of application and their relation to effectiveness was suggested, including much work on the use of concentrated sprays.

The fundamental chemistry, especially of the newer economic poisons, is of primary importance. The mechanism of action of the various types of economic poisons and the relation of structure to toxicity of insects are of fundamental interest. Chemical versus biological methods of evaluation should be presented. Performance methods of evaluation of these chemicals have been given careful consideration by several workers. Emphasis was placed by several workers on the need for much additional information on various aspects of the problem regarding the use of DDT, 2,4-D, and other pesticides. There is direct importance in studies on the metabolism of DDT.

The toxicity and the physiological action of insecticides, fungicides, rodenticides, and herbicides on plants are of basic importance. The toxicity of treated plants to animals, and the toxicity of treated plants and animals to humans and to wildlife are of practical concern. A long-range consideration of the effect of sprays on both plant and human nutrition and its relation to public health is of direct concern. The hazards in field application and methods of protecting operators should be reported in detail and further research should be emphasized.

The relative toxicity, especially of the newer compounds, to various pests and species is of practical importance, as are also differences in species tolerance to DDT and other sprays. This is also of direct concern in relation to parasites and predators. There is interest in synergistic action and the effect of spreaders, stickers, and related products. The effect and use of repellents and attractants have received attention.

The spray residue problem has been emphasized for 30 years. It should receive continued intensive attention from a variety of viewpoints, including its importance in both

fresh and processed fruits and vegetables. The possibility of soil contamination, which was earlier emphasized with the lead arsenate sprays, should also be explored in evaluating new sprays. Information already accumulated regarding this problem should be presented in the immediate future.

It has been stated that 40% of the economic poisons are used in the Far West. It, therefore, seemed fitting that an extensive Symposium on Economic Poisons should be organized for the western meeting (San Francisco, March–April 1949) of the AMERICAN CHEMICAL SOCIETY. This was followed by the Atlantic City symposium, September 1949. A symposium on fungicides will be presented at the Philadelphia meeting in April 1950, and another on oil sprays at the September 1950 meeting of the Society.

Stanley B. Freeborn of the University of California, in the September 1948 issue of *Agricultural Chemicals*, has forcefully discussed research needs from the standpoint of the experiment stations, and pleads for an intensive attack on pesticidal problems through an organized fundamental type of research. Such an attack needs continued expansion to all research in the broad field of economic poisons as well as in agriculture.

The National Research Council has recognized the importance of the food protection problem and the need for an organization to provide technical counsel on the utilization of new materials in the production and processing of foods. The desirability of cooperative action by industry, government, and other research organizations in providing the scientific guidance required for the protection of the food supply has been emphasized.

The field of these very important chemicals covers a very broad scope. An organized consideration of all phases of the problem is needed in developing a research program. From this the most critical research needs should be selected and emphasized. The following outline presents one type of an organized picture of the breadth and scope of the problem, and the variety of research and educational needs. Chemistry plays a major role in this work.

Research Program on Economic Poisons

I. Safety and Hazards
  1. Toxicology and Pharmacology
    A. A more detailed outline appears in *Food and Drug Quarterly*, September 1949, including chemical and biochemical studies and acute, chronic, and allergic toxicity
  2. Medical and Public Health
    A. Symptoms and diagnosis, acute and chronic
    B. Antidotes
    C. Methods of treatment
  3. Use and Application
    A. Precautions and safety measures
    B. Formulation
    C. Dusting
      (1) Machine
      (2) Airplane
    D. Bait exposure stations for mammal poison
    E. Secondary poisoning hazards
  4. Consumption, Foods and Feeds
    A. Spray residue
      (1) Spray load at harvest
      (2) Removal methods
        (*a*) Fresh fruits and vegetables
        (*b*) Processed foods
      (3) Relation of effectiveness and limitation of application to residue load
      (4) Keeping quality and flavor
      (5) Processing breakdown
      (6) Effect on nutritive values of human and animal food
  5. Soils and Plants
    A. Soil toxicity
    B. Plant toxicity and nutrition
      (A and B include immediate and delayed injury)
  6. Manufacturing, Safety and Precautions
  7. Chemistry and Analytical Methods
    A. Fundamental

B. Methods of analysis for trace amounts in food and toxicological material
8. Education
A. Public press
B. Distributors
C. Research men, extension, county agents
D. User, professional and general
E. Physicians
F. Manufacturer and field representatives
II. Effectiveness and Efficiency of Pest Control
1. Entomological, Rodenticidal, Fungicidal, Herbicidal
A. Effectiveness of newer pesticides for different pests under different climatic conditions in different areas
B. Efficiency of spray application
C. Limitations in use, effectiveness, safety
D. Species and developed resistance
E. Ecological relationships
2. Chemical
A. Formulation and compatibility
B. Coverage
C. Mode of action
III. Engineering
1. Design of Equipment for Efficient and Safe Application
A. Ground equipment
(1) Stationary and portable
(2) Pumps, spray nozzles, pipe lines, pressures
B. Airplane equipment
2. Development of Methods of Application
3. Decontamination

This is a very broad and extensive program that should be of both direct scientific and practical value to manufacturers, food processors and the food industry, food consumers and general public, and governmental and industrial research. It should emphasize the value and real benefit to mankind of the use of the newer economic poisons in pest control, even though their improper use may in some cases be somewhat hazardous, necessitating regulation and adequate precautions. Undue emphasis may have been given to certain hazards in the use of pest control chemicals and insufficient emphasis to the value and benefits resulting from their use. Further public emphasis should be placed on the important role which these chemicals have played in safeguarding the health of man and animals, and on the important contribution which they have made in reducing tremendous economic losses due to pests of various kinds. Attention is directed to the papers in this volume which do place emphasis on the value and benefit to mankind.

More rapid, effective, and sound progress will be made in the introduction of new economic poisons if we proceed cautiously. Too hasty release of such products may boomerang and delay the progress of pest control for many years, with an adverse effect on the various groups involved. The inauguration and establishment of a research foundation to make possible a more comprehensive and organized study of the multiplicity of problems involved in this rapidly expanding field is needed. Such a research foundation would act as a coordinating agency and as an organizational clearinghouse for all research in this field, and thus markedly promote efficiency and maximum productiveness of time and funds.

Such a foundation would (1) collect, organize, and interpret information through numerous subcommittees; (2) determine the most critical needs in a research program and point out where priorities should be placed; (3) arrange a subsidy program to promote research in the critical areas; and (4) encourage educational programs through special committees. Other essential parts of the program as it is progressively developed may be implemented as rapidly as facilities and funds permit.

Subdivision on Economic Poisons

Interest in economic poisons has resulted in the formation of a new subdivision within the Division of Agricultural and Food Chemistry of the AMERICAN CHEMICAL SOCIETY.

This was authorized at the Atlantic City meeting (September 1949) of the Society, and organization of the subdivision has subsequently been effected.

The purpose of the subdivision as outlined by the committee is to promote interest, research, and publications on the chemistry of pest control materials, and to provide a means for the exchange of information and ideas in this field.

The interests of this group can be defined broadly to include all phases of the chemistry of pest control materials. This encompasses chemical work on substances used to control, mitigate, or destroy pests of all kinds, such as insecticides, insect attractants and repellents, fungicides, herbicides, bactericides, rodenticides, and substances for related uses. Work dealing mainly with the application of these materials and their biological properties properly belongs in the fields of entomologists, plant pathologists, plant physiologists, bacteriologists, agronomists, rodent control specialists, etc., and are not of primary interest to the new subdivision.

The group will furnish a forum for research workers in chemistry and other scientific fields to discuss problems of mutual interest, and develop a better concept of the many problems involved through the exchange of information and viewpoints.

There has been much discussion regarding the name which should be used to identify the new subdivision and the field of work covered. A referendum is now in progress to assist in the selection of a suitable name which is both inclusive and exclusive. Because a decision will not be made until the fall (1950) meeting of the Society, the use of the name "Economic Poisons" is being continued for the subdivision, at least until that time.

Thanks and appreciation are expressed to the many individuals and groups whose generous cooperation and help have made possible the organization of both the symposia and the new subdivision of the Society.

The subdivision on economic poisons appreciates the privilege of presenting the papers included in the San Francisco and the Atlantic City symposia as the first number of the new AMERICAN CHEMICAL SOCIETY ADVANCES IN CHEMISTRY SERIES.

World Use of Economic Poisons

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This paper points out a few simple but fundamental reasons why expansion of the world use of economic poisons is being seriously retarded. These remarks are based on personal observations in twenty different countries of North America, South America, Europe, and Africa, and on contacts with technical men in as many more countries.

It is obvious that the world use of economic poisons can be greatly expanded; no attempt will be made to say how much. The use of pesticides is frequently associated with the spectacular nature of a pest or the urgency of meeting its attacks. Grasshopper or locust campaigns are, generally speaking, easy to sell to the administrative powers because these periodic pests destroy all the crops attacked. Quick action must be taken to save the crops. On the other hand, continual annual crop losses from other pests may, over a period of years, equal or exceed those caused by locusts, but these losses are accepted as a matter of course because only a part of the crop is lost each year. In 1945 three Central American countries imported 38,500,000 pounds of copper sulfate and only 75,000 pounds of all other pesticides. The copper sulfate was used almost exclusively in the preparation of Bordeaux mixture for the control of the sigatoka disease of bananas. Without this control measure, the bananas could not have been grown profitably.

During 1948 in the same countries the imports of certain other pesticides increased greatly because of the need for locust control. It is to be expected that, after the locust outbreak subsides or is controlled, these imports will drop again.

The use of economic poisons has expanded in the field of public health since their indispensability has been shown in the control of insect-borne diseases. The organic insecticides, especially DDT, are now being used extensively in the control of malaria, yellow fever, dysentery, and other diseases. Their use in this field will certainly expand for years to come.

Lea Hitchner of the National Agricultural Chemicals Association estimates an annual use of $200,000,000 in pesticides for the United States. In spite of the fact that the United States has been using more pesticides than any other country, the amount used has increased steadily. There is still a likelihood of expansion here.

The introduction of the Colorado potato beetle into various European countries has created a potential market for insecticides. Its recent spread into Germany and Poland has been followed by a marked increase in the use of various kinds. The past history of this insect indicates that steadily increasing amounts of insecticides will be needed annually in order to control it.

More examples could be given to show potentially greater use of economic poisons, but all would show the same trend.

The manner in which the world chemical industry quickly produced vast tonnages of DDT during the war was amazing. Even more so was the efficiency achieved in production, so that entomologists had an insecticide available at a price which permitted its

use. The chemical industry is still far ahead of the entomologists. New insecticides are being produced more rapidly than the entomologists can test them. The same can be said for other pesticides used to combat plant pathogens or weeds. The new chemicals must be properly tested and evaluated before being released as pesticides. Much cooperative work is needed. After all, DDT was only a chemical entity for over 60 years before its insecticidal properties were discovered. Today, in most of the world, application of the presently known good pesticides is needed far more than discovery of other new ones.

Work of the FAO

These observations relating to the use of economic poisons have been made through the work of the Food and Agriculture Organization of the United Nations. FAO is an autonomous body of 58 nations concerned with all phases of food, agriculture, forestry, and fisheries throughout the world. It was conceived because, in peace or war, the majority of the world's peoples are not amply fed and on the premise that food is basic to human well-being and world peace.

FAO is not a relief agency. It does not purchase or distribute food products, although it is much concerned with food deficits and food surpluses. Neither is it a research agency, though it does try to enlist the interest of scientists in its fields and coordinate their efforts in the solution of pressing problems. Finally, of course, it is not a political organization, but operates as an international extension agency on a strictly impartial basis.

At the present time many nations are sharing their food with other less fortunate nations—less fortunate because of the devastation of war, drought, poor crops, or other reasons. There seems to be no other immediate solution. By sharing the scientific knowledge now available for increasing food production, the devastated and underdeveloped countries can be enabled to increase their own food production. It is true that some nations will probably always be partially dependent on imports of food, but improved methods of production, processing, and storing will reduce the degree of dependency. The dissemination of technical information is a major responsibility of FAO. It may be done in various ways, according to the problem.

FAO is primarily concerned with problems relating to food. Entomology is so closely associated with the production and saving of food that this phase of work is obviously important to the Agriculture Division of FAO. The field of agricultural entomology in the world is so broad that an extremely large staff would be required if FAO attempted to cover the entire field. The entomological staff is small and its coverage is limited. However, it is not the purpose of the staff to conduct research or to participate in action programs. A few specific problems of world-wide interest have been selected for study. These include the pests of stored food, the Colorado potato beetle, the Moroccan locust, and the field insects of China. In addition, advice may be given on control of introduced insects. The technical knowledge of America and western Europe is drawn upon freely. The task of FAO is to assist the member nations, especially those least developed; consequently, this section becomes a kind of international entomological extension service.

Stored Food

Attention has been focused by FAO on the tremendous losses in stored food over the world caused by insects, rodents, and fungi. International meetings have been held on the subject in Washington, D. C., London, England, Florence, Italy, and Cali, Colombia, where technicians from many countries have gathered to study the problem and exchange information on means of solving it. In addition, field trips have been made to different countries to study grain storage. As a result of these meetings and field trips some progress has been attained.

The potential use of economic poisons in this specialized field may be stated as follows: The use of fumigants will increase, but no faster than the construction of improved storage facilities which will permit the efficient use of fumigants. Residual insecticides will increase in use for many years, but the total tonnage will not be high. In a few countries,

insecticides are being added directly to grains to ensure protection against insect attack. A trend in this direction could result in a great increase in the use of economic poisons. Two factors are delaying it at this time—cost and hazard to the consumers. The millers resist any addition of foreign material to the grain because they have to take it out again. In the small subsistence-producing nations this method appears to hold much promise. A general use of rodenticides in grain storage plants would not require many tons of chemicals and would not be much of a factor in expanding the world use of economic poisons. The heating of grain in storage has been controlled by the use of certain chemicals added to the grain, but the cost is still too high for practical use. The fungi are controlled by drying and there is little likelihood of a market here for economic poisons for many years to come.

Costs

Further remarks regarding costs of insecticides or inadequacy of equipment used are generally applicable to the whole field of pesticides and are not limited to their use in grain storage only.

Why is the world use of economic poisons being retarded? The price paid for the pesticide by the ultimate consumer is frequently prohibitive in importing countries. The quality of the pesticides obtained from the major producing countries is excellent, but this quality is of little value if the price prevents its use. A few examples may be quoted to demonstrate this point.

In one European country where field insecticides are not generally used but are very promising, 50% wettable DDT of American manufacture was purchased in midyear 1948. The retailer was a large cooperative with presumably favorable prices to consumers, yet the price to the consumer in that country, at the official rate of exchange, was roughly $1.50 per pound. At the same time this identical material was selling f.a.s. New York at $0.23 per pound. It is granted that the price per pound must be higher to compensate for freight, handling, selling costs, etc., but there is no justification for an increase of 500%. The inevitable result is either the refusal of the imported material as an aid to agriculture or, in case of emergencies, a trend toward establishment of small local manufacturing plants which are made secure in their inefficiency by protective tariffs. The end result is the same to the consumer—prohibitive prices. The example given for Europe can be repeated for numerous other countries and on other economic poisons. The differential may be as great in a few places. It is not found on all poisons. The correction of this one factor lies mainly in the sales organizations responsible for supplies to the foreign markets.

Equipment for Pesticide Application

Pesticides and the equipment with which they are applied are so fundamentally associated that it is astonishing to see how often these two items are separated. Pesticides are frequently sold in a foreign area with complete disregard of the available means of application; yet the whole future market depends mainly upon the proper distribution of the materials. The want of suitable equipment can be a very serious factor in retarding the use of economic poisons.

There are many examples of equipment being sold in areas for which it is not suited. The unsuitability may be a lack of trained operators, a lack of basic knowledge of the equipment's possibilities, equipment inappropriate to the terrain, or, occasionally, equipment so highly specialized that it cannot be adapted to the general use for which it is needed. In general, simple equipment can best be used to demonstrate the value of the use of pesticides.

One specific piece of equipment, which had to be mounted on a vehicle, was extensively sold for locust control in an underdeveloped country. The roads in this area were not passable to trucks and the machine was mounted on a jeep. The machine was too heavy for a jeep, so the net result was a broken-down jeep and a piece of specialized equipment shaken to pieces by travel over almost impassable terrain. Add to this the complete lack

of operators with mechanical training and a lack of appreciation for dosage per acre, and the final result is either of two things: When the equipment is working the amount of insecticide applied per acre greatly exceeds the needs, and when the equipment is not working a large crew of workers and other vehicles are inactive for long periods. Simple one-man dusters would have done a far better job at a greatly reduced cost. The value of the insecticide also would have been better demonstrated.

In some areas where grapes are raised on a large scale the use of small one-man sprayers is well established for the control of disease.

In Southern Europe, for instance, these sprayers have been designed for the use of inexpensive fungicides, typified by home-mixed Bordeaux mixture. Neither the nozzles nor the guns are suitable for the use of high-priced organic insecticides or fungicides. In order to get the proper application of the new pesticides, suitable nozzles and guns must be used. At present the flow rates are too high and the spray patterns are far too coarse to give desirable coverage with such insecticides as wettable DDT or benzene hexachloride. The guns are frequently a plain quarter-turn valve, rather than a simple gun with a mechanism for permitting the quick release and cut-off of flow. The only justification offered for a quarter-turn valve in place of a gun is the very minor saving in the original cost. This is not good economics, because the savings in the original investment are quickly lost by the wasted pesticides of a few days' field operation. Theoretically, when spraying fruit trees the operator always turns off his quarter-turn valve when walking from tree to tree. In practice, however, this is frequently not done because both the operator's hands are occupied otherwise.

It becomes only too obvious that many of the designing engineers have not used, under practical conditions, the equipment they have designed. Equipment of this type is being sent into South America. There are many other small factors that affect the operation of pesticide-applying equipment. There is, unfortunately, a tendency on the part of many people to forget the basic reason for spraying—the control of plant pests. The chemical industry should keep in mind that pesticides, no matter how excellent their quality, or how abundant their quantity, are useless in a warehouse and are of value only after they are applied in the field. The means of application is therefore a very fundamental consideration in the expansion of economic poisons. In recent years there has been a great increase in the degree of cooperation between the engineers designing equipment and the scientists who are concerned with application.

The lack of technicians in underdeveloped countries is a serious handicap in the immediate expansion of the use of economic poisons. The salesmen representing the producers commonly tend to exaggerate the merits of their products when selling in the foreign market. Claims are made without scientific investigation to substantiate them. Such practices are particularly harmful because of the lack of technicians. Help is needed from technicians who can work on the problems in other countries, not at home. There is, for example, a very large potential market for an insecticide that will control leaf-cutting ants. These insects are one of the major entomological problems from Mexico to the Argentine. A simple cheap method of control is needed. There are several promising leads, but almost nothing is being done to find such a method.

The labels of pesticides going into foreign markets should receive careful attention to guard against misunderstanding the directions. There is likely to be a language difficulty, and even in the foreign language the directions should be clear and concise. And the change to the metric system should not be overlooked.

Much misunderstanding can be avoided by sending technical men to give first-hand information where export is desired. Some customer service in foreign markets by technicians would pay excellent dividends in the increased use of pesticides.

The world use of economic poisons can be expanded. There are simple basic factors retarding this expansion: prohibitive consumer prices, lack of equipment with which to make proper field application, lack of skilled personnel in countries needing the benefits of economic poisons, exaggerated claims of nontechnical salesmen, and difficulties involved in labeling the export products.

Insecticides in Agriculture

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There is general agreement that destructive insects cause tremendous losses; the figure currently used is $4,000,000,000 for an average year. Insect control presents many problems, the simplest of which is the correct concentration and rate of application. For development of insecticides cooperation of chemists, entomologists, and toxicologists is essential.

Estimates of losses caused by insects have been assembled by different workers at various times and in various ways, but none have attempted to consider all the destructive species. Not all insects are destructive; in fact, many kinds are beneficial. No summary has been made of the value of insects.

All estimates of losses have dealt largely with the destructiveness of the better known important species that attack crops and products needed by man. The losses reported in earlier estimates were restricted to comparatively few species. Large as these were, they were much less than the currently used figure of $4,000,000,000 for an average year. The wide difference between the estimated losses of today and those of some fifty years ago is due to many factors. Some introduced insects have become important destructive pests since the earlier estimates were made. Agricultural conditions have changed during this period and many native species are now causing heavy losses to crops which replaced their native hosts. In storing and handling our agricultural products we have not taken steps to prevent losses. Some current practices make the products more susceptible to attack by insects and encourage multiplication of the pests. The public has become insect-conscious and rejects products which are infested. This increases loss and raises the standard for control. Wormy apples, insect fragments in cereals and canned products, are no longer acceptable. Many other changes in our habits and methods of agriculture could be cited to explain the wide difference in estimates that have been made of the losses that insects cause to agriculture.

Irrespective of the method used in preparing estimates of these losses, all investigators agree that the losses are tremendous. A few examples to illustrate this destructiveness have been taken from a report of hearings before a Congressional committee (*1*).

Crop	Pest	Period	Average Annual Loss Dollars
Cotton	Boll weevil	1909–29	256,015,000
		1946	319,349,000
Corn	Earworm	1945	140,000,000
	Corn borer	1946	37,000,000
Stored small grains	Weevils and moths	1947	600,000,000
Balsam, fir, and spruce	Spruce budworm	1910–20	4,500,000
Ponderosa pine	Black Hills beetle	1947	15,000,000
Apples	Codling moth	1940–44	50,000,000
Citrus	California red scale	1943–44	10,000,000
Beans	Mexican bean beetle	1944	5,502,000
Onions	Onion thrips	1944	14,500,000
Tobacco	Hornworm	1944	84,073,000
Cattle	Cattle grubs	1940–44	160,000,000

Benefits from Insect Control

Important as estimates of losses may be, information on the prevention of losses is of greater interest and significance. Measures developed by entomologists for the control of many important pests involve the use of several different procedures: following certain farm practices, using cultural methods, distributing natural enemies, applying insecticides, and using mechanical devices.

Information on how control may be attained has been disseminated by many means, and an effort has been made to encourage farmers and others to use them. The extent of use has varied greatly and it is well recognized that full advantage is not taken of what is now known on how to prevent losses caused by insects. A few illustrations of controls requiring the use of insecticides will show the benefits that have been derived where control measures have been applied.

In 1947 growers in Louisiana who applied measures to control wireworms increased the yield of cane by 20 tons per acre and the acre yield in sugar by 3500 pounds.

In 1947 the yield of potatoes treated with DDT insecticides increased as much as 30% above the yield in untreated fields.

In Utah the production of alfalfa hay has been nearly doubled in areas where insecticides were applied; and in areas where alfalfa seed was produced the yield has increased by 600% by the timely application of insecticides. In the latter case it meant the difference between a loss and handsome profit.

In 1948 the use of DDT insecticides for the control of the hornfly so increased the production of milk and meat that the county agents in several states estimated the benefits to be worth $54,000,000.

The 1946 cooperative control program against grasshoppers saved crops on 5,666,000 acres at an estimated value of $41,000,000. For each dollar spent $52 worth of crops was saved.

The aerial application of DDT insecticides to 413,000 acres protected 1,500,000,000 board feet of lumber at an estimated stumpage value of $4,600,000.

The estimated cost of protecting apples from codling moth is $25,000,000—it permits marketing a crop with an average annual value (1931–35) of $110,000,000. Without control the crop would be largely unmarketable.

In the years 1947 and 1948 the growers of lima beans in a single county in California increased their returns by $7,500,000 by controlling wireworms.

Cost of Insect Control

Satisfactory figures on the annual expenditure by growers for insecticides are not available. This varies from season to season, depending on several factors in addition to the intensity of insect infestations. It has been estimated that for the calendar year 1934 farmers and others paid about $25,000,000 for the more commonly used insecticides; for the same materials the cost in 1948 would have been approximately $28,000,000. During this 14-year interval the use of agricultural insecticides had more than doubled. The estimated expenditure for insecticides in 1948 approximated $60,000,000. Interest in and appreciation of the benefits of insect control are thus apparent.

Expenditures for insecticides alone are not an accurate index of the cost of insect control. Many control practices do not involve the use of chemicals. Where chemicals are used considerable labor is required. To this must be added operation, maintenance, and investment costs of equipment needed to apply them. When these are taken into account the cost of control increases several times. In 1938 Hyslop (*2*) estimated that the cost of controlling the more important insect pests was $142,927,000.

Problems of Insect Control

The control of insects is no easy or simple task. Even for those species that are well known and for which control measures are fairly standardized, many things have to be considered. In cases where the suitable insecticide is known, there is need for accuracy in using the correct concentration and rate of application. That is usually the simplest part of the operation. Timing the application may mean the difference between success and

failure. Proper application is also essential. Not infrequently the task of control is further complicated by the presence of several pests. This may require knowledge of the compatibility of several materials, some of which may be applied at the same time, others a few days later. With these and other factors, and the variability that is associated with all biological activity, there is need to keep in mind the effect the materials may have on the plant or animal and the product which is being protected. Are the materials safe and usable for the purpose for which they were intended? is an ever-present question.

Entomologists have had these and various other questions before them in developing measures for control. They are fully aware that answers cannot be determined in short order, and that the solution of a problem for one area does not mean that the answer is applicable to all parts of the country. Experimental work has to be done when the insects are present and during the limited active period of the stage of development when the treatment may be applied. This frequently means that experimental work can be done only during a few weeks within a year.

Standard control practices developed within these limitations have taken all the various factors into consideration. Entomologists recognize the inadequacy of many of the recommended control measures. They have initiated the effort to develop new and more effective insecticides and welcomed the interest and assistance of others. The recent accelerated development of new insecticides has opened new fields and done much to stimulate public interest in insect control. Much more needs to be learned than to know that the new materials will kill insects, before their true worth can be determined. We must determine many things. Knowledge of the effect on beneficial insects, wild life, soil, and machines is important. Of greater importance is: Are they safe for the operator and for those who handle and use the product?

Cooperation in Development of Insecticides

Many of the new insecticidal chemicals are complex organic materials with long and complicated names. There is need for simple common names. Without simplified terminology costly losses may result from relatively minor errors in nomenclature. Coined names must be protected if they are to remain available for general use, and must be defined with sufficient accuracy to assure that a standard product will be marketed. Selection of such names requires cooperative consideration by many agencies. Chemists can make important contributions to this undertaking.

The complexity of the new insecticidal chemicals brings many other problems. Synthetic organic chemicals are not effective against all pests. There is a marked selectiveness in action even between closely related species of insects. Some insects have already developed resistance to some of the newer materials. The idea of insects developing resistance to certain chemicals is not new. The over-all principle is well established in a few cases. The early development of flies resistant to DDT, a chemical which had been highly and universally effective for fly control, came as a surprise. Other cases of resistance to DDT are being indicated, and at least one kind of mite has developed resistance against another of the newer chemicals—parathion.

With chemicals that are as highly toxic to insects as some of the newer ones are, other problems arise in the development of methods for their use. When only a small amount of a chemical is needed, ways must be devised to apply it. The first of these is to determine how the chemical can be formulated into an insecticide. This is not a task for the chemist alone, because not all solvents, carriers, and diluents, even though suitable from the physical-chemical point of view, are equally effective entomologically. Here the chemist and entomologist must work together. The more closely and understandingly they cooperate the sooner the answer will be developed.

When the new materials are developed, there is still the need to determine that they can be effectively and properly applied. This requires investigations in the field of application equipment—sprayers, dusters, etc. The standard machines for handling large volumes of insecticides are not suitable where the dosage is reduced—as it can be—to amounts as low as a gallon of finished insecticide per acre.

The development of new insecticides means even more. It requires appraisal of the effect of the material on the operator in all stages of use. It involves knowledge of the amount of residue that may remain on, or in, the part of the product that is used as food for man or beast and the effect of such residue on their health. Leadership in such studies belongs to the toxicologist. The chemist, however, has a very important relation to these problems. He must supply the method for analysis and for removal of insecticidal residues. For some of the new insecticidal chemicals the entomologist has accurate information on their effect on insects. Suitable, satisfactory methods of analyses of the chemical and its residues await determination.

Outlook for Insect Control

The field of insect control is expanding; the need for it is better recognized and appreciated. Agricultural development will in future depend on pest control much more than in the past, and it will be a salient part of the production and protection of agricultural commodities. Chemicals are essential for the control of many major pests. It is highly important that chemists whose part in the cooperative effort is of increasing importance, increase their interest in and understanding of the wide variety of problems. Entomologists welcome the opportunity to work closer with the chemists. The author, for one, is delighted with the expanding opportunities for developing the cooperative teams needed to find sound, safe, effective, economical answers to the numerous problems of insect control.

Seasonal Market for Insecticides

In general, the use of insecticides is seasonal, and the demand for them fluctuates greatly because the intensity of insect infestation differs widely in various years. Peak seasons of infestations may be separated by years. The market for insecticides is, therefore, highly fluctuating; and success in the business of producing and marketing insecticides depends in no small measure on the ability to have the insecticide available at the time and the place needed. Production and marketing of insecticides offer a greater challenge to management and judgment than do many other fields in the production of chemicals.

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Some Problems in the Use of Newer Economic Poisons

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Some of the economic problems that confront the chemical industry and some practical problems of agriculture and society in general, resulting from the use of newer economic poisons, are discussed. Above all else, more facts are needed upon which to base both present procedures and future studies.

No adequate discussion of even a single major problem associated with the newer chemicals can be offered without neglecting equally important and related problems. Actually, the titles of the papers presented in this symposium reflect the complexity of the situation resulting from the rapid development of sharp chemical tools for combating pests.

This paper discusses, first, some of the economic problems that confront the chemical industry and, secondly, some practical problems of agriculture and society in general, which are associated with these newer products. There may be little that is new in such a procedure, other than to bring well known facts into a relationship that appears to be somewhat vague. There is a recognized need for a clearer understanding between research workers in public agencies and the men whose task it is to make available the products that are needed in a progressive society. The struggle to maintain a working equilibrium becomes more intense as the numbers of products multiply and the speed of development is increased.

Economic Problems

The costs of developing and producing new economic poisons are well known to the men of industry. To the average research worker, particularly in agriculture, these costs are merely words. They are, however, definite segments of the problem of availability and use. No social contribution has been made by either institutional research worker or chemical manufacturer until people have found a use for a product and are willing to purchase it because it will do something safely, more efficiently, at less cost, or more agreeably than compounds previously used. The newer insecticides and fungicides have these qualifications.

Basic manufacturers of chemicals and local formulators have some mutual problems and each group has difficulties peculiar to itself. The costs of screening new products, trying to find one useful compound among thousands that prove to be useless, the expenses of pilot plant operations, conversion to plant production, product control, getting product acceptance, working out details of insurance, and safety precautions as well as general advertising, sales, and administrative costs give the basic manufacturer many a headache. The continuous fluctuation in prices of raw materials, machinery made obsolete by new products or processes, new packaging requirements, provisions for meeting the nearly 500 laws under which the industry operates, all contribute to the ultimate cost that a consumer must pay.

The local formulator who purchases concentrated products from the manufacturer has his problems. More frequently than not the advent of a new product catches him with a stock of some older chemical which the new one will replace. Lead and calcium arsenates, rotenone, pyrethrum, sabadilla, and others all have lost some usage to the newer economic poisons. Costs such as these cannot be passed on to consumers because of that grand American institution, competition.

Concentrates of new chemicals are always expensive, but they frequently change rapidly in price. Picture the position of a small but locally important mixer of insecticides remote from sources of supply, who suddenly finds himself with a substantial inventory of a new chemical purchased at 15 to 20% above a newly announced price. His competitive position becomes what is known as "poor."

With many of the newer economic poisons manufactured in the East, west coast formulators find themselves at a disadvantage with respect to both freight rates and supplies. . Everyone realizes the almost impossible task of estimating the amounts of insecticides or fungicides that will be used in a given year, particularly where new compounds are concerned. There is yet to be discovered a formula by which the annual guesstimate of "how much" can be solved. The end result is that most smaller, local mixers play conservative and sacrifice possible savings in carload prices, supplies, and sales, by purchasing l.c.l. lots. This is, of course, doubly true where costly compounds are concerned. These factors all enter into the ultimate price to the consumer.

The local manufacturer shares with the basic supplier the problems of machinery changes, safety precautions for operating personnel, developing useful local formulations, product control, product acceptance, and the usual administrative, advertising, sales, and delivery cost. Furthermore, these secondary manufacturers, despite ever-increasing help from the more progressive manufacturers, must work out many problems of labels, containers, and compliance with local laws or regulations concerning manufacture and sale of new formulations. All this takes manpower, time, and money. If these local institutions are to continue filling the long-established needs which their services have merited, as evidenced by the demand for the goods they produce, the costs of those services must be reflected in the price the consumer pays. The increasing demand for personnel adequately trained to cope with the use of new chemicals does not lessen the costs.

All these facts and many more are well known to any commercial man in the insecticide or fungicide industry today. When agricultural scientists and advisers talk with growers, they frequently overlook many of these factors. On the other hand, the men of industry frequently forget some of the complex problems associated with the widespread use of potent chemicals.

The processes of nature cannot be hurried and most of the knowledge we have is relatively fragmentary. Practically every known agricultural practice is subject to continuous scientific scrutiny, with the result that periodic revisions are almost the rule. If our oldest concepts are so subject to change, how very little we must know about these five-year-old marvels. It is natural for everyone involved to be concerned with the possible hazards which may be incurred as a result of volume use of newer economic poisons.

Harmful Residues

One of the first considerations in the use of any chemical is possible accumulations of harmful residues in soils. Evidence proves beyond any doubt that many of the newer compounds remain in the soil for at least 5 years. How much longer they may persist, time alone can determine. We know that both selenium and molybdenum can be picked up from soils by plants, which thus become extremely toxic to animals, even when plants themselves are apparently unharmed. Often a given piece of land may be treated safely as far as one crop is concerned but another crop may be injured. For example, potatoes will tolerate large amounts of DDT in the soil as a means of controlling wireworms which are extremely destructive; melons, on the other hand, are severely injured by excessive DDT in the soil. There can be no assurance in many cases as to the ultimate use of any

piece of land, so the implications are clear. A similar situation exists in the case of sweet potatoes, which may be treated with benzene hexachloride (hexachlorocyclohexane) soil applications, whereas white potatoes are very susceptible to taste changes from even small amounts of the chemical in the soil. The long-time effects of chemicals in soils on microorganisms are of sufficiently serious import to slow down the most enthusiastic sales manager.

The widespread use of economic poisons has a definite impact on the animal complex on the face of the earth which provides our sustenance. Already we have seen the use of DDT for codling moth control on apples result in a relatively minor pest becoming a serious threat. The same material used as a wonder spray for fly control now fails, after a couple years of common usage, with the appearance of new, resistant strains of flies. Bees and other pollinating insects as well as helpful predators or parasites may be decimated and their important aid be lost by untimely or improper use of most of the newer insecticides.

The danger to domestic animals, including pets, is an important hazard in the use of all newer economic poisons. Drift dusts or sprays from carelessly applied materials may set back the useful development of many valuable chemicals. Only recently have authorities finally decided that we should slow down on the use of DDT on cows until we know more about the occurrence of the chemical in milk, butter, and steaks. Our sportsmen and, incidentally, a major economic factor in our pleasant way of life—the fish, game, and wildlife activities—are part and parcel of the problem of chemical usage in forests and streams. We need continued and expanded investigations of the effects of the newer pesticides on wild life.

There is a tremendous financial investment in our agriculture. The place which this country occupies in world leadership today is due in part to our food-producing capacity. To maintain that position at a time when portentous predictions are rife regarding an overpopulated world, we need ever-improved chemical aids, but we must, at the same time, impair neither the quantity nor quality of our orchards and vineyards. Frequently it takes 10 to 20 years or even more before we see the ultimate effects of continued usage of chemicals on plants. The financial and social losses which can result from improper or unduly hasty use of chemicals should temper profits with caution.

Effect on Man

Finally, new and little-known chemicals present hazards to man himself, no less complex than the biological enigmas of agriculture. Plant workers, commercial pest control operators, dealers and feed store employees, growers, farm hands, food industry workers, and consumers all have the mutual problem of both acute and chronic toxicity. Within the past two years we have seen marked strides in the fields of study which concern themselves with the toxicity to man of all the newer economic poisons. It is self-evident that a compound designed to kill one living organism will affect other living things. What we most need to know is the margin of safety between those organisms which we would kill and those which we would keep unharmed. Most of the newer economic poisons are actually less toxic than those which we have used in volume for years. Cyanide, arsenic, strychnine, and nicotine have a much more fatal ring to the average person than DDT, benzene hexachloride, chlordan, or parathion. It is what we do not know rather than what we know which concerns us most about the newer compounds.

Investigation, Education, Regulation

Obviously, some definite responsibilities must be met. They can best be met by investigation, education, and regulation in that order. Investigation is a job for both industry and government. It is the cornerstone upon which education and regulation must be laid. Agricultural workers in soil sciences, pathology, and entomology are well in the forefront from the standpoint of investigation of the newer chemicals. They are aware of the values of such farm aids and the need for them. The plant physiologists have not kept abreast of the effects of these same compounds on plants. The establishment of both

laboratories of their own and fellowships at institutions proves the sincere interest of industry in helping to solve the many problems involved. There is a great need for more work on the pharmacology and toxicology of the new economic poisons with respect to both man and animals.

The problem of educating the users of these new tools is a joint one, facing both industry and the public instructional agencies. If and when clear-cut answers to the many questions that arise are forthcoming from investigators, the educational aspects of the problems at hand will be solved with great rapidity. You cannot inform others until you yourself know the answers.

It is impossible to legislate safety. The death rate from automobile accidents should prove that. Furthermore, no one can regulate or legislate wisely with insufficient facts at hand. Specific legislation regarding individual compounds is unnecessary and ill advised; such rules and regulations will create more problems than they will solve. There is a definite need for some control over unscrupulous or misinformed persons who, perhaps unwittingly, would expose themselves and the public to needless waste or hazards. All reputable industrial groups recognize this and would be the first to demand such protection for themselves. There now are adequate laws for this purpose; they need unification and simplification, not multiplication.

The problems of proper usage of our newer, sharper chemical tools require closer integration of efforts and a clearer understanding among the many groups involved. Above all else we need more facts upon which to base both present procedures and future studies. Continued and increasing contact and exchange of ideas together with intelligent planning of effort can speed our knowledge and our progress. There is more opportunity and more satisfaction in our personal share of any task if we try to understand how our work meshes with that of other groups.

Labeling Requirements for Insecticides and Other Economic Poisons under Federal Law

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The Federal Insecticide, Fungicide, and Rodenticide Act affords added protection against the dangers inherent in the use of newer means of pest control. The manufacturer has greater responsibility for furnishing specific information as to how the product can be used effectively but without damage to the user, crops or animals, or the public.

The Federal Insecticide, Fungicide, and Rodenticide Act regulates the marketing in interstate commerce of insecticides, fungicides, rodenticides, and herbicides. Instruments and contrivances intended for trapping, destroying, repelling, or mitigating insects or rodents, or destroying, repelling, or mitigating fungi, are also covered by the act. This law, which was enacted on June 25, 1947, superseded the Insecticide Act of 1910. The old law covered only insecticides and fungicides and did not control the marketing of such products as effectively as does the new law. The new legislation became effective for devices on June 25, 1947, for rodenticides and herbicides on December 25, 1947, and for insecticides and fungicides on June 25, 1948.

Tremendous progress has been made in the development of pest-control measures in recent years, and with it has come an increased interest by the general public in pest control. People are beginning to realize that it is no longer necessary to put up with pests that annoy man and carry disease, or continue to suffer heavy economic losses caused by the many pests that attack livestock, growing crops, and forest and other agricultural products. Research has provided better tools and methods than were ever before available for combating these pests and research is continuing to improve them. Unfortunately, however, many of the chemicals used in pest control are highly toxic to man and animals. Great care must be exercised in handling the more toxic materials to prevent injury to man, useful animals, and vegetation. Contamination of foods with harmful residues must at all times be avoided. No doubt highly toxic substances will always have an important place in pest control operations, but they need to be used with great care, precision, and discrimination.

The new law is intended to afford the public added protection to avoid the dangers inherent in the use of these new and more effective means of pest control. It places upon the manufacturer greater responsibility for furnishing the user specific information as to how the product shall be used to be effective in controlling pests, and at the same time cause no damage to the user or his crops or animals.

Containers of insecticides and other economic poisons must bear labels showing the name or brand of the article, the net contents, the name and address of the manufacturer or distributor, an ingredient declaration, an appropriate warning or caution statement, including the word "POISON" (in red), the skull and crossbones, and an antidote statement on highly toxic materials. Adequate directions for use must accompany each economic poison. Devices subject to the act are not required to bear labels; but if they

do, no false or misleading representations may be made on labels or in accompanying literature.

Registration of Economic Poisons

Before an insecticide or other economic poison may be marketed legally in interstate commerce, it must be registered with the United States Department of Agriculture. This procedure brings the product to the attention of the department before it is tried out on the public. It gives the department an opportunity to examine the proposed claims and to question those which are likely to cause injury, before the product goes on the market, instead of waiting, as under the old law, until the product has been marketed, a sample of it analyzed and tested (which might take many months), and court action instituted.

To obtain registration, an application, together with the formula and proposed labeling, must be submitted to the department. This material is closely scrutinized by scientific personnel in the various fields involved and, if the proposed claims appear warranted and the submitted material is otherwise in compliance with the law, a notice of registration is issued. If the article is such as not to warrant the proposed claims, or if it and the labeling or other material required to be submitted do not comply with the law, the applicant is notified of the deficiencies and given an opportunity to make necessary corrections. If he then insists, in writing, that such corrections are not necessary and requests that the article be registered, the law requires that it be done under protest. Because of the higher penalties that are imposed if a person or firm is found guilty in court of marketing a misbranded or adulterated product that has been registered under protest, there is little interest on the part of manufacturers and distributors in obtaining this type of registration.

Even though a product is registered unconditionally, the responsibility of the person or firm marketing it is in no way changed thereby. Under the law, registration cannot be used as a defense for the commission of any crime prohibited by the law. Furthermore, registration can be canceled at any time if, upon the basis of later information, it appears that the labeling, previously accepted, does not give the protection intended by the law. It is the policy of the Insecticide Division, when labeling for a new economic poison is submitted for registration, to require reasonably conclusive evidence that the material can be used safely and effectively if the accompanying directions and precautions are followed. The marketing of pest-control materials before the hazards involved in their intended uses are known can be a dangerous procedure.

If reasonable doubt exists as to effectiveness or safety when proposed directions and precautions are followed, preparations should be handled only on an experimental basis. Shipments for experimental use only are exempt from the penalties of the law if used under the supervision of any federal or state agency authorized by law to conduct research in the field of economic poisons, or by others if a permit has been obtained from the department before shipment.

Before a new chemical is first offered for federal registration, the manufacturer has often carried on considerable research and spent large sums of money developing it to the point where he considers it ready to market. Generally, some tests have been made by federal and state research agencies, but these tests have not been conducted on a large enough scale or over a sufficient period of time to permit specific recommendations as to all the potential uses for the product or the care needed in handling it. In most instances there is considerable information available from one source or another concerning the material's effectiveness for some purposes. The acute oral toxicity to the usual laboratory animal is frequently fairly well known, but adequate data concerning chronic toxicity are in many cases lacking. Chronic toxicity studies require long periods to complete and are very costly.

When sufficient information is available concerning a substance to justify releasing it to the general public, labeling containing adequate directions for use and necessary precautionary statements should be prepared and affixed to each package. Users of economic poisons should be educated to follow directions and observe precautions in every instance

unless otherwise advised by competent authorities. Many of the new complex economic poison formulations must be handled like precision instruments. Careless use invariably leads to trouble.

Even when the manufacturer has carried out extended studies of his product, practical use and further experimental work will sometimes uncover faults or previously unsuspected dangers. In such cases, appropriate changes should be made on labels without delay.

Degree of Toxicity

The regulations for the enforcement of the Federal Insecticide, Fungicide, and Rodenticide Act set specific dosages by which formulations must be judged to determine the exact labeling requirements for only the "highly toxic" class of substances. Briefly, the highly toxic category is limited to that group of the economic poisons which will kill 50% or more of the laboratory animals (mice, rats, or rabbits) when the substance is administered at the dosages and in any one of the manners described below, when ten or more animals of each species are used for assay:

1. Orally, 50 mg. per kg.
2. By inhalation, 200 parts of a poison per 1,000,000 parts of air by continuous inhalation for 1 hour or less.
3. By skin contact (rabbits only), 200 mg. per kg. by continuous contact with bare skin for 24 hours or less.

Economic poisons which have acute toxicity of varying degrees below the highly toxic category have been segregated somewhat arbitrarily into three additional groups. The first might well be called the one requiring a "WARNING: MAY BE FATAL IF SWALLOWED" type of label, and includes mixtures which are less hazardous than those in the "highly toxic" group but are still toxic enough to be somewhat hazardous to handle. Many economic poison formulas which are not toxic enough to require the word "POISON" (in red), the skull and crossbones, and an antidote statement are capable of causing injury and must carry suitable precautionary labeling for public protection. The second group is one in which formulations having minor hazards are placed. This class may be characterized by "CAUTION: HARMFUL IF SWALLOWED" type of designation. The final group is the rather small class of compounds which have a relatively low toxicity and require no precautionary statements. In this class are a number of products for which claims of absolute safety cannot be used logically because some injury to beneficial plants or animals remains.

These major categories are based largely on acute toxicity records, but the significance of chronic poison hazards to the adequacy of "warning" and "directions-for-use" labeling is given full consideration whenever it is a factor for public protection.

No specific wording for precautionary labeling has been felt to be necessary or advisable, but certain patterns of suitable statements are being prepared by a number of groups, notable among which are the Labels and Precautionary Information Committee of the Manufacturing Chemists' Association and the Committee on Toxicity and Antidotes of the Association of Economic Poisons Control Officials. The Insecticide Division maintains close liaison with these groups and others interested in the problem, and acceptable recommendations are being worked out. Labeling will, of course, never be a static proposition, because initial warnings and directions for use will be subject to change as more precise information on economic poison formulations becomes available.

Federal and State Regulations

One of the difficulties encountered by producers of economic poisons has been the preparation of labeling which will comply with the laws and regulations of both the Federal Government and the various states in which the product is marketed. This difficulty has been aggravated by differences in interpretation by different enforcement officials. The Insecticide, Fungicide, and Rodenticide Act recognizes this difficulty and authorizes the

administrative authorities to cooperate with state regulatory agencies in carrying out the provisions of the law and in securing uniformity of regulations. Full advantage is being taken of this authorization through both cooperative arrangements for enforcement work and discussion with representative groups of state officials. In consultation with the Executive Committee of the Association of Economic Poisons Control Officials, a set of interpretations of the federal regulations has been prepared and published, so that the manufacturer and all others concerned can be governed accordingly. The closer cooperation between federal and state officials is tending to iron out differences and make the marketing of useful economic poisons on a nation-wide basis a much easier job.

Registration and Labeling of Economic Poisons in California

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In almost 50 years of regulating the sale of economic poisons, the state of California has developed legislation to prevent sale of worthless products, to provide for adequate labeling, and to assure users that the products correspond to guarantee. Each product must be registered before it can be sold legally in the state, and conformity to guarantee is determined by analysis of samples.

Because the protection afforded users of economic poisons under the federal law applies only to materials shipped in interstate commerce, state laws must provide adequate protection to users of economic poisons shipped solely within the state, and provide cooperative enforcement of the federal law.

California, one of the first states to recognize the need for control, enacted legislation in 1901 governing the sale of insecticides. This was later broadened to include, under the term "economic poisons," all materials used for pest control. In the almost 50 years of regulating these materials in California the law, which is the Economic Poisons Article of the Agricultural Code, has been modified from time to time, but the basic purpose has been to prevent sale of worthless products, to provide for adequate labeling, and to assure users that the products correspond to guarantee.

Registration

The law provides that each product must be registered before it can be sold legally in the state. Registration acts as a screen by which worthless and untried products are withheld from the market. Registration may be refused, after hearing, for any economic poison which is of little or no value for the purpose for which it is intended, or which is detrimental to vegetation (except weeds), to domestic animals, or to the public health and safety when properly used, and there may be required such practical demonstration as may be necessary to determine the facts.

When only a few standard chemicals were used in pest control, it was relatively easy by referring to published information to determine whether a product could be expected to do what was claimed for it. With the development of the many new chemicals, such as DDT, benzene hexachloride, chlordan, tetraethyl pyrophosphate, and parathion, it has been necessary to adjust procedures to evaluate their usefulness and hazards. Although the experiment stations and other official agencies are still relied upon for unbiased data concerning these new materials, there has developed a trend for manufacturers to carry on their own research. Many manufacturers have extensive investigational staffs and the data they present are given careful consideration in connection with registration of a new chemical. A manufacturer must not experiment at the expense of a user, but there is no objection to his giving away free samples for trial to develop information.

To be of most value to users of economic poisons, the label must carry adequate directions for use, proper statement of ingredients, and any necessary cautions. The directions for use must not only inform the user how to mix the material and the proper dosage to apply against a particular pest, but also must list the pests against which it is effective. It is important that the ingredient statement show both the name and percentage of each active ingredient, in order that a user may determine the relative worth of the products offered him. Both the federal and state laws permit options whereby the manufacturer is not required to disclose the amount of each active ingredient, but a few manufacturers take advantage of this, as it is good salesmanship to point out how much of a particular ingredient is present.

Precautions

Not all pest control materials are as poisonous to human beings as strychnine, sodium arsenite, hydrogen cyanide, and mercuric chloride, but almost every one presents some hazard when it is improperly handled. There are many types of hazards, and all must be considered if accidents and damage are to be avoided. There may be dangers during application—for example, a material may be flammable or it may produce vapors or mists that are toxic to the operator. There may be dangers to the area treated—for example, it may injure valuable plants, or livestock, or wildlife. Danger is still possible after the material is applied. Residues on forage crops or food crops may be deleterious, or water supplies may be contaminated. Furthermore, danger may still lurk long after the job has been done. Disposition of empty containers presents a very serious danger in the case of some distinctly poisonous chemicals such as sodium fluoroacetate, sodium arsenite solution, and lead arsenate. Particular care is necessary in disposing of empty containers which have held 2,4-D herbicides, to avoid damage to valuable plants. Care is needed to make certain that leftover portions of material are not set aside in improperly labeled containers or stored where they may cause injury years later. An unlabeled container of a hazardous chemical is like a loaded gun—or worse, it is like a gun that isn't known to be loaded.

Conformance with Guarantee

When an economic poisons product is accepted for registration, the manufacturer is authorized to sell it in the state. Official samples are drawn by inspectors to determine if a product corresponds to guarantee. Approximately 8000 different economic poisons are registered for sale in the state and it is possible, with present facilities, to sample and analyze only about one fifth of these materials in any one year. Samples are always drawn from original containers that have not previously been opened, and they may be drawn from supplies in the hands of manufacturers, dealers, or users. Reports of analyses are sent to the registered manufacturer, any dealer involved, and any user involved. At the end of each year, reports of all samples are published in a special publication of the department. Copies are available without charge upon request.

Analysis of Samples

The problems of regulatory chemistry were relatively simple in the early days, or at least they now seem so in retrospect. The analytical chemist was faced with only a few compounds—Paris green, lead arsenate, lime-sulfur solution, nicotine dusts, and petroleum oil sprays. The complexity of chemicals used for pest control began to increase more rapidly 10 or 15 years ago, particularly with the development of many organic compounds designed for specific uses. Then the intensified research conducted during the war increased the number and types of pest control materials almost explosively. Twenty years ago there were 1600 products registered for sale in California, ten years ago there were 3600, and today there are 8000. Applications for registration of approximately ten new products are filed every day.

Although many of the new products contain but one active ingredient mixed with a solvent or a dust diluent, it is not uncommon for a product to contain five or six active in-

gredients and several inert ingredients. To complicate matters still further, the accelerated development and production of pest control materials frequently present the analytical chemist with a complex mixture to analyze before anything has been published concerning the chemistry of the new ingredients it contains. The "unknown" samples in a quantitative chemistry class were unknown mixtures of known compounds. An official sample of a pest control product is actually an unknown mixture of unknown compounds. The guaranteed analysis offers a suggestion of the composition, but the chemist cannot assume that it is correct.

Each development in pest control materials made by the research chemist constitutes a challenge to the scientific skill and ingenuity of the analytical chemist. Methods devised for examination of certain mixtures of ingredients are seldom of sufficient general interest to warrant publication, although typewritten copies circulate freely between regulatory offices and the manufacturers concerned. The technical literature of analytical chemistry is continually consulted for assistance in examination of pest control materials, and the author's laboratory regularly contributes data on problems of general interest. During the past year or two, papers have been published in *Analytical Chemistry* on analysis of products containing DDT, benzene hexachloride, 2,4-dichlorophenoxyacetic acid, and phenothiazine. Papers have been published in the *Journal of the Association of Official Agricultural Chemists* by chemists serving as referees in development of analytical techniques. Some methods of less general interest are included in an annual publication issued by the bureau, presenting results of analyses of official samples of economic poisons.

Bioassay

Examination of official samples is usually restricted to chemical analysis and determination of significant physical characteristics—for instance, the guaranteed fineness of some dusts, and the boiling range and viscosity of petroleum spray oils. A bioassay laboratory is also maintained for examination of products for which chemical analysis is not possible or practical—for example, no chemical method is yet available for examination of red squill, commonly used as a rat poison. The potency of such products can be determined only by feeding carefully apportioned dosages to a series of white rats. Colonies of houseflies, cockroaches, clothes moths, and other similar pests are maintained for testing the efficacy of different types of products. Cooperation of the chemical laboratory and the bioassay laboratory has not only been found effective in examining products officially sampled, but it has been of service in developing analytical methods—for example, bioassay with houseflies may readily determine traces of toxic chemicals left behind in the separation of components by crystallization, distillation, or other means.

Misuse

Economic poisons are probably more closely regulated than any other class of material generally sold. In addition to registration required before a product can be sold, and continuous sampling and investigation after it is once put on the market, each reported case of injury is studied to determine if the material was at fault or if there was misrepresentation made in recommendations for use of the product. Two cases of reported injury that were of particular interest come to mind.

A grower reported that he had purchased lime-sulfur solution, but that it was not labeled and the odor was not quite right. He had sprayed seventeen acres of peaches and wondered if there was something wrong with the material, as it had seemed to burn his face more than usual when he sprayed it. Investigation revealed that he had accidentally been sold an unlabeled drum of sodium arsenite solution. Luckily, he had done a poor job of spraying, it had rained, and the injury was not as serious as would be anticipated when sodium arsenite solution is used at the rate normal for lime-sulfur dormant spray. The seller of the unlabeled material paid reasonable damages to the farmer and, after settling in court, will probably never again sell an unlabeled drum of a pest control material.

Another interesting case is that of a manufacturer who recommended use of 2,4-D weed killer as a selective spray for control of weeds in young carrots. He had previously

restricted sale of his material for control of weeds in lawns, but wished to extend his business. Apparently he believed that if petroleum oil selective weed killers could be used on carrots, 2,4-D should also be suitable. Twenty acres of carrots were a total loss and a hearing was called for the manufacturer to show cause why his product should not be refused registration for having been sold for a purpose for which it was not intended. The manufacturer paid the farmer for his damage, which amounted to about $2000 and promised henceforth to obey all provisions of the law, and the matter was settled.

Sale of a deficient or misbranded economic poison is a violation of law, for which the seller is subject to prosecution as a misdemeanor. The maximum penalty is a fine of $500 or 6 months in jail. Repeated violations are cause for revocation of registration. This is a very drastic penalty and is seldom required.

Summary

Each insecticide, fungicide, rodenticide, herbicide, or other pest control product must be registered with the State Bureau of Chemistry as an economic poison before being offered for sale in California. Registration may be refused, after hearing, for a product that is of little or no value for the purpose intended, or that is detrimental to vegetation (except weeds), to domestic animals, or to the public health and safety, when properly used.

Labels should bear adequate directions for use, proper statement of ingredients, and any precautions or warnings necessary for proper use of the product. New chemicals for pest control are not permitted to be sold until data are developed to demonstrate that they can be used in accordance with proper directions without undue hazard.

About 8000 different economic poisons are registered for sale in the state. Samples are drawn where materials are found offered for sale throughout the state and analyses are made to determine conformity to guarantee.

State and Municipal Health Department Requirements for Use of Common Residual Insecticide Sprays

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A questionnaire requesting the regulations applicable to the use of DDT and chlordan in food-manufacturing establishments was sent to 244 city and state health departments. It yielded 188 or 75% replies, 164 or 87% of which revealed the absence of any regulations of use of these products. Four state and twenty city health departments reported specific regulations. No regulatory actions were reported as resulting from the use of residual sprays and no instance of an injury or unfavorable results directly attributable to the use of residual sprays was reported. DDT and chlordan sprays may be used legally under carefully controlled conditions where there is no possibility of the food product becoming contaminated, except in Tennessee.

There is much confusion among food manufacturers concerning municipal and state restrictions on the use of DDT and chlordan residual sprays in their plants.

The attitude of the Federal Food and Drug Administration revolves basically upon two sections of the federal law: Section 402(*a*), "A food shall be deemed to be adulterated if it bears or contains any poisonous or deleterious substance..." or "if it bears or contains any added poisonous or added deleterious substance which is unsafe within the meaning of Section 406...." The applicable portion of Section 406 is: "Any poisonous or deleterious substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice, shall be deemed to be unsafe for the purposes of the application of Clause 2 of Section 402(*a*); but when such substance is so required or cannot be so avoided, the administrator shall promulgate regulations...."

No valid argument can be presented alleging that DDT and chlordan spray in manufactured food products cannot be avoided. Therefore, this requires an interpretation of the Federal Food and Drug viewpoint to be that no DDT or chlordan will be tolerated in a manufactured food product. On the other hand, nothing in this federal act prohibits the use of DDT and chlordan sprays, provided they do not actually contaminate the product.

But in addition to considering the federal viewpoint, food manufacturers must also follow state and municipal regulations. To determine the attitude of such agencies, 244 questionnaires were sent to all state health departments and to health departments of cities whose population exceeds 50,000. The inquiries were:

1. Does your department have any regulations or restrictions concerning the use of residual sprays in food manufacturing establishments?
2. Name the residual sprays regulated.
3. Describe briefly the type of regulation.
4. Have these laws or regulations actually been invoked in any regulatory action?

5. Has your department any record of injury or experience with unfavorable effects resulting from the use of these materials in food manufacturing establishments?

6. If you have not done so already, are you considering issuing any regulations for the use of these products?

Of the 188 replies received, 158 stated that these agencies have no regulations governing the use of DDT and chlordan, but 38 added that they are considering the issuance of such regulations. Presumably, each department in this group of 158 depends upon the "standard adulterations clause," embodied in most food laws, which prohibits the presence of any poisonous, deleterious, filthy, or decomposed substance in a food product, as the means of regulating the use of residual sprays.

Control of Residual Sprays

Of the thirty departments which state they do have regulations controlling the use of residual sprays, six specifically stated that they rely upon the standard adulteration section of their food law. The remaining 24 agencies, including four state departments, have the following regulations:

The state of California has a spray residue law (Agricultural Code, Section 1011) which sets tolerances for certain toxic substances including DDT. In addition, the use of all residual sprays in food manufacturing establishments is prohibited if its use can be avoided by good manufacturing procedures (California Health and Safety Code, Section 6471). Administratively, all such materials are viewed with disfavor except those which have been proved harmless.

The cities of Berkeley, Long Beach, San Jose, Glendale, Pasadena, and Sacramento follow the state regulations. California apparently permits the use of DDT and presumably other residuals under carefully controlled conditions, as evidenced by the existence of a California State Department of Public Health poster entitled "Use of DDT in Food Establishments." This poster suggests that DDT be employed under carefully controlled conditions, which include the use of a nonmisting compressed air spray, limited to wetting surfaces remote from food supplies, utensils, and open flames under adequate ventilation. The oil solutions must not be allowed to remain in contact with the skin.

Although Colorado relies on standard contamination laws, the city and county of Denver specifically limit the use of residual sprays to those conditions in which foodstuffs, utensils, and equipment are covered and protected. Misting sprays may not be used in food preparation areas but are permitted in dining rooms of eating establishments.

Indianapolis regulates the use of DDT by requiring that food and utensils be protected during spraying. This is required under the authority vested in the health officer to regulate the use of harmful substances, including residual sprays.

In Wichita, Kan., residual sprays may be used after notifying the City Health Department and taking protective measures approved by the health department.

Detroit, Mich., controls the use of DDT by a regulation which requires that all food be removed from the area being sprayed.

Kansas City, Mo., Health Department restricts permission for the use of residual sprays to DDT until further studies have been made.

Nevada's Department of Health prohibits the use of sprays on all surfaces with which the food product may come in contact. This includes even pyrethrum sprays.

In Charlotte, N. C., all residual sprays are regulated, in that they may be used only after establishments have closed for the day. All food must be protected during the spraying operation.

The Portland, Ore., Bureau of Health prohibits the use of toxic chemicals that might come in contact with food or food surfaces.

The New York State Department of Agriculture and Markets relies on the standard adulteration section of its law, but additionally advises that the department is "very fussy" regarding the use of poisonous material in food-handling establishments, particularly where poisons might come in contact with the food. In New York City the standard adulteration section also applies and additional requirements are aimed at offering protection to the operator during the spraying project.

Akron, Ohio, permits the use of "DDT or other efficient and safe insecticide . . . in and around the premises . . . of food-selling establishments and, when used, all food shall be so protected that it will not be contaminated. . . ."

The city of Hamilton, Ohio, considers that all these substances are regulated under its law, which forbids the use of poisonous substances where persons, animals, or fowl may be affected.

Tennessee's Department of Agriculture prohibits the use of DDT because its Food and Drug Law considers a food adulterated if it may have become contaminated. This is possible, it is alleged, when DDT is used. Knoxville's Health Bureau, on the other hand, permits the use of residual sprays, provided the food is "protected from contamination, and the food must be pure and wholesome."

The Dallas, Tex., Health Department permits the use of DDT and chlordan, under the restriction that it not come in contact with food or utensils.

The Milwaukee, Wis., Health Department regulates the use of DDT, chlordan, and the gamma isomer of benzene hexachloride by permitting their use only when food, food working surfaces, and equipment are covered during the spraying process so as to prevent spray from being deposited thereon.

In each of the above instances, except the state of Tennessee, the regulations are designed to restrict the use of residual sprays in such a way that they will not contaminate the food. This is a positive approach, with the same viewpoint as those departments which consider their adulteration law as controlling. The noted exception may well be based on a misunderstanding aimed at restricting the indiscriminate spraying of materials containing residual sprays in a fog or mist, as is the common practice with the harmless type of sprays, essentially pyrethrum. The proper application of residual sprays contemplates the use of nonmisting, nondripping spray heads directed at nonproduct zone surfaces, which accomplishes the job more efficiently than a paint brush. As a matter of fact, the Federal Food and Drug Administration has recently been quoted as frowning upon the use of DDT in dairy barns. This has been taken as a discouraging indication by those interested in the use of residual sprays. However, it is almost certain that this opinion contemplates use under conditions peculiar to dairy barns.

No state or city had actually taken any regulatory action except Tennessee, where the food products in two establishments were condemned, presumably after a residual spray had been applied. The details of the spraying were not given.

In reply to the request for a record of injury or experience involving unfavorable effects from the use of these materials, there were several nonspecific comments but no definite instances of ill effects.

Use of Residual Spray Materials in a Typical Food Industry

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The plant inspection program of the American baking industry has shown that 80% of sanitation problems can be avoided by good housekeeping. Proper use of residual spray material, such as DDT and chlordan, will control casual invaders—roaches, ant, flies, silverfish, dermestids, fungus beetles, and meal worms—without contamination of food products.

Following passage of the Federal Food, Drug, and Cosmetic Act of 1938, the baking industry established a broad program of education in sanitation among its manufacturing members. Much of this program has centered about the activities of the American Institute of Baking, which is the scientific advisory foundation maintained by the baking industry to accomplish just this objective.

The first concrete idea materialized from the industry's program was the realization that approximately 80% of sanitation problems can be avoided in a baking establishment if good housekeeping is maintained. This means: (1) proper building maintenance, with provision for adequate rodentproofing, screening, and removal of interior structural harborages for insects as well as rodents; (2) good storage practices, which include the development of an inspection system for all incoming ingredients, storage away from walls and in stacks of such size as to permit easy inspection, proper turnover of ingredients, and proper handling of damaged goods; and (3) generally good housekeeping practices, adequate cleanup of flour-handling equipment, overhead proofers, and removal of flour dust throughout the plant, cleaning of machinery and working areas to remove accretions, proper cleaning of mobile equipment, proper maintenance of floors, maintenance of generally good appearance, and removal of unused equipment from production or ingredient storage areas.

Unfortunately, no bakery so far has been able to maintain these criteria of good housekeeping to absolute perfection, although it is not possible to say that 100% maintenance of such criteria would keep a bakery absolutely free from insect infestation. The American Institute of Baking, in advising the industry, feels that it must regard baking establishments as subject to constant danger from casual invaders. This theory has been well borne out by the facts developed during the plant inspection program.

At present, the American Institute of Baking is inspecting some 150 to 200 bakeries per year. Such inspections involve painstaking appraisal of the conditions of infestation within the plants over a period of 3 or 4 days by an individual scientifically trained by years of experience in this work. During the year 1948 approximately 100 bakeries were so inspected.

Invading Insects

Results of these inspections have shown that the following insects must definitely be regarded as a casual invasion threat:

1. Roaches, of all four common varieties. The Oriental variety comes from outside the plant and invades individually wherever possible by getting in through broken screens, open doors, etc. The brown-banded roach has no particular habitat and flies into the plant in areas where it is common. The German and American roaches ordinarily come in with cartons of ingredients.
2. The common ants, particularly the Pharoah and Thief varieties, are common invaders of a bakery.
3. Flies of all types are common invaders. However, the housefly and vinegar fly or gnat are the two most common varieties. These will invade the plant under their own power.
4. Silverfish and firebrats come into the plant in cartons and incoming paper stocks. They are particularly likely to infest returnable cartons from old deliveries.
5. Dermestids. The two most common invaders are the carpet beetle and the drugstore beetle or cigaret beetle. The latter, although not exactly identical species, are comparable in their habitat and habits. They are all likely to fly into the plant.
6. Fungus beetles, another family that feeds upon decomposed flour and other cereal products, are brought into the plant in ingredient containers or they may fly in.
7. Meal worms. The three most common are the light meal worm, dark meal worm, and lesser meal worm.

Many other insects are occasional casual invaders; the ones described are merely those most commonly seen. Ordinarily, when one or two specimens of an insect species invade a clean bakery, nothing is seen of them until they have bred and developed into a definite focus of subsequent infection; in other words, until there is a colony in the place where the original invader sought harborage. It has been common practice to seek out the colonies visually and spray them with a contact spray. This contact spray killed the adults and larva which it touched, but all too often left eggs and pupae to develop unharmed.

Experience in the baking industry since World War II has shown that proper use of residual spray materials such as DDT and chlordan will most effectively control these casual invaders. When housekeeping in a bakery is maintained as near perfect as possible, the application of a residual layer of toxic insecticide on areas upon which casual invaders are most likely to travel in seeking harborage will effectively kill the invader individual before it has an opportunity to nest. Experience has borne out this theory in a general way, and specific data are now being collected which will describe it in more scientific terms.

Application of Sprays

Both DDT and chlordan are used in colorless, odorless, deobase-type solvent: DDT in 5% solution and chlordan in 2% solution. The oil solvent is used because it is a nonconductor and because experience has shown that the crystals from a film of oil solvent solution adhere more firmly to the surface sprayed. A pressure-type spray tank, either a hand pump or mechanical source of air pressure, is used, with a special nozzle which gives a fan-shaped nonmisting spray. A special dripless valve is used. Several valves and nozzles which meet these requirements are on the market. The appropriate areas are sprayed with this fan-shaped painting spray stream, so that the surface glistens with the wet film but there is not sufficient quantity to run down. Experience has shown that this will leave approximately 200 mg. of DDT per square foot or an equivalent amount of chlordan. This procedure is recommended by the U. S. Public Health Service in its spraying technique for residences and food establishments treated in its program of spraying DDT only for malaria control. It has not as yet recommended chlordan for this use. Specifications for the sprayer nozzles procedure can be obtained from this source.

The following areas of the bakery are treated with such a spray: screens, the window and door jambs, areas of wall immediately adjacent to windows and doors, all walls up to a height of 3 feet, the corners and areas of the floor next to the wall to a distance of 3 feet from it, light cords, electrical switchboxes, connection boxes and electrical motors, space behind sinks, space below the floor joists and the basements, where the ends of the joist are sprayed for a distance of 2 feet from the wall, bottom of the storage skids, the lockers, be-

hind and on the top and in the insides, the areas around urinals and stools in lavatories, and floor drains generally.

Objection has been raised to the use of DDT and chlordan in food establishments because of the possibility that mists may spread onto the product zone of equipment and onto surfaces of food or ingredient mixes. Experience has shown that the use of the paint spray nozzle effectively prevents this. As large a pressure is used as will lay a flat stream of liquid; there is no mist and should be no drip. Admittedly, other sprays are used in baking establishments, and special precautions must be taken to keep DDT or chlordan solutions from being confused with these.

A sound sanitation program for a baking establishment provides that only specific employees apply the DDT or chlordan spray. The spray materials are kept properly labeled and locked up with the spraying equipment, and only employees authorized to use it have keys to this equipment. The employees applying the equipment should be provided with rubber gloves and masks, although experience has shown that they will not consistently use masks and do not use rubber gloves. They are instructed, when undertaking an assignment of this character, to be sure that there is adequate ventilation in the room and all windows are open, and they are told to wash their hands immediately, should any spray solution spill on them. So far, in many thousands of applications, no ill effects have been reported in baking establishments following this procedure.

In its program of bakery sanitation, the institute feels that it is allowing for control of insect infestation to the extent of about 20% of its effort in the use of these sprays. They give a tool that protects from possibility of casual invasion. This feeling of security is checked upon constantly by reinspections and plants that have followed this procedure faithfully, combined with the good housekeeping required, have had no infestations.

Infestation of the interior of flour handling and other bakery equipment, which might arise from an entirely different source than the casual invader, requires different techniques and this problem is not a part of this paper.

Conclusions

The baking industry feels that it needs residual sprays of the character of chlordan and DDT. It has no fear of contamination of the finished product, for there is far less danger from contamination here than there has been in the past from such poisons as sodium fluoride powders, which were used for many years without adverse publicity, despite infrequent food poisonings from their use.

Residual sprays such as chlordan and DDT are needed in the production of bakery goods and are an essential factor in the production of these goods free of insect sources of filth. However, there is no need to establish a tolerance or acceptance of any amount of DDT in bakery goods. No residual sprays are to be employed in any way in any area where they could contaminate either ingredients or the finished product. This is in contrast to the agricultural uses of these products, where they are necessarily incorporated in a slight degree in the finished product. Whatever is decided regarding agricultural use should not enter into the question of use in food-manufacturing establishments.

Pharmacology and Toxicology of Parathion

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To determine the toxicity of parathion to warm-blooded animals, tests were made with mice, rats, guinea pigs, rabbits, and dogs. Greatest hazards are associated with insecticidal use, but symptoms of toxicity may be detected well in advance of severe toxic actions.

Parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate) is primarily of interest as an insecticide. Preliminary reports on some of the pharmacological and toxicological aspects of this organic phosphate have been presented by DuBois, Doull, and Coon (*4*), Hagan and Woodard (*6*), and Hazleton and Godfrey (*8*). A method for its chemical estimation has been presented by Averell and Norris (*2*). These preliminary investigations indicated that parathion functioned as a cholinesterase inhibitor, was rapidly absorbed following dermal application, and was relatively toxic to warm-blooded animals, and atropine was only moderately effective as an antidote. More recently Lehman (*9*) presented a comparison of DDT and several other insecticides, including parathion. As indicated in the paper, many of the data were preliminary at the time of presentation and are subject to re-evaluation with additional experience. DuBois *et al.* (*5*) have contributed studies directed primarily at the anticholinesterase activity of parathion (93% purity).

The purpose of the present paper is to present certain aspects of the pharmacology and toxicology in greater detail and to report the results of chronic toxicity experiments. The material used in these investigations was Thiophos 3422 parathion supplied by the American Cyanamid Company. The material was of technical grade, 95 to 97% purity, specific gravity 1.2537. Parathion is a heavy brown-colored liquid with a characteristic odor. It is almost insoluble in water but is soluble in organic solvents, including propylene glycol. Wherever the term "wettable powder" is used it indicates a preparation of parathion adsorbed onto Attapulgus clay to represent the percentage indicated. "Dusts" are prepared from the wettable powder by further dilution with pyrophyllite.

Experimental

Acute Toxicity. The LD_{50} following oral administration of parathion, either in propylene glycol solutions or in aqueous suspensions of the 15% wettable powder, has been determined for rats, mice, and guinea pigs. The lethal dose was approximated for rabbits and dogs. The results of these experiments are summarized in Table I. Statistical evaluation was by the method of Wilcoxon and Litchfield (*11*).

These data indicate that female rats are significantly more susceptible to the action of parathion than are males, in either sex the propylene glycol solution is more toxic than the wettable powder, in propylene glycol the slopes of the dose response curves for the two sexes deviate from parallelism, and guinea pigs and rabbits are relatively resistant to its toxic action. In mice and guinea pigs the sex difference was not great and the data were combined. Following oral administration of lethal doses of parathion the animals exhibited peripheral muscular twitching followed by labored breathing, salivation, lach-

rymation, convulsions, depression, and death. Prompt and apparently complete recovery from nonlethal doses is indicative of reversibility of the action. Depending on the dosage and the preparation, death may result in from 1 to 24 hours. In general, animals that survive more than 24 hours may be expected to recover, and there is no apparent latent toxicity.

Technical parathion held in the conjunctival sac of rabbit eyes produces local irritation and a marked myosis which is spontaneously reversible in 24 hours. No systemic effects were observed.

For these experiments mongrel male dogs were given parathion orally by means of capsules containing the 15% wettable powder. A single dose of 25 mg. per kg. was lethal in about 3 to 5 hours. Ten milligrams per kilogram caused severe symptoms of toxicity and refusal to eat, and after three daily doses this animal succumbed. After 5 mg. per kg. one dog showed such severe effects that the dose was not repeated, and the animal succumbed in about 36 hours. These three dogs are included in Table I as acute deaths. The symptoms were comparable to those seen in other species, and autopsy revealed only gastrointestinal irritation and pulmonary hemorrhage.

Table I. Summary of Acute Oral Toxicity of Parathion in Mice, Rats, Guinea Pigs, Rabbits, and Dogs

(Values in body of table represent number of deaths/number tested. Maximum observation period was 48 hours. All doses are as parathion)

Animal	Mouse		Rat				Guinea Pig	Rabbit	Dog
Solvent, mg./ml.	Propylene glycol 1 and 10	15% wettable powder 1 and 2	Propylene glycol 1		15% wettable powder 1		Propylene glycol 10 and 100	15% wettable powder 1	15% wettable powder capsule
Sex	M and F	M and F	M	F	M	F	M and F	M	M
Dose, Mg./Kg.									
1.0	...	...	0/6	4/11	...	0/10	...	..	0/1
1.5	...	0/6	...	...	...	...	...	..	...
2.0	3/20	...	1/14	6/11	...	2/10	...	..	0/1
3.0	5/20	...	2/14	7/11	...	9/17	...	..	0/1
4.0	...	...	5/7	4/4	...	10/17	...	..	..
5.0	11/38	0/11	7/14	..	...	4/7	...	..	1/1
7.5	...	0/2	4/6	..	1/10	...	3/8	..	..
8.0	10/16	...	...	..	...	...	...	..	..
10.0	25/26	0/20	6/7	..	10/19	6/7	7/13	0/1	1/1
12.0	15/20	...	..	..	...	...	...	..	..
12.5	...	...	...	..	4/10	...	...	...	..
15.0	16/20	6/32	2/2	..	14/20	7/7	14/17	0/1	..
17.5	...	...	...	..	...	...	6/12	...	..
20.0	10/10	25/45	5/5	..	16/20	...	9/11	0/1	..
22.5	...	5/13	..	..	9/10	...	...	...	...
25.0	3/3	33/34	...	..	7/8	...	...	0/1	2/2
30.0	...	10/12	2/2	..	9/11	...	...	..	..
35.0	...	6/6	..	..	...	...	...	..	..
40.0	...	...	..	..	...	...	2/2	..	..
50.0	...	...	..	..	...	...	2/2	..	..
LD_{50}, mg./kg.[a]	6.0	21.0	5.0	1.75	12.5	3.5	9.3	..	..

[a] Method of Wilcoxon and Litchfield (*11*).

Dermal Absorption. To determine the toxicity of parathion following dermal application, the method of Draize, Woodard, and Calvery (*3*) was followed. Variables considered in the design of these experiments were concentration as a factor of area, solvent, exposure time, and number of exposures. In some cases the wettable powder was applied in the dry form, while in other cases sufficient water was added to produce a viscid paste. All doses in the table are presented as milligrams per kilogram of parathion, regardless of the concentration or solvent.

As the concentration of parathion in the propylene glycol solutions is increased, it follows that the area covered by the solution is decreased. That this is a factor in toxicity is indicated by the greater toxicity of the 10 mg. per ml. solution than the 50 mg. per ml. solution. This relationship appears to be true also of the various dry preparations, in that the 1% powder is somewhat more toxic than the 15%. The addition of water to convert the powder to paste does not appreciably influence the toxicity. In comparable concentrations the wettable powder formulation is less toxic than the propylene glycol solution.

This is evidenced by a definite shift to higher doses, as shown in Table II. Acute symptoms develop within a few hours, the most obvious being salivation, lachrymation, exophthalmia, peripheral tremors, and diarrhea. When these are severe the animal usually succumbs within 12 to 18 hours following application.

Table II. Toxicity of Parathion Following Dermal Application to Rabbits

(Skin of abdominal area was closely clipped and parathion preparation applied under rubber sheeting. All doses are as parathion. "Dust suspension" is an aqueous suspension of 15% wettable powder comparable to a spraying application of 3 pounds per 100 gallons)

Dose, Mg./Kg.	Exposure Time, Hours	No. of Exposures	Technical	Propylene Glycol, Mg./Ml.			1% Dust		15% Dust		25% Dust		Dust Suspension, 3 to 800	
				10	50	200	Dry	Plaster	Dry	Plaster	Dry	Plaster	15%	25%
10.0	24	1	..	0/1	..	..	0/1	..	..	..	..	..	..	..
15.0	..	..	..	..	..	..	..	..	..	..	..	..	0/4	..
20.0	24	1	..	..	..	..	0/1	..	..	..	..	..	..	..
25.0	24	1	..	1/1	0/3	..	..	..	..	..	..	..	..	0/4
37.5	24	1	0/1	..	..	..	..	..	..	..	..	..	..	..
40.0	1	5	..	..	..	..	0/3	0/3	..	..	..	..	..	..
40.0	24	1	..	..	..	1/2	..	..	..	..	..	..	..	..
50.0	24	1	0/1	1/1	0/3	..	..	..	..	..	..	..	..	..
65.0	24	1	1/1	..	..	..	..	..	..	..	..	..	..	..
100.0	24	1	..	..	1/3	..	1/2	..	..	..	..	..	..	..
150.0	24	1	..	..	1/2	..	..	..	..	0/3	..	..	..	..
200.0	24	1	..	..	..	..	1/1	..	..	..	..	..	..	..
250.0	24	1	..	..	..	..	..	..	..	..	..	0/3	..	..
300.0	24	1	..	..	..	..	..	..	0/1	1/1	..	..	..	..
500.0	1	1–4	..	..	..	..	..	..	..	..	3/4	1/3	..	..
600.0	1	3–5	..	..	..	..	..	..	2/3	0/3	..	..	..	..
750.0	24	1	..	..	..	..	..	..	1/1	..	..	..	..	..
1000.0	1	1	..	..	..	..	..	..	..	..	3/3	2/3	..	..

Chronic Toxicity. RATS. In these experiments albino rats of a standard strain were placed in individual cages with wire-mesh floors elevated above the droppings. Water was offered ad lib. The basic control diet consisted of a commercial, finely powdered dog meal. Parathion was added to the basic diet by incorporating carefully prepared parathion dust in the appropriate quantities. To ensure thorough mixing, each batch was mixed in a McLellan batch mixer. Each week the animals were weighed and the food consumed during the previous week was determined. The residual food was discarded and a freshly prepared food was offered. Occasional assays of the residual food indicated that the loss of parathion during the week was negligible. Initially, dietary levels as high as 1000 p.p.m. were offered, but the rats refused to accept levels of 250 p.p.m. or higher, and died of starvation with no detectable food consumption.

With 10 male rats serving as control, similar groups of rats were placed on dietary levels of 50 and 100 p.p.m. From the group receiving 100 p.p.m. two rats were lost after 8 and 17 days, respectively, and this group was, therefore, considered to have been represented by eight animals. At 50 p.p.m. the animals showed no evidence of toxicity, but at 100 p.p.m. there were occasional periods of peripheral tremors and irritability during the first several weeks of the experiment. Thereafter the animals appeared to be normal. These experiments were continued for 2 years. Summaries of body weights, food consumption, and survival are presented in Tables III and IV. The mortality indicated in Table IV is relatively low for all groups. When survival is calculated on the basis of the number of days each rat lived in terms of the theoretical survival of all rats for the 2-year period, the results indicate 87% survival for the controls, 95% survival for the 50 p.p.m. group, and 93% for the 100 p.p.m. group. This indicates that the majority of the animals were lost relatively late in the 2-year period. It is probable that the difference in either mortality or survival is not significant.

With the upper tolerable limits thus established, three additional groups of 20 male rats each were started. One group served as control, one group received 10 p.p.m., and one 25 p.p.m. Shortly after the second series of experiments was started a minor epidemic of respiratory disease was responsible for several deaths, particularly in the control group, and these animals were eliminated from further consideration, because experiments of this type are designed to cover infantile rats in the 60- to 70-gram weight range and it was impossible to replace them. After 93 weeks these feeding experiments were continuing without apparent undesirable effects. The data are also summarized in Table III.

In view of the observed difference in acute susceptibility between rats of the two sexes, it became desirable to study the chronic toxicity of parathion in female rats. To initiate such studies, two or three immature females were placed in a cage with one male and allowed to remain until pregnancy became obvious, at which time they were placed in individual cages but continued on the diet until birth of offspring. The results from this series are also summarized in Table III, the blank areas representing the period of parturition and rearing of litters. All females of the control and 10 p.p.m. groups produced living litters, and all but one of the 50 p.p.m. group. As soon as the litters were weaned, the parent females were placed in individual cages and returned to the original parathion diet. At the time of report there had been no significant mortality, although the groups receiving parathion showed some retardation of growth. This appears to be associated with a reduction in food intake and there is no gross evidence of toxicity.

Female rats were maintained over a considerable period of time on a diet containing 100 p.p.m. but continually manifested evidence of toxicity and presented an unthrifty appearance. It is evident, therefore, that chronic toxicity follows a sex pattern comparable to the acute toxicity.

Table III. Summary of Body Weight and Food Consumption after Various Intervals of Feeding

(Parathion was added to diets in form of carefully prepared dust. Dietary levels in p.p.m. are as parathion. All values are average for number of survivors. Omissions in female series cover periods of parturition and lactation)

	Males						Males						Females					
	Control		10 P.P.M.		25 P.P.M.		Control		50 P.P.M.		100 P.P.M.		Control		10 P.P.M.		50 P.P.M.	
Week No.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.	Wt., g.	Food, g./wk.
0	66	..	69	..	62	..	66	..	70	..	69	..	63	..	61	..	64	..
4	..	..	..	..	..	..	..	..	..	..	..	..	128	..	125	..	108	..
6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	139	..
7	..	..	..	..	..	..	..	..	..	..	..	..	160	..	167	..	..	..
8	226	143	242	158	228	141	240	151	249	154	222	147	..	..	..	..	..	..
14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	180	..
15	..	..	..	..	..	..	..	..	..	..	..	..	191	..	183	..	182	110
16	296	137	300	140	294	138	301	134	311	147	290	139	194	129	187	108	185	141
24	331	144	350	155	342	149	327	133	334	150	326	150	217	115	206	115	201	105
32	340	110	384	132	365	130	347	124	353	135	352	135	229	143	214	127	205	108
40	380	121	399	130	385	128	360	113	373	129	371	133	253	125	226	123	207	126
48	378	126	399	140	385	139	374	115	388	123	375	123	257	141	236	112	210	116
56	394	130	412	129	394	122	379	110	391	117	377	123	261	120	241	106	212	101
64	391	159	424	162	392	176	380	123	393	142	377	136	280	125	253	88	222	93
72	413	149	432	135	417	149	391	129	401	148	398	141	..	..	..	..	..	..
80	426	127	436	117	432	122	393	133	404	161	395	151	..	..	..	..	..	..
88	427	134	450	128	434	128	398	121	412	136	402	133	..	..	..	..	..	..
96	..	..	..	..	..	..	407	105	418	113	397	111	..	..	..	..	..	..
104	..	..	..	..	..	..	407	111	425	119	385	121	..	..	..	..	..	..
Survival	7/10		11/16		14/16		6/10		8/10		5/8		4/6		9/9		5/8	

Table IV. Summary of Survival and Food Consumption Data for Rats Receiving Basic Laboratory Diet, and Basic Laboratory Diet to Which Were Added 50 and 100 P.P.M. Parathion

Parathion, P.P.M.	No. of Rats		% of Mortality	Average Weight, G.		Rat Days		
	Start	Finish		Start	Finish	Theoretical	Actual	% of survival
Control	10	6	40	66	407	7290	6154	87
50	10	8	20	70	425	7290	6934	95
100	8	5	38	69	385	5832	5415	93

	Total Food and Parathion Consumed, G.	Food Consumed		Parathion Consumed	
		Total, g.	Av./rat/day, g.	Total, g.	Av./rat/day, g.
Control	.....	105,534	17.15	...	...
50	132,955	132,948.35	19.17	6.65	0.001
100	103,284	103,273.67	19.07	10.33	0.002

Upon completion of the 2-year experimental period all surviving male rats in the initial groups receiving 50 and 100 p.p.m., and the corresponding control group, were sacrificed by exsanguination through a cardiac puncture following light ether anesthesia. An autopsy was performed on each rat immediately following death. The liver, kidneys,

adrenals, testes, spleen, heart, lungs, and brain were removed and weighed. In addition to these tissues, the thyroid glands were examined and perirenal and peritoneal fat was removed. In most of the animals the nasal and ear passages were exposed for examination. A portion of the blood was oxalated for hematological examination. Samples of the tissues were placed in formalin for histopathological examination and the remainder, plus blood and fat, were saved for chemical analysis.

These animals at the time of sacrificing presented an extremely good general appearance for rats of their advanced age. There were a few cases of improper balance which is suggestive of middle ear infection, and a few animals showed gross evidence of nasal or upper respiratory tract infection, but none was of a severe nature. Some of the animals on the 50 and 100 p.p.m. levels exhibited slight alopecia on the lower portions of the body and legs; however, the skin over these areas appeared to be in excellent condition with no evidence of scales or dermatitis, and the condition was of such a mild nature that it was not visible when the animals were in their normal position. Gross examination at autopsy revealed the majority of the animals to be in very good condition, although there were occasional variations from the normal in specific organs. The most general observation, which occurred without exception, was the presence of hemorrhagic and consolidated areas of the lungs, a condition always observed in animals of this age, and gross examination revealed no significant difference in the appearance of the various groups. No peritoneal fluid was found in any of the animals. The incidence of pleural fluid was negligible in the control and 50 p.p.m. groups. The 100 p.p.m. group showed some pleural fluid in four of the five survivors. In two of these there was evidence of upper respiratory tract infection. The incidence of pus in the ear was relatively high but apparently not associated with the outward symptoms of disturbance in equilibrium noted, for one animal showing disturbed equilibrium was entirely free of ear infection while the other showed the presence of a greenish pus.

Correlation of Data

In general, there is reasonably good correlation in the data on individual organ weight for each animal. The range of weight and average for the liver, kidneys, adrenals, testes, spleen, and brain appeared to be comparable for all groups. There was evidence of slight cardiac enlargement in the parathion-treated groups since, when expressed as per cent of body weight, the control was 0.0027% while the 50 and 100 p.p.m. groups were 0.0035 and 0.0036%, respectively. The range and average for the lungs were comparable with the exception of those from one rat in the 50 p.p.m. group, which showed a localized abscess in one lobe and multiple abscesses throughout the entire left inferior lobe.

The hematological findings appeared to be well within normal ranges encountered in the experience of this laboratory.

Histopathological examinations of random selected samples of lung, adrenal, testicle, spleen, stomach, heart, intestine, liver, esophagus, brain, colon, and kidney were made. The lung samples showed evidence of congestion. Occasional testicular atrophy was observed, both grossly and microscopically. All these changes, as well as any observed in the other tissues, appear to be within normal range considering the age and consequent senility of the animals.

Chemical analysis of the above tissues, plus abdominal fat and blood, by the method of Averell and Norris (*2*) was essentially negative. Occasional positive values were observed in the control rats, as well as those on the parathion diets.

To test for tissue storage of parathion, female rats on 50 and 100 p.p.m. diet, and appropriate control rats, were allowed water while food was withdrawn. During the withdrawal period the behavior of the treated and control rats was typical of starvation with no evidence of parathion toxicity. Death was neither hastened nor delayed.

Dogs. For chronic feeding experiments mongrel male dogs were given oral doses of the 15% wettable powder in hard gelatin capsules, 6 days per week. It was found necessary to administer the dosage either with the meal or afterward because prior administration resulted in loss of appetite and refusal to eat. The animals were weighed each week

and the daily dosage was adjusted according to the weight. At intervals during the period blood samples were obtained for red and white counts, differentials, and hemoglobin determination. Although these showed some fluctuation, the values in general were within normal limits. At 2 mg. per kg. daily the dog lived for 3 weeks but continually showed toxic symptoms and was difficult to feed. At 1 and 3 mg. per kg. per day the animals survived for 90 days. During the early stages of this period the animals were nervous and irritable and the dog receiving 1 mg. per kg. exhibited urinary retention and leucocytosis. During the last month both dogs were normal in behavior. Upon completion of the 90-day period, the animals were sacrificed for pathological examination and chemical analysis of the tissue for the presence of parathion. Neither dog showed evidence of gross pathology, absence of pulmonary hemorrhage being particularly noticeable. Histopathological examination revealed degenerative changes and cloudy swelling in the liver, more severe with the lower dose. Other organs were normal except for evidence suggestive of a pneumonic condition in the lungs.

Fate. Preliminary investigations directed at adapting the method of Averell and Norris (*2*) to the analysis of animal tissues indicated that if precautions were taken to avoid emulsions the method could be used satisfactorily. Tissue samples of about 5 grams were most convenient, and the usual reagent and tissue blanks were run simultaneously. Following the administration of an acutely lethal intravenous dose to a dog it was found that parathion could be recovered from the urine, liver, bile, kidney, spleen, and lung.

Following oral administration of a lethal dose to a dog (25 mg. per kg. wettable powder) tissues taken immediately after death analyzed as follows: no parathion recovered from bladder bile, liver, kidney, abdominal fat, saliva, or intestine; small quantities (2 to 7 p.p.m.) of parathion recovered from oxalated blood, spleen, lung, brain, and spinal cord. The urinary bladder was strongly contracted and no urine could be collected. The results of these two experiments indicate a universal distribution of parathion following acutely lethal doses.

Male and female rats fed on 10, 50, and 100 p.p.m. food for several months showed no storage of parathion in the abdominal fat, nor was any recovered from the lung, liver, spleen, brain, whole blood, or kidney.

Two dogs which had received parathion orally for 90 days were sacrificed 24 hours after the last dose; the analyses were essentially negative. Small quantities were found in the voided and bladder urine of both dogs, while the oxalated blood, serum, brain, liver, and lungs were completely negative. For the dog which had received 1 mg. per kg. of parathion per day the bile, spinal cord, spleen, kidney, and intestine were also negative; the dog which had received 3 mg. per kg. per day showed traces in the spleen, kidney, and intestine and a value of 13 p.p.m. for the spinal cord, although the brain was negative. These results are in good agreement with those obtained following oral administration of acutely toxic doses, except for the one value for spinal cord, which does not seem to be in agreement with the other tissues.

Discussion

A discussion of the results reported should take into consideration both the pharmacological and toxicological aspects per se, and their interpretation in terms of the health hazards presented by parathion.

There is satisfactory agreement between the acute toxicities reported by various laboratories and investigators, although results in Table I tend to indicate that parathion is somewhat more toxic than the results reported elsewhere. Factors which may influence the acute toxicity are route of administration, the vehicle used in administration, and the sex.

In common with other organic phosphates, parathion is readily absorbed following application to the skin. Dry formulation materially reduced the toxicity. These observations are not in agreement with the data of Lehman (*9*) so far as the toxic dose is concerned. Because parathion is almost insoluble in water, no comparison can be made between the aqueous solution and the dry powder. The dry formulation of parathion is less

toxic than the propylene glycol solution and moistening the dry preparation does not materially influence its toxicity.

Long-term feeding experiments using rats and dogs contribute data of material significance in both directly and indirectly evaluating the hazards of parathion exposure. During the first few weeks of the chronic feeding experiments in rats reported above, each animal was consuming daily a quantity of parathion which, calculated on body weight, is equivalent to one or more lethal doses. As noted, this level produced some toxic symptoms. As weight increased the percentage of lethal dose intake was reduced proportionately and these symptoms completely disappeared. It is apparent, therefore, that following oral administration rats are capable of disposing of at least one lethal dose per day through such mechanisms as excretion, destruction, and storage. Chemical analyses of the urine and of the tissues at autopsy indicate that neither urinary excretion nor tissue storage is a significant factor in the disposition of parathion. This observation has been substantiated by Lehman (*10*), who reports that parathion is not stored in the tissues to an appreciable extent and that it is rapidly destroyed by the tissues of the body. The results of chronic feeding to dogs also substantiate these observations, and in neither species could any significant histopathological damage be detected.

Because the results of these chronic feeding experiments at maximum tolerated levels do not reveal any influence of parathion on behavior, survival, weight gain, food consumption, or tissue pathology in male animals and only minor influence in female rats, they are at considerable variance from the observations of DuBois *et al.*, following repeated intraperitoneal injection. Selection of the intraperitoneal route of administration and female rats as the basis for generalization on the hazards of parathion does not appear to be justified. Such a design does not take into consideration the difference in fate following absorption of drugs by the oral and intraperitoneal routes, and all observers have noted the particular susceptibility of the female rats, whereas in other animals this sex difference is not so apparent. Both the LD_{50} and the slope of the dose response curve are significantly different for male and female rats following oral administration of parathion in propylene glycol. Although the above authors do not give confidence limits for their LD_{50}'s following intraperitoneal administration, the confidence limits following administration of parathion in propylene glycol orally to female rats are such that 75% of the calculated LD_{50} would be well within the confidence limits and therefore not significant. When the daily dose was reduced to 12.5% of the calculated LD_{50} they reported no deaths after 20 days of administration. This is in line with other observations on the ability of the rat to metabolize parathion and would not be indicative of cumulative hazards under any reasonable conditions of exposure.

Evaluation

From a practical standpoint it is entirely possible that parathion may be of clinical interest, but certainly its greatest hazards are associated with its insecticidal use. In this respect it is interesting to evaluate parathion according to a series of criteria postulated by Lehman (*9*):

Parathion is relatively toxic to insect life (*1*) as well as to mammals; therefore, under conditions of usage it has a favorable ratio.

Administration over a long period of time by means of treated food produces no evidence of toxicity or accumulation in doses which are acutely tolerated. Although a sex variation was observed in rats, this is no greater following chronic exposure than following acute toxicity.

Parathion is uniformly distributed throughout the body following acutely lethal doses, but there is no evidence of storage in any of the tissues studied even after long terms of feeding.

Both clinical and biological tests are available for the detection of parathion in fractional parts per million, thus making the contamination of foodstuffs or the presence of residues readily detectable.

Although published clinical reports on parathion are not available, field reports (*7*) indicate that the symptoms of toxicity are detected well in advance of severe toxic actions.

These symptoms are consistent with the pharmacological action and usually appear in the order of nausea, chest pains, blurred vision. Removal from exposure and symptomatic treatment have thus far been followed by rapid abatement of symptoms.

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Pharmacology and Toxicology of Some Important Economic Poisons

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Some of the more important pharmacologic and toxicologic principles that have been highlighted by government, commercial, and university laboratories are outlined. Cooperative research by chemists and toxicologists is needed, directed toward the development of economic poisons possessing a greater margin of safety for man and animals.

The pharmacology and toxicology of certain economic poisons have been developed to a degree which surpasses investigations of any other class of nonmedicinal compounds. In certain instances more is known concerning the site and mechanism of action, the absorption, distribution, and excretion of these substances than is known concerning some of the more commonly used pharmaceutical compounds. This has come about as a result of the conscientious recognition of the public health hazards which are inherent in the economic poisons.

This review outlines some of the more important pharmacologic and toxicologic principles which have been highlighted by the work of the government, commercial, and university laboratories that have been concerned with this problem (*3*, *5*, *13*, *16*).

Portals of Entry

As far as man and animals are concerned, the economic poisons exert their harmful and deleterious effect after absorption and distribution through the blood stream. Relatively few agents are irritating or corrosive in their action, and their effect on the intact skin may be considered secondary (*5*, *8*). Toxic action of economic poisons is exerted by alteration in physiologic or biochemical activity of various systems, organs, and cells.

The portals of entry through which the poisons gain access to the body are determined largely by the nature of the exposure. The use of sprays and aerosols in dispersing media has greatly increased the hazards of absorption through the respiratory tract. Absorption is influenced markedly by the physical properties of the particular compound. When the portal of entry is via the gastroenteric route, the compounds with high water solubility such as soluble arsenates, strychnine, thallium, and 1080 are much more hazardous. Absorption through the skin depends on high lipide solubility, and the ratio of fat-water solubilities may also be important with respect to gut absorption and transfer by the blood stream.

Accidental poisoning in children not infrequently occurs. Economic poisons, not properly labeled, are of great concern both for children and adults. The more extensive problem of poisoning in man and domestic animals, however, arises from the ingestion of residual poisons remaining on agricultural products, on weeds, and in impregnated soil (*1*, *6*).

Percutaneous absorption has been shown to be a definite hazard with the organic phosphates. In animals, fantastically small quantities applied to the intact skin or the mucous membranes result in absorption with fatal consequence. The work of Horton and others has indicated the inadvisability of incorporating benzene hexachloride (hexachlorocyclohexane) in clothing for miticidal purposes. As Woodard has pointed out, it is of paramount importance to determine the absorption, metabolism, excretion, and storage of a toxic compound in mammals. Once having gained access into the body, the economic poison is carried by the blood stream and distributed throughout the tissues in a manner regulated by the agent's physical and chemical activity. Eventually an equilibrium is established between absorption on the one hand and storage, detoxication, and excretion on the other. The relative rate of this reaction determines, in part, the toxicity of the insecticide or other poison. The majority of the fat-soluble compounds which are halogenated are absorbed through the skin or across mucous membranes, are distributed to the liver and other parenchymal organs, and may be stored in the body fat in a quantity which is determined by the characteristics of the lipoid matter of the animal and the dosage of the compound (*6*).

Toxicity

It is a first principle of toxicology that no chemical substance is a poison at all concentrations; toxicity occurs only when a critical concentration is reached within vital cells. Whether or not an economic poison will exert a particular deleterious effect depends on the relative rates of absorption as compared with detoxication and elimination, its inherent toxicity, and the physiologic status of the organism.

Poisoning may be acute, delayed, subacute, or chronic, depending upon the intensity and duration of exposure and the susceptibility of the species. Quantities of DDT may be stored in the body in amounts which, if taken in an acute dose, would be rapidly toxic. This is true of lead and other agents, as well. However, conversely, the organic phosphates and nicotine, which exert an extremely deleterious effect when absorbed in large quantities over a short period of time may, when absorbed in lesser quantities over a considerable time, exert no appreciable untoward effects. Furthermore, the summation of repeated minimal injuries may result in eventual serious damage to an organ or cell. The more highly developed cells of the body, such as are found in the central and peripheral nervous systems and the conducting mechanism of the heart, are more sensitive to the toxic effects of economic poisons than are the less specialized cells of muscles, fat, and bone. It has been demonstrated with several of these agents that gross effects, such as retardation of growth and normal activity, may not occur at absorption levels which produce significant tissue damage. This emphasizes the necessity for more complete toxicologic studies, with special reference to histologic-pathologic changes.

Tolerance in insects to increasing amounts of DDT, BHC, and several of the inorganic agents, such as arsenic, is known to occur. However, in animals, this phenomenon has not been demonstrated to the same degree. Ability of the cells of insects to function in the environment of a toxic agent, without alteration of cellular function, is one of the most striking of biologic phenomena.

This review does not attempt a discussion of detailed pharmacologic knowledge which has arisen concerning many of the principal agents. However, a simple approach can be made to the toxicology of these agents by classifying them according to the vital systems, upon which they exert their primary effect. If a toxic agent exerts a significant effect upon a particular tissue, organ, or system, it will cause one of the following changes: (1) stimulation, (2) depression, or (3) degeneration. A summary of the principal effects resulting from the more common economic poisons is given in Table I.

It is true that the degree of system damage may be determined in part by the nature of the exposure; thus, in mammals, high concentrations of DDT may produce central nervous system stimulation and cardiac irregularities, while low concentrations, absorbed over a period of time, may produce damage primarily to parenchymal tissues (*3*). Generally, similar chemical types exert approximately similar qualitative physiologic

effects and pathologic changes; however, pathologic changes in tissues are in addition occasionally specific for particular agents. An example of this is seen when the deleterious effects of DDT on the liver and muscle are compared with the action of DDD on the adrenal glands and of chlordan on the optic nerve.

Table I. Chief Effects of Economic Poisons on Tissues, Organs, and Systems of Man

Organ or System	Effect		
	Stimulation	Depression	Degeneration
Central nervous system	Nicotine, strychnine, arsenic, halogenated hydrocarbons, organic phosphates, dinitrophenols, fluoroacetate (1080)	Nicotine	Arsenic, methyl bromide
Peripheral nervous system	Nicotine, organic phosphates	....	.....
Lung	Organic phosphates, ethylene dibromide, ethylene dichloride, ethylene chlorohydrin	....	.....
Liver	.....	....	Arsenic, phosphorus, DDT, benzene hexachloride, chlordan, toxaphene, carbon tetrachloride, DD, ethylene chlorohydrin
Heart	DDT, fluoroacetate (1080)	....	Halogenated hydrocarbons
Kidneys	.....	....	Arsenic, methoxychlor, benzene hexachloride, ethylene chlorohydrin, carbon tetrachloride, DDT phenols
Gastroenteric tract	.....	....	Arsenic, phosphorus, fluorides, petroleum oils, calcium oxide and hydroxide, phenols
Muscles	Organic phosphates	....	.....
Blood pigment	.....	....	Chlorates
Bone	.....	....	Fluorides, phosphorus
Hair	.....	....	Arsenic
Body as a whole	Dinitrophenols	....	.....

Exposure to a toxic agent does not necessarily result in poisoning. A definite concentration must result in vital tissues before irreversible toxic effects occur. The most effective method of treating poisoning is to prevent its occurrence. This having failed, the sine qua non is removal of the unabsorbed portion, or alteration chemically or physically so that the absorbed portion does not remain in contact with cells in toxic amounts. Unfortunately, most of the antidotal treatment of poisoning from agents of economic importance is symptomatic—that is, physiologic in nature. For example, the acute, excitatory effects of the halogenated hydrocarbons on the central nervous and cardiovascular systems predominate in acute poisoning. Phenobarbital has proved to be useful for control of experimentally induced poisoning in animals and is apparently the drug of choice in human therapy (*10*). Procaine may be of value in the treatment of cardiac arrhythmias which may arise in man following exposure to 1080.

At present, no effective agent has been devised to combat the hyperglycemia and depletion of liver glycogen which result from acute poisoning with ANTU (*4*). The intense pulmonary edema and pleural effusion seen with this compound may respond to oxygen under increased pressures. Fortunately, the minimum toxic dose is high in man, in contrast to other species. The organic phosphate esters are among the most potentially dangerous economic poisons with which we have to deal. Fortunately, specific antidotes against the phosphates are now available. The inhibition of choline esterase and resulting excessive parasympathetic nervous stimulation which are caused by these agents may be successfully combated by means of magnesium salts and atropine (*9*).

Unfortunately, it has not been possible to develop an ideal economic poison which has a low order of toxicity for man and is effective over a wide variety of field conditions. Cooperative research on the part of chemists and toxicologists should be directed toward the development of economic poisons possessing a greater margin of safety for man and animals.

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New Safe Insecticides

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In the search for safe insecticides, hundreds of products have been prepared and subjected to screening tests against insects. This paper presents information on six materials related to piperonyl butoxide, tested in combination with pyrethrins.

World food shortages lend added weight to the importance of safe insecticides. More than 5% of all cereal food is destroyed in storage by insects. The millions of tons lost each year are equal roughly to the volume of grains in international trade. Protection of stored grains is only one aspect of the use of insecticides in food conservation. Control of insects on growing crops, on livestock, in food processing plants, in storage, and in the household assumes new significance in view of the necessity for more adequate production of foodstuffs.

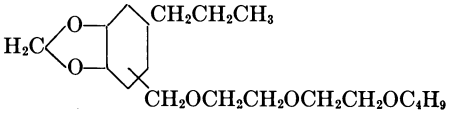
Insecticides that combine high efficiency against insects with complete safety to man and other warm-blooded animals are therefore a mighty weapon to ensure food supplies for the world. During World War II popular interest in insecticides was centered on the spectacular usefulness of DDT in combating abnormal exposure of troops and civilian populations to insect pests. This important and versatile material has become almost a household synonym for "insecticide." Now that we are engaged in a war against hunger, however, public health authorities are becoming increasingly conscious of the fact that the protection of food products properly demands a high degree of safety in any insecticides that are used for this purpose, a property that most of the existing commercial synthetic products do not possess.

The ideal insecticide should meet the following exacting list of requirements:

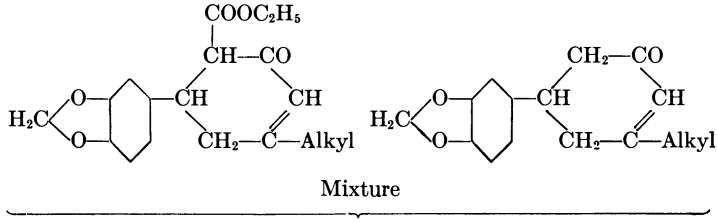
Toxicity to undesirable insects and lack of toxicity under conditions of use to desirable insects
Complete safety for man and other warm-blooded animals
Rapid knockdown action toward insects with minimum recovery
Stability on storage and exposure
Solubility in suitable solvents
Freedom from irritation, undesirable odor, color, and staining properties
Volatility low enough to ensure effectiveness over an adequate period of time
Cost in economic range

In the search for safe insecticides the authors have prepared hundreds of new products and subjected them to preliminary screening tests against insects. That part of their work dealing with methylenedioxyphenyl derivatives was prompted by the original fundamental studies of O. F. Hedenburg, with whom they have collaborated in this field. Two materials of this type—piperonyl butoxide (I) and piperonyl cyclonene (II)—have recently been introduced commercially. These products have definite insecticidal properties in themselves, but show their maximum efficiency toward insects and other arthropods when used in combination with pyrethrins. Furthermore, they are at least as nontoxic

toward warm-blooded animals as pyrethrum, which is recognized as the safest insecticide in common use.



I
Piperonyl butoxide (*7*)
(Propylpiperonyl)(butyl) diethylene glycol ether



Mixture

II
Piperonyl cyclonene (*3*, *8*)

In continuing efforts toward the development of other nontoxic insecticides, many new products are being synthesized and tested. The direction of the synthetic work is guided by the theory that the insecticidal activity of a given substance is due to the combined influence of a toxic nucleus and modifying auxiliary groups. To illustrate the theory, this paper presents information on six materials related to piperonyl butoxide, when tested in combination with pyrethrins.

Screening Tests on Insecticides

Experimental results obtained on these products by various screening procedures are summarized in Tables I to IV. Detailed description of these tests on insects and discussion of their relative practical usefulness for evaluating insecticides will be given in a subsequent publication (*5*). In this screening program on seven materials and the necessary standards of reference about 38,000 houseflies and over 5000 other insects were used.

These tests not only represent different techniques, but also supply some basic information regarding the use of the insecticides under practical conditions. Because they are screening tests, it is desirable to use insects that are commonly employed by many different laboratories, in order to give a comparative evaluation of the materials. This pro-

Table I. Tests against Houseflies by Peet-Grady Methods

Test solutions. 300 mg. of material + 30 mg. of pyrethrins per 100 ml.
Standard solutions. 30 and 100 mg. of pyrethrins per 100 ml.
100 houseflies per test. Averages of 19 tests

Material	% Knockdown 3 min.	5 min.	10 min.	% Mortality (of Flies Down) in 24 Hours
(Propylpiperonyl)(butyl)diethylene glycol ether (I) (piperonyl butoxide)	46	65	93	74
Modification of auxotoxic group				
(Propylpiperonyl)(methyl) ethylene glycol ether (V)	49	68	93	74
(Propylpiperonyl)(methyl) ether (VI)	35	55	78	27
(Propylpiperonyl)(decyl) ether (VII)	31	47	68	24
(Propylpiperonyl)(benzyl) ether (VIII)	35	51	73	26
Modification of toxophoric nucleus				
(Methylnaphthylmethyl)(butyl) diethylene glycol ether (III)	29	43	66	14
(Dichlorobenzyl)(butyl) diethylene glycol ether (IV)	37	51	79	17
Pyrethrins, 30 mg. per 100 ml.	27	44	68	19
Pyrethrins, 100 mg. per 100 ml.	60		92	43

Table II. Tests against Houseflies

Peet-Grady method. Test solutions. 300 mg. of material plus 30 mg. of pyrethrins per 100 ml.
Standard solution. 100 mg. of pyrethrins per 100 ml.
100 houseflies per test. Averages of 19 tests

Turntable method. Test solutions. 150 mg. of material plus 15 mg. of pyrethrins per 100 ml.
Standard solution. 100 mg. of pyrethrins per 100 ml.
100 houseflies per test. Averages of 16 tests

Loop method. Test solutions. 300 mg. of material plus 30 mg. of pyrethrins per 100 ml.
Standard solution. 175 mg. of pyrethrins per 100 ml.
50 houseflies per test. Averages of 6 tests
Dosage 0.00023 ml. per housefly

Residue method. Test deposits. 10 mg. of material plus 1 mg. of pyrethrins per square foot
Standard deposit. 20 mg. of pyrethrins per square foot
100 houseflies per test. Averages of 4 tests

Material	% Mortality in 24 Hours			
	Peet-Grady	Turntable	Loop	Residue
(Propylpiperonyl)(butyl) diethylene glycol ether (I) (piperonyl butoxide)	74	69	98	74
Modification of auxotoxic group				
(Propylpiperonyl)(methyl) ethylene glycol ether (V)	74	81	99	93
(Propylpiperonyl)(methyl) ether (VI)	27	8	28	<1
(Propylpiperonyl)(decyl) ether (VII)	24	7	13	32
(Propylpiperonyl)(benzyl) ether (VIII)	26	6	25	1
Modification of toxophoric nucleus				
(Methylnaphthylmethyl)(butyl) diethylene glycol ether (III)	14	6	16	<1
(Dichlorobenzyl)(butyl) diethylene glycol ether (IV)	17	4	18	1
Pyrethrins, 100 mg. per 100 ml.	43	35		
Pyrethrins, 175 mg. per 100 ml.			53	
Pyrethrins, 20 mg. per square foot				47

Toble III. Tests by Residue Method

German cockroaches. Test deposits. 20 mg. of material plus 2 mg. of pyrethrins per square foot
Standard deposit. 5 mg. of pyrethrins per square foot
10 insects per test. Averages of 10 tests

Red flour beetles. Test deposits. 10 mg. of material plus 1 mg. of pyrethrins per square foot
Standard deposit. 5 mg. of pyrethrins per square foot
20 insects per test. Averages of 10 tests

Material	German Cockroaches, % Mortality in 24 Hours	Red Flour Beetles, % Knockdown in 24 Hours
(Propylpiperonyl)(butyl) diethylene glycol ether (I) (piperonyl butoxide)	77	75
Modification of auxotoxic group		
(Propylpiperonyl)(methyl) ethylene glycol ether (V)	80	79
(Propylpiperonyl)(methyl) ether (VI)	21	34
(Propylpiperonyl)(decyl) ether (VII)	37	75
(Propylpiperonyl)(benzyl) ether (VIII)	52	52
Modification of toxophoric nucleus		
(Methylnaphthylmethyl)(butyl) diethylene glycol ether (III)	28	23
(Dichlorobenzyl)(butyl) diethylene glycol ether (IV)	27	67
Pyrethrins, 5 mg. per square foot	22	85

Table IV. Tests against Mexican Bean Beetle Larvae by Settling Tower Method

Test dusts. 0.5% material + 0.05% pyrethrins + 0.25% rotenone
Standard dust. 0.75% rotenone
10 larvae per test. Averages of 12 tests
Dosage of dust. 0.283 gram per square foot

Material	48 Hours		72 Hours	
	% dead	% dead and moribund	% dead	% dead and moribund
Piperonyl cyclonene (II)	78	98	90	96
(Propylpiperonyl)(butyl) diethylene glycol ether (I) (piperonyl butoxide)	43	83	60	84
Modification of auxotoxic group				
(Propylpiperonyl)(methyl) ethylene glycol ether (V)	52	94	73	93
(Propylpiperonyl)(methyl) ether (VI)	28	56	32	58
(Propylpiperonyl)(decyl) ether (VII)	14	32	19	32
(Propylpiperonyl)(benzyl) ether (VIII)	22	44	29	43
Modification of toxophoric nucleus				
(Methylnaphthylmethyl)(butyl) diethylene glycol ether (III)	19	43	29	38
(Dichlorobenzyl)(butyl) diethylene glycol ether (IV)	8	30	15	25
Rotenone, 0.75%	18	40	32	45

cedure is necessary to provide a standard measure of the effectiveness of a given substance, and to give other workers data that they can compare with their results on other insecticides.

The majority of the tests were carried out against houseflies, because they are the most commonly used insects in standard test procedures, and they are comparatively easy to rear in the laboratory.

Four tests were employed against houseflies: the standard Peet-Grady method (*1*); the turntable method (*2*); the loop method, in which the material is applied to individual female houseflies, by means of a calibrated wire loop (*9*); and a residual deposit test, in which flies are exposed to glass plates 25 hours after they have been sprayed with acetone solutions of the products.

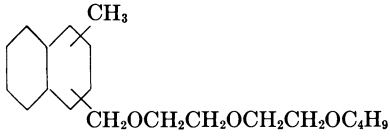
Because of the importance of safe insecticides in the protection of foods, additional tests have been run against German cockroaches, red flour beetles, and Mexican bean beetle larvae. The residual deposit test was used on the first two insects, and a dust test on the bean beetles. The procedure for the latter method is to incorporate the materials into dusts which are distributed on bean leaves (*6*); piperonyl cyclonene, which is superior to butoxide against this insect, was included in this series of tests.

Chemical Structure and Insecticidal Activity

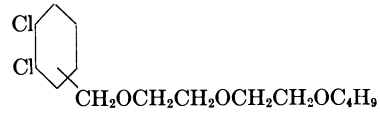
Experimental work carried out in these laboratories during recent years has been based on the theory that insecticides owe their activity to a toxic nucleus—the toxophore—the properties of which may be modified by auxiliary radicals—the auxotoxes. This nomenclature is suggested by the names of analogous functions in dyestuffs, the chromophore and auxochrome groups.

A striking illustration of the effect of chemical structure on insecticidal properties is provided by the data given in this paper on compounds related to piperonyl butoxide. According to the above theory, the methylenedioxyphenyl nucleus present in this substance is the toxophore. The materials selected for comparison show the reduction in toxicity produced, first, by modifying the toxophore, and, second, by substituting different groups for the auxotox radical.

At the present time, from the point of view of toxicity to insects combined with low toxicity to warm-blooded animals, there is no sound chemical basis for the selection of toxophoric nuclei. This point is made clear by the data presented for the methylnaphthylmethyl (III) and dichlorobenzyl (IV) analogs of piperonyl butoxide, both of which contain the diethylene glycol butyl ether group, which is considered to be the auxotoxic group according to the theory stated above. Each of these nuclei would be expected to show strong insecticidal properties in structures of this type, but the findings prove that these products are generally ineffective. Until our knowledge of the influence of structure on insecticidal activity has been greatly extended, the discovery of potent new toxophoric nuclei seems bound to depend on chance.



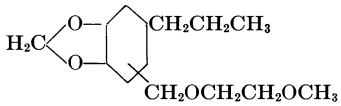
III



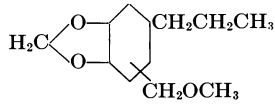
IV

The improvement of a toxophoric nucleus by modification of the auxotoxic groups comes through painstaking research. This aspect of the development of a valuable insecticide is illustrated by the comparison of four materials (V, VI, VII, and VIII) closely related to piperonyl butoxide, selected from a large number of homologous series which the authors have studied. These substances are analogs of piperonyl butoxide in which the diethylene glycol butyl ether auxotoxic group has been replaced by ethylene glycol methyl ether, methyl ether, decyl ether, and benzyl ether groups. Generalizations regarding the effect of different auxotoxic groups cannot be made safely, because their influence may

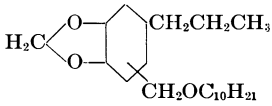
well be dependent on the nature of the toxophoric nucleus. Product V is shown by the authors' data to have insecticidal activity approximating that of piperonyl butoxide, but has the disadvantage of being more volatile. Products VI, VII, and VIII are generally low in activity.



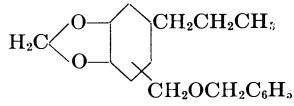
V



VI



VII



VIII

Although compounds containing the methylenedioxyphenyl group are usually considered solely as synergists or activators when used in combination with pyrethrins, materials of the type of piperonyl butoxide also have definite insecticidal properties in themselves—for example, against houseflies by the Peet-Grady method piperonyl butoxide alone at 2 grams per 100 ml. gave an average 24-hour mortality of 75% in 20 tests, and by the turntable method at 5 grams per 100 ml. gave an average 24-hour mortality of 78% in 20 tests. By comparing these results with those in Table II, it will also be seen that combinations of piperonyl butoxide with pyrethrins exhibit a marked degree of activation. Thus, products such as these are true insecticides which also have the property of showing activation when combined with materials such as pyrethrins. These substances are normally used in combination with pyrethrins in order to obtain maximum efficiency at an economical cost level.

Application Studies on Insecticides

When a given material has been demonstrated by screening techniques to have potential value as an insecticide, there then arises the still broader question of how to evaluate its usefulness for specific applications under practical conditions. Then this information must be extended by development of suitable commercial formulations, with due regard to their effectiveness and safety in the hands of the average user, who is the ultimate link in the efficient use of insecticides.

Finally, in order to prove the safety of a given substance to man and other warm-blooded animals, exhaustive study must be made of its acute and—most important—its chronic toxicity. In the case of piperonyl butoxide, for example, about 3 years have already been spent on these studies, and the results of the acute toxicity tests have been published (*4*). A 2-year study of chronic toxicity is nearing completion and the results, which have demonstrated the high degree of safety of piperonyl butoxide both alone and in combination with pyrethrins, will be published at an early date. These experiments with laboratory animals have involved the daily ingestion of doses in food at different levels throughout the life of the animals or for periods of 1 to 2 years, and also in successive generations. In addition to observations of the general health of the animal, information on gross and microscopic pathology has been accumulated, the latter through the agency of a competent outside expert. Not the least important aspect of all such studies is wholehearted cooperation with federal and practicing toxicologists specializing in this field.

Acknowledgment

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Chemicals, Inc., who conducted the biological tests and assisted in the compilation of the data.

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Spray Residues on Food Crops and Their Relation to Total Food Consumption

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It may be estimated conservatively that less than 10% of the food we eat is contaminated with harmful spray residues. These observations, correlated with the relatively low amounts of various pesticide residues found on food crops and the immeasurable trace that may be on a single service of the food, emphasize the little danger to health from spray residues.

The wide publicity given DDT and the many new pesticides that have been developed during the past few years has made the public aware of the widespread use of spray chemicals. Many misstatements about the poisonous properties of these new products have caused abnormal concern and fear over the possible presence of excessive amounts of spray residues on food crops. This concern is a natural one, because the public does not realize the very small amount of the insecticidal residue that may remain on a single service of food, which usually does not exceed 0.25 pound in weight. Nor does the public realize that only a relatively small percentage of the food we eat has ever been treated with a spray or dust of a specific pesticide.

Most pesticides—that is, insecticides and fungicides—are poisonous to humans as well as to the pest that they control if ingested in sufficient quantity. This is true of the older insecticides such as nicotine, the arsenicals, and the fluorine compounds that have been in common use for many years, as well as the new organic compounds such as DDT. Spray residues on food products must be kept below amounts that may result in a health hazard to man. The possible hazard to human health, however, from eating food that carries excessive amounts of spray residues is one not of acute toxicity because of the deadly poisonous nature of the spray chemical, but of chronic complications. There never is enough pesticide remaining on a single service of food to cause a temporary illness, let alone a fatality. This may be exemplified from the observations of Neal and von Oettingen (*1*), who fed a man subject a single dose of 770 mg. of DDT in olive oil at one time and 475 mg. of DDT at another time. A thorough physical examination before and after the ingestion of the DDT failed to reveal any untoward effects whatsoever. By comparison, 770 mg. is more than 1500 times the amount of DDT that may be found on a service of food that has received spray treatment during the growing season.

The danger to health from food carrying trace amounts of a pesticide is from the cumulative effect of eating poison spray continuously over a period of time. Our problem then is to adjust and recommend spray programs for the control of each pest, so that there will not be enough of the spray chemical on the food crops to cause health complications. This is not an impossible task. Entomologists and plant pathologists must learn the minimum amount of each pesticide that will give effective control of the pest, having in mind the need of the longest period of time possible between the last application of the pesticide and harvest. This time period will permit maximum losses of the pesticide by decomposition, volatilization, and erosion and will also lower the proportional amounts

because of increase in weight of the food crop by growth after the last spray treatment. A few results showing correlation of these factors are given in Oregon Experiment Station circulars (*2*). Furthermore, limited studies indicate that the processing treatment given prior to freezing or canning lowers the residue on some food crops.

Investigations of amounts of pesticide residues on crops have been under way for several years at the Oregon Agricultural Experiment Station. Analytical data on the amounts of spray residues on food crops before and after one or more spray or dust applications, on the food at harvest, and after the usual heat and washing treatment given prior to freezing or canning show that relatively small quantities of pesticides are present on the food as it is consumed by the public. Residue studies of the newer organic insecticides include DDT, DDD, DMDT, and parathion as they may be used to control pests on various fruits and vegetables. Table I indicates the amounts of spray residues remaining on several crops, after specifically controlled spray or dust treatments have been applied.

Table I. Spray Residues on Food Crops at Harvest

			DDT, P.P.M.
Apples	Hood River, Oregon fruit district	34% of samples	1.0
		28% of samples	2.0
		18% of samples	3.0
		20% of samples above	3.0
Pears	Medford, Oregon fruit district	30% of samples	1.0
		34% of samples	2.0
		13% of samples	3.0
		22% of samples above	3.0
Asparagus	5.0% DDT dust, 30 lb. per acre, 4 days before harvest		6.4
	Same dust treatment, then washed and blanched for freezing		2.6
Beans	3.0% DDT dust, 30 lb. per acre, 4 days before harvest		1.1
	Same treatment, canned by usual cannery process		0.7
Corn	5.0% DDT dust, 30 lb. per acre, 2 weeks before harvest		Trace
Peas	3.0% DDT dust, 50 lb. per acre, 1 week before harvest		Trace
Tomatoes	3.0% DDT dust, 30 lb. per acre, 7 weeks before harvest		Negative
	3.0% DDT dust, 30 lb. per acre, 1 week before harvest		1.8
			Parathion, P.P.M.
Apples	0.5 lb. of 25% parathion to 100 gal., after 2nd application		2.9
	0.5 lb. of 25% parathion to 100 gal., 2 applications, last one 4 weeks before harvest		0.09
	0.5 lb. of 25% parathion to 100 gal., 2 applications, last one 9 weeks before harvest		0.01
Beans	0.5% parathion dust, 50 lb. per acre, 7 days before harvest		0.08
Cauliflower	0.5% parathion dust, 50 lb. per acre, 7 days before harvest		0.21
	Same dust treatment, then washed and blanched for freezing		0.04

The determination of DDT residue on apples grown in the Hood River fruit district and on pears at Medford is carried on in branch laboratories established in those areas. The majority of samples selected for analyses are suspected of carrying higher amounts of residue than the average because of the spray program used or because the last application of insecticide was made within a few weeks of harvest. As indicated by Table I, about 80% of all the samples analyzed carried 3.0 p.p.m. or less of DDT during the past harvest season. Only about 20% of the samples showed residues above 3.0 p.p.m.; six samples showed residue deposits slightly above 7.0 p.p.m.

The amounts of pesticide residues on vegetables after dust treatments are variable, depending upon the surface area of the vegetable. Italian broccoli and leafy vegetables will retain more residue than beans on a weight basis. The few analyses given in Table I indicate that vegetables properly treated with DDT dust carry relatively small amounts of the insecticide at harvest time. A further lowering of the amount of residue has been found after the washing and blanching prior to freezing. For example, asparagus found to carry 6.4 p.p.m. of DDT at harvest shows only 2.6 p.p.m. after washing and blanching. A similar decrease was observed after beans received the blanching treatment. Corn and peas that had received heavy treatments of DDT dust during the growing season showed a trace of the insecticide due to contamination by the mechanical handling of the vegetables prior to canning. Analyses after canning or freezing gave negative results for the same vegetables.

Determination of parathion deposits on fruits and vegetables indicates such small amounts present that there should not be a residue problem with this insecticide. Parathion apparently volatilizes or decomposes so rapidly that less than 0.10 p.p.m. of residue

remains on the food crop at harvest. Furthermore, limited studies indicate that when vegetables carrying parathion residue are blanched or otherwise heated, more than 75% of the parathion is decomposed by the treatment. Further studies on the effects of blanching and canning will reveal to what extent various other pesticides may undergo decomposition.

Chronic health complications that may develop from eating food that contains traces of harmful spray residues depend upon the amount present and the extent to which daily or recurrent ingestion of the contaminated food continues. Not all foods included in the daily diet carry spray residue. Based upon statistics obtained from *The Western Canner and Packer* and the U. S. Department of Agriculture (*3*, *4*) approximate percentages of various foods eaten per person in the United States are given in Table II.

Table II. Percentage of Foods Eaten per Person in United States

	%
Not contaminated with spray	
Flour, cereal, bread, etc.	19.5
Meats, fish, poultry	11.9
Fats, butter, margarine, etc.	5.6
Sugars, candy, jellies, etc.	5.7
Potatoes	20.5
Potatoes, sweet	2.7
Peas	1.4
Corn	3.9
Carrots	0.9
Beets	0.3
Pumpkin	0.3
Onions	1.8
Cucumbers	0.6
	75.1
Usually sprayed	
Beans	2.5
Tomatoes	5.8
Cabbage	2.4
Asparagus	0.3
Peppers	0.2
Cauliflower and broccoli	0.3
Spinach, lettuce, etc.	0.3
Apples	2.9
Pears	1.1
Oranges	4.9
Grapefruit	2.5
Other fruits	1.7
	24.9

About 75% of the food we eat contains no spray residue whatsoever; the other 25% may or may not have been sprayed during its production. Some food crops may have been treated with DDT in one part of the country, but with nicotine or rotenone insecticide in another production area. Consequently, possibly half or even less of a certain crop grown throughout the United States may have received spray or dust treatments with this one pesticide. Again, a large proportion of crops such as apples, pears, and peaches are peeled before canning or freezing and therefore no spray residue is present in the product. Furthermore, a large proportion of the citrus fruits are ingested as juice and the consumer is not exposed to the residues on the peel. Accordingly, it may be estimated that less than 10% of the food we eat is contaminated in any way with harmful spray residues. These statistics, correlated with the relatively low amounts of various pesticide residues found on food crops and the immeasurable trace that may be on a single service of the food, emphasize that there is little, if any, danger to health from spray residues.

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Public Health Aspects of Agricultural Chemicals

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A well planned public health program is needed for the safe exploitation of agricultural chemicals. This should embody research on toxicity, cooperation of public and private agencies for exchange of information and promulgation of control measures, labeling of economic poisons, adequate inspection, and education in occupational and consumer health.

In 1900, rural people were generally healthier than people who lived in the city (*7*). Fifty years ago the length of life among white males in the rural areas was ten years, and among white females, seven and a half years, longer than that of the same groups who lived in the city. Since this time, health conditions have improved markedly in urban centers but more slowly in rural areas, so that today the life expectancy of rural and urban populations in the United States is approximately the same. Undoubtedly a major reason for this development is the improvement of medical care and public health services in the cities, while the rural areas have lagged in this respect. Coincidentally, agriculture and allied activities have become highly industrialized. Today the farm is a "factory in the field."

It is perhaps not generally known that accidental deaths are much higher in the rural areas than in the cities. For example, in 1948 there were 55 accidental deaths per 100,000 agricultural workers as contrasted with 16 per 100,000 manufacturing workers. In addition to accidents, the farmer is the special target of many other occupationally associated conditions, such as the diseases transmitted by contact with plants and animals; conditions resulting from excessive exposure to sunlight, such as actinic dermatitis and skin cancer; and skeletal difficulties, such as arthritis, myositis, and others resulting from strenuous physical labor plus exposure to the elements. Finally, with the enormous strides made in recent years in the sciences, farming has now become a mass production industry, using practically every type of chemical. Today no significant agricultural production is achieved without the aid of chemical adjuncts.

Importance of Agricultural Chemicals

Reports of occupational diseases sent to the California Department of Public Health reveal strikingly that it is "one world" so far as hazards to health among occupational groups are concerned. The same chemical may cause illness to the factory worker who manufactures it, to the truckers and stevedores who handle and transport it, to the farmer who uses the material, to the packers and canners of the food treated with it, and to the person who consumes the foods that may be contaminated with it.

There are 80,000 kinds of insects in North America, at least 5000 of which are known to be of economic importance. The estimated damage to food products caused by insects annually in the United States amounts to $2,000,000,000. The insecticide industry in the

United States has a total value of about $15,000,000. In 1948, 35,000,000 pounds were produced of one insecticide alone—DDT (*9*). There are about 25,000 plant diseases in the United States, including 10,000 of economic importance, and these cost the nation about $4,000,000,000 a year. The annual wholesale value of all agricultural chemicals in the United States is about $185,000,000. The Bureau of Chemistry of the State Department of Agriculture in California reported during the fiscal year 1946–47 the registration of 7717 economic poisons.

Pesticides are only one group of chemicals in use in agriculture. There are now hormones either to accelerate or retard the growth of plants. Livestock are being fattened with the aid of chemicals. Soil and seeds are treated with chemicals. New fertilizers are being developed. Many other applications are being discovered.

At the outset, one must note the fact that the public health values of the agricultural chemicals far outweigh the health hazards in their use. The conquest of the insect-borne plagues with the aid of insecticides can now be foreseen. Agricultural chemicals make possible greatly increased production and preservation of food. The Malthusian philosophy of starvation is being discredited effectively with the aid of modern chemistry. Society can and should use the agricultural chemicals with complete safety to health.

It can be assumed that almost any material that is poisonous to insects is poisonous to man, the main variables being the dose and relative susceptibilities of the species. The agricultural chemicals, like other chemicals, reach the body by inhalation, ingestion, and contact with the skin and mucous membranes. They may cause acute and chronic diseases which vary from superficial skin and mucous membrane inflammations to serious and sometimes fatal systemic conditions.

Other speakers on this program describe the toxicological and pharmacological action of various of these poisons. It is appropriate, however, that a representative of a public health department should discuss the public health aspects. When illness and death occur as a result of overexposure to these poisons, the public health industrial hygienist is called upon to investigate and devise controls. The Bureau of Adult Health of the California State Health Department has in recent years investigated and devised controls for a great variety of economic poisons. These include fumigants such as methyl bromide and the cyanides, preservatives such as sulfur dioxide, the pesticides, and others such as ethylene chlorohydrin used to accelerate the sprouting of seed potatoes. From another aspect, the Bureau of Food and Drugs, also a part of the State Health Department, is vitally interested in the prevention of adulteration of foods by these poisons. It is essential today that the public health agencies cooperate very closely with the agricultural agencies, the farmer, the manufacturer of chemicals, and others concerned in the proper handling of this problem.

Hazards to Public Health

The arsenicals are today not the public health problem they once were. However, many lessons were learned from the experience with them. In 1940, the U. S. Public Health Service reported its study on the effect of lead arsenate exposure on orchardists and consumers of sprayed fruit (*8*). It found, in a large group of consumers of the apples treated with lead arsenate, that there was a slight increase in the amount of lead and arsenic in the blood and urine, although no harmful effects from this could be demonstrated at that time. However, lead arsenate constituted a definite health hazard to sprayers in the apple orchards of Washington (*1*). The U. S. Public Health Service investigators (*8*) stated, "Although sprayers who apply lead arsenate spray intermittently did not appear to be adversely affected, top consideration should be given to the protection of the health of men who mix or apply lead arsenate sprays every working day of the season." They recommended that protective equipment be provided for mixing tanks at which people work on a full-time basis. Other investigators recommended also that the orchard industry set up an "authority" with responsibility for providing an adequate health protection program for orchard workers (*1*). These recommendations apply essentially to similar operations for any of the economic poisons.

With the exception of nicotine, the agricultural chemicals derived from plants are probably among the least hazardous to man (*6*). Nicotine itself is one of the most efficient insecticides. Nicotine can cause serious poisoning or death if swallowed, inhaled, or absorbed through the skin.

The halogenated hydrocarbons comprise the largest significant group of pesticides. Most extensive investigation of toxicology has been done with DDT and it illustrates well the ramifications of a public health problem with agricultural chemicals. DDT can cause toxic manifestations by any route of administration, and a number of cases of poisoning have been reported when large amounts of the material have been ingested. It is possible, too, that many reported cases of DDT poisoning were in reality caused by the solvent in which the material was prepared rather than by the active insecticide itself.

But a more important health aspect of DDT is the fact that it is stored in the body fat at a level four to ten times that of the dietary intake. It is secreted in the milk of cows, goats, dogs, and rats (*2*). Other animals fed this milk show toxic symptoms. The DDT in the milk appears to be concentrated in the butterfat portion and therefore is transferred to the butter. So today the American people are consuming DDT in their milk and butter as well as in other agricultural produce. This may be especially important in the case of infants and children because of their high consumption of milk. In animals the withdrawal of food with consequent consumption of body fat produced characteristic DDT nervous symptoms. This effect might be important in cases of human starvation or illness in which there is an appreciable storage of DDT in the body. Howell (*4*) recently reported finding 17 p.p.m. in a lipoma removed from an individual who had worked with DDT and ingested it in milk and other foods over a period of about four years. No clinical effects were noticed in the patient. One can only speculate on the possible hazard should such an individual be forced by starvation or illness to metabolize his body fat.

Organic Phosphates

These observations have motivated the U. S. Department of Agriculture and Bureau of Food and Drugs recently to take steps banning the use of DDT in spraying of dairy barns and of dairy and meat cattle.

The organic phosphates are the newest large group of insecticides, the major compounds being tetraethyl pyrophosphate and parathion. These have all been shown to be extremely poisonous to animals, and in the brief period in which they have been used they have demonstrated their toxicity to human beings. In the first half of 1949, there were at least five fatalities reported, one in California. Persons exposed to these materials may display marked contraction of the pupils to the point of blindness, shortness of breath with a feeling of tightness of the chest, headache, and other symptoms depending upon the severity of exposure. These effects result, in part at least, from the destruction of cholinesterase by parathion with consequent building up in the body of excessive amounts of acetylcholine.

Because the phosphates are readily absorbed through the skin and are hazardous from exposure by any route, prevention of poisoning includes avoiding contact with the bare skin and avoidance of inhalation of the chemicals. It is recommended also that workers change their clothes completely and bathe with soap and water after every use of this material. Particular caution is indicated on the part of pilots engaged in airplane spraying because of the effects of the organic phosphates on the eyes. Parathion presents special problems because of its translocation into the plant.

The agricultural chemicals in general are more dangerous to handle in solution than in wettable powder or dust form. Parathion and nicotine are the only exceptions to this as they seem to be about equally toxic in wet or dry form.

One overriding problem is the lack of knowledge concerning the effects of exposure to small amounts of these chemicals over a long period of time. Cancer must be regarded as a possible hazard in the handling of some of the agricultural chemicals and the solvents in which they are prepared. Cancer of the skin has been reported in persons in contact with arsenicals used in fruit spraying and sheep dipping (*3*, *5*). Multiple organ malignancies

were observed in animals exposed to 2-acetaminofluorine, a material originally under investigation for use as an insecticide (*10*).

Public Health Program for Control of Economic Poisons

A well planned public health program is needed for the safe exploitation of the agricultural chemicals. Such a program should embody the following major features:

Research. Many economic poisons are released on the market prior to adequate research in their toxicity. Too often human injury is the stimulus to research which should have been done before the material was used. There is need for the training of more personnel in the fields of toxicology, pharmacology, and occupational health. There is an urgent need for the augmentation and improvement of present research facilities. There is a need for the better coordination and free exchange of information between private and public research groups. All the above could be greatly facilitated by the establishment of a national science foundation with adequate financial support and broad representation of the groups concerned.

Cooperation of Private and Public Agencies. The federal, state, and local public health agencies, the agricultural agencies, and the chemical manufacturers and distributors should maintain a close liaison for the exchange of information and the prompt promulgation of public education and control measures.

Legal Controls. These include a labeling law requiring all economic poisons to be clearly labeled according to content and amount of each ingredient, with appropriate statements on first aid measures and precautions; and an adequate system of inspection for the presence of poisonous residues on the surface of, or within, the foods placed on the market. It is recommended also that the states adopt legislation consistent with the federal laws.

Education in the occupational health and consumer health aspects must be extended. Public health agencies in collaboration with the agricultural and food and drug manufacturers' agencies should disseminate appropriate educational material for farmers and allied workers, for the employees engaged in handling and manufacturing economic poisons, for the medical and allied professions, and for the public generally.

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Benefits and Hazards of Insecticides to Public Health

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Striking improvements in the control of insect-borne diseases in recent years are largely due to DDT. Improvements in agricultural insect control are due to inorganic insecticides, insecticides of vegetable origin, DDT, and an ever-widening range of new organic insecticides. Use of promising new insecticides must wait on knowledge of their toxicity.

The effectiveness of some of the new insecticidal agents against an extremely wide variety of disease vectors has made possible the control of many important diseases which previously defied all practical efforts at control and has brought within the realm of possibility the ultimate eradication of certain diseases.

Diseases which careful scientific study indicates are subject to control by insecticides include malaria, plague, epidemic typhus, murine typhus, and enteritis due to *Shigella*.

Diseases which will probably be subject to control by insecticides but have not yet been adequately tested include sandfly fever, dengue, urban yellow fever, bartonellosis, cutaneous leishmaniasis, Chagas' disease, filariasis, trench fever, and louse-born relapsing fever. Some of the virus encephalitides, sleeping sickness, and visceral leishmaniasis may also be susceptible of control.

Control of Malaria and Plague

More data showing epidemiological statistics for insecticide-treated areas and for control areas have been published on malaria than on any other disease. With few exceptions, these data indicate a remarkable degree of control surpassing anything reported previously. For example, after a single year of operations, Viswanathan and Rao (*31*), working in Bombay Province, India, were able to report control of 74% or better based on spleen and parasite rates of children and adults in sprayed as compared to control villages in the Kanara District and 30% or better in the less severely affected Dharwar District. The infant parasite rate, which more nearly reflects new cases of malaria, was reduced by 94% in the Kanara District and apparently by 100% in the Dharwar District. (These examples are very incomplete. Since the preparation of this paper, a much more extensive résumé of disease control with insecticides has been presented at the meetings of the American Society of Tropical Medicine, Memphis, Tenn., November 6 to 9, 1949. The résumé will be published.)

Essentially similar results have been reported in more limited studies in Panama, where a reduction in the parasite rate from 52% in the control area to 14.8 in the treated area was obtained (*27*), and in Puerto Rico, where the rate was reduced from 5.8 to 0.9% in one year (*12*, *25*). In Panama and Costa Rica, Macready (*15*) reported that the malaria rate of hospitalized employees was reduced on the average by 53% for each 1000 admissions. In Peru, the parasite rate was less than 1% after treatment, where it had

ranged from 11 to 28% before the use of DDT (*4*). In a small area of the Volturno River Valley in Italy, the parasite rate was reduced from 21 to 1% in one year (*1*). An improvement of about 50% was noted in Veneto Province in Italy following treatment with DDT (*23*). Good results have been reported in New Guinea (*2*) and in the New Hebrides Islands (*33*), especially among military personnel. In Mauritius, in the Indian Ocean, a carefully controlled study showed the parasite rate of the general population to be reduced by two thirds and that of the children by one half (*26*).

In the United States, the incidence of malaria is low and it has been falling gradually for many years. However, the current large program of house spraying conducted by the states in cooperation with the Communicable Disease Center is believed to be increasing that rate of fall. Coupled with auxiliary techniques, the house spraying program, if continued, has an excellent chance of eradicating malaria from the continental United States in the not too distant future.

The first control of plague through the use of DDT was accomplished by the U. S. Army in Dakar in November and December 1944 and in Casablanca in July 1945 (*8*). The outbreak in Dakar had been active since April 20, 1944, and its complete control was apparently almost entirely due to the universal application of DDT in the native quarters to persons, beds, floors, walls, and premises generally. Following this experience, DDT was used more promptly in Casablanca. Macchiavello (*13*, *14*) considers, on the basis of his experience in Peru, that the use of DDT followed by the use of 1080 (sodium fluoroacetate) may be the method of choice in the control of epidemics of bubonic plague. Pollock (*20*) using DDT alone successfully controlled an epidemic of plague in Haifa in July 1947.

Control of Typhus

Probably the most dramatic accomplishment in public health in the last quarter of a century was the control of major epidemics of louse-borne typhus in densely populated, heavily infested populations under wartime conditions. This accomplishment was made possible by a great body of research before World War II and by the efforts of at least a dozen military and civilian agencies during that war. Methods of louse control with MYL (pyrethrins, 0.2%; *N*-isobutylundecylenamide, 2.0%; 2,4-dinitroanisole, 2.0%; and phenol S, 0.25%) and DDT powders were first field tested in the Near Eastern and African theaters and finally proved in the epidemics in Naples (*3*, *24*), in German concentration camps (*5*, *7*), and in Korea and Japan (*21*). Although immunization was of value especially in protecting expert personnel carrying out control work, it has been pointed out by Bayne-Jones and others that it was the judicious use of insecticidal powders that actually stopped the epidemics. It has been estimated that there were 5,000,000 cases of typhus in Russia alone following World War I. By contrast, the number of civilian cases in the Near East, North Africa, Italy, Germany, Korea, and Japan during World War II appears to have been less than 500,000.

The murine typhus control programs of the individual states, in cooperation with the United States Public Health Service, probably represent the largest unified attack on murine typhus ever undertaken. Since actual operations were begun in September 1945, a total of 1413 tons of 10% DDT dust has been applied in 1,105,006 premises treatments in 156 counties of 10 states. Control of the tropical rat flea, *Xenopsylla cheopsis*, the chief vector of murine typhus, has been 80% or better under field conditions. As a result of the programs, murine typhus has decreased more rapidly in the treated counties where it was originally more frequent. There has, during the same period, been a decrease in the officially untreated counties which originally suffered less from the disease. This may be explained, in part, by DDT treatment, ratproofing, rat eradication, and general sanitation carried on in the "untreated" counties by the local government without state support or by private enterprise and, in part, by a natural decline in the disease due to unidentified causes. The decrease in murine typhus in 61 counties of 9 states dusted each year, 1946 to 1948, was 84% for the first 10 months of 1948 based on the first 10 months of 1945 before treatment was begun. The comparable figure for the officially untreated counties was 61%. The rapid control of small outbreaks of typhus transmitted by lice but presumably of the murine variety has been reported (*6*, *17*).

Control of Other Diseases

The control of diarrheal diseases through the control of flies by insecticides has been noted in association with several large scale programs—for example, the antimalaria program in Greece (*30*) and the antisandfly program in Malta (*22*). The only program of fly control by insecticides, in which the effects on diarrheal diseases were carefully followed and subjected to statistical analysis, was carried out by the Public Health Service in towns in the lower Rio Grande Valley. This study showed that a significant reduction in the amount of infection, disease, and death resulted from the degree of fly control which was obtained. The effect on infections due to *Shigella* was greater than that on infections due to *Salmonella* (*32*).

Several other diseases have already apparently responded to vector control with insecticides, although the conditions in which the outbreaks occurred did not permit complete statistical evaluation of the control. Control of sandfly fever in Malta was reported by Semple (*22*). Hertig and Fairchild (*9*) have noted the virtual cessation of new cases of both cutaneous leishmaniasis and bartonellosis following sandfly control with DDT in Peru. There is every reason to suppose that dengue, urban yellow fever, and filariasis would respond to control of their mosquito vectors and that Chagas' disease and trench fever and louse-borne relapsing fever would also respond. With somewhat more reservation, one may predict the control of some of the virus encephalitides, trypanosomal sleeping sickness, and visceral leishmaniasis.

Public health officers are concerned with the control of arthropods as vectors of human disease. Important as disease control may be, food production is even more important. Biologically, organisms are more often and more completely limited by their food supply than by disease. The total health of our population is affected by anything which reduces our food supply. The chemical control of food-destroying insects is, therefore, a necessary part of any survey of the benefits of insecticides to public health. Of course, the methodology and conduct of such control are not the responsibility of the public health profession, but the necessity of such control must be considered here, just as the necessity for maintaining the health of farmers and their families must be considered in a comprehensive outline of agriculture. Just how much human food is saved from insect destruction is difficult to estimate. It is thought that 10% or more of the total crop in this country with an annual value of about $13,000,000,000 is lost through insect damage (*11*, *16*). The damage to stored food products alone is said to average 5% even in countries with well developed technical services (*10*). The earlier estimates of losses may be too low in view of the increased production which may now actually be obtained by the judicious use of insecticides (*18*).

The toxicity of a substance is its capacity for causing injury, whereas the hazard of a substance is the probability that such injury will actually occur. Factors that influence the capacity of a given compound to cause injury include its residual action; its acute toxicity; its subacute and chronic toxicity (including not only the accumulation of the toxin or its metabolites in the tissues but also the accumulation of tissue damage with or without the accumulation of the toxin); its solvent and particle sizes, as used; its relation to diet; and the timing of its application. The toxicity of a compound may vary for different species, for different ages, and for the two sexes. Factors that influence hazard include not only all the aspects of toxicity but also the method of use, the degree of warning, such as odor given by the compound itself; the degree of education of the public; and many other subtle factors.

There is nothing basically new in our present problem, but there are a great variety of new insecticidal compounds and formulations available, and there is a greatly increased demand for their use.

Just as the benefits of insecticides to public welfare fall into two classes, so also do the hazards fall into two groups: first, those which are traditionally and legally the responsibilities of local, state, and federal health officers, and, second, those which are traditionally and legally the responsibilities of other authorities. The two groups are often impossible to separate. Of direct interest to the public health professions are the following:

Acute poisoning in the household by actual ingestion or food contamination
Acute poisoning of handlers and operators
Acute poisoning in industry
Subacute or chronic poisoning of consumers through residues in fruit, vegetables, meat, or dairy products or through improper household use
Subacute or chronic poisoning of handlers and operators
Subacute or chronic poisoning in industry
(Health officers may also be notified when household pets are poisoned.)

Of equal importance to the public health are hazards of acute or chronic poisoning of farm animals and poultry; insecticide residues in fruit, vegetables, meat, or dairy products; phytotoxicity; reduction of soil fertility; and disturbance of the balance of nature through selective destruction of wildlife. These latter hazards are largely the responsibilities of agriculturalists and conservationists but are mentioned here for completeness.

The great interdependence of public health and agriculture can be no better illustrated than by the problem of insecticide residues in food. Compounds, vehicles, and spraying schedules adapted for insect control must be selected by the agriculturalist in such a way as to ensure an acceptable product at harvest. However, once the product is harvested and offered for human food, it becomes the concern of various public health agencies. The health of the agricultural operator who applied the insecticide is also a matter for medical and public health attention.

The benefits which have been pointed to in disease control are largely due to DDT. The benefits which have been pointed to in agricultural insect control are due to the inorganic insecticides, to insecticides of vegetable origin, to DDT, and to an ever-widening range of new organic insecticides. In both fields, and particularly in disease control, the use of several promising new insecticides must be severely limited or entirely omitted because so little is known of their toxicity that they cannot be used with confidence.

At least the following groups are interested, in one capacity or another, in the toxicity and hazard presented by insecticides: the public health and medical profession; the chemical industry; the agricultural industry, including farmers and state and federal departments of agriculture (registration and labeling); the Food and Drug Administration and similar state authorities (prohibition of contaminated food in commerce); the food industry, including canners, frozen food packers, and green grocers; the armed services; various research groups; and the public generally.

Broadly speaking, the most important factors in the safe use of economic poisons are adequate information and appropriate care by all persons having any contact whatsoever with the poisons. Safety may be promoted but not ensured by voluntary control, such as the self-discipline of industry; by legal control, such as regulation of sale, labeling, and distribution; and by economic control, such as the refusal of food processors to buy from farmers or dealers food containing excessive residues for which no adequate method of decontamination is known.

The principal features of the Federal Insecticide, Fungicide, and Rodenticide Act have been briefly and clearly presented by Perry (*19*); the full text and interpretations of the act are also available (*28*, *29*).

Conclusions

Because the benefits of insecticides to the public health both in a restricted and in a broad sense are very great, it should be our purpose to use the new as well as the older compounds in such a way as to avoid injury and at the same time obtain the maximum benefits.

Some think that efforts should be made to simplify the nomenclature and to encourage industry to do proper toxicological research and supply methods for analysis before a new compound is submitted for registration. The study of antidotes should be encouraged. It is clear that research in the health hazards as well as the benefits of insecticides must be intensified and that the efforts of different laboratories should be better coordinated. Education of industry, of handlers and operators, of the medical and public health profession, and of the public must be advanced.

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The Physician's Role in the Pesticide Problem

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The host of new synthetic organic pesticides presents a variety of problems to the practicing physician because of the lack of basic fundamental information on these chemicals. The American Medical Association has organized a committee on pesticides to consider the following problems of economic poisons and to coordinate information and make it available to physicians and other interested persons or groups: safe standards of use, development of prophylactic and antidotal measures, voluntary industry controls, standardization of nomenclature, and professional and public education.

The introduction of the newer economic poisons has created potential health hazards which, although not entirely new, are of such magnitude that the practicing physician must give serious consideration to them in order to safeguard the public welfare. The seriousness and extent of these problems are reflected in the increasing amount of attention which is being devoted to them over the radio and in the press. Unfortunately some of this publicity has been ill-advised and not always in accordance with the facts. Consequently, in this atmosphere of misinformation and misunderstanding, considerable confusion on the part of physicians, as well as the general public, regarding the safety of these materials has resulted. This confusion, if allowed to go unattended, may jeopardize the benefits to the health and economic welfare of the country which a more enlightened attitude would ensure. Since chemists have played such a prominent part in the development of pesticides, the public health problems associated with this situation and the approach undertaken by the medical profession to assist in their resolution may be of interest.

Practitioner's Problems

The bewildering host of new synthetic organic chemicals presents a variety of complex problems to the medical practitioner, many of which are entirely new to him. The basis for these problems lies in the lack of essential information about the many ways in which pesticides seem to influence physiologic function and the apparent indifference and carelessness with which many of these new and untried compounds are handled and used.

Frequently the physician is called upon to render professional advice or service on health problems involving these toxicants, only to find that there is little or no information upon which to base a rational conclusion. In other instances, he must piece together facts from a variety of widely scattered sources. Too often this information is of a limited nature and not applicable to the situation at hand or is so controversial and inconclusive in character as to render a reliable judgment impossible. As a consequence, the physician must resort to symptomatic measures which are often unsatisfactory because of the difficulty of relating symptoms to causative agent. At the same time, he is

faced with the problem of warning the public without alarming it about the dangers inherent in these materials.

These and related problems are being encountered by the practicing physician with increasing frequency because of the wider and oftentimes injudicious use of the newer pesticidal toxicants. The need for information has been apparent for some time. However, the equal need for the coordination and integration of the existent information, so that it may become readily available to all who should have it, has not received adequate attention to date.

Toxicology Problems

The wide gaps in present knowledge of the physiologic and phytologic properties of the new organic toxicants are all too apparent to anyone familiar with these poisons. Physicians need fundamental information on the mechanism of action, rate of destruction or excretion, and the long-term effects of the compounds on tissue structures. Medical practitioners have little information on secondary biological factors such as diet, susceptibility of age groups, individual sensitivity, and the presence of pre-existing organic changes in relation to their influence on the ultimate toxicity of these chemicals. In addition, many factors other than the acute or chronic toxic properties of the basic ingredient must also be evaluated. Such factors as the toxicity of the carrier, diluents and other so-called inert ingredients, the existence of additive or potentiating activity of these materials in combination with the basic ingredient, particle size in the case of aerosols, and the intrinsic dangers of different types of application demand further study. Until this information is available, the medical profession will take a dim view of those preparations which have labels implying that the product may be used with relative freedom and safety, but at the same time carry a disclaimer which states, in part, that the buyer assumes all the risks of handling and use whether in accordance with directions for use or not.

That much of this information is actively being sought in private, industrial, state, and governmental research laboratories is to the credit of those individuals who have recognized the need and are actively seeking solutions to these pressing questions. The medical profession recognizes that the orderly accumulation of information of this nature is both time-consuming and expensive. However, it also recognizes that in most instances the acquisition of this information should have preceded, rather than followed, the release of the chemical. Undoubtedly many of the toxicologic problems which have been encountered could not have been anticipated. Nevertheless, the absence of such information should have tempered the enthusiasm with which some of these materials were introduced.

In common with other aspects of the toxicology problem, physicians are embarrassed by the absence of adequate information to guide them in the assessment of the hazards of food contamination. Untried agents of complex composition have been rapidly introduced in a multitude of different formulations for agricultural purposes. Oftentimes these preparations were employed in excessive amounts far beyond the indications for use set forth by the manufacturer. This resulted in a series of economic misadventures which caused a wave of hysteria and overrestrictive legislation. Exaggerated claims for usefulness by some salesmen and distributors have compounded the initial harm to a point where the potential usefulness of the newer agricultural chemicals is being seriously threatened.

The medical profession views with equal alarm the abuses that have occurred with these materials and the retaliatory measures which threaten overregulation. These abuses have compelled food processors to reject fruit and vegetable crops which have been treated with certain of the newer materials. It has also prompted health officials to confer with government and technical workers on means of establishing measures for the protection of the public.

The economic and health significance of this problem is so acute that immediate control measures are imperative. What the nature of control measures should be can

only be determined by the willingness with which all groups enter into the solution of the problem. Voluntary control should and can be the measure of choice provided standards for the development, release, and distribution of pesticides are adopted which offer the same type of protection to the consumer that exists for drugs and other inherently dangerous substances. Uniform standards for the pretesting of new compounds which would thoroughly evaluate their relative safety and effectiveness under all circumstances of ordinary use must be established. Factors such as the effects on nutritive value, appearance, palatability, and storage qualities of food products, acute and chronic toxicity, practical methods for the detection and decontamination of residues, and the medicolegal aspects of quality control, labeling, and tolerances must be investigated. Advertising claims and promotional activities should be confined within the limits that the available information indicates are justifiable and reasonable.

This type of control should extend not only to agricultural chemicals but for all types of bulk and packaged preparations which are offered for sale as pesticides. No better standard than the old, well worn axiom, "it's better to be safe than to be sorry," could be used by the pesticide industry in the development and distribution of its products.

Education of the Public

Aside from the apalling lack of basic toxicologic information on pesticides, the need for public enlightenment in these matters is one of the most pressing problems which confronts the practicing physician. The education of the consumer, be he farmer, industrial handler, or domestic user of economic poisons, to their intrinsic toxic properties is of immediate importance. The consumer must be psychologically conditioned to the fact that he is using inherently dangerous compounds. Individually, he must be trained to read labels and follow directions for use to avoid unnecessary risks in handling or application in the home or in his work. He must be educated in order to discount the claims of overenthusiastic salesmen and certain newspaper scientists' reports of new worlds conquered. He must also be informed that new compounds are not "miracle workers" or "wonder drugs," but highly selective chemicals which are specific in their action. Lastly, the consumer must be told where to seek reliable information when questions about use confront him.

The problems of educating the public in such matters are many and diverse. It will require not only the support of medical people but that of all individuals who have a social conscience. The interest and sincere efforts of all scientific workers are needed to simplify and hasten this much needed program of public education.

Committee on Pesticides

The practicing physician, because of his unique position in community life, has the opportunity to create a better understanding of the uses and limitations of these pesticides. Recognizing the physician's responsibility in this aspect of the problem, the American Medical Association has held two meetings at its headquarter offices to determine in what way the association and its membership can best make a contribution. A conference on the health hazards of pesticides was called on November 3, 1948, to assess the health hazards created by the newer economic poisons and to exchange ideas and information relative to the public health problems they have created. About forty individuals attended this meeting representing government agencies, academic centers, trade associations, manufacturers, toxicologic laboratories, and state public health departments.

It was exceedingly apparent from this meeting that there is much to be learned about the uses and limitations of pesticides. However, it was more apparent that an equal need exists for the coordination and integration of the existent information so that it may become readily available to physicians and others. To fill this gap and to provide this much needed service, a committee on pesticides was proposed.

An exploratory meeting of this committee was recently held to determine in what

manner it could best serve physicians and the public. The following projects seemed worthy for immediate consideration:

1. PROMOTION OF SAFE STANDARD OF USE. It becomes increasingly apparent that the criteria of safety by which the older pesticides were judged do not always hold for the newer preparations which have been introduced. New methods of application, new fields of usefulness, and more frequent use by untrained individuals have increased the need for establishing standards for the measurement of the immediate and long-term hazards to the user of pesticides. Considerable concern has been expressed in government and medical circles about the expanded use of the newer pesticides without a full knowledge of their potential dangers. Consequently, it is imperative that the areas of probable health hazards be outlined and precautions broadcast relative to their use under these circumstances.

2. FOSTER DEVELOPMENT OF PROPHYLACTIC AND ANTIDOTAL MEASURES. The stimulation of laboratory and clinical research on prophylactic and treatment measures for many of the newer economic poisons is much needed at the present time. Considerable laboratory and animal work remains to be done before investigations can be undertaken. The therapeutic trials committee of the Council on Pharmacy and Chemistry of the AMA has had considerable experience in the organization of impartial clinical trials on remedial agents, and its knowledge and facilities would be offered freely in such matters. However, at the present time, the field of study is so broad that these investigations must be restricted to individual agents which show promise of wide acceptance and use.

3. STIMULATE VOLUNTARY CONTROL. As previously indicated, voluntary control is the desired method for regulating problems which have their roots in industry. It is desired that this approach be undertaken and its many possibilities exhausted before resort is made to other measures. Sincere efforts in this direction will win the cooperation and support of all other interested groups. The committee will aid and encourage this approach by bringing health problems to industry's attention and will suggest possible ways for their correction.

4. ASSIST IN STANDARDIZATION OF NOMENCLATURE. The profusion of trade names, initials, numbers, and combinations thereof for pesticides contributes to misuse and errors in the handling of these preparations. The coining of common or generic names for pesticides is a recent and commendable innovation in this field of science. It presents many problems which require the services of many groups. The many years of experience of the Council on Pharmacy and Chemistry in the development of generic names for drugs is at the disposal of the committee in its consideration of nomenclature problems.

5. ACCUMULATE AND EVALUATE NEW INFORMATION. The compilation and appraisal of new information bearing on the health aspects of pesticides will be undertaken by the committee. In this phase of its work it will keep in close contact with state and government health services in an effort to establish comprehensive records of poisonings and the circumstances of their occurrence; it will suggest means to avoid recurrence. The assembled information will be available to all who have a use for such data.

6. UNDERTAKE AN INTENSIVE EDUCATIONAL PROGRAM. A program of information on health benefits derived from the wise use of pesticides and the health hazards associated with their misuse is greatly needed. The educational facilities of the American Medical Association, consisting of ten professional journals and the lay publication, *Today's Health*, will be available to the committee. In addition, the inquiry service which is conducted by the various departments, councils, and bureaus of the American Medical Association will be coordinated in order that letters of committee interest will receive their mutual attention.

Preliminary work on several of these projects has already been in progress for over a year. These initial efforts will be expanded and intensified when the committee on pesticides becomes activated in the near future. However, pursuance of this program will require the support and cooperation of all segments of science and industry. Advisory bodies from the various interested groups will be called upon for recommendations and suggestions on problems which fall within their field. In turn, the assistance of this committee will be available, not only to the medical profession, but to all other groups in any direction in which the committee may be useful.

Determining New Insecticides in Formulations and Residues

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Three groups of synthetic organic insecticides have come into commercial use in the past few years—chlorinated hydrocarbons, phosphorus-containing compounds, and piperonyl derivatives. Methods for determining composition and purity of commercial grades have been developed, but only a few procedures are available for their detection and estimation in dusts, oil solutions, emulsion concentrates, and aerosols. When more than one insecticide is present in a mixture, even less is known concerning accurate determination of each component. More precise methods for determining their amount and possible degradation products are needed.

The ultimate usefulness in economic entomology of a compound possessing outstanding insecticidal properties can be determined only after numerous problems have been solved. These problems include determining the acute and chronic toxicity of the product to man, to cattle, and to other farm animals. The effect of the product on vegetation, soils, beneficial insects, and wildlife must also be ascertained. Solutions to these and related problems can be expedited by physical and chemical studies of the product. Methods of analysis are especially important. Not only is it necessary to have methods that will permit the analysis of the technical or commercial grade of the insecticide, but procedures should be developed for the detection and estimation of the product in dusts, wettable powders, solutions, emulsion concentrates, and aerosols. Methods are also needed for determining the product in combination with other insecticides and fungicides and in spray residues. Because some new insecticides are absorbed in animal tissues and found in dairy products, quantitative methods must be found for determining the insecticide in them.

The importance of methods of analysis for new insecticides is evidenced by the fact that during the past two years industry and government have cooperated in developing methods for two of them—tetraethyl pyrophosphate and benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) (*37*, *45*).

Among the more important synthetic organic compounds that have been developed commercially as insecticides during the past several years are certain chlorinated hydrocarbons, phosphorus-containing compounds, and piperonyl derivatives. The chlorinated hydrocarbons that have been of most practical interest are DDT, TDE (DDD), methoxychlor (methoxy analog of DDT), benzene hexachloride, chlordan, and toxaphene (chlorinated camphene). Although methoxychlor contains oxygen, it is included in this group because of its close relationship to DDT. Of the organic phosphorus-containing products, hexaethyl tetraphosphate, tetraethyl pyrophosphate, and parathion have received greatest attention. The more important piperonyl compounds are piperonyl cyclonene, piperonyl butoxide, and propyl isome.

Numerous investigators have reported the entomological results obtained with these synthetic organic compounds against many different insects, both agricultural and household. In this paper some of the analytical procedures that have been developed for these compounds and formulations of them are assembled and reviewed.

The methods of analysis for the chlorinated hydrocarbons may be divided into five classes—determination of total organic chlorine, determination of hydrolyzable or labile chlorine, colorimetric methods, physical methods, and bioassays. The last mentioned is beyond the scope of this manuscript and is not considered.

Determinations of Total Organic Chlorine

Chlorine makes up 50% or more of the weight of all the chlorinated hydrocarbons under consideration except methoxychlor, which contains 30%. Therefore, methods based on the determination of total chlorine appear appropriate. Such methods are not specific, however, and any other chlorine-containing organic compound will interfere.

In one procedure that has been widely used, the sample, after suitable treatment, is refluxed with sodium and isopropyl alcohol, after which the solution is diluted with water and the inorganic chloride is determined by standard methods (*13*, *54*). The method has been adopted by the Association of Official Agricultural Chemists (*29*, *30*) as a tentative one for technical DDT and for dusts, oil solutions, and aqueous emulsions of DDT, for use in the absence of other chlorine-containing compounds. The National Association of Insecticide and Disinfectant Manufacturers has also accepted the total-chlorine method for the analysis of these preparations (*28*). Essentially the same procedures have been described by Donovan (*22*), of the Insecticide Division of the Production and Marketing Administration, for technical DDT and various commercial DDT products containing no other compounds interfering with the chlorine determination.

Carter (*12*), of the Bureau of Entomology and Plant Quarantine, has adapted the total-chlorine method to the analysis of mixtures of DDT and benzene hexachloride in the following manner: After determination of total chlorine in the mixture, the DDT is estimated by the Schechter-Haller colorimetric method (*47*), half this value is subtracted from the total chlorine (because DDT contains 50% of chlorine), and the difference is calculated as benzene hexachloride. This procedure gives no indication of the amount of the gamma isomer of benzene hexachloride.

The total-chlorine method has been used extensively in the determination of spray residues of the chlorinated hydrocarbons (*56*). Usually the kind of insecticide applied has been known, and by means of the proper factor the chlorine values could be calculated to the insecticide originally used. This calculation is not entirely valid, as the determinations do not differentiate between the insecticide and its degradation products or other contaminants containing organic chlorine. The values obtained by the total-chlorine method are useful, however, because they indicate the magnitude of the residue and the analysis can be made in a short time with standard laboratory equipment.

A procedure that has been widely used for spray residues is the separation of the residue from the sample by extraction with an organic solvent, usually benzene. After most of the solvent has been removed, the residue is treated with sodium and isopropyl alcohol and the chloride ion is estimated by standard methods. Carter (*10*) has determined in this manner DDT residues on a number of crops, and he has recommended the adoption by the Association of Official Agricultural Chemists of the method as a tentative one for DDT (*11*). Koblitsky and Chisholm (*42*) have determined DDT in soil samples by the sodium–isopropyl alcohol procedure after removing the DDT by extraction with an azeotropic mixture of two volumes of benzene and one volume of isopropyl alcohol.

The total-chlorine method for determining residues of benzene hexachloride, chlordan, and toxaphene has also been used (*55*) in experiments where it was known that these insecticides had been applied. With benzene hexachloride, which is known to give off-flavor to some crops, it has not been demonstrated that a relation between organic chlorine values and off-flavor exists. In fact, in most cases where off-flavor was attributed to benzene hexachloride, it has not been possible to detect organically bound chlorine.

The total-chlorine method for residues of the chlorinated hydrocarbons has also been applied to animal tissues, milk, and dairy products (*9*). As in the spray-residue determinations, the method does not differentiate between the insecticide and metabolites.

Recently it was shown that when DDT, benzene hexachloride, or toxaphene is fed or applied to cattle, such organic chlorine residue as may be present in the fatty tissues consists essentially of unchanged insecticide. Carter (*12*) demonstrated their presence by separating the fats and other oxygenated products with sulfuric acid–sodium sulfate mixture and determining total chlorine. In experiments with DDT Schechter (*46*) demonstrated its presence in fatty tissue and in butterfat by the Schechter-Haller colorimetric method (*47*). The residues were then tested for toxicity to houseflies in comparison with the known insecticides of the same concentration. In both cases the known insecticide gave the same mortality as the residue.

Determinations of Hydrolyzable Chlorine

All six of the chlorinated hydrocarbons yield part of their chlorine on treatment with ethanolic alkali, but methods of analysis involving this reaction have been developed only for DDT and benzene hexachloride. This type of determination is somewhat more specific and subject to less interference than the total-chlorine method, but it must be used with discretion. When it is applied to DDT, one of the chlorine atoms is removed to form hydrogen chloride; with benzene hexachloride 3 moles of hydrogen chloride are formed. Gunther (*34*) was the first to develop a method using this principle for the determination of DDT in dust and spray residues. The original procedure has been modified by substituting 4.5 *N* ammoniacal methanol for potassium hydroxide in ethyl alcohol and carrying out the reaction at 45° C. instead of refluxing the solution (*5*). This modification has been successfully employed by Gunther (*33*) for the determination of DDT residues on many different crops.

LaClair (*44*) and Soloway *et al.* (*50*) have modified the dehydrohalogenation reaction to permit the determination of *p,p'*-DDT in dusts and oil solutions containing technical DDT. The reaction between the base and the halide is carried out at 20° to 30° C., as Cristol (*16*) has found that under proper conditions at this temperature the *p,p'*-DDT reacts completely, whereas the *o,p'*-DDT and most of the impurities react only slightly. The Association of Official Agricultural Chemists' (*3*) tentative method for the determination of the purity of *p,p'*-DDT, which employs the dehydrohalogenation procedure, has been modified by Fleck (*30*) so that the reaction is carried out at 25° C. instead of under reflux conditions.

Unlike DDT, TDE does not lose one mole of hydrogen chloride when heated with ferric chloride, but rearranges to form an isomeric compound (*31*). It may be possible to develop this observation into an analytical method to differentiate between the two products or to detect the one in the presence of the other.

The dehydrohalogenation reaction has been applied by Goldenson and Sass (*32*) to the determination of benzene hexachloride in impregnated cloth. Howard (*39*) proposed the reaction for the determination of benzene hexachloride residues in food, and Barlow (*6*) used it for benzene hexachloride in the blood of cattle.

LaClair (*43*) has employed the dehydrohalogenation reaction to determine the gamma isomer of benzene hexachloride in the technical product and in dust mixtures. Two identical samples are dissolved in 95% ethyl alcohol and treated with 1 *N* ethanolic potassium hydroxide at 0° C. for 15 and 50 minutes, respectively. The 15-minute period is sufficient to dehydrochlorinate most of the alpha and the delta isomers without appreciably affecting the gamma. In 50 minutes the gamma isomer is also dehydrochlorinated. The beta isomer does not react under these conditions, and usually the epsilon isomer is present in quantities too small to interfere seriously.

Colorimetric Methods

Methods based on color reactions have been published for several of the chlorinated hydrocarbon insecticides. Although most colorimetric methods are much more specific

than chlorine determinations, care must be exercised in their use, as analogs and closely related compounds may produce the same or similar colors. In general, colorimetric methods are used mostly for spray-residue determinations and for qualitative tests of the insecticide in various formulations.

More experimental work has been done with DDT than with all the other five chlorinated hydrocarbons combined, probably because DDT was the first of the group found to have insecticidal value. Carter (*10*) has summarized the several colorimetric methods for DDT. The one proposed by Stiff and Castillo (*51*), as modified by Claborn (*14*), and the one by Schechter and Haller (*47*) have probably been most widely used. In the Stiff and Castillo method, when the DDT is heated in pyridine solution containing xanthydrol and potassium hydroxide, a red color develops which is proportional to the quantity of DDT present. The reaction is sensitive to 10 micrograms. As TDE does not give a color with this reagent, Claborn (*14*) has proposed the reaction for the determination of DDT in the presence of TDE. He has also shown that for the development of the color the amount of water in the pyridine is critical.

The colorimetric method for DDT developed by Schechter *et al.* (*49*) is based on intensive nitration of DDT with the formation of a tetranitro derivative. The product obtained with the *p,p'* isomer gives an intense blue color upon addition of sodium methylate, whereas the *o,p'* derivative produces a violet-red color. As the method permits the estimation of as little as 10 micrograms and none of the degradation or metabolic products of DDT interfere, it is especially useful for work on spray residues and biological specimens. Modifications of the method that are suitable for determining DDT in fatty materials such as milk, butter, meat, and eggs have been described by Schechter *et al.* (*48*) and Clifford (*15*). Tressler (*53*) has adapted it for determining DDT or its residues in canned foods. The nitration method is more specific for DDT than any other proposed. With the exception of TDE, which gives an almost identical color, none of the other chlorinated hydrocarbons interferes.

Recently a colorimetric test for methoxychlor residues was proposed by Fairing (*27*). The methoxychlor sample is treated with alcoholic potassium hydroxide, the reaction product is extracted with ether, the ether is removed, and the residue is treated with concentrated sulfuric acid. An intense cherry-red color is developed. No other insecticide has been found to interfere, and the reaction is sensitive to about 5 micrograms of methoxychlor.

Satisfactory colorimetric methods for benzene hexachloride, chlordan, and toxaphene are not available and are urgently needed.

Davidow (*19*), of the Food and Drug Administration, has described a colorimetric method applicable to technical chlordan. The method is based on the observation that when technical chlordan is heated with a mixture of diethanolamine and methanolic potassium hydroxide, a purple color is produced. When known amounts of this insecticide were added to cabbage, pears, and fresh and rancid rat fat, recoveries of 74 to 104% of the insecticide were obtained. However, because two crystalline isomers of chlordan isolated from the technical product do not give a colored reaction product with the reagent, further investigation of the method is being made. The red color obtained when technical chlordan is heated with pyridine, alcoholic alkali, and ethylene glycol monoethyl ether, as described by Ard (*2*), likewise fails with the crystalline isomers of this insecticide.

A method that is stated to be applicable to residues of benzene hexachloride (*20*) is based on the fact that benzene hexachloride yields essentially 1,2,4-trichlorobenzene on dehydrohalogenation with alkali. This product possesses a characteristic absorption band in the ultraviolet, which permits its quantitative determination.

Physicochemical Methods

Methods utilizing characteristic physical properties have been developed for several chlorinated hydrocarbon insecticides. Daasch (*18*) has used infrared spectroscopy for the analysis of benzene hexachloride. By this means it is possible to determine the gamma-isomer content, as well as that of the other isomers of technical benzene hexachloride, provided the product is substantially free of the higher chlorinated cyclohexanes.

The gamma isomer of benzene hexachloride can also be determined by polarography (*24*, *40*). The method is based on the fact that, under the conditions used, the gamma isomer is the only one of the five isomers that is reduced at the dropping mercury electrode.

Both the infrared spectroscopic method and the polarographic method require special instruments. When instruments for both are available, the latter method seems to be preferred. Neither method has been found to be applicable to spray residues.

Physical methods based on absorption spectra in the infrared or ultraviolet have also been suggested for determining DDT. The method proposed by Herriott (*38*) for the *p,p'* isomer is based on the fact that in ethyl alcohol solutions it absorbs ultraviolet light very slightly at a wave length of 250 mμ. After dehydrochlorination by dilute alcoholic sodium hydroxide the reaction product absorbs strongly at this wave length. The increase in absorption is measured and compared with the value obtained with a similarly treated standard solution. Downing *et al.* (*23*) have shown that infrared spectroscopy can be used for the chemical characterization of technical DDT, including detection of the several isomers and impurities, and the quantitative estimation of DDT content. Neither method appears to have had widespread application.

A method for determining the gamma isomer of benzene hexachloride by partition chromatography has been developed by Aepli *et al.* (*1*). Nitromethane and *n*-hexane are used as the partition solvents, and silicic acid is the supporting medium. The method appears to be useful for routine product analyses. An accuracy of about 2% of the actual gamma isomer present is claimed.

A cryoscopic method for determining the gamma isomer of benzene hexachloride, developed by Bowen and Pogorelskin (*8*), is based on the fact that the freezing point of a compound is lowered by the presence of dissolved impurities. The method is useful for benzene hexachloride preparations of higher gamma-isomer content than the usual technical grade containing 10 to 12% of the gamma isomer. The method is rapid and requires only simple, readily available equipment.

Cristol *et al.* (*17*) have based a method for the determination of *p,p'*-DDT in technical DDT on the fact that the *p,p'* isomer is almost insoluble in 70% aqueous ethyl alcohol and the *o,p'* isomer is soluble. The method has not been tried with mixtures of the other chlorinated hydrocarbons.

A mass-isotope dilution method for determining the gamma isomer of benzene hexachloride, in which gamma-hexadeuterobenzene hexachloride is used as a tracer molecule and the dilution is determined by use of infrared spectrophotometry, has been developed by Trenner *et al.* (*52*). Impurities have no effect on the accuracy of this method.

Analysis of Organic Phosphorus Compounds

Of the three organic phosphorus insecticides—hexaethyl tetraphosphate, tetraethyl pyrophosphate, and parathion—the first two have been shown to be mixtures (*36*) that contain tetraethyl pyrophosphate as the principal active ingredient. Several methods have been proposed for the determination of this compound in the commercial products (*25*, *35*). All are based on the separation of the tetraethyl pyrophosphate from the related ethyl phosphates, followed by its hydrolysis to diethyl orthophosphoric acid and titration with standard alkali. Both hexaethyl tetraphosphate and tetraethyl pyrophosphate are soluble in water and are rapidly hydrolyzed to monoethyl and diethyl orthophosphoric acid. This rapid hydrolysis to nontoxic products greatly limits the duration of the insecticidal effectiveness of tetraethyl pyrophosphate, but it also eliminates the danger of toxic residues on the crops treated.

The only method that has been described for the assay of technical grades of parathion and its formulations is that of Bowen and Edwards (*7*). The method makes use of the polarograph. The electrolysis is carried out in an acetone-water solution with potassium chloride as the electrolyte and gelatin as the suppressor. An accuracy of ±1% is obtained.

For spray residues of parathion Averell and Norris (*4*) have developed a method that is sensitive to about 20 micrograms. The method is based on the reduction of the nitro

group to the amine, diazotization, and coupling with *N*-(1-naphthyl)ethylenediamine, which gives an intense magenta color with an absorption peak at 555 mμ. Edwards (*26*) has found that commercial samples of benzene, which is commonly used for stripping residues from plant material, give an identical color by the method. The interfering substance can be removed by distillation of the benzene. The oxygen analog of parathion, diethyl *p*-nitrophenyl phosphate, gives the same color as parathion by the method. This oxygen analog is stated to be much more toxic to warm-blooded animals than parathion, and it has been suggested that parathion is converted to the oxygen analog on exposure to the air. However, no definite evidence has been adduced that this compound accompanies spray residues of parathion, and laboratory experiments have failed to confirm the reaction.

Analysis of Piperonyl Compounds

Of the three piperonyl compounds that have received considerable commercial attention as insecticides, a method of analysis is available only for piperonyl butoxide (*41*). This product gives a blue color on treatment with a reagent comprising tannic acid in a mixture of phosphoric and acetic acids. Satisfactory results can be obtained in the presence of small amounts of pyrethrins, but larger amounts tend to obscure the color. A modification of the method (*21*) which overcomes this difficulty is the removal of the pyrethrins by saponification with alcoholic sodium hydroxide prior to carrying out the test.

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Mass-Production Techniques for Estimation of Parathion Residues

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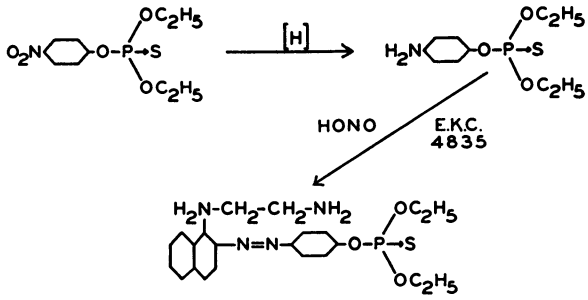
The magenta color reaction for parathion has been adapted to mass-production techniques for quantitative estimation of parathion residues in and on certain fruits, vegetables, and miscellaneous substrates.

Experimental batches of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate) were made available to many entomological research centers in early 1947 (through the courtesy of the American Cyanamid Company), for evaluation as to insecticidal potential in an integrated program of testing against many species of insects and mites. Requisites of entomological practice in the field include companion chemical estimations of deposits afforded by the various dosages, adjuvant mixtures, and additive combinations. Details of a very sensitive method for the chemical estimation of parathion, as developed by Averell and Norris (*1*), were made available at the same time. The presumed gross chemistry of the analytical procedure is shown in Figure 1.



Brilliant magenta dye

Figure 1. Presumed Chemistry of Color Reaction for Parathion

E.K.C. 4835 is *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Recent chromatographic evidence indicates that the final colored reaction product from technical grade parathion may be a mixture of at least three and possibly four components.

The resultant magenta color is remarkably intense per unit of parathion; this, and other characteristics, indicated the ready adaptability of this method to the quantitative estimation of parathion in and on fruits, vegetables, foliage, and woody tissues, and in soil. The present paper reports upon endeavors to define or elaborate upon the parameters

restricting the applications of this method, and upon certain refinements pertinent to mass production techniques for parathion estimations, as based upon 2 years' work and nearly 3000 estimations upon approximately 50 different substrates. These techniques are designed for the efficient handling of large numbers of analyses involving a variety of substrates.

Analytical Procedures

From 8 to 30 pounds [or 120 leaves, if foliage (*4*)] of the field sample are used; penetration samples are dissected or otherwise suitably treated so as to afford several pounds of the desired anatomical portion of the field sample. [The size of the field sample and its method of collection vary tremendously for different materials—e.g., olives *vs.* alfalfa hay. These two parameters are under statistical investigation, but the paucity of reliable and sufficiently extensive data precludes formal publication of field sampling and processing techniques for materials other than leaves (*4*). A manual of processing procedure (*5*) has been mimeographed for guidance of personnel, based upon empirical observations; as data are accumulated, it will be revised and expanded periodically.]

After being coarsely chopped or shredded, the material so obtained is sampled by quarters to afford 454 grams of analytical sample. This pound of sample is placed in a special stainless steel container with 908 ml. of technical benzene and 25 ml. of 10% hydrochloric acid solution, then minced thoroughly with a high-speed cutter on a drill press, as previously described (*3*). After being capped tightly with No. 300 M.S.T. cellophane un-

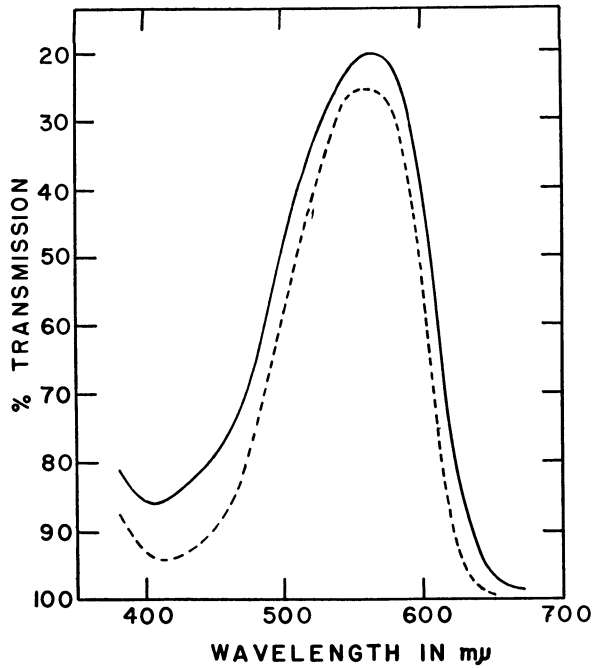


Figure 2. Dyed Parathion and Dyed Aniline

—— Parathion, $\lambda_{max.}$ 560 mμ, $\epsilon_{max.}$ 39,091
– – – Aniline, $\lambda_{max.}$ 560 mμ, $\epsilon_{max.}$ 40,859
In 60% ethyl alcohol, pH 1.2

der a cork gasket (*3*), the stainless steel can is tumbled end over end at 58 r.p.m. for 30 minutes. ["Strip" samples are tumbled from 2 to 30 minutes, depending upon their nature; leaf and certain other penetration samples may be tumbled for as long as 4 hours (see *5* for details).] From 200 to 300 ml. of the resulting benzene extract are then col-

lected by decantation through Sharkskin filter paper, and stored in a tightly closed 500-ml. bottle at 3° C. for subsequent estimation of parathion content.

Within 48 hours, the sample is analyzed. To the bottle, at room temperature, are added 20 grams of an intimately ground 2 to 1 mixture of clay (Attaclay, available from the Attapulgus Clay Company, Philadelphia, Pa.) and Hyflo Super-Cel, with shaking. After the resulting suspension has settled, an aliquot (usually 100 ml. This volume will vary with the amount of parathion present in the original sample, and must be predetermined for each sample type and field treatment.) of the clear supernate is pipetted into a 500-ml. bottle, and evaporated quickly to a moist residue at 80° to 90° C. with the aid of a gentle current of impinging air.

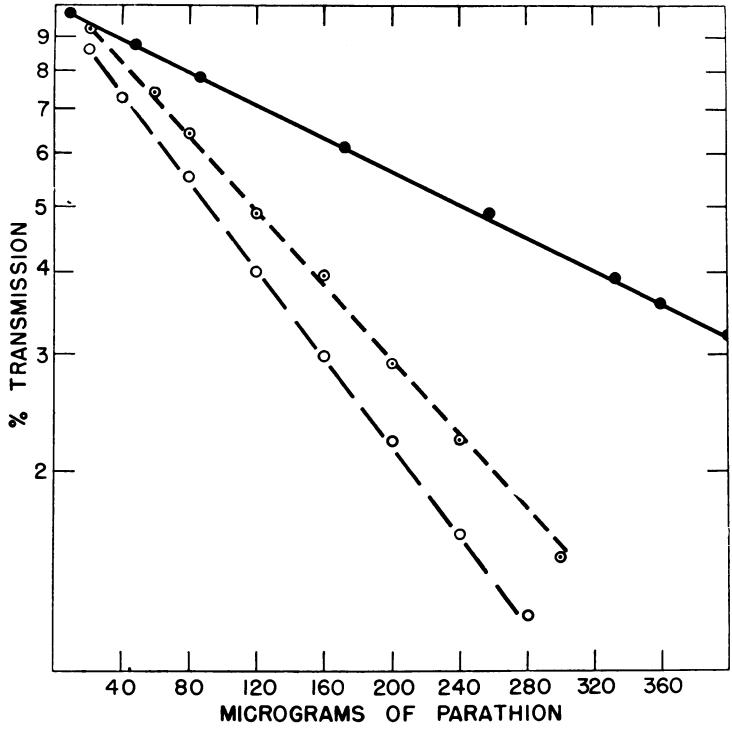


Figure 3. Standard Curves for Dyed Parathion

— — In 20% ethyl alcohol (1)
——— In 60% ethyl alcohol
– – – In acetone

For 20% ethyl alcohol, $E = -304.2 \times +607.6$; for 60% ethyl alcohol, $E = -824.3 \times +1647.2$; for acetone, $E = -350.7 \times +715.5$. Ordinate is % transmittance $\times 10^{-1}$

To the bottle are then added 10 ml. of 95% ethyl alcohol, 10 ml. of water, 2 ml. of 5 *N* hydrochloric acid solution, and approximately 0.5 gram of powdered zinc, and the resulting mixture is maintained at 80° to 90° C. for 10 minutes. This reduction mixture is filtered at once through Sharkskin paper into a 100-ml. volumetric flask, and the filter cake is washed with three 7-ml. portions of water. Exactly 1 ml. of 0.25% sodium nitrite solution is then added to the combined filtrates with shaking, followed after exactly 10 minutes by 1.0 ml. of 2.5% ammonium sulfamate solution with shaking. After another 10-minute period 2.0 ml. of a 1% solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Eastman Kodak Company, No. 4835) are added, with shaking. The magenta color indicative of parathion develops at this stage; after a 10-minute development 50 ml. of 95% ethyl alcohol and 2 ml. of 3 *N* hydrochloric acid solution are added, and the mixture is adjusted to volume with water. If cloudy, the final mixture is extracted in the volumetric flask with two 5-ml. portions of petroleum ether (30° to 60° or 60° to 70° C.).

The final optically clear solution is evaluated as to intensity of color at 555 mμ, and with slit width at 0.02 to 0.04 mm., on a Beckman spectrophotometer adjusted to 100% transmittance with an untreated sample freshly processed in parallel with the unknown sample. From a standard calibration curve (see Figure 3), the concentration of parathion may readily be ascertained, and an appropriate factor converts this value to micrograms of parathion present in the original field sample.

Calibration

The original procedure of Averell and Norris (*1*) may be followed, using either parathion distilled under high vacuum or reduced parathion repeatedly recrystallized from dilute hydrochloric acid solution. The use of ordinary technical grade parathion is not recommended for precise comparison purposes, because it has been the authors' experience that batch and manufacturer variation in both quality and composition of product may be considerable. High-vacuum distillation of parathion requires equipment not always available, and the securing of sufficiently pure crystalline reduced parathion, without seed material, is likely to be a time-consuming and difficult process. For precise calibration and the preparation of a standard curve, therefore, carefully purified aniline or aniline hydrochloride may be used, inasmuch as the visible absorption spectrum obtained thereby (after dyeing) is spectrophotometrically indistinguishable from that of reduced parathion (after dyeing), as shown in Figure 2.

The resultant magenta color attains 87.50% of its maximum intensity at 555 mμ within 6 minutes at room temperature, 92.61% within 9 minutes, 96.03% within 15 minutes, and 99.43% within 30 minutes. At 50% transmittance the ratio of purified aniline to technical grade parathion is 0.958; as the purity of the parathion is increased this ratio approaches unity. Thus, the determination of this ratio could conceivably be used as a criterion of purity for technical grade parathion.

Typical standard curves for a technical grade of parathion are shown in Figure 3. Beer's law is followed within the range 10 to 400 micrograms of parathion per milliliter of dyed solution. Deviation becomes apparent outside this range, under the conditions of test.

Table I. Typical Blank Values of Various Fruits and Vegetables

(Parts per million of parathion)

Apples, Rome Beauty	Surface	0.00
	Pulp	0.01
Beans, string	Total	0.01
Cabbage	Total	0.20
Carrots	Tubers	0.04
	Foliage	0.00
Celery	Total	0.12
Corn, sweet	Kernels	0.04
	Husks	0.02
Grapes, seedless (Thompson)	Total	0.03
Hay, alfalfa	Air dried	0.00
Lemons, Eureka	Peel	0.00
	Pulp	0.00
Olives, Mission	Seeded	0.06
Oranges, navel	Peel	0.10
	Pulp	0.00
	Processed peel (stock feed)	0.50
	Orange oil	2.50
	Twigs	0.09
Oranges, Valencia	Peel	0.35
	Pulp	0.07
	Processed peel (stock feed)	0.30
	Orange oil	1.60
Strawberries	Fruits plus caps	0.10
Walnuts (unhusked)	Surface	0.10

For consistent, comparable results it is essential that a fresh sample blank be processed and otherwise be treated identically with each current batch of field samples. Whenever possible, this blank specimen should be from the same locality and should have the same previous spray (or other) history as the actual samples. Frequently, fruit and vegetable parts possess benzene-extractable pigments or other substances not removable by the decolorizing treatment utilized. In order to eliminate such background interfer-

ences, the dyed solution from the sample blank is used to preset the spectrophotometer at 100% transmittance immediately prior to running the actual samples corresponding to that blank. When the field sample is sufficiently large, there is ordinarily little if any difference between the colors at 555 mμ developed or present in blank samples from the same locality; blank samples from different localities may, however, exhibit demonstrably different absorption characteristics at this wave length when processed as described. In Table I are listed some typical blank values (sample blank transmittance corrected for reagent blank transmittance at 555 mμ, converted to parts per million of parathion).

Preparation of Samples

As pointed out by Averell and Norris (*1*), this colorimetric procedure for estimating parathion is extremely sensitive—for example, as little as 2 to 3 micrograms of parathion per sample will afford a visible color, and the presence of as little as 0.2 microgram of parathion per sample is spectrophotometrically demonstrable. It thus becomes essential to avoid contamination of the sample at every step. Extreme measures are required to accomplish this end, for on exploratory material a negative or borderline sample could easily be trace-contaminated so as to appear to contain misleading quantities of parathion. The following aids are recommended in eliminating contamination:

Scrub all utensils, containers, working surfaces, and equipment with warm 10% trisodium phosphate solution after each sample. Wherever possible, rinse each utensil and piece of equipment with acetone and benzene. Because parathion residues adhere so tenaciously to glass surfaces, subject all glassware to the following sequence of washes: warm 10% trisodium phosphate solution, distilled water, acetone, and benzene. Rinse stored glassware with benzene again just prior to use.

Never bring containers of parathion or parathion dusts into the room where these preparations or analyses are being performed. If parathion-containing extracts are spilled, hose down the floor at once.

Cover all table areas and other working surfaces with wrapping paper or other disposable material; replace after processing each sample.

Keep blank field samples separate from treated samples, both when in transit from the field and when in refrigerated storage awaiting processing.

Separate component parts of some field samples in the field—e.g., carrots from carrot tops —if they are to be analyzed separately for parathion content. As an illustration of this point, airplane-dusted carrots were put through a commercial washer and divided into two lots. One lot was topped and stored 4 days in a paper bag; the untopped lot was treated similarly but topped during the normal processing. The tops analyzed 1.2 p.p.m. of parathion. The field-topped carrots analyzed nil parathion, whereas the lot untopped during storage analyzed 0.2 p.p.m. of parathion.

Exercise scrupulous care during dissection of the samples to avoid even trace carry-in contamination. Specific techniques must be evolved and verified for each type of fruit or vegetable [see Carman *et al.* (*2*) and Gunther (*5*)]. To illustrate, the following procedure has been found satisfactory for apple penetrations. A clean, sharp knife is forced axially one third through the apple, and the fruit is cracked open by twisting the knife. That portion of the apple pulp untouched by the knife is scraped out with the aid of a sharpened melon baller. With care, the flesh can be scraped gently from areas inside the skin so as to leave ultimately an intact paper-thin translucent shell of skin freed from adhering pulp. Accidental puncture of the skin with the scraping tool means discarding the remainder of that apple and recleansing the tool.

Unless proved otherwise, even refrigerated (3° C.) storage of field samples may not lessen the disappearance (migration, degradation, metabolization, or other routes) of parathion contained in certain tissues—for example, frequently 10 to 20% losses of parathion content have been experienced after storage at 3° C. for 5 days. Expeditious processing of all samples is thereby indicated.

Size of Subsample. Although it is experimentally obvious that, with many fruits and vegetables, an arbitrary 8- to 30-pound field sample is hardly adequate for true representation of a field plot, practical considerations in large scale field operations delimit the size of the sample.

Exploratory studies indicate that 64 mature Valencia oranges per sample, when picked in a consistent manner with 16 fruits from each of 4 randomized normal trees, suffice to give fair (10%) agreement between replicates; such a sample ordinarily weighs from 18 to 24 pounds. Very cursory tests with Thompson seedless grapes demonstrate

several hundred per cent variation between the deposits on 5-pound replicated samples of small bunches selected at random throughout the treated vineyard.

Nonetheless, when such a field sample is suitably subdivided into pooled component parts and subsampled by quarters into pound portions, there will in general be excellent analytical agreement between the pound subsamples or "aliquots." Subsamples of less than a pound may contain insufficient parathion to afford readily obtained aliquots of extract containing amounts of the insecticide optimum to the accuracy of the present method. Compounded dusts and other concentrated forms, of course, require less material but also extraordinarily painstaking sampling and subsampling techniques so as to maintain the bulk homogeneity originally present. The inhomogeneity of aged insecticide-containing dust mixtures will be dealt with in a subsequent report.

Choice of Solvent. As indicated by Averell and Norris (*1*), and independently confirmed by the authors, technical benzene is a superior stripping solvent for parathion residues. It is almost completely miscible with technical grade parathion at room temperatures, it is universally available and low in cost, it is readily volatile, it fails to contribute to storage decomposition (*6*), it is a good solvent for plant oils and waxes, and it is immiscible with water. On the other hand, benzene is highly flammable and its vapors are very toxic to human beings, especially as a chronic toxicant even in small doses.

Because of these hazardous features, certain commonly used solvents other than benzene were investigated for this purpose.

Solubility tests with purified parathion indicated that it is at least 30 volume % soluble in technical grade acetone, benzene, carbon tetrachloride, dioxane, ethyl acetate, and ethyl alcohol. Cost and other considerations (*1*) limited the field to acetone, benzene, and dioxane. Residues remaining from the evaporation of dioxane interfered with the development of the desired deep magenta color, affording instead a pale yellow-orange. When 50- to 200-ml. volumes of the other two solvents containing from 76 to 279 micrograms of technical grade parathion at five levels were evaporated and run through the analytical procedure, benzene afforded slightly more reliable results with a recovery range of 82 to 96% (average 89%), whereas the recoveries from acetone were within the range 76 to 95% (average 86%). In both sets of data, as the quantity of parathion increased from 76 to 279 micrograms—regardless of volume of solvent—the analytical recovery decreased in an irregular manner.

These facts suggest that variable recoveries of parathion from the evaporation procedure, as used routinely, should be expected. In general, however, the recovery data did not demonstrate a clear-cut distinction between acetone and benzene for the purpose at hand. A slow steady loss of parathion in proportion to the volume of either solvent evaporated (*1*) was not noted, which would indicate that the rate of evaporation is also important. The final decision as to solvent was determined by certain incidental properties of the parathion.

Wet Extraction Techniques. It may readily be demonstrated that suitably dispersed parathion is short-lived. For example, a thin film of a 25% wettable powder exposed in a laboratory environment may show little or no parathion, by analysis, after 14 days (*7*); ground citrus peel containing 12 p.p.m. of field-applied parathion will analyze considerably less insecticide after being dried to constant weight at 65° C. in a convection oven; and there is marked dissipation of parathion from treated fruits even at 3° C. Such observations indicated that the drying and extraction techniques (*4*) previously used for residue and penetration studies could not be employed with parathion. The wet extraction techniques herein described were therefore devised to circumvent drying operations. Acetone as the solvent was thereby eliminated from consideration because most of the contemplated field samples were high in water content and would thus have required excessive quantities of solvent for adequate extraction—e.g., orange juice, plums, ripe peaches, etc.

As a rule of thumb, 2 ml. of benzene per gram of analytical sample serve to afford adequate extraction with minimum emulsification difficulties, so that the addition of emulsion breakers is usually unnecessary in order to obtain sufficient clear extract for analysis. Experiences have indicated that occasionally less benzene will suffice; on the

other hand, Thompson seedless grapes and ripe freestone peaches require 4 ml. per gram. The optimum volume should be determined for each type of material (*5*).

Purifying the Benzene. Occasionally a drum of technical grade benzene is encountered, the contents of which will develop a pronounced pink color when subjected to the analytical procedure. A typical transmittance–wave-length curve from a 250-ml. specimen of such processed benzene is shown in Figure 4. By comparison with

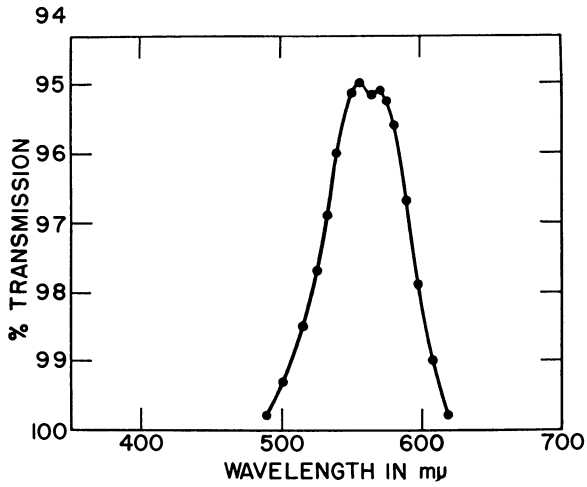


Figure 4. Typical Transmittance–Wave-Length Curve for Processed Unknown Constituent of Technical Grade Benzene

Figure 2, it will be seen that the major maxima coincide at 550 to 560 mμ. The nature of this interfering contaminant is not known, but it is readily extractable into aqueous hydrochloric acid (*7*). To eliminate the tedium of testing and tracing every lot of benzene, 25 ml. of 10% hydrochloric acid solution are routinely added to the sample just prior to mincing. This procedure has effectively removed such solvent interference from consideration.

Table II. Interference of Liner Materials with Analysis

Material	Indicated Micrograms of Parathion
Stock solution	77.2
Cellophane	77.2
Lucite	Dissolved (no color)
Neoprene	240.5
Nylon	30.1
Oilcloth, white	No color
Rubber	No color
Velon	50.0
Vinyl acetate (polymer)	66.5
Waxed paper	Disintegrated (no color)

Subdividing the Sample. Wet processing requires unusually thorough subdivision of the sample, to permit efficient extraction by a water-immiscible solvent. The machine developed for this purpose has been described (*3*); its design was based upon that of the familiar and efficient Waring Blendor. Originally, samples were minced in added water, but emulsions difficult to break were frequently formed during the subsequent stripping process, whereas if the sample were minced directly in benzene as the fluid medium these emulsification phenomena were minimized. Adequate mincing in benzene is a hazardous operation and should not be entrusted to untrained personnel. Forced ventilation of the entire mincing area is essential; benzene vapors are both toxic and flammable.

Container Closure. The stainless steel containers (*3*) of material minced in ben-

zene are capped with a double thickness of No. 300 M.S.T. cellophane topped with a buffer gasket of 0.125-inch cork sheeting, and then the steel lid is bolted on. Many types of liner material were tested. Cork liners were unsatisfactory because of contamination, and metal foils were too expensive for large-scale operations. A number of materials were evaluated by being immersed in 100 ml. of a stock solution of parathion in benzene for 1 hour, with subsequent analysis of the resulting benzene extracts. Some of these materials, and the analytical results obtained, are presented in Table II.

The No. 300 M.S.T. cellophane is sufficiently thick to resist reasonable tearing and is waterproof; each cellophane liner is used only once, then discarded. The authors have found it convenient to purchase this cellophane in precut 6 × 12 inch sheets in lots of 10,000 (available from the Zellerbach Paper Company, Los Angeles, Calif.).

Down-Draft Hood. To protect personnel from benzene vapors, it is recommended that all the pouring, container closing, and final decantation and bottling operations be performed on an efficient down-draft hood (*4*). To increase efficiency in inserting and removing the bolts on the steel cans, a reversible air-driven screwdriver has been suspended on a counterweight over the down-draft hood.

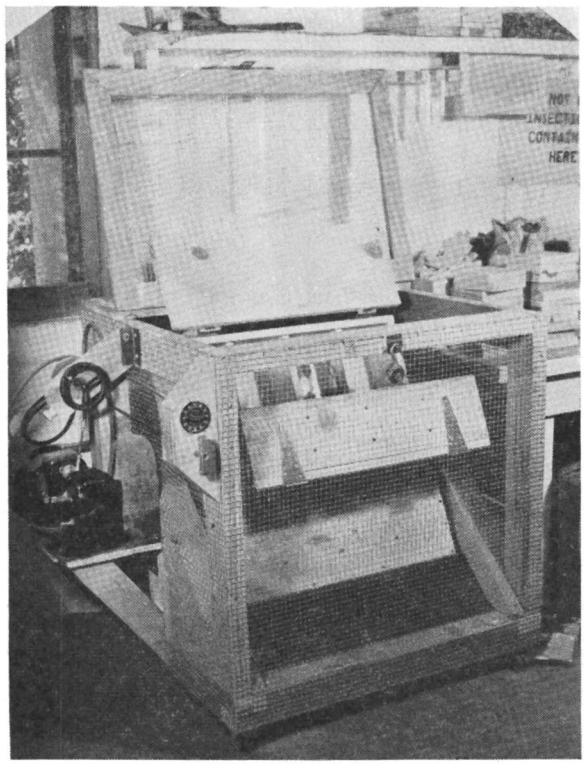


Figure 5. Drum-Type Stripping Machine with Automatic Controls

Automatic Stripper. A photograph of the drum-type stripper is shown in Figure 5.

Each of the four vanes of the drum contains four compartments, 6 × 6 × 10 inches in inside dimensions. One of the steel cans fits snugly into such a compartment; the wooden blocks stacked to the right of the apparatus in the photograph are used to wedge 2-quart Mason jars (for leaf stripping) into the compartments. Each vane lid, opening to the front, is fastened shut by two suitcase catches. The drum itself is fastened by means of welded collars to a 1-inch steel shaft suspended on 4-inch bronze sleeve bearings. This shaft is coupled to a heavy-duty, 30-to-1 gear reducer, which in turn is driven through a

coupling by a 0.5-hp., 1725 r.p.m., explosionproof motor. Thus, the drum revolves at 58 r.p.m., a speed sufficient to induce a violent surge effect within the stripping container. Accessories include a remote-control, explosionproof switch, and a built-in electric time clock. This last feature is very convenient, for with it the operator can set the stripper to run any predetermined length of time without attention. The pulley system visible above the driving mechanism allows the operator to hand-position the drum for loading and unloading the vanes.

Efficiency of Stripper. There are extant nearly as many stripping devices or manual stripping procedures as there are entomological laboratories. Regardless of the means whereby residue stripping is achieved, consistent and assiduous application of any one technique by one group of workers will presumably lead to comparable analytical results within that laboratory. However, this constancy will not hold when the attempt is made to compare analytical results originating in different laboratories where different techniques are employed, because the efficiency of the stripping operation will delimit the ultimate analytical value even though the analytical techniques themselves are standard. If rigid comparisons of data from various groups are to be valid, therefore, stripping techniques must be standardized.

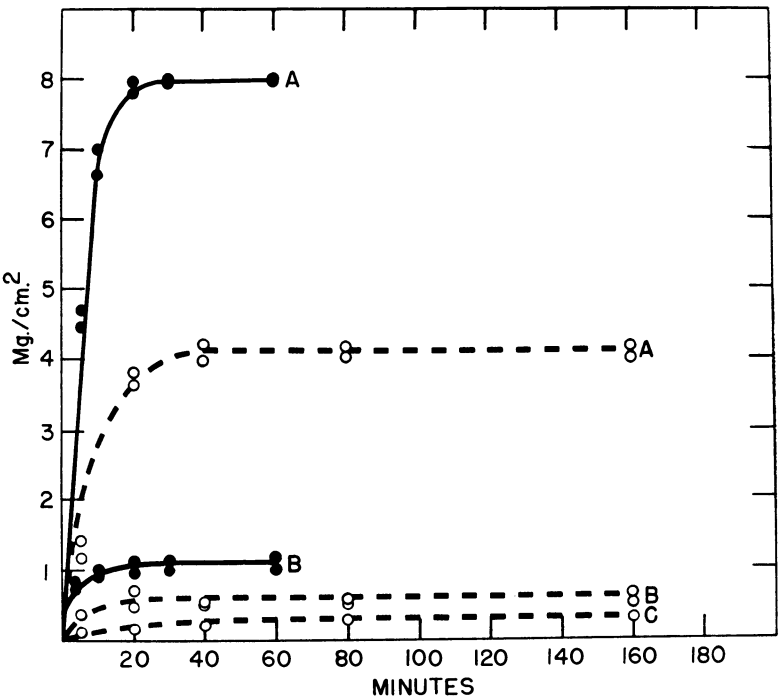


Figure 6. Time-Efficiency of Stripping Apparatus in Extracting Parathion from Orange Leaves

——— After 7 days; – – – After 11 days
A. Original strip; B. First restrip; C. Second restrip

Adequate comparisons of the efficiencies of the many types of stripping devices in existence have never been made; actually, such comparisons are not necessary, nor is it necessary to consider the purchase or construction by each group of a standardized piece of stripping equipment. Rather, it is proposed that the efficiency of each such apparatus be determined as the time of stripping required to attain equilibrium of concentration of the insecticide between extracting solvent and substrate—i.e., when the plot of quantity of insecticide in solvent *vs.* time levels off. Then, if all stripping operations in a given ap-

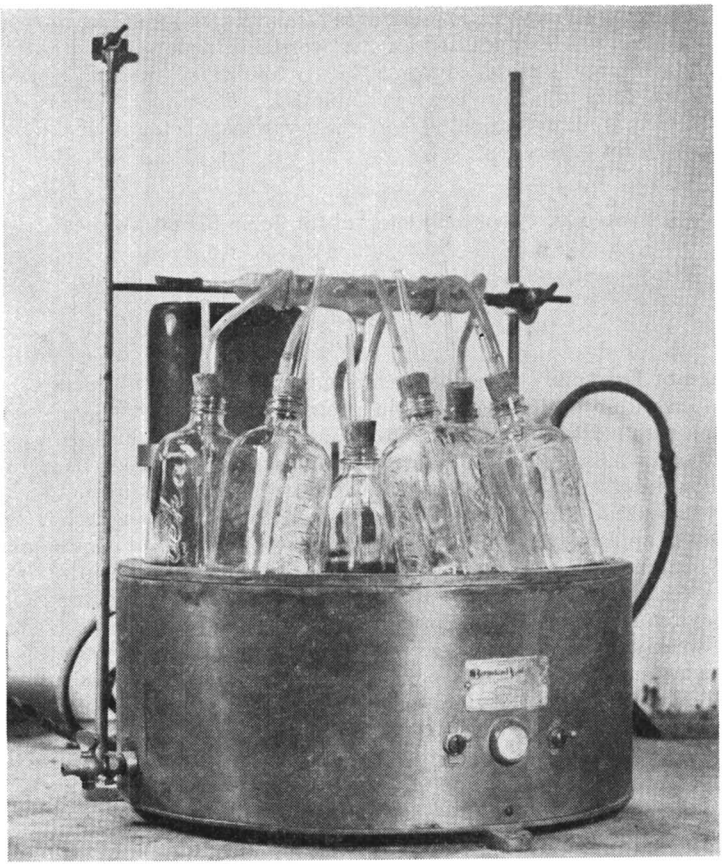


Figure 7. Apparatus for Evaporation and Reduction Steps

paratus were extended to twice this minimum time, all samples in all apparatus or procedures would be positively overstripped and thus in comparable condition. This statement is based upon the fact that the ratio of solvent to solute in question is ordinarily so large (of the order of 1,000,000 to 1) that an eventual equilibrium is unavoidable.

To this end, the authors have determined the time-efficiency of their apparatus, as demonstrated by the data plots in Figure 6.

The substrate was Valencia orange leaves, with 2500 leaves per sample selected in a carefully prescribed manner (*4*). The trees involved were field sprayed in a conventional manner with 4 pounds of a 25% wettable powder of parathion, then sampled after 7 days and again after 11 days. Each sample was mixed thoroughly and subsampled into 125-leaf units in 2-quart Mason jars. To all units were added 250 ml. of benzene each, and they were sealed, stripped for various lengths of time, then restripped with fresh benzene, again for various lengths of time. The strip solutions were analyzed in the usual manner.

From Figure 6 it is apparent that the stripping apparatus approaches the equilibrium distribution within 15 to 20 minutes; therefore in accordance with their proposed standardization, the authors utilize 30- to 40-minute stripping intervals in routine practice. It is further demonstrated in this figure that one strip per leaf sample is adequate for routine purposes, with the initial equilibrium at approximately 80% extraction.

These curves also demonstrate the incidental 50% loss of parathion after the additional 4 days in the field for the second sample.

Similar data have been secured for the other plant parts studied to date. Minced

citrus peel, for example, requires 30 minutes to attain the 80% equilibrium condition and, therefore, 1 hour is routinely utilized for the overstrip standardization. Residues on 2-pound analytical samples of whole Rome Beauty apples are apparently extracted well within a 30-minute interval, as shown in Table III. These four samples were stripped separately, to afford an indication of the typical variation between field deposits, under the conditions of the experiment.

Table III. Stripping Interval for Rome Beauty Apples

	Parathion, P.P.M., after	
Sample No.	30 min.	180 min.
B-196	7.1	7.7
B-192	13.6	13.0

Storage of Extracts. After being filtered, the decanted extracts are stored, in bottles capped with waxed paper liners, in a refrigerator set for 3° C. Even at this low temperature, with most of the benzene frozen, a loss of the contained parathion must be assumed to occur unless proved otherwise Pure parathion in benzene solution can be stored without loss almost indefinitely in the dark at this temperature, but technical grade parathion in a benzene extract of plant material may behave differently. For example, benzene extracts of apple pulp (total) have lost 30% of their contained parathion in 5 days at 3° C., and benzene solutions of orange oil have exhibited 20% losses in 10 days. On the other hand, certain leaf extracts are apparently stable at this temperature. Room-temperature environments for any but pure benzene solutions are not to be recommended. Because of weekly surges of sample receipts, it is frequently necessary to store large numbers of samples and sample extracts for short periods of time. Under such circumstances, mechanical failure of the refrigerators could be serious. The authors have therefore fitted their refrigerators with red warning lights and thermostats set to turn the lights on at 10° C.

Because of the unknown nature of this possible disappearance of parathion during refrigerated storage, it is strongly recommended that all extracts be analyzed within 48 hours after preparation.

Analysis of Samples

The actual analytical procedure is essentially that of Averell and Norris (*1*), the major modifications being the addition of steps to eliminate the interfering amounts of proteinaceous and waxy or oily extractants resulting from general utilization of larger samples. Some minor modifications have necessarily been introduced in adapting their procedure to mass-production efficiency. To preserve the continuity of the present report, however, the detailed analytical method has been presented in its entirety.

Decolorization. This procedure, adapted from (*1*), will remove most green and some orange-to-red substances from the benzene solutions, but will not remove appreciable parathion in the concentrations commonly encountered.

Evaporation of Samples. Six samples of usually 250 ml. each are simultaneously evaporated to a moist residue in a water bath, shown in Figure 7, which is maintained at 80° to 90° C. The exhaust manifold is connected with the house vacuum. With the assemblies shown a gentle stream of air is drawn over the surface of the evaporating solution and out the vacuum line; the air inlet tube is lowered frequently so as to maintain a rippled surface on the benzene extract. In this manner, 250 ml. of benzene can be evaporated completely in 10 minutes or less.

Although Averell and Norris (*1*) suggest that the final evaporation of the last 10 ml. be accomplished with the aid of a jet of air at room temperature, so as to avoid excessive losses of parathion, the apparatus shown in Figure 7 accomplishes the final evaporation without such "excessive" losses. This conclusion is supported by the data in Table IV, with losses of about 20% in all cases. Data for both acetone and benzene are again included to re-emphasize the interchangeability of the two solvents for this utility. The two

volumes of solvent were chosen to represent the extreme range most frequently encountered in these laboratories.

Table IV. Evaporation Losses of Parathion with Water Bath at 80° to 90° C. vs. Air Jet at Room Temperature

Parathion, Micrograms	Solvent	Solvent, Ml.	How Evaporated	% Loss
112.1	Acetone	1.0	Air jet	21.9
112.1	Acetone	1.0	Air jet	23.9
106.0	Benzene	1.0	Air jet	19.2
106.0	Benzene	1.0	Air jet	20.6
112.1	Acetone	250	Water bath	18.0
112.1	Acetone	250	Water bath	16.9
106.0	Benzene	250	Water bath	19.2
106.0	Benzene	250	Water bath	20.4

Reduction of Parathion. This reduction is carried out in the same kind of bottle and in the same bath as shown in Figure 7. Such a tall bottle, in contrast to a beaker or short flask, maintains reflux conditions and thus prevents excessive losses of alcohol. As indicated by Averell and Norris (*1*) and verified by the authors, this procedure will reduce quantitatively several milligrams of parathion in the time specified. Ordinarily six reductions are carried on simultaneously.

Development of Color. This procedure is exactly as described by Averell and Norris (*1*).

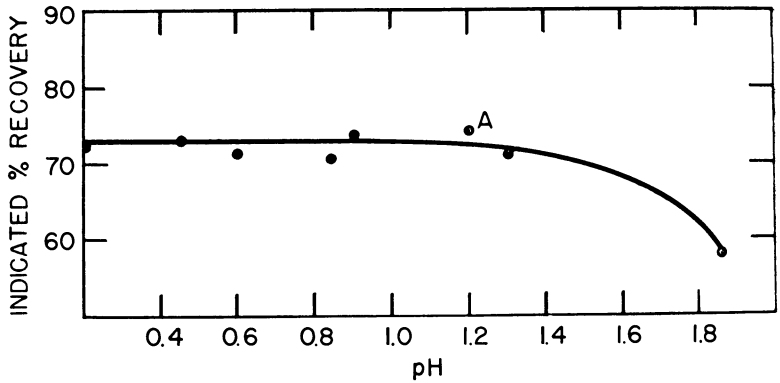


Figure 8. Effect of Varying pH on Intensity of Color of Dyed Parathion

A. pH of original dyed sample without dilution

Removal of Proteinaceous "Clouds." With the unchanged method of Averell and Norris (*1*) the final colored solution of 50.0 ml. may contain as much as 20% ethyl alcohol. Many plant materials, particularly apple pulp, yield proteinaceous substances to the benzene during extraction; if the aliquot of extract represents more than 40 to 50 grams of sample, these substances will be present in this final solution as a cloud of solid particles. These substances, whatever their nature, appear to be completely soluble in 60% ethyl alcohol. Thus, the authors recommend the addition of 50 ml. more of ethyl alcohol at this stage, the addition of more hydrochloric acid to maintain pH, and final dilution to 100.0 ml. with water. The resulting solution will be optically clear except for oily or waxy substances, which are removed in the next step.

Removal of Oils and Waxes. Two extractions with 5-ml. portions of petroleum ether (30° to 60° C. or 60° to 70° C.) have sufficed to eliminate interfering oils and waxes in suspension. Such extractions are conveniently carried out in the 100-ml. volumetric flask after final dilution and mixing. For the evaluation of color only a small portion of the 100 ml. will be utilized; therefore, if necessary, some of this solution can be discarded

at this point so as to accommodate the 5 ml. of petroleum ether in the volumetric flask. After being vigorously mixed by shaking with the colored solution, the petroleum ether is conveniently removed with the aid of a small pipet and rubber bulb.

Neither visible nor spectrophotometric evidence has ever been secured to indicate that any of the dye is transferred to the petroleum ether. If, however, for other reasons it is deemed undesirable to extract oils and waxes at this stage, the extraction may be performed similarly just prior to the addition of the sodium nitrite solution. Extraction at this earlier stage is best accomplished with 30° to 60° C. petroleum ether, for the 60° to 70° C. fraction will remove significant amounts of the reduced parathion hydrochloride. This point is illustrated by the data in Table V. In each instance, 194 micrograms of the reduced amine in 43 ml. of water at pH 3.1 were extracted twice with 5-ml. portions of the petroleum ether, then color development and evaluation were carried out in the usual manner.

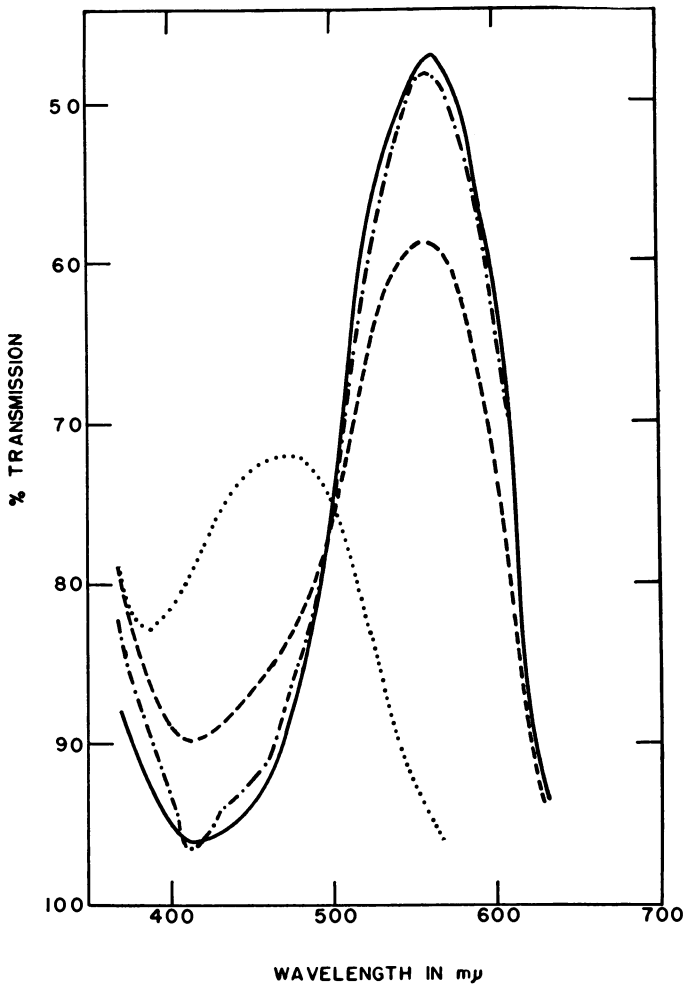


Figure 9. Transmittance–Wave-Length Curves for Dyed Parathion in 20% Ethyl Alcohol

——— pH 2.25; —·—· pH 1.68; — — — pH 1.40; · · · pH 10.9

pH and Color. Averell and Norris (*1*) stressed the fact that this colored solution is pH sensitive, although it would seem to be well buffered. The authors have evaluated certain effects of minor changes in pH, and have found that the absorption maximum is not shifted from pH 0.2 to pH 2.25, and that the intensity of color is not affected appreciably within the pH range 0.2 to 1.4. When a solution containing 104.6 micrograms of parathion as the magenta dye was adjusted to various pH values, and the transmittance was determined at 555 mμ, the transmittance held essentially constant below pH 1.4, as shown in Figure 8.

Table V. Extraction of Reduced Parathion Hydrochloride by Petroleum Ethers from Aqueous Solutions

Petroleum Ether, ° C.	% Loss
30–60	6.4
	6.4
60–70	20.6
	20.0

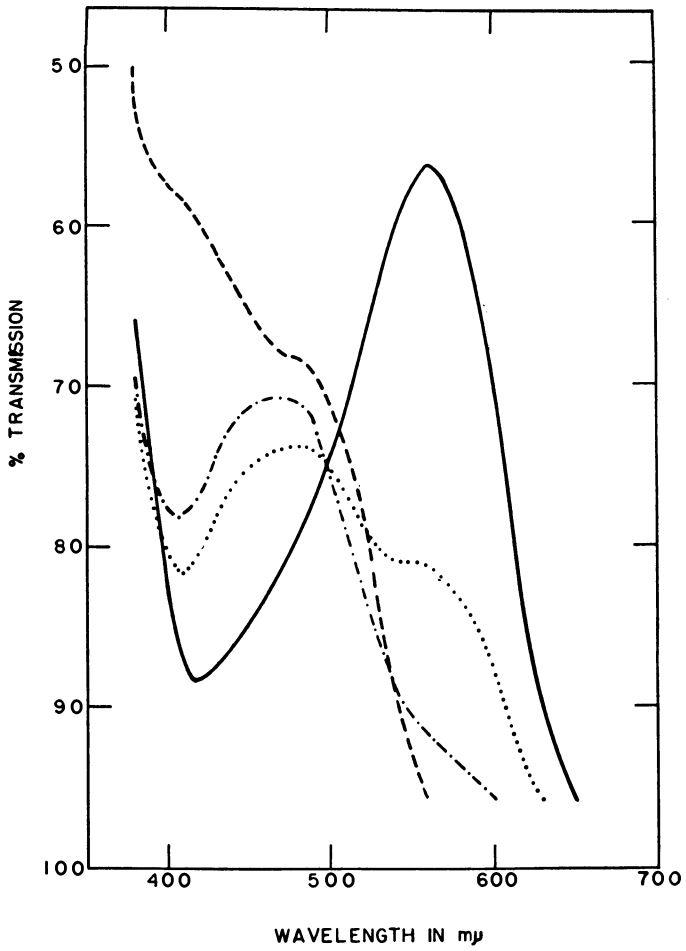


Figure 10. Transmittance–Wave-Length Curves for Dyed Parathion in 60% Ethyl Alcohol

——— pH 1.90; · · · pH 2.30; –·–· pH 2.80; – – – pH 10.5

Tautomerism of the Dye. The complete visible absorption spectra were secured for dyed parathion at several pH values in both 20 and 60% ethyl alcohol. These curves are plotted in Figures 9 and 10. The appearance of an isobestic point in both instances indicates that tautomeric forms of the dye are involved (*8*).

20% *vs.* 60% Ethyl Alcohol. As may be seen from Figures 9 and 10, the absorption maxima in both are at 558 mμ.

Interfering Substances

Any nitro- or amino-aromatic compound may be considered a potential source of interference until demonstrated otherwise. For example, DN-111 in benzene was processed in the usual manner. During the reduction step a pink color developed which was insoluble in petroleum ether; addition in proper sequence of the dye-producing reagents resulted in the development of a deep purple color within 10 minutes.

Dyed aniline is spectrophotometrically indistinguishable from dyed parathion, within the visible range. However, when purified cyclohexylamine (7.6×10^{-5} *M*) is subjected to the analytical procedure there is no color development.

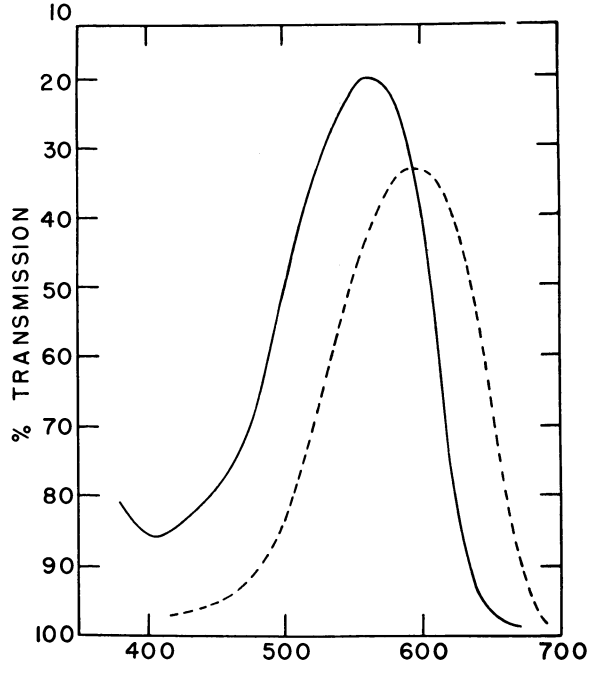


Figure 11. Transmittance–Wave-Length Curves for Dyed Parathion and p-Nitrophenol in 60% Ethyl Alcohol at pH 1.2

—— Parathion; – – – *p*-Nitrophenol

p-Nitrophenol also interferes, as may be seen from Figure 11. Dyed parathion exhibits its maximum absorption at 558 mμ ($\epsilon_{max.}$ 39,091) after 10 minutes, whereas dyed *p*-nitrophenol peaks at 594 mμ ($\epsilon_{max.}$ 9600). The latter dye requires more than a 10-minute development period to attain maximum intensity of color, but some color is developed within 10 minutes. There is sufficient overlap of the two curves to warrant the use of a spectrophotometer in routinely evaluating the colored solutions obtained from technical grade parathion. To this end, the authors recommend that the wave length 555 mμ be

adopted for the colorimetric estimation of parathion, with particular reference to parathion in and on plant products. Even though the *p*-nitrophenol color may not develop fully before colorimetric evaluation of a sample, the use of a spectrophotometer will minimize this possibly variable interference.

Acknowledgment

This work and any practicable results derived therefrom have been encouraged and expedited throughout by the American Cyanamid Company. Additionally, the authors wish to express appreciation for aid in various phases of the work to Mrs. M. Elliot Miller, Charlotte McHale, Jacquelyn Sellers, L. D. Anderson, M. M. Barnes, G. E. Carman, J. H. Barkley, J. C. Ortega, C. R. Shafer, and H. U. Meyer of these laboratories, and to W. E. Baier and C. W. Wilson of the California Fruit Growers Exchange, Ontario.

Summary

The magenta color reaction for parathion has been adapted to mass-production techniques for quantitative estimation of parathion residues in and on certain fruits, vegetables, and miscellaneous substrates. From examination of the mechanisms of this color reaction, and from experiences gained in running nearly 3000 analyses on 50 different substrates, it has become apparent for such applications that:

1. Storage of field samples at room or refrigerator temperatures (3° C.) may be equivalent to leaving in the field with regard to persistence and migration of the contained parathion.
2. Eight pounds of field sample quartered to 1 pound of parts are adequate for many of the materials examined.
3. Benzene is the best single solvent for stripping or extracting the contained parathion.
4. Wet processing techniques, with usually 2 ml. of benzene per gram, are essential.
5. The resulting benzene strips or extracts frequently will not tolerate storage even in the frozen state.
6. Rapid removal of solvent at low temperatures is essential.
7. Chlorophyll and some red constituents should be removed prior to color development.
8. Final dilution to 60% ethyl alcohol eliminates protein clouds and extraction with petroleum ether removes oily and waxy substances.
9. Reasonably close pH control is indicated.
10. Spectrophotometric evaluation of color at 555 mμ is recommended.

The efficiency of the machinery employed is discussed, pertinent supporting analytical data are presented, and the sensitivity of the color reaction, leading to unbelievably easy contamination, is stressed. Overprocessing in all steps is recommended as a means of nullifying inherent variations in the efficiencies of different types of processing apparatus used by other workers.

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PAPER 627, University of California Citrus Experiment Station.

Mass Estimation of DDT Surface and Penetration Residues

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Techniques for processing large numbers of DDT surface and/or penetration samples whereby precision is in no way sacrificed for speed, involve the utilization of several mechanical devices, including a revolving drum-type stripping machine and an adapted drill press. The efficiency of these mechanized techniques in terms of per cent residue removed and number of samples processed per day is given and compared with the efficiency of hand processing as previously done in this laboratory. The dehydrohalogenation method is the primary analytical procedure used and the permanent setup expediting the analysis of 40 to 50 samples per day is described. Results of analyses of duplicate surface and penetration samples agree within 0.5 microgram per sq. cm. or 0.5 p.p.m.

With the advent of DDT as an important insecticidal material, much work has been required to study its persistence in the field and its tolerance by animals and plants. For the past 5 years this laboratory has been engaged in estimating residues on approximately 20,000 surface and penetration samples. These included a wide variety of materials, such as alfalfa, fruits, vegetables, nuts, leaves, twigs, soil, peat moss, wooden slats, galvanized iron, and tar paper. Techniques had to be developed to handle this large volume and variety of samples routinely and expeditiously.

Hand Processing

Until 1947 all samples were processed by hand, with benzene as the stripping solvent because of its high efficiency and ready availability. With leaf surface residues, the measured leaves were placed in a 2-quart wide-mouthed Mason jar with 150 ml. of benzene. A gasket of three thicknesses of heavy waxed paper was used under the tightly screwed lid. (The wax dissolved from the exposed part of the gasket does not interfere with the subsequent analysis.) The jar was then shaken 20 times vertically, inverted, and again shaken 20 times vertically, and finally shaken 20 times horizontally with rotation. The benzene extract so obtained was filtered, 100 ml. more of benzene were added to the jar of leaves, and the process was repeated. All filtering operations were done over a specially constructed down-draft slotted floor hood (*3*), as the normal updraft hood does not remove the heavy benzene vapors adequately, and discomfort and injury to the technician may result. The recovery of DDT was 98 to 100%, as indicated by restripping the sample and analyzing the second strip solution for DDT. An experienced technician could process 35 samples a day by this method.

With fruit surfaces, 8 pounds of fruit weighed to the nearest ounce were washed by a jet of benzene from a calibrated wash bottle, using 15 ml. per fruit. The very fine stream was directed at the fruit while it was rotated on an ice pick over a 6-inch funnel fitted with

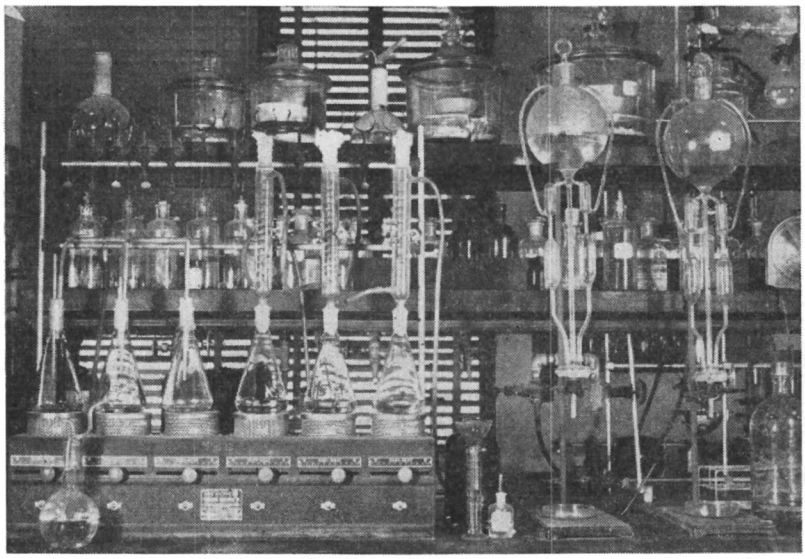

Figure 1. Permanent Apparatus Assembly for Quantitative DDT Estimations

fluted Sharkskin filter paper. The recovery of DDT was the same as for leaf surfaces and again 35 samples could be turned out per day.

In fruit penetration studies 8 pounds of fruit were first thoroughly scrubbed with warm 10% trisodium phosphate solution and then rinsed thoroughly with distilled water. Citrus fruits, if depth of penetration into the peel was of interest, were peeled in longitudinal sections with a buttonhook peeler and the albedo or white portion was separated from the flavedo or colored portion. The separated peel was placed in pie tins lined with waxed paper and dried in a forced draft oven at 65° C. for 16 hours. The dried peel was then crushed and steeped for 48 hours in a measured volume of benzene sufficient to cover the sample. If, on the other hand, only the total amount of DDT in the peel was of interest, the fruit was halved and juiced on a power juicer. The pulp was removed, the peel sliced, and the sample dried and treated as before. Thin-skinned fruits, such as apples, pears, and avocados, were peeled with a vegetable peeler, cores or seeds were removed, and the pulp was sliced in thin slices. Pulp and peel were then dried and treated in the same way as the citrus peel. The steeping completed, the samples were filtered through Sharkskin filter paper and the volume of benzene recovered was noted.

The recovery of DDT by this method was 93 to 96% (*3*), as confirmed by exhaustively extracting a drained sample with benzene and analyzing the extract for DDT. Two samples per operator could be prepared for drying in 1 day; however, 3 days elapse between the start and finish of the processing when done in this way.

When the penetration study was conducted on leaves, they were dried, crushed, and extracted exhaustively with benzene in a Soxhlet apparatus in the usual manner.

Mechanical Processing

For the past 2 years the output of analyses has been increased greatly, particularly with penetration studies, by utilization of wet-processing techniques and development of several new pieces of apparatus. This equipment includes the revolving drum-type stripping machine and especially adapted drill press used also for parathion studies. A detailed description of this press has been published (*2*), and the stripping machine and accessory equipment have been described (*5*).

Leaves are stripped for 30 minutes in the usual 2-quart wide-mouthed Mason jars. Instead of a waxed paper gasket, however, 2 squares of No. 300 M.S.T. cellophane topped by a square of white nylon are used, as the benzene would disintegrate the wax paper during this longer stripping period. The entire 250 ml. of benzene are added at once, the

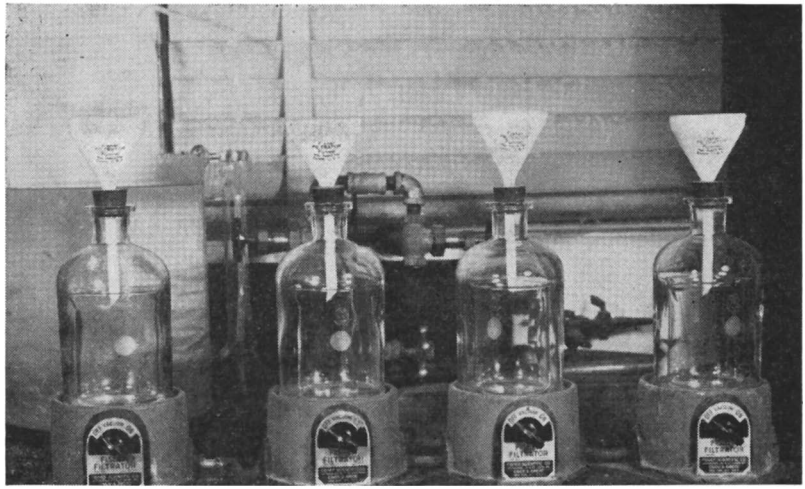

Figure 2. Fisher Filtrator Assembly for Quantitative DDT Estimations

lids tightened, and the jars wedged firmly in the compartments by means of wooden blocks. The rest of the processing is carried out as before. Actually the stripping operation is 95% complete in 5 minutes. All samples are overprocessed, however, so that results will be comparable to those of other workers who overstrip regardless of their stripping method.

By using the stripping machine 60 samples may be processed per day as compared with 35 per day by hand stripping. Recovery of DDT was shown to be the same in both cases.

In fruit and vegetable penetration studies, after the samples have been washed and peeled in a manner prescribed for each type, they are placed in the metal cans originally developed for parathion studies and minced on the drill press. A double layer of cellophane again serves as the contact gasket. These cans are also used now in stripping off fruit surface residues. A 1-pound sample of peel segments taken by quarters from the peel of an 8-pound sample of oranges is minced for 1 minute with 2 ml. of benzene per gram of peel and stripped for 30 minutes on the mechanical stripping machine. This again represents overstripping. It has been found empirically that this amount of benzene is sufficient for most fruits and vegetables. After stripping, the samples are filtered through Sharkskin filter paper and the volume of benzene recovered is noted. With these improved techniques 16 samples can be completely processed in one day by one operator. Recovery of DDT is the same as for dry processing.

Soil samples are handled by shaking 2 pounds of soil, the moisture content of which has been determined for subsequent correction to dry weight, with 1.5 ml. of benzene per gram of soil for 1 hour in the metal cans on the mechanical stripper.

Benzene solutions of DDT may be stored indefinitely at 3° C. Leaves and fruit or vegetables awaiting processing for DDT estimation may be stored at 3° C. as long as the fruit and leaves are in good condition.

Analytical Procedures

The analytical procedures used for quantitatively estimating the DDT are the dehydrohalogenation method (*4*) developed by Gunther and the modification of Baier *et al.* (*1*) involving the use of 4.5 *N* ammonia in methanol instead of 1 *N* alcoholic potassium hydroxide as the dehydrohalogenating agent. The latter method is used for samples containing large quantities of oils, such as occur in certain penetration studies and for samples containing sulfur. Because the modified method involves a 16-hour incubation period, it is used only when the other method proves inadequate.

A permanent setup has been made to expedite the handling of a large number of analyses (*3*).

The benzene strip or extract solutions are evaporated nearly to dryness in 500-ml. standard-taper Erlenmeyer flasks on 3 units of a 6-unit variable heat extraction apparatus hot plate (see Figure 1). Evaporation is hastened by directing a jet of air at the surface of the benzene, gentle enough to avoid spattering when maintained 0.5 inch above the surface of the liquid. The benzene vapors are removed through a manifold connected to the house vacuum. On this apparatus 250 ml. of sample can be reduced to a volume of about 5 ml. in 10 minutes.

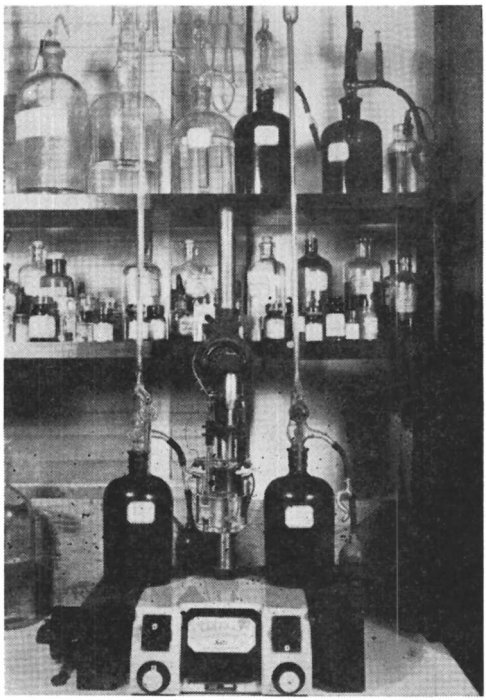

Figure 3. Titrator Assembly for Quantitative DDT Estimations

To the moist residue from the evaporation are added approximately 50 ml. of 1 *N* ethanolic potassium hydroxide solution from a graduated cylinder. The flasks are placed on the 3 remaining hot plates of the apparatus, fitted with reflux condensers, and allowed to reflux gently for exactly 15 minutes as timed by a stop clock. Three digestions and 3 evaporations may be carried on simultaneously (see Figure 1). The digestion completed, the flasks are disconnected from the condensers and 100 ml. of distilled water are added rapidly to each from an automatic Machlett pipet in order to stop the reaction. Three drops of phenolphthalein indicator solution are added and the solution is neutralized with 2 *N* nitric acid solution from another automatic pipet (Figure 1). Exactly 25 ml. of a saturated solution of C.P. barium nitrate are added with swirling, to precipitate any fatty acids resulting from saponification as barium salts.

After standing at room temperature for 5 minutes or longer to allow some coagulation of the barium salts, the flask contents are filtered with gentle suction through a double thickness of Sharkskin filter paper on a battery of 4 Fisher Filtrators into 400-ml. beakers (Figure 2). In order to remove water-soluble chlorides, it is imperative that the filter paper be washed thoroughly prior to filtering operations. Two drops of concentrated sulfuric acid are added to the clear or faintly turbid filtrate and the samples are titrated electrometrically on a Leitz G. & D. electrotitrator (Figure 3). This method of titration has the advantage of giving accurate and easily determined end points, no matter what

the color of the solution. It is easily possible for one operator to titrate 40 to 50 samples per day.

Conclusion

Through the use of these techniques, accuracy has not been sacrificed for speed. Results from analyses of duplicate surface and penetration samples agree within 0.5 p.p.m. or 0.5 microgram per sq. cm., as the case may be. Obviously, the larger the amount of DDT present the greater the accuracy. Using all this special equipment, the cost per analysis is about $1.25, including labor and normal depreciation of equipment. The advantages of mechanized over hand processing are so great, however, that the equipment is considered well worth while.

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PAPER 626, University of California Citrus Experiment Station, Riverside, Calif.

Microbioassay of Insecticide Residues in Plant and Animal Tissues

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Small shell vials closed by a screen top have been used as exposure chambers in evaluating the toxicity of organic insecticides to houseflies and in bioassaying for contamination of plant or animal matter with these substances. Treatment of a chloroform extract containing hexachlorocyclohexane or DDT with strong sulfuric acid and passage of the chloroform extract containing parathion through an absorption column greatly diminish the interference due to extractives from the plant or animal tissues. Data for LD_{50} to female houseflies are given for parathion, γ-hexachlorocyclohexane, and *p,p'*-DDT in terms of micrograms per vial and per square centimeter. Female flies require approximately twice the dosage needed for males. The small vial method is extensively used in following the development of resistance.

Several organic insecticides have come into wide use recently before chemical methods for their analysis had been developed, and in some cases interfering reactions have prevented use of known analytical methods in the presence of plant or animal tissues. The importance from the legal and public health viewpoints of minute traces of residual insecticides in foodstuffs makes quantitative determination imperative. Hence, in this emergency recourse has been taken to methods of bioassay. This, of course, is a very old practice with many drugs of plant or animal origin, but until a few years ago bioassay with insecticides was limited to the so-called screening of chemicals to find those having possible insecticidal value and to the standardization of fly sprays. Neither of these uses necessarily involved determination of the amount of insecticide present.

With the advent of DDT, determining its persistence on walls sprayed for control of mosquitoes and flies became very important. A simple method of analysis involving exposure of mosquitoes to the sprayed surface and comparison of mortality to that caused by known deposits of DDT was widely used (*9*). For the determination of DDT residues in plants and animals, total organic chloride provided a fairly satisfactory analytical method from the start, and the colorimetric method of Schechter and Haller (*6*) is extremely delicate and widely applicable. This is not the case with hexachlorocyclohexane, often called benzene hexachloride or BHC, whose highly insecticidal gamma isomer has defied chemical analysis when present among the other isomers or other organic compounds containing chlorine.

A pioneer study of the distribution of this substance in the tissues of rats to which it had been fed was made by Laug (*3*). He evaporated ether extracts in Erlenmeyer flasks, so that a deposit was left over the bottom. Female houseflies were confined in the flasks, and the mortalities after 20 hours were compared to those obtained with known amounts of γ-hexachlorocyclohexane. Because most of the inner surface of the flask was untreated, the flies were out of contact with the toxicant during an unknown fraction of the exposure period. The exposure period was so long that the insects had to be fed dur-

ing the period to prevent injury from lack of food and water. Laug found that some "tissue mortality" was caused by extractives from liver and other organs, and he noted that in the presence of much fat the toxic effect of γ-hexachlorocyclohexane was so decreased that less than 10 p.p.m. could not be estimated with certainty. It seemed that these two difficulties might be greatly lessened by using a much smaller exposure chamber whose entire inner surface could be treated, and that the interference from fat or organ extractives might be lessened by appropriate treatment. The method described was designed with these two objectives in mind.

Apparatus and Procedures

The exposure chamber is a flat-bottomed shell vial, 2.0 cm. in diameter by 4.0 cm. long. The open end is heated in the flame and lightly pressed upon a flat surface to give a rolled edge projecting both outward and inward. On the outside this holds in place a metal cover (Figure 1), and on the inside it prevents spilling of the insecticidal solution

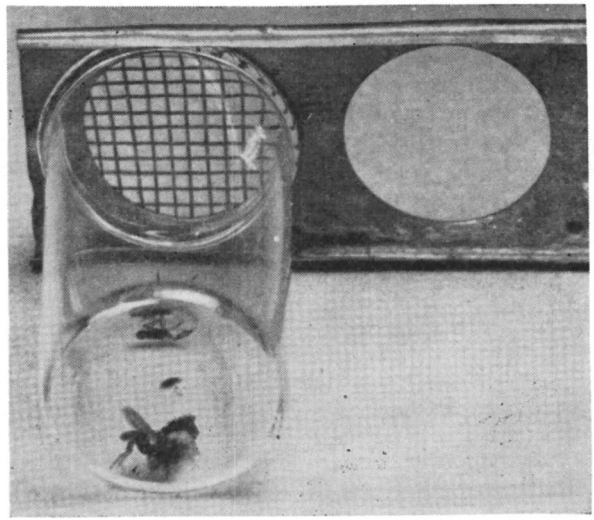

Figure 1. Small Vial Exposure Chamber with Screen Top in Place

when the vial is rolled as described below. The metal cover is a thin sheet of copper or aluminum 6 cm. long and just wide enough for its curled sides to fit snugly around the outer rolled edge of the vial but permitting the vial to slide when pushed. Two circular holes 2 cm. across are in the cover. One is open and soldered; over the other is a piece of sufficiently fine wire screen to restrain the test insect. Such chambers have the important advantage that all trace of toxicant from a previous test can be removed readily by solvents and detergents, and because they are both cheap and easy to make, any number desired can be kept on hand.

The inner surface of a vial is treated with the insecticidal coating by introducing a measured volume, usually 5 or 10 ml., of standard solution or tissue extract and placing the vial in an air bath oven at 70° C. with care to avoid local overheating until the volume is reduced to about 1 ml. The vial is then removed and rolled by hand while the remaining solvent evaporates, care being taken to secure an even distribution over the sides and bottom. As soon as the vial is cool, the cover is put on with the open hole over the vial. Flies or other test insects may then be introduced as desired.

This exposure chamber is not restricted to any species of test insect and the extract of tissue suspected of containing a given insecticide may, of course, be prepared in any desired manner. The use of the common housefly, *Musca domestica*, as test insect and

procedures that have been found useful in bioassay of γ-hexachlorocyclohexane and of parathion may be taken as examples.

A colony of houseflies is maintained in the laboratory in general conformity with the official procedure for the Peet-Grady test (*5*). When needed, a group between the ages of 3 and 5 days is anesthetized by chilling or with carbon dioxide, and females are taken in a group of 25 and allowed to revive completely in a test tube plugged with cotton and laid on its side. Then the plug is removed and the mouth of the test tube is quickly placed just within the open hole in the cover of a treated vial. By a quick shake the flies are thrown into the vial. The cover is slid to bring the screen over the vial, and the assembly is laid on its side for the duration of the exposure. The practice is to lay the vials parallel to the source of light. This is not important with houseflies, but may be critical with insects that are strongly phototropic. Observation has shown that the flies crawl over all parts of the surface to which they have access, but they tend to remain on the lower side wall. No tendency to go to the untreated screen has been noted. Because the screen has an over-all area almost exactly one tenth of the total area, the loss in sensitivity on this account is slight.

After exposure for 30 minutes, each group of flies is released into a small observation cage and given sugar and water. To avoid environmental effects they should be kept at the same temperature, humidity, and light conditions as those of the rearing room, but to avoid possible effects upon the breeding stock from volatile toxicants all exposed flies should be kept in a separate room. After 24 hours, mortality is determined. With a small proportion of the individuals this must be somewhat arbitrary, according to the criteria that insects too helpless to crawl are counted as dead, and those that crawl or fly more or less normally are counted as surviving.

Schechter *et al.* (*7*) in their studies on DDT in milk found that interfering extractives are largely removed by treatment with strong sulfuric acid. Furman and Hoskins (*2*) found the same treatment effective when hexachlorocyclohexane was the contaminating substance, and it has been applied in later work to numerous extracts of plants and animals. The procedure now used when either DDT or hexachlorocyclohexane is suspected is as follows:

The chloroform extract obtained in any desired manner—e.g., by mixing in a Waring Blendor—is shaken thoroughly with an equal volume of 10% sodium sulfate in concentrated sulfuric acid. After the acid layer is withdrawn, the treatment is repeated twice with a 1 to 1 mixture of concentrated and 20% fuming sulfuric acids. Rinsing with water, then with saturated sodium carbonate solution, and finally with water again gives a chloroform solution that can be evaporated in the test vial. The temperature is not allowed to rise, because much charring will occur and there is danger of destroying part of the insecticide.

Parathion is a relatively unstable compound and the drastic treatment with sulfuric acid cannot be used. Interfering extractives from some plants can be removed without loss of parathion by passing the chloroform solution through a short column containing 2 parts of Attaclay plus 1 part of Hyflo Super-Cel, followed by rinsing with an equal volume of pure chloroform. DDT also passes through such a column without loss. The solution is then evaporated in a test vial as described before.

Results

To illustrate the results obtained by this method, a few data are selected from investigations on the contamination of chicken flesh with γ-hexachlorocyclohexane and of cabbage with parathion. These cases are especially interesting because with the chicken flesh the liquid fats alter contact with the hexachlorocyclohexane and also cause considerable mortality of flies and because with cabbage chemical analysis of parathion is difficult on account of very high blanks with untreated samples when the usual colorimetric method is used.

Figure 2 shows the standard dosage-mortality lines for γ-hexachlorocyclohexane (line *A*), parathion (line *C*), and *p,p'*-DDT (line *F*) plotted according to the probit–log dosage procedure of Bliss (*1*). Line *B* is the corresponding dosage-mortality line resulting when

γ-hexachlorocyclohexane in the presence of chloroform extract of chicken flesh is put through the acid treatment. The small displacement to the left, signifying greater effect from a given quantity of insecticide than when the material is tested alone, may be due to

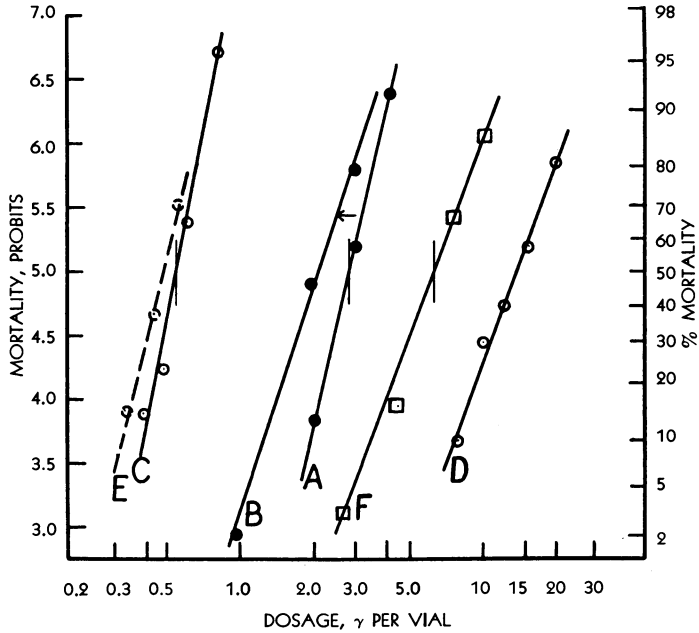

Figure 2. Dosage-Mortality Lines for Female Houseflies with γ-BHC, Parathion, and *p,p'*-DDT

A. γ-BHC
B. γ-BHC plus extract of chicken flesh after acid treatment
C. Parathion
D. Parathion plus cabbage extract
E. Parathion plus cabbage extract after passage through absorption column
F. *p,p'*-DDT

some extractives not removed by the sulfuric acid, for Laug (*3*) noted that fats reduced evaporation of the comparatively volatile γ-hexachlorocyclohexane. The arrow indicates the mortality found with 5 ml. of an extract of chicken flesh corresponding to the presence of 2.5 micrograms of γ-hexachlorocyclohexane. Because this was an aliquot of 100 ml. used in extracting 75 grams of flesh the original contamination may be calculated to be 2.5 × 100/5 ÷ 75 = 0.67 p.p.m. of γ-hexachlorocyclohexane. Direct exposure of flies to a chloroform extract of contaminated chicken flesh gave no definite result because they became smeared in the liquid fat. This effect was completely eliminated by the acid treatment.

In the case of parathion plus cabbage extractives the dosage-mortality line (*D*) is so far to the right of that for straight parathion that analytical results based upon the latter as a standard would be entirely erroneous. Thus the LD_{50}'s are 0.56 and 14.0 micrograms per vial, respectively, or a 25-fold greater dosage is required in the presence of the cabbage extractive. Of course, line *D* could be used as the standard for analysis, but probable differences among various kinds of cabbage make this an unsafe procedure. The great loss in sensitivity is another serious objection to use of line *D* for reference. Passage of a chloroform extractive solution plus parathion through the adsorption column described above resulted in line *E*, which can be used as the reference line. Actually, it agrees closely with the original standard dosage-mortality line, *C*. Using this method, no

difficulty has been encountered in assaying parathion residues on cabbage and allied plants. The residues never exceeded a few hundredths part per million.

A device in which insects may be exposed to known amounts of a chemical may be used in ascertaining comparative toxicities—e.g., in screening. Figure 2 gives a simple example of this, for it shows that the LD_{50}'s for parathion, γ-hexachlorocyclohexane, and *p,p'*-DDT to adult female houseflies are 0.56, 2.7, and 6.7 micrograms per vial, respectively. Because the treated surface has an area of 28.0 sq. cm., these dosages amount to 0.020, 0.096, and 0.239 microgram per sq. cm., respectively. When an attempt was made to determine the LD_{50}'s for the other isomers of hexachlorocyclohexane no mortality resulted with deposits up to 10,000 micrograms per vial. These deposits are easily visible, and, in fact, were partly flaked from the glass surface by the activity of the confined flies.

Male houseflies are more susceptible than females to certain insecticides—e.g., pyrethrins (*8*). Such differences may be determined with ease and precision by the small vial method. Thus with *p,p'*-DDT the LD_{50}'s for male and female houseflies are 3.7 and 6.7 micrograms per vial, respectively. With γ-hexachlorocyclohexane they are 1.4 and 2.7 micrograms per vial. Thus the females require approximately twice the dosage needed for the males. This difference is only partly accounted for on a weight basis, for the average weights of the flies used were: males 12 mg. and females 18 mg. The reproducibility achieved with the small vial method may be illustrated by a typical set of data obtained with γ-hexachlorocyclohexane and each sex of houseflies. Successive trials gave the following mortalities.

γ-BHC, γ/Vial	Mortality	
	Males	Females
1	8, 11, 18, 21, 3, 8, 9, 10, 10, 13 Av. 11.1	..
1.5	40, 56, 62, 66, 47, 54 Av. 54.2	..
2	88, 91, 93, 94, 97, 89, 93, 93, 95 Av. 91.4	5, 11, 14, 5, 8, 15 Av. 9.7
3	..	63, 71, 77, 52, 58, 59, 66 Av. 63.7
4	..	85, 91, 93, 90, 93, 93, 95 Av. 91.4

A matter of very great interest is the increase in resistance to DDT that has been shown recently by houseflies in various parts of the world. This is often so marked that practical use of DDT is no longer feasible. An especially resistant strain, found in southern California by March and Metcalf, is called the Bellflower strain for purposes of identification (*4*). They reported that no residual deposit of DDT gave 100% kill. By the small vial method not over 25% kill of these female flies could be obtained with several thousand micrograms per vial. Further tests with various naturally occurring and selected resistant races are in progress.

Discussion

The methods described for handling chicken flesh and cabbage have been used for several other products in which hexachlorocyclohexane or parathion contamination was suspected, but doubtless occasions will arise in which these procedures will be inapplicable. Modification in the preparation of extracts, however, does not affect the usefulness of the small exposure chamber. Because by this small vial method a test may be made with amounts of a few micrograms or less, the term "microbioassay" is appropriate. The effect of failure to extract the toxicant completely from a tissue is diminished by adding known amounts to representative samples of the same kind of uncontaminated tissue when determining the reference dosage-mortality line. There is always the possibility that a chemical present in some part of the body after ingestion will be harder to extract than when freshly mixed with the tissue. Hence, precautions such as fine subdivision, long extraction, and use of a really good solvent should always be taken.

With some insecticides the physical state of a residual deposit has a pronounced effect upon its toxic effect. This is especially true of technical DDT, which tends to remain in viscous droplets when released from solution in volatile solvents. These may remain

for weeks if undisturbed, but they crystallize instantly when touched by a moving insect. The deposit within a small vial may be observed closely, under a microscope if desired, and this phenomenon may be followed in detail.

The flaking off of very heavy deposits—e.g., of α-, β-, or γ-hexachlorocyclohexane or of p,p'-DDT—sets a limit to the amounts to which insects can be exposed in the vials. In this respect the present procedure differs sharply from those in which the toxicant is applied to a piece of filter paper or other porous material. Under such conditions enormous amounts may be employed, but a large percentage is completely removed from contact with the test insects and dosages so determined for 50% or any other mortality have little meaning.

Accessibility of the deposit to the insects by contact is the chief feature of the small vial method, but fumigant action cannot be eliminated entirely. In the case of DDT this has been found to be unimportant, for flies kept in the vials out of contact with the surface are not affected. But with γ-hexachlorocyclohexane or parathion there is a noticeable toxic effect. If the vials are stood upright, laid on the side, or hung upside down, there is a decrease in the mortality produced in the order given. The position on the side has been adopted because it avoids extremes and because practical use of an insecticide often involves limited but not accentuated ventilation.

Acknowledgment

The valuable assistance of O. H. Fullmer is gratefully acknowledged.

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CONTRIBUTION from the laboratory of insect physiology and toxicology, Division of Entomology and Parasitology, University of California.

Toxicity of Spray Residue of Fresh and Processed Fruits and Vegetables

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Bioassay with mosquito larvae for the detection of insecticide residues in fresh and processed fruits and vegetables is feasible, subject to the limitation that the untreated natural product is in itself nontoxic to the larvae at the dilutions tested.

The purpose of this work was to determine the toxicity to mosquito larvae of insecticide spray residues. That certain insecticides are translocated in plants (*4*, *5*) adds impetus to this study. Fresh orchard fruit sprayed or dusted with preparations containing parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate), tetraethyl pyrophosphate (TEPP, HEPP), DDD [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane], DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane], chlorinated camphene, and basic lead arsenate were shipped from California to Yonkers, N. Y., by air express for bioassay.

Materials and Methods

As mosquito larvae are relatively easy to kill with insecticides, any toxic spray residue is likely to be detected. Two species of mosquito larvae were used, the yellow fever mosquito (*Aedes aegypti* L.) and the southern house mosquito (*Culex quinquefasciatus* Say). Tests with the southern house mosquito were made essentially according to the method of Campbell, Sullivan, and Smith (*1*), except for the kind of food supplied and size of containers used.

The eggs (furnished through the courtesy of C. H. Bradley) were shipped weekly from Orlando, Fla., to Yonkers, N. Y., via air mail, and as soon as received, they were placed in tap water in 4-liter beakers at room temperature. The eggs hatched within 24 hours. When the larvae had hatched, powdered dog biscuit was added at the rate of 100 mg. per liter. The following day 125 mg. of blood albumen were dissolved in 150 ml. of water, and added at the rate of 125 mg. per liter to beakers containing larvae. Thereafter powdered dog biscuit and blood albumen were fed on alternate days in amounts specified above. Larvae 5 days old were used for testing. Test tubes (25-ml. capacity) containing ten larvae each in the solution to be tested and the controls in tap water alone were placed in an oven at 30° ± 1° C. overnight (20 hours).

The solutions containing larvae were poured into porcelain dishes and living and dead larvae were counted. Tests were run in duplicate—i.e., two tubes containing 10 larvae each. If any dead larvae were found in a check, the tests were repeated. Tests were made at four or more concentrations. Each series of tests was repeated on a different day.

Larvae of the yellow fever mosquito, which are shallow feeders, were reared at room temperature by methods similar to those used in culturing *Anopheles* mosquito larvae (*2*, *6*). Filter papers containing the eggs (furnished through the courtesy of R. E. Heal, Merck & Co., Inc., Rahway, N. J.) were placed in tap water in shallow porcelain pans (12 × 7 inches, and 2 inches deep). The eggs hatched within 24 hours. When the larvae had hatched, powdered dog biscuit was added daily at the rate of 100 mg. per liter of water.

Larvae 3 days old were used in testing. Tests were conducted in the same manner as with the southern house mosquito.

The fresh fruit was ground in a meat chopper, and the juice was pressed out and diluted with tap water at ratios of 1 to 7.5, 1 to 15, and 1 to 30. The processed baby food was also diluted with tap water at the same ratios as the ground fresh fruit.

Results

Apricots, prunes, and peaches from sprayed trees were tested on the larvae of *Aedes aegypti* for the toxicity due to spray residues. The apricots were sprayed on April 1 and May 6 and the fruit was harvested on or about August 9. The samples were tested during the month of August at dilutions of 1 to 7.5, 1 to 15, and 1 to 30. Apricot samples from trees sprayed with DDT (50% wettable) at the rate of 1.5 pounds per 100 gallons of water were toxic at all three dilutions tested. Samples from trees sprayed with DDD (50% wettable) at the rate of 2 pounds per 100 gallons of water were toxic at dilutions of 1 to 7.5 and 1 to 15. Apricot samples from trees sprayed with parathion (25% wettable) at the rate of 2 to 3 pounds per 100 gallons of water were also toxic at dilutions of 1 to 7.5 and 1 to 15. The peach trees (cling) were sprayed on June 16 with a single application of tetraethyl pyrophosphate, and the fruit was harvested on or about July 2. Tests were made during the month of July. Both treated and check peaches were unripe when tested. It was found that unripe unsprayed peaches were toxic to mosquito larvae at dilutions of 1 to 7.5 and 1 to 15, but at the dilution of 1 to 30 neither the treated nor the the check peaches showed toxicity. It was not possible to distinguish between the toxicity of treated and check at dilutions of 1 to 7.5 and 1 to 15 by the method of ranking described by Wilcoxon (7).

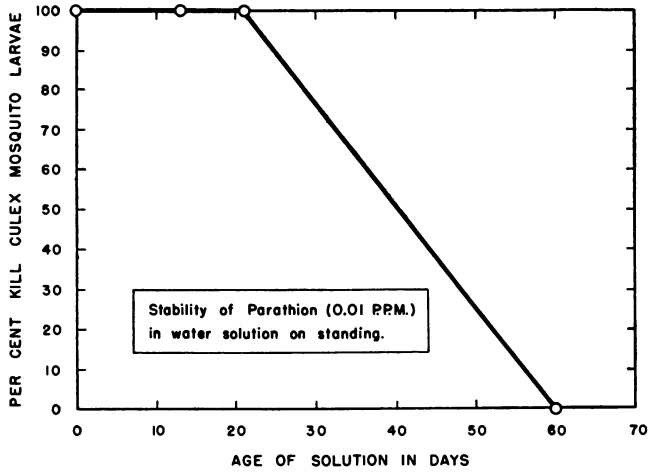

Figure 1. Stability of Parathion

In addition to the tests made on peaches and apricots, samples of prunes from trees that had been sprayed with parathion, DDT, DDD, basic lead arsenate, and toxaphene at the rate of from 1 to 2 pounds of these insecticides per 100 gallons of water were tested on larvae of *Aedes aegypti*. The trees had been sprayed on April 20 and June 16, 1948. The fruit was harvested on or about September 10. Prunes from trees that had been treated with 1 quart of tetraethyl pyrophosphate and 12 pounds of sulfur dust per acre on June 15, and harvested about July 6, were tested on larvae of the above named species. None of the prune samples tested in this study exhibited any significant toxicity to mosquito larvae as compared with the unsprayed check.

Strained processed peas and peaches containing known quantities of parathion ranging in concentrations from 0.25 to 10 p.p.m. were diluted with tap water at ratios of 1 to 7.5, 1 to 15, and 1 to 30, and tested on larvae of *Aedes aegypti*. Kills of 100% were obtained at all concentrations tested. The average LD_{50} of aqueous parathion solutions was 0.003 p.p.m. [a value comparable to that obtained by Gleissner (*3*)], as compared with 0.055 p.p.m. for processed peas and 0.0053 p.p.m. for processed peaches. Strained processed peas containing known quantities of gamma isomer of benzene hexachloride (γ-hexachlorocyclohexane) were nontoxic, whereas strained processed peaches gave kills of 100% at the same dilutions. The pH values of the processed peas and peaches used in these tests were 6.0 and 3.7, respectively, indicating that the toxin is not destroyed in an acid medium. The LD_{50} of an aqueous solution of the gamma isomer of benzene hexachloride was approximately 0.1 p.p.m.

An aqueous solution containing 1 part in 200,000,000 of parathion gave 50% kill to southern house mosquito larvae (*Culex quinquefasciatus* Say). Parathion solutions did not lose any toxicity on standing for a month at room temperature (Figure 1). At the end of 2 months, however, the solutions lost their toxicity to mosquito larvae.

Table I. Toxicity to *Aedes aegypti* Larvae of Processed Baby Food[a]

(Known quantities of insecticides added before processing)

Insecticide	Concn. before Dilution, P.P.M.	Dilution Ratio 1:7.5	1:15	1:30	1:7.5	1:15	1:30
		Peas, Strained, % Dead			Peaches, Strained, % Dead		
TEPP	10	4	0	9	0	0	6
	5	6	6	5	4	0	0
	2	0	0	0	20	0	0
BHC	10	0	0	0	100	100	100
	5	0	0	0	100	100	100
	2	0	7	0	100	100	100
Methoxychlor	10	56	15	0	14	0	0
	5	5	0	0	10	4	0
	2	0	0	0	5	0	0
Check	..	0	0	0	6	0	0

[a] % moisture in strained peas ranged from 83.7 to 87.5; total solids, 12.6 to 16.3. % moisture in strained peaches ranged from 63.3 to 73.5; total solids, 26.5 to 39.7. Moisture and total solids determinations by Theo Svolos.

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Some Poisonous Residue Factors in Use of Two New Organic Insecticides

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Laboratory and field research work has developed methods for using two new organic insecticides with freedom from poison residue. With pure gamma isomer of hexachlorocyclohexane proper formulation and timing of application are the essence of success. On some crops applications must be made before fruits or heads form; on others applications may be made to within 2 or 3 weeks of harvest, and in others, within a few days of harvest. Commercial usage with proper timing and application has resulted in no poison residues or undesirable taste residues. With proper usage, dosage, and application the pure gamma isomer of hexachlorocyclohexane leaves no residues that would constitute a health hazard or produce off-flavors in food products. The unstable nature of tetraethyl pyrophosphate makes treatment of crops up to harvest time possible. This chemical is therefore an effective agricultural insecticide against many pests.

The introduction of the use of DDT (dichlorodiphenyltrichloroethane) as an insecticide during World War II initiated a revolution in the problems of insecticide residues. The resulting changes have been so far-reaching that today, only a few years later, most major insecticides are new synthetic organic chemicals. Prior to the introduction of DDT the major insecticides were inorganic chemicals, except for some few relatively expensive organics of plant origin such as nicotine, pyrethrum, and rotenone.

Two of the most important synthetic organic insecticides which have been introduced following DDT are the technically pure gamma isomer of hexachlorocyclohexane (lindane) and tetraethyl pyrophosphate. The technically pure gamma isomer was developed as the result of investigational work on the insecticide, benzene hexachloride, which was first used in England and France during World War II and was used in the United States on agricultural crops at the same time. It was found to have insecticidal value equal to DDT in most respects and better in others. Considerable residue action was evident, and it was toxic to a wider range of insects than was DDT, and had vapor action not evident with DDT.

Of five or more isomers, the only one that is appreciably insecticidally active is the gamma isomer, which occurs in various percentages, usually 12 to 13%, depending on methods of manufacture. This mixture of isomers results in a compound of strong and persistent odor, mostly due to the beta isomer, which odor is retained by some fruits, vegetables, and animal tissues after treatment for insect infestations.

Research work soon showed the possibility of producing the pure or technically pure gamma isomer. This production was finally accomplished on a large commercial scale and the compound has now been introduced as a large-volume insecticide on the American market.

Tetraethyl pyrophosphate was first manufactured in Germany about 1940 as hexa-

ethyl tetraphosphate or the hexaethyl ester of tetraphosphoric acid. This compound and its manufacturing methods were discovered in Germany by chemists with the allied occupation forces and the information was brought to the United States. Research work on this chemical finally indicated that it contained as the active agent tetraethyl diphosphates which are generally described as tetraethyl pyrophosphate, and this is now well established in the insecticide and chemical industry.

As the value of these two new chemicals for insecticides became more evident, the need for extended experimental and test work was definitely established. It was necessary to determine chemical formulas, work out analytical methods, obtain knowledge of various physical and chemical characteristics, and complete evaluation of insecticidal action as well as toxicity and effect of residues. Toxicity was concerned with not only insects but humans and other warm-blooded animals. Residual studies included information on persistence and type and amount of residue. This information, once accumulated, must be correlated with similar information on other insecticides.

Pure Gamma Isomer of Hexachlorocyclohexane

Investigational work on the pure gamma isomer of hexachlorocyclohexane involved both laboratory and field tests. The pure gamma isomer was found to have retained practically all of the insecticidal value of the parent commercial benzene hexachloride containing 12 to 13% gamma isomer. The three-way action of contact poison, stomach poison, and vapor action poison was also evident with the pure gamma isomer. In addition, methods of manufacture were found which retained the insecticidal action of the parent compound and yet removed the objectionable odor, thus making available a fine chemical for killing insects.

According to Lehman (*2*), toxicity tests in the laboratory with small animals such as rats and dogs indicated that the refined or technically pure gamma isomer has a mean lethal dose of 125 mg. per kg. of body weight. This gives it an acute oral toxicity about twice that of DDT. Nicotine is twelve times and tetraethyl pyrophosphate sixty times as toxic as gamma isomer. On the same basis arsenic is about four times as toxic. According to Lehman, pure gamma isomer shows much less tendency toward storage in body tissues than does DDT. Pure gamma isomer is stored in body fat at a level about equal to the amount of dietary intake; DDT is stored in body fat at a level four to ten times that of the dietary intake. The pure gamma isomer of hexachlorocyclohexane shows practically no dermal toxicity on skin application. Its chronic toxicity is four times less than DDT.

Laboratory studies showed the pure gamma isomer of hexachlorocyclohexane to have residual life equivalent to that of the normal mixed isomers. The material does not break down in normal storage, as do mixed isomers.

It was also established in laboratory work that this product could be readily formulated into dusts, wettable powder, or liquid formulations. Liquid formulations were more readily made with this than with the commercial benzene hexachloride, because of the higher concentration of the gamma isomer.

Field test work with technically pure gamma isomer of hexachlorocyclohexane has been extensive and involved and is being continued. It was necessary to know such factors as insecticidal value in field applications as compared to other insecticides, as well as residual life, residue from the poison standpoint, and residual taste or odor factors. These factors have been worked out on numerous crops and some of the results are dealt with in this paper. Because the pure gamma isomer was found to be effective on insects in the soil as well as on insect infestations on plants, its residual life in soil of all types and effects on tuber and root crops were also of major importance.

The residual toxicity of the pure gamma isomer was found to be equivalent to that of ordinary commercial benzene hexachloride. Commercial usage has shown that the residual action is effective for a longer time with dust or wettable powder spray applications than with emulsive solvent-type formulations. The over-all residual life of the chemical is on the order of 4 to 8 days as compared to 14 to 21 days for DDT. This is, of course, adequate residual life for good insect control in most cases, and the shorter

life and the smaller amount used make the pure gamma isomer especially important because of freedom from residue problems. Despite the fact that it has a low vapor pressure, 0.00001 mm. at ordinary temperature and 0.2 mm. at the temperature of boiling water (*1*), it is an effective fumigant and its volatility is one of the major reasons for its disappearance from a plant crop (Figure 1). More important than this is the high potency to insects, which is approximately ten times that of DDT. This means that lower dosages are used, thus greatly reducing any residue problem.

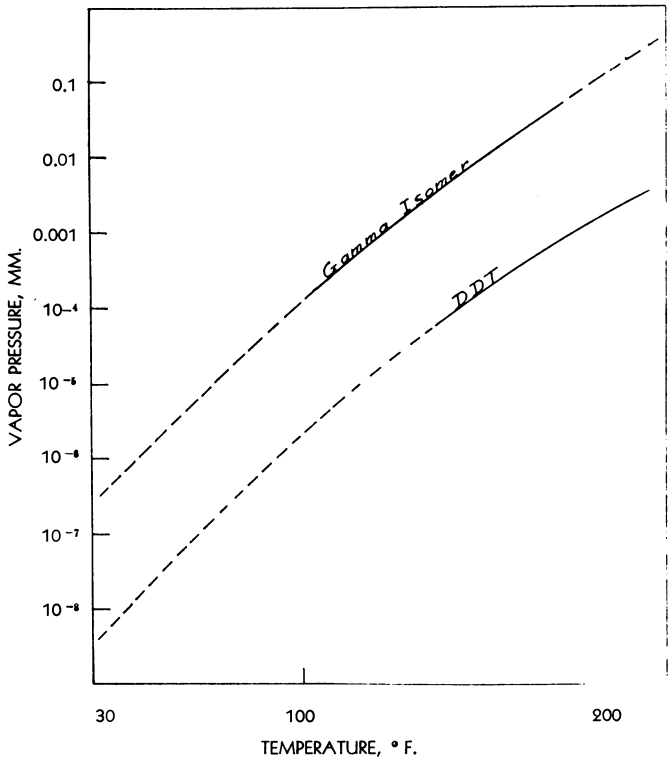

Figure 1. Vapor Pressure of Gamma Isomer of Hexachlorocyclohexane and DDT

To date, within the scope of the writers' information, there has been no residual deposit or poison residue recovered from treated fruits or vegetables, where proper formulations and amounts of the pure gamma isomer have been used not later than 2 weeks prior to crop harvest. Likewise, there is no known record of poisoning to man or animals from applying the insecticide or eating food treated with the insecticide. Freedom from poisonous residue and undesirable taste in the use of the pure gamma isomer of hexachlorocyclohexane is achieved therfeore by proper formulation, timing, and application of insecticide treatments.

The pure gamma isomer has been found to be an excellent soil insecticide for control of most common soil insects. Persistence of the material in the soil is longer than on plant surfaces. The possibility of flavoring of root and tuber crops grown in infested soil is also a problem and this has been worked out in the following manner: First, light dosages such as 0.125 to 0.25 pound of gamma isomer per acre may be used in soils to be planted to potatoes, which are probably the most sensitive crop as regards imparting of flavor, or to other tuber crops. Such treatment has not been found to impart unfavorable taste to most root crops. Second, the soil may be treated with a heavier dosage, such

as 0.25 to 0.5 pound of gamma isomer per acre, and a nonaffected crop planted for that season. So far this procedure has not produced undesirable results in most tuber or root crops grown during the season following treatment (Table I).

Table I. Pure Gamma Isomer Taste Test on Potatoes

Gamma Isomer, Lb./Acre	Objectionable Taste							
	Taster 1	Taster 2	Taster 3	Taster 4	Taster 5	Taster 6	Taster 7	Taster 8
0.125	None	None	None	None	Slight	None	None	None
0.25	None	None	Slight	Slight	None	Slight	None	None
Check untreated (second year)	None	None	None	None	None	None	None	None

The application of pure gamma isomer to plants for insect control has created similar problems, in that undesirable taste might be imparted to mature fruits or vegetables (Table II).

Table II. Pure Gamma Isomer Taste Test on Canned Tomatoes

Gamma Isomer, Lb./Acre	Objectionable Taste			
	Taster 1	Taster 2	Taster 3	Taster 4
0.25	None	None	None	None
0.5	None	None	Slight	None
Check untreated	None	None	None	None

This factor is handled by applying the chemical not later than 30 days before harvest on crops that might hold some residual taste. On other crops it has been possible to use the chemical to within 2 weeks of harvest without retaining undesirable taste. This point is largely one of varieties, so that it becomes necessary to specify on labels which crops must be treated before fruits or heads form, which can be treated up to 2 or 3 weeks prior to harvest, and which have no particular time limit. The following fruits and vegetables are among those satisfactorily treated.

In the eastern United States apples of the Delicious variety treated 10 days prior to harvest with pure gamma isomer showed no trace of off-flavor at harvest time. Dusts and sprays applied to carrot seedlings produced no traces of off-flavor in the mature vegetables. Several tasters could not differentiate between peaches sprayed within a few days of harvest and check fruit. Celery in Florida sprayed twice, once within 6 weeks of harvest and once within 30 days of harvest, was canned and put through a severe series of tests.

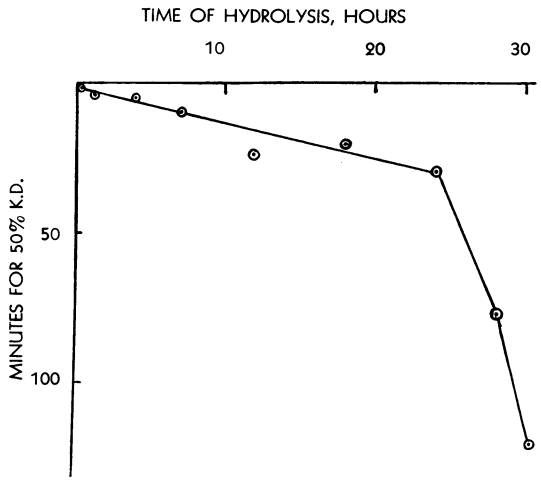

Figure 2. Tetraethyl Pyrophosphate Persistence Test

Filter papers treated with solution and exposed to *Drosophila*

Reports by the National Canners Association showed negative results on both taste tests and analytical tests for residual chlorides. Asparagus treated and canned in a manner comparable to the celery also gave completely negative results.

It has thus been possible to work out satisfactory applications of this chemical on many crops without the danger of poison or other undesirable residues on the harvested crops.

The use of pure gamma isomer of hexachlorocyclohexane on livestock has also been worked out. It has been found possible to use the wettable powder formulation dispersed in water as a spray on livestock for control of flies, lice, and ticks. Proper dosage and application must be used, of course, but this is again indicative of the safety factor of this insecticide.

Tetraethyl Pyrophosphate

Early work in the laboratory and in the field soon established the fact that there would be no poison residue factor with this chemical, owing to its rapid decomposition. Within a relatively few hours the chemical broke down into diethyl phosphoric acid

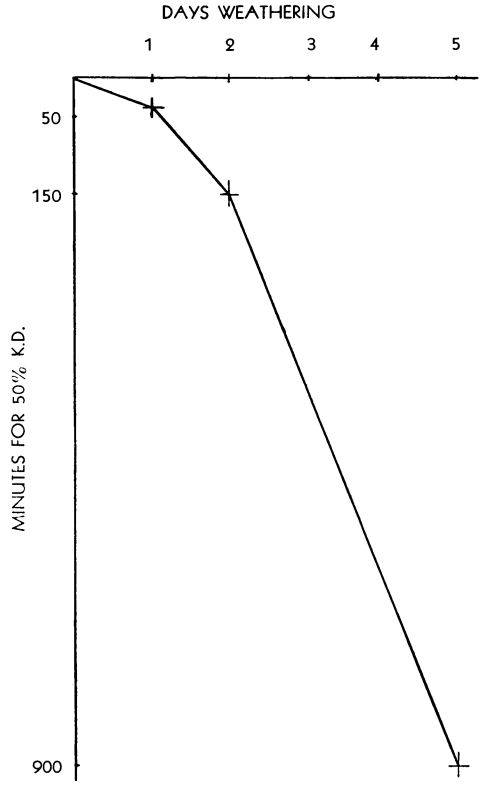

Figure 3. Tetraethyl Pyrophosphate Persistence Test

Fly cage sprayed with tetraethyl pyrophosphate and weathered in laboratory. Flies introduced at stated intervals

and finally ethyl alcohol and phosphoric acid, both of which are nonpoisonous in relatively large amounts, especially in view of the low dosage used (Figures 2 and 3). Although this chemical was found to be highly toxic in its pure form to both insects and warm-blooded animals, the rapid hydrolysis on exposure to air or moisture eliminated the

poison residue problem. This has been verified by field usage experience and laboratory tests.

In the laboratory the chemical was put into water at the ordinary spray dilution of 1 to 800 and after 24 hours' standing the treated water was used as drinking water for test animals. There were no reactions, evidence of poison, or undesirable effects on any animals as a result of these tests, even with long feeding periods. It was not possible to differentiate between test animals and check animals by any of the customary tests.

Formulations of tetraethyl pyrophosphate as an emulsive concentrate proved to be relatively complex, considering the apparent ease of formulation. Because of the unstable nature of the chemical, dust formulations were considered impossible. It was found after extensive research work that a dust which would be stable for 10 days to 2 weeks could be made with a specially selected and processed filler.

Field test work with the chemical has consisted of many tests and a large number of commercial applications in both spray and dust forms. Insecticidal action has been satisfactory in all cases where materials have been properly applied. No toxic residue has been found on any treated plants or food crops, which include most varieties of crops.

A sample of hops which had been treated with tetraethyl pyrophosphate showed a negative chemical analysis. The plant material was also extracted and the extract added to the drinking water of test animals and sensitive insects. The animals and insects that drank this treated water for several days showed no reaction. With the sensitive insects it would have been possible to detect even a few parts per million. In addition, there have been extensive commercial field applications of the chemical in dust and spray form to crops such as apples, pears, grapes, celery, broccoli, Brussels sprouts, and others up to within a few days of harvest; there has been no detectable poison residue on any of the crops. The lack of poison residue with use of tetraethyl pyrophosphate is due to the fact that it hydrolyzes within a few hours of application, breaking down into transient nonresidual and nonpoisonous chemicals. Thus it is possible to use tetraethyl pyrophosphate well up to harvest time of food products without danger of residual poison on crops. The fact that the chemical is used in extremely small amounts is a definite advantage in respect to freedom from poison residue.

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Selenium Residue on and in the Peel of Washington Apples

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The average selenium residue on and in the peel of 30 samples of Jonathan, Delicious, and Winesap apples with no history of selenium sprays was found to be 0.001 p.p.m. The selenium residue on and in the peel of Jonathan and Delicious apples that had received one or more sprays of selenium ranged from 0.020 to 0.155 p.p.m. The addition of 1 quart of light grade petroleum oil increased the selenium residue. When single-tree plots were sprayed twice with selenium sprays significantly more selenium on and in the peel was found than in the unsprayed fruit, but there was significant difference due to variety or to position of sample on tree.

The literature on the distribution of "natural" selenium in the soils, the absorption by vegetation, the toxicity of compounds of either natural or applied selenium to man and animals, and the use of selenium as a spray for insect control is voluminous and no attempt is made to cover it here. The literature has been well reviewed fairly recently (*2*). Very little has been published on the increase in selenium content of apple peelings, due to the application of selenium-bearing sprays.

A proprietary selenium compound, known as Selocide, has been proposed for the control of Pacific mite, *Tetranychus pacificus* McG., and European red mite, *Paratetranychus pilosus* Can. and Fanz., in the apple-producing areas of the state of Washington. Selocide has been used on an experimental basis and to a limited extent on a commercial basis.

Selocide is reported (*4*) to be a 30% solution of a mixture of potassium hydroxide, ammonium hydroxide, sulfur, and selenium in the proportions corresponding to the empirical formula $(KNH_4S)_5Se$. The commercial material contains 48 grams of selenium per liter, or approximately 6.4 ounces per gallon. The reactions that occur when a concentrated solution of Selocide is diluted with water to prepare a spray mixture, in the presence of carbon dioxide and oxygen of the air, may be represented as follows:

$$4(KNH_4S)_5Se + 10CO_2 \text{ (from air)} + 15O_2 \text{ (from air)} + H_2O \longrightarrow$$

$$10K_2CO_3 \text{ (soluble)} + 20NH_3 + 10H_2S + 10SO_2 + 4Se \text{ (metallic)} + H_2O$$

The Selocide spray residue remaining on the fruit is red metallic selenium (*3*).

Selocide is not compatible with acid lead arsenate ($PbHAsO_4$) under normal spray program conditions, but is compatible with cryolite (Na_3AlF_6) (*5*). Selocide is compatible with DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] and has been used with DDT for the control of mites and Codling moth, *Carpocapsa pomonella* L., during the 1947 and 1948 growing seasons.

Methods and Procedure

Selenium on and in the peel of apples was determined by the procedure of the Association of Official Agricultural Chemists (*1*). Samples of approximately 1 kg. of whole fruit were hand-peeled with a Devault peeler and the parings were digested as directed (*1*, 29.60).

Samples, with no selenium on or in the fruit, were prepared for analysis and known quantities of selenium were added. These were analyzed by the above noted methods. The quantity of selenium recovered and the percentage recovery are presented in Table I.

Table I. Recovery of Selenium Added to Apple Parings

Added, γ	Recovered, γ	Recovery, %
60	59.8	99.6
60	58.8	98.0
80	78.4	98.0
80	82.2	102.8
120	119.0	99.7
120	121.2	101.0
200	198.0	99.0
200	194.2	99.4
	Average recovery (8)	99.4
	Range	98.0 to 102.8

Selenium in Unsprayed Fruit

To establish the level of selenium on and in the peel of fruit not sprayed with selenium, samples were collected from thirty different commercial growers whose insect-control program contained no selenium sprays. Samples of approximately one-half box (0.5 bushel) were taken at random from incoming trucks at the fruit packing warehouses. Only fruit from growers whose spray programs were definitely known by the field representatives of the warehouses were selected for sampling. Ten samples per variety, of Jonathan, Delicious, and Winesap from ten different growers, were taken for analysis. The half-box samples were taken to the laboratory and subsampled for analysis. All subsamples were properly labeled and stored at 32° F. for 3 to 4 months before being analyzed.

The selenium residue on and in the peel of the non-selenium-sprayed fruit is presented in Table II.

Table II. Selenium Residue on and in Peel of Non-Selenium-Sprayed Fruit

Variety[a]	No. of Fruit per Sample	Total Weight of Fruit per Sample, Grams	Average Selenium[b], P.P.M.	Selenium Range, P.P.M.
Jonathan	7 to 11	1000 to 1102	0.001	0.000 to 0.005
Delicious	5 to 8	1020 to 1160	0.0006	0.000 to 0.004
Winesap	6 to 11	982 to 1112	0.001	0.000 to 0.004

[a] 10 samples per variety analyzed in duplicate.
[b] Whole fruit basis.

The samples reported in Table II carried a very low residue level of selenium on and in the peel. The average value of 0.001 p.p.m. for the thirty samples was less than the variation within varieties and less than the variation between duplicates. The selenium residue of 0.001 p.p.m., the average, can be considered as a possible trace of selenium on and in the peel.

Selenium in Sprayed Fruit

To establish the level of selenium on and in the peel of selenium-sprayed apples, samples were collected from five commercial Jonathan apple orchards and eight commercial Delicious apple orchards by representatives of the packing warehouses and submitted to the laboratory for analysis. The sampling and analytical procedure used for these samples was identical to that used for the samples reported in Table II. Sufficient fruit was available for duplicate analysis of most of the samples.

The results obtained from these samples and the dates of the selenium sprays are presented in Table III.

Table III. Selenium Residue on and in Peel of Selenium-Sprayed Fruit

Sample Weight, Grams	No. of Fruit per Sample	Selenium, P.P.M.[a]	Average Selenium, P.P.M.	Dates Selenium Sprays Applied[b]
		Jonathan Apples		
1052	8	0.03	0.03	May 5
1002	7	0.02		
1016	6	0.03	0.03	June 1
1039	7	0.05		
924	7	0.03		
1082	7	0.06	0.05	May 6 and June 18
1010	8	0.07		
1050	7	0.08	0.07	May 5 and 28
1090	8	0.06		
1036	7	0.07	0.06	May 6 and June 18
		Delicious Apples		
1004	8	0.03	0.03	May 5
1178	6	0.03		
1102	6	0.04	0.04	May 28
1048	9	0.04		
1070	9	0.02	0.03	May 5 and June 1
1064	7	0.05		
1058	7	0.07	0.06	May 5 and June 28
1028	5	0.09		
1072	5	0.03	0.06	May 5 and June 1 and 28
1011	7	0.11		
1062	8	0.10	0.10	May 5 and July 9
1128	6	0.09		
1090	6	0.12		
1118	6	0.12	0.11	May 13 and June 14[c]
1022	6	0.16		
1020	6	0.14		
1072	6	0.13	0.14	May 13 and June 14[c]
	Av. (27)	0.07		
	Range	0.02 to 0.16		

[a] Whole fruit basis.
[b] All spray programs consisted of 1 pint of Selocide plus DDT per 100 gallons.
[c] Sprays applied on June 14 had 1 quart of light grade petroleum oil per 100 gallons (California state specification).

Statistical analysis of the data in Table III shows the increase in selenium on and in the peel, over the samples in Table II, to be highly significant. The amount required for significance at the 0.01 level was found to be 0.003 p.p.m. The selenium residue on and in the peel is roughly proportional to the number of applications and the length of time between spraying and harvesting. The addition of 1 quart of light grade petroleum oil significantly increased the selenium spray residue remaining on and in the peel.

Effect of Variety and Location of Sample on Tree

Single-tree plots of Jonathan, Delicious, and Winesap apples were sprayed on June 2 and 28 with 1 pint of Selocide and 1 pound of actual DDT per 100 gallons. Samples of one full box were selected from the north, south, east, and west sides and from the top of the tree to determine the effect of prevailing winds, amount of sunshine, etc., on the selenium residue. These samples were subsampled in the laboratory, stored, and analyzed by the same procedure as the samples in Table II. The results are presented in Table IV.

Table IV. Effect of Variety and Location of Sample on Tree

Variety[a]	No. of Fruit per Sample	Weight of Fruit per Sample, Grams	Av. Selenium Residue, P.P.M.[b]	Range in Selenium Residue, P.P.M.
Jonathan	8 to 11	1016 to 1138	0.16	0.14 to 0.17
Delicious	6 to 9	1006 to 1126	0.12	0.10 to 0.16
Winesap	7 to 12	1000 to 1140	0.11	0.08 to 0.17
			Av. (30) 0.13	
			Range 0.06 to 0.17	

[a] 5 samples per variety analyzed in duplicate.
[b] Whole fruit basis.

Statistical analysis of the data in Table IV shows the selenium residue on and in the peel to be highly significant over the residue on and in the peel of nonsprayed apples. There is no significant difference between varieties. Selenium residue is evenly distributed on the trees.

Acknowledgment

Appreciation is expressed to the McLaughlin-Gormley-King Company for assistance in conducting this work.

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Fruit Surface Residues of DDT and Parathion at Harvest

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Surface residues of DDT and parathion at various times during the season and at harvest were determined for apples, pears, peaches, oranges, and lemons. Low level surface residues of parathion on apples were not carried over into cider. Harvest residues on fresh fruit are distinguished from residues present in food at the time of consumption which are included under the designation ultimate residues.

With reference to the surface deposits accruing from orchard application of insecticides, certain characteristics which enhance their value as implements in the chemical control of many species of insects—physical persistency and chemical stability—may also be conducive to the contamination of the harvested fruits with potentially deleterious residues.

Supplementary to the need for data on the acute and chronic toxicity of these insecticides to man and domesticated animals is the requirement for information concerning the magnitudes of the deposits that are present on or in foodstuffs as harvest or ultimate residues following commercial usage.

The widespread commercial use of DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane] and the potentialities of the more recently developed parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate) have placed emphasis on such investigations concerning these compounds.

The Food and Drug Administration has not as yet held hearings concerning the establishment of formal tolerances for DDT or parathion on fresh produce or in processed foods. For apples and pears, an informal tolerance for DDT of 7 p.p.m. has been announced (*3*). [These hearings were in progress at the time of publication.]

Scope, Definitions, and Methods

This investigation is a portion of a general experimental program being carried out by the University of California Citrus Experiment Station on the fate of insecticide residues (*2*) and methods of removing them (*5*). The discussion presented herein involves tree fruits and is largely restricted to a consideration of "surface residues." This term has been defined (*5*) to refer to residues present above the cuticle (extrasurface residues) and to deposits that may be incorporated in the cuticle (cuticular residues). The quantities reported as surface residues are those present in the solvent following standardized extraction procedures and include both extrasurface and cuticular residues. Little is known as to what extent insecticide residues may be redistributed through the epidermal layer in the process of solvent extraction or stripping.

While most of the data presented are representative of harvest residues on fresh fruit, some consideration is also given to residues present in processed food. It is considered

appropriate to refer to residues present in food at the time of consumption as "ultimate residues" (*5*).

DDT was determined by the dehydrohalogenation method (*4*). Parathion analyses were made by the magenta color reaction of Averell and Norris (*1*) as modified by Gunther and Blinn (*6*). Two compounds or degradation products thereof which may cause the development of interference colors in the magenta color reaction may be encountered in surface residues resulting from commercial spray or dust applications. The first of these, dicyclohexylamine dinitro-*o*-cyclohexylphenate, is in widespread commercial use, whereas the second, a dinitrocaprylphenylcrotonate, is involved at present only in experimental and semicommercial usage. Blank corrections were provided for all sets of analyses.

Samples for estimates of surface residue parameters on apples and pears were taken from three trees selected for representative size and shape among those of the experimental orchard. Each of the three samples consisted of 30 fruits. Six fruits were taken from each of four tree quadrants composing three fourths of the tree height and six from the top one fourth of the tree. These samples were generally taken before and after the penultimate and ultimate orchard applications and at harvest. Samples for analyses of cider were taken from juice expressed with a hydraulic cider press. Samples of fruit for pressing were selected at harvest from a series for which parallel analyses for surface and pulp residues were made. Each of the triplicated peach samples was constituted by selecting three fruits from each of eight trees. Citrus varieties were sampled by selecting one fruit from each quadrant of seven trees. Replicate samples were taken from other sets of seven trees. Sample routing and manipulation have been described (*6*, *7*).

Table I. Surface Residues of DDT on Rome Beauty Apples at Harvest

Technical Compound[a], Lb./100 Gal.	No. of Applications	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M.
1	3	75	1.0
0.5	4	50	1.5
0.5	4	50	0.3
0.5	5	40	2.0
0.5	6	40	3.8
1	6	60	5.2

[a] As wettable powder containing 50% technical DDT.
[b] Since final application.

Table II. Surface Residues of DDT on Bartlett Pears at Harvest

Technical Compound[a], Lb./100 Gal.	No. of Applications	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M.
2	1	110	0.9
1	1	110	0.4
1	2	70	2.2
0.5	2	70	1.6
0.5	2	85	0.9
0.5	3	40	1.2
0.5	4	14	2.7

[a] As wettable powder containing 50% technical DDT.
[b] Since final application.

Results

The amounts of surface deposits resulting from commercial and experimental applications of DDT and parathion were ascertained on apples, pears, peaches, oranges, and lemons. Applications were made with conventional high pressure spraying equipment, utilizing manually operated guns or semiautomatic booms, and with two types of air blast sprayers.

Surface residues of DDT on apples resulting from experimental applications applied in schedules comparable to commercial usage ranged from less than 0.5 to 2.0 p.p.m. at harvest. Schedules of application involving higher spray concentrations and greater frequency of application than are at present required in commercial practice resulted in most cases in residues of less than 7 p.p.m. Typical harvest residues are presented in Table I.

On pears, experimental applications of DDT applied in commercial schedules resulted in surface residues ranging from less than 0.5 to approximately 3 p.p.m. Typical harvest residues are shown in Table II.

Intervals between application and sampling of apples retaining weathered surface residues of parathion ranged between 21 and 70 days. At harvest, these residues were 0.05 p.p.m. or less, following each of 13 varied schedules of application (Table III). (These values are derived from samples containing an optimum amount of parathion for analytical precision.) Low level surface residues on apples were not carried over as ultimate residues in cider (Table III). This fact supports the contention that there is close association of the parathion deposits with the waxlike coating of the fruit.

Table III. Surface Residues of Parathion on Apples

Variety	Technical Compound[a], Ounces/100 Gal.	Application	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M. Pretreatment	Fresh Weight, Entire Fruit, P.P.M. Posttreatment	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M. Surface	Fresh Weight, Entire Fruit, P.P.M. Cider
Conventional Sprayer								
Rome Beauty	1	3rd	21	0.02	0.3	..	..	..
		4th	25	0.01	0.3	41	0.02[c]	..
	2	3rd	21	0.04	0.4	..	..	..
		4th	25	0.02	0.4	41	0.02[c]	..
	4	3rd	21	0.09	1.3	..	..	..
		4th	25	0.09	1.0	41	0.05[c]	..
	2	1st	..	..	0.5	70	Trace[c]	..
	4	1st	..	..	1.2	70	Trace[c]	..
Delicious	1	2nd	27	0.01	0.5	..	..	..
		3rd	36	0.01	0.3	29	0.01[c]	..
	2	2nd	27	0.02	0.8	..	..	..
		3rd	36	0.01	0.6	29	0.01[c]	..
	2	3rd	27	0.03	1.4	..	..	..
		4th	36	0.02	0.6	29	0.02[c]	0.00[d]
	4	2nd	27	0.09	2.2	..	..	..
		3rd	36	0.05	0.9	29	0.05[c]	0.00[d]
Speed Sprayer								
Delicious (400 gal./acre)	2	1st	..	..	0.3	22	0.01	..
Delicious (200 gal./acre)	4	1st	..	..	0.4	22	0.01	..
	4	1st	..	..	0.5	22	0.04	..
Delicious (100 gal./acre)	8	1st	..	..	0.2	22	0.01	..

[a] As wettable powder supplied by American Cyanamid Co. containing 25% technical parathion.
[b] Since previous treatment.
[c] Harvest.
[d] Values reported for cider are from duplicate analyses of juice expressed from entire fruits of same series bearing indicated surface residues at harvest.

On pears (Table IV) parathion residues resulting from ten varied schedules did not exceed 0.08 p.p.m. at harvest. (These values are derived from samples containing an optimum amount of parathion for analytical precision.) Intervals between the terminal application and harvest ranged from 7 to 33 days.

Surface residues of parathion on peaches were 4- to 15-fold higher than for comparable schedules on apples or pears, possibly because of the higher initial deposits retained on the more retentive surfaces of these fruits. Surface residues of DDT on peaches were also higher than those which would be expected to result from comparable schedules on apples and pears. Typical residue values for peaches are shown in Table V.

As compared with the residues of DDT present within citrus peel (*2*), the relative significance of surface residues of DDT on citrus fruits is diminished by the fact that these are readily removed in large proportion by the usual packinghouse processing (*5*).

On oranges, typical surface residues of DDT at harvest ranged from less than 0.5 to approximately 2.5 p.p.m. following single applications. Under conditions of multiple applications and shorter intervals between treatment and sampling, a higher range of surface residues was found. Equivalent or larger amounts were generally present within the peel (*2*). Representative surface residues are presented in Tables VI and VII.

Table IV. Surface Residues of Parathion on Bartlett Pears

Technical Compound[a], Ounces/100 Gal.	Application	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M. Pretreatment	Fresh Weight, Entire Fruit, P.P.M. Posttreatment	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M.
			Conventional Sprayer, Single Gun			
2	2nd	28	0.01	0.3	7	0.08[c]
2	3rd	27	0.01	0.5	..	..
	4th	21	0.03	0.7	33	0.01[c]
4	3rd	27	0.02	0.8	..	..
	4th	21	0.04	1.1	33	0.02[c]
8	3rd	27	0.03	2.6	..	..
	4th	21	0.08	2.1	33	0.04[c]
			Conventional Sprayer, Broom Guns			
2	2nd	23	0.01	0.3	7	0.06[c]
			Conventional Sprayer, Fixed Nozzle Vertical Boom			
2	4th	22	0.01	0.2	28	0.02[c]
			Spray Duster (Master Fan Type)			
8	1st	..	..	0.2	..	..
(200 gal./acre)	2nd	27	0.03	0.3	22	Trace[c]
			Speed Sprayer			
1	1st	..	..	0.1	25	0.00
2	1st	..	..	0.2	25	Trace
4	1st	..	..	0.3	25	Trace

[a] As wettable powder containing 25% technical parathion.
[b] Since previous treatment.
[c] Harvest.

Table V. Attenuation of Posttreatment Surface Residues of Parathion and DDT on Lucas Beauty Peaches

	Technical Compound, Ounces/100 Gal.	Interval, Days 0	7	14	30
		Fresh Weight, Entire Fruit, P.P.M.			
Parathion[a]	4	4.5	1.5	1.0	0.2[b]
Parathion	8	10.9	3.7	1.8	0.6[b]
DDT[c]	8	16.1	7.6	4.4	2.7[b]

[a] As wettable powder containing 25% technical parathion.
[b] Harvest.
[c] As wettable powder containing 50% DDT.

Table VI. Attenuation of Posttreatment Surface Residues of DDT on Citrus Following Single Applications

Type Formulation	Technical Compound, Lb./100 Gal.	Interval, Days[a] 2	141	2	141	2	141
		Fresh Weight, Entire Fruit, P.P.M.					
		Navels		Valencias		Lemons	
Wettable powder	2.0	17.1	2.1[b]	16.2	...	23.9	1.7
Kerosene solution	2.0	8.9	2.3[b]	4.9	...	14.0	2.3
Kerosene solution	1.0	2.8	1.5[b]	3.0	...	3.2	0.2
Light medium oil solution	1.2	2.2	0.2[b]	2.1	...	2.7	1.3

[a] Application, 10/1/45.
[b] Harvest.

Table VII. Surface Residues of DDT on Valencia Oranges at Harvest Following Sequence Applications

Applications[a] 5/9/46	6/7/46	8/9/46	Interval, Days[b]	Fresh Weight, Entire Fruit, P.P.M.
S	..	..	117	2.3
S	½ S	..	88	5.8
S	S	..	88	8.4
S	..	S	25	6.3

[a] Kerosene-Velsicol AR-60 (95-5) 3 gallons, technical DDT 2 lb., and blood albumin spreader 0.25 lb. per 100 gallons of water applied as full coverage spray (S) or outside coverage spray (½ S).
[b] Since application.

On lemons, surface residues at harvest ranged from 2 to 3 p.p.m. (Table VIII), somewhat larger amounts being generally present within the peel (*2*).

Table VIII. Attenuation of Surface Residues of DDT on Eureka Lemons

Technical Compound[a], Lb./100 Gal.	Interval, Days				
	1	23	35	44	84
	Fresh Weight, Entire Fruit, P.P.M.				
2	15.8	6.4	...	2.9[b]	...
2	....	...	7.8	...	2.3[b]

[a] Kerosene-Velsicol AR-60 (95-5) 3 gallons, technical DDT 2 lb., and blood albumin spreader 0.25 lb. per 100 gallons of water.
[b] Harvest.

Studies on oranges (Table IX) have shown that surface residues of parathion are also of relatively low magnitude in comparison with the quantities found within the peel (*2*). Surface residues on these fruits decreased rapidly (*5*), reaching values of 0.01 p.p.m. or less at harvest following a single application at required dosages.

Table IX. Surface Residues of Parathion on Navel Oranges at Harvest

Technical Compound[a], Lb./100 Gal.	No. of Applications	Interval, Days	
		110	140
		Fresh Weight, Entire Fruit, P.P.M.	
1	1	..	0.01
1.25	1	..	0.02
1.5	1	..	0.09
1	2	0.20	..

[a] As wettable powder containing 25% parathion. First application 9/15/47, second application 10/15/47.

Acknowledgment

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DDT and Parathion Spray Residues on Apples

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The tests reported were conducted in 1948 on apples growing in the Yakima Valley in the Pacific Northwest and in the Mississippi Valley, to determine the magnitude of parathion and DDT spray residues at harvest. The climates and spray schedules differ markedly in the two areas; consequently, spray residues also differ, and are larger in the Mississippi Valley than in the Yakima Valley.

The use of insecticides on the edible parts of plants presents the problem of removal or avoidance of excessive toxic residues on the harvested crops. This paper shows the magnitude of residues that may result from the application of sprays containing parathion and DDT insecticides.

In 1948 tests were made on apples growing in the Yakima Valley in the Pacific Northwest and in the Mississippi Valley. The climates of these two areas differ strikingly. The Mississippi Valley is characterized by moderately heavy precipitation during the growing season, the average rainfall being 3.5 to 4.3 inches per month. The Yakima Valley, on the other hand, is very arid, the average precipitation being less than 1 inch per month during the summer, and is completely dependent upon irrigation for orchard growth. Spray schedules also differ in the two areas. In the Mississippi Valley the orchards are sprayed more often, and the interval between the last spray and harvest is shorter, than in the Yakima Valley. Spray residues at harvest may be expected to differ also.

Methods

Spray schedules applied on experimental plots at the Yakima, Wash., and Vincennes, Ind., laboratories of the United States Bureau of Entomology and Plant Quarantine were studied to determine the magnitude of parathion and DDT spray residues at harvest. The parathion sprays were prepared from 25% wettable powder and the DDT sprays from 50% wettable powder, except in one series of tests, when a 25% DDT wettable powder was used. All spray treatments were planned and made by members of the Division of Fruit Insect Investigations. Conventional hydraulic sprayers were used in this work.

The studies in the Yakima Valley were made on two varieties of apples and those in the Mississippi Valley on five varieties. Duplicate samples of 1500 and 2000 grams of fruit were employed for each analysis at Vincennes and samples of 1000 to 1500 grams at Yakima.

Parathion analyses were made by the method of Averell and Norris (*1*). DDT residues were determined at Vincennes by the total-chlorine method of Wichman *et al.* (*4*), and at Yakima by the colorimetric method of Stiff and Castillo (*3*), as modified by the Food and Drug Administration (*2*).

Parathion Residues

Table I shows the parathion residues on Delicious and Winesap apples in the Yakima Valley immediately after the last spray application and at intervals until harvest. Parathion was used at two concentrations, both as close as possible to the minimum necessary to give the desired control. Consequently, the residues found were comparatively low and dropped to 0.1 p.p.m. or less in approximately 2 weeks.

Table I. Parathion Residues on Delicious and Winesap Apples, Yakima Valley

Plot No.	Treatment (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, lb.	No. of Sprays	Parathion Found, P.P.M. Delicious Apples July 29	Aug. 16	Oct. 11	Winesap Apples Aug. 5	Aug. 11	Aug. 17	Oct. 18
7	1.25	1	2							
	1.25	...	1	0.90	0.09	0.07	..	..	..	..
	..	1	1							
	1.25	0.5	3	..	..	..	1.10	0.36	0.10	0.03
10	..	1	1							
	1.25	...	2	0.40	0.05	0.05	..	..	..	..
11	0.6	1	1							
	0.6	0.5	1							
	0.6	...	1	0.30	..	0.03	..	..	..	..
	..	1	1							
	0.6	0.5	3	..	..	..	0.90	0.24	0.03	0.02
12	..	1	1							
	1.25	0.5	1							
	1.25	...	1	..	..	0.04	..	..	..	..

On Delicious apples the initial residues were 0.9 and 0.4 p.p.m. in plots 7 and 10, respectively, both of which were sprayed with 1.25 ounces of parathion. The difference between the two plots was consistent throughout the individual trees sampled. The spray mixture used on plot 7 also contained DDT, while that used on plot 10 contained only parathion. These plots showed the same relative magnitude of residues 18 days after spraying, and at harvest, 74 days after the spraying. Plot 11, sprayed with 0.6 ounce of parathion, showed an initial residue of 0.3 p.p.m.

Initial residues on the Winesap apples were somewhat higher than those on Delicious. Six days later approximately one third of the residue had been lost, and 12 days after the spraying the deposits were down to 0.10 and 0.03 p.p.m. for the respective strengths. At harvest little more than a trace of parathion was found on the fruit.

Table II shows the parathion residues on Golden Delicious apples in the Mississippi Valley immediately after the final spray application and after 25 and 38 days of weathering. Five plots received six parathion sprays and a sixth plot received parathion in only the last two sprays.

Table II. Parathion Residues on Golden Delicious Apples, Mississippi Valley

(Six sprays on all plots; final spray Aug. 5)

Plot No.	Treatment (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, ounces	Parathion Found, P.P.M. Aug. 5	Aug. 30	Sept. 13
27	1	8	1.09	0.19	0.10
30	2	4	3.49	0.24	0.15
25	4	8	3.04	0.37	0.20
28	4	..	3.40	0.36	0.15
24	8	..	6.14	0.88	0.42
21	..	12	3.20	0.42	0.25
	(2 sprays)				
	4	12			
	(4 sprays)				

With the exception of plot 30, the residues after the final spraying show a good correlation with the amount of parathion applied. The loss of residue was very rapid, however. The results in plot 21, which received parathion only in the last two sprays, as

compared with those in plots 25 and 28, which received the same amount of parathion in all six sprays, show that the first four parathion sprays had little effect on the residue at harvest.

Table III shows the parathion residues on Jonathan and Starking Delicious apples from seven spray plots in the Mississippi Valley. Identical treatments were used on both varieties. The Starking variety showed a slightly lower parathion residue than the Jonathan. The difference was not great, however. In general, the residue after the final spraying was proportional to the concentration of parathion in the spray mixture. The exception is plot 4, sprayed with 2 ounces of parathion with nicotine-bentonite-oil, which shows a residue approximately equal to that obtained on plots sprayed with 4 ounces of parathion, alone or in combination with DDT (plots 11 and 12). The residue 2 weeks after spraying was only one quarter to one third of that found immediately after spraying, and 46 days after spraying only one plot (No. 14 sprayed with 8 ounces of parathion) showed a residue significantly in excess of 0.1 p.p.m.

Table III. Parathion Residues on Jonathan and Starking Delicious Apples, Mississippi Valley

(Six sprays, except where otherwise indicated)

Plot No.	Treatment[a] (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, lb.	Parathion Found, P.P.M. Jonathan Apples July 30	Aug. 13	Sept. 14	Starking Delicious Apples July 30	Aug. 13	Sept. 14
9	1	1	0.53	0.12	0.03	0.32	0.07	0.03
11	4	0.5	1.41	0.41	0.11	1.22	0.24	0.07
12	4	0.5	1.23	0.33	0.09	0.99	0.23	0.04
	(Sprays 3 and 6)							
	..	1						
	(In sprays 1, 2, 4, 5)							
15	..	1.5	1.65	0.39	0.11	1.22	0.20	0.07
	(In sprays 1, 2, 4)							
	..	1						
	(In spray 6)							
	4	..						
	(In sprays 4 and 6)							
	(Sprays 3 and 5 o nitted)							
14	8	..	3.80	1.06	0.28	3.39	0.73	0.20
4	2		0.99	0.23	0.02	1.08	0.20	0.03
	(With nicotine-bentonite-oil)							
13	2	12	0.10	0.00	0.00	0.12	0.01	0.00
	(In sprays 1 to 4)							
	..	1						
	(In sprays 5 and 6)							

[a] Final parathion spray on July 28 except plot 13, which received final spray on June 16.

Plots 12 and 13 afford a comparison of the harvest residues from first- and second-brood sprays. On plot 13, which was sprayed on May 11 and 20 and June 3 and 16, the parathion residue was only 0.1 p.p.m. on July 30 and none in subsequent samplings. On plot 12, which received two sprays on July 7 and 28, the residue on Jonathan apples was 1.23 p.p.m. on July 30 and 0.09 on September 14.

Table IV gives the data from seven plots of Winesap and Rome Beauty apples in the Mississippi Valley. The spray schedules are similar to those used for the plots included in Table III, except that an additional parathion spray was applied on plots 9, 11, 14, and 4 on August 19, and the final harvest sample was taken on October 5. Only on the plot that was sprayed seven times with the 8-ounce strength of parathion (plot 14) did the spray residue at harvest approximate 0.1 p.p.m.

The data presented indicate that parathion spray residues weather very rapidly on apples. Sprays applied to the fruit prior to July 1 would not be expected to leave more than a trace of parathion on the fruit at harvest. The harvest residues vary in magnitude according to the concentration of parathion in the spray mixture. Adhesives such as nicotine-bentonite-oil, although increasing the initial residue of a given spray mixture, do not show an appreciable effect on the final harvest residue. When the concentration of parathion is not greater than 4 ounces in 100 gallons of spray, and the final spraying is done not less than 40 days before harvest, the parathion residue at harvest, under the conditions of the experiments, does not exceed 0.1 p.p.m.

Table IV. Parathion Residues on Rome Beauty and Winesap Apples, Mississippi Valley

(Seven sprays, except where otherwise indicated)

Plot No.	Treatment (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, lb.	Date of Final Parathion Spray	Parathion Found, P.P.M. Rome Beauty Apples July 30	Aug. 20	Oct. 5	Winesap Apples July 30	Aug. 20	Oct. 5
9	1	1	Aug. 19	..	0.45	0.01	..	0.72	0.02
11	4	8	Aug. 19	..	0.28	0.01	..	0.46	0.04
12	4	0.5	July 28	1.16	0.14	0.01	1.28	0.16	0.03
	(In sprays 3 and 6)								
	..	1							
	(In sprays 1, 2, 4, 5, 7)								
15	..	1.5	July 29	1.55	0.14	0.04	1.83	0.27	0.06
	(In sprays 1, 2, 4)								
	..	1							
	(In spray 6)								
	4	..							
	(In sprays 4 and 6, sprays 3, 5, 7 omitted)								
14	8	..	Aug. 19	..	0.49	0.09	..	1.03	0.12
4	2	..	Aug. 19	..	1.13	0.05	..	1.39	0.05
	(With nicotine-bentonite-oil)								
13	2	0.75	June 16	0.10	0.00	0.00	0.12	0.01	0.00
	(In sprays 1 to 4)								
	..	1							
	(In sprays 5, 6, 7)								

DDT Residues

Table V shows the DDT spray residues at harvest on Delicious and Winesap apples in the Yakima Valley. Most of the Delicious plots received two sprays containing DDT, the last one 97 days before harvest. The Winesap plots received four sprays containing DDT, the last spray 74 days before harvest. The harvest residues in the Winesap plots were significantly higher than those in the Delicious plots, but were less than one half the proposed tolerance of 7 p.p.m. of DDT.

Table V. DDT Residues on Delicious and Winesap Apples, Yakima Valley

(One to three sprays)

Plot No.	Treatment (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, lb.	No. of Sprays	Date of Last DDT Spray	DDT Found, P.P.M. Delicious apples, Oct. 11	Winesap apples, Oct. 18
4	..	1	2	July 7	1.0	...
7	1.25	1	1			
	1.25	0.5	1			
	1.25	...	1	July 7	1.0	...
	..	1	1			
	1.25	0.5	3	Aug. 5	...	2.7
10	1.25	1	1			
	(1 lb. benzene hexachloride technical + 2 quarts oil)		2	June 16	1.4	...
11	0.6	1	1			
	0.6	0.5	1			
	0.6	...	1	July 7	1.5	...
	..	1	1			
	0.6	0.5	3	Aug. 5	...	3.3
12	..	1	1			
	(1 lb. benzene hexachloride + 2 quarts oil)					
	1.25	0.5	1			
	1.25	...	1	July 7	1.7	...

Table VI shows the DDT residues at harvest on plots in a Golden Delicious orchard in the Mississippi Valley. All plots received six sprays containing DDT.

The harvest residue in plot 1, in which a 50% DDT wettable powder was used, was only slightly less than where the same amount of DDT in a 25% wettable powder was applied in plot 2. Reduction of the concentration of actual DDT in the spray mixture (plots 3 and 4) resulted in lower residues at harvest.

Table VII shows the residues of DDT at harvest in the Mississippi Valley on Jonathan and Starking Delicious apples on which a six-spray schedule was used. All plots except plot 8 were sprayed six times with DDT at 8 ounces to 1 pound per 100 gallons. Plot 8 received only four sprays, three containing 1.5 pounds and one containing 1 pound

Table VI. DDT Residues on Golden Delicious Apples, Mississippi Valley

(Six sprays on all plots; final spray Aug. 5; analyses Sept. 13)

Plot No.[a]	Treatment (Active Ingredient in 100 Gal.)[b] Parathion, ounces	DDT, ounces	DDT Found, P.P.M.
1	..	12	7.9
	(No adhesive or spreader used on 1 plot)		
2	..	12	8.8
3	..	8	7.1
	(No adhesive or spreader)		
4	2	4	3.7

[a] Plots 1 and 3 represent averages of 3 plots each.
[b] 50% wettable powder used in plot 1 and 25% wettable powder in other plots.

of DDT. In plots 2 and 3, adhesives were included in all sprays, and harvest residues approximated 10 p.p.m. of DDT even though only 8 ounces of DDT were used. In all other plots the harvest residues were less than 7 p.p.m. of DDT except in plots 7 and 8, in which they were 7.8 and 7.3 p.p.m., respectively, on the Starking apples. In general, the harvest residues were proportional to the amount of DDT applied in the last cover spray.

Table VII. DDT Residues on Jonathan and Starking Delicious Apples, Mississippi Valley

(Six sprays on all plots; final spray on July 28)

Plot No.	Treatment (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, ounces	DDT Found, P.P.M. Jonathan apples, Aug. 31	Starking Delicious apples, Sept. 2
2	..	8	7.8	10.2
	(With lead arsenate–Bordeaux-oil or nicotine-bentonite-oil)			
3	..	8	10.3	10.0
	(With nicotine-bentonite-oil)			
4	..	1	6.4	6.8
	(Av. of 5 plots, no adhesive or spreader)			
5	4	8	3.7	4.7
6	..	1	4.3	5.6
	(In sprays 1, 2, 4, 5)			
	4	8		
	(In sprays 3 and 6)			
7	2	12	5.6	7.8
	(In sprays 1 to 4)			
	..	1		
	(In sprays 5 and 6)			
8	..	1.5	6.5	7.3
	(In sprays 1, 2, 4)			
	4	..		
	(In spray 6, sprays 3 and 5 omitted)			

Table VIII. DDT Residues on Rome Beauty and Winesap Apples, Mississippi Valley

(Seven sprays on all plots; final spray on Aug. 19; analyzed on Oct. 5)

Plot No.	Treatment (Active Ingredient in 100 Gal.) Parathion, ounces	DDT, ounces	DDT Found, P.P.M. Rome Beauty apples	Winesap apples
2	..	8	9.1	11.8
	(With lead arsenate–Bordeaux-oil or nicotine-bentonite-oil)			
3	..	8	12.1	11.1
	(With nicotine-bentonite-oil)			
4	..	16	9.5	10.4
	(Av. of 5 plots, no adhesive or spreader)			
5	4	8	6.7	6.9
6	..	16	8.6	9.8
	(In sprays 1 2, 4, 5, 7)			
	4	8		
	(In sprays 3 and 6)			
7	2	12	11.3	11.2
	(In sprays 1 to 4)			
	..	16		
	(In sprays 5, 6, 7)			
8	..	24	5.1	6.8
	(In sprays 1, 2, 4)			
	4	16		
	(In spray 6, sprays 3, 5, 7 omitted)			

Table VIII shows the residues of DDT at harvest on Rome Beauty and Winesap apples in the Mississippi Valley. The plot treatments are the same as for Jonathan and Starking Delicious apples (Table VII) except that a seven-spray schedule was used. The residues at harvest shown in Table VIII are greater than those in Table VII. A comparison shows that when six cover sprays of DDT are applied without adhesives the harvest residues are approximately 7 p.p.m. or slightly more. If, however, seven cover sprays are applied, the residues may exceed 9 p.p.m. of DDT, unless the concentration is reduced to less than 1 pound of DDT in 100 gallons.

Summary

In 1948 spray schedules used experimentally in the Yakima Valley in the Pacific Northwest and in the Mississippi Valley were studied to determine the magnitude of the parathion spray residues during the interval between the last spray and harvest. DDT residues were also studied and results of analyses made at harvest are reported.

The spray schedules studied in the Yakima Valley included at least three sprays containing not more than 1.25 ounces of parathion in 100 gallons. Harvest residues were less than 0.1 p.p.m. of parathion.

In the Mississippi Valley the studies included sprays containing as much as 8 ounces of parathion in 100 gallons. When 4 ounces or less of parathion were used, and no spray was applied less than 40 days before harvest, parathion residues were generally less than 0.2 p.p.m. Increasing the concentration of parathion in the spray mixture or decreasing the time interval between the last spray and harvest sometimes resulted in heavier residues.

Spray schedules using 1 pound or less of DDT in 100 gallons in one to four sprays were studied in the Yakima Valley. DDT residues were well below the proposed tolerance of 7 p.p.m. in all treatments studied. A four-spray schedule with 74 days between the last spray and harvest resulted in a residue of only 3.3 p.p.m. of DDT at harvest.

Spray schedules with as much as 1.5 pounds of DDT in 100 gallons were studied in the Mississippi Valley. The number of sprays containing DDT was as high as seven, six being applied in most of the treatments. A six-spray schedule in which 1 pound of DDT was used, without any adhesive, resulted in harvest residues approximating or slightly in excess of 7 p.p.m. of DDT. When seven sprays were used, DDT residues in some treatments were considerably in excess of 7 p.p.m.

The use of adhesives, such as nicotine-bentonite-oil or lead arsenate–Bordeaux-oil, increased the DDT residues at harvest.

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Parathion Spray Residue on Soft Fruits, Apples, and Pears

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The amount of parathion spray residue on soft fruits is roughly proportional to the length of time between date of application and date of analysis. Parathion spray residue was lost from the surface of Delicious apples at the rate of 80 to 85% in 12 to 13 days and 93 to 100% in 30 to 32 days. The rate of loss was the same for 1-pound as for 4-pound concentrations. Fifty-five samples, collected from commercial orchards, were analyzed. No significant relationship was found between the number of days between spraying and analysis and the parathion residue. There was no significant difference in parathion residue due to the concentration of the spray mixture used. All residues were only a fraction of 1 p.p.m.

Parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate) is an ester of thiophosphoric acid with the empirical formula $C_{10}H_{14}NO_5PS$. It is a high boiling deep-brown to yellow liquid, some samples of which possess a characteristic odor. Its boiling point has been calculated to be 375° C. or higher, at 760 mm. pressure; its refractive index is n_D^{25} 1.15360; specific gravity is 1.26. The vapor pressure is 0.0006 mm. of mercury at 24° C. The technical grade has a purity of approximately 95%.

Parathion is very slightly soluble in water (20 parts per million), but is completely miscible in many organic solvents including esters, alcohols, ketones, ethers, aromatic and alkylated aromatic hydrocarbons, and animal and vegetable oils. It is practically insoluble in such paraffinic hydrocarbons as petroleum ether, kerosene, and refined spray oils (about 2%) unless a mutual solvent is used (*1*).

Parathion has been recommended in the state of Washington (*3*) for the control of various species of mites and orchard aphids. Wettable powders, containing 15 or 25% of parathion, have been used at concentrations ranging from 0.25 to 4 pounds per 100 gallons of water on an experimental basis and from 0.25 to 0.5 pound on a commercial basis.

Methods and Procedure

Parathion spray residue on the surface of the fruit was determined by the method of Averell and Norris (*2*). Samples of sufficient size of firm fruit completely to fill 1-gallon wide-mouthed glass jars were selected for analysis.

The parathion was removed from the surface of the fruit with benzene (redistilled) in an end-over-end type tumbling machine at a speed of 72 revolutions per minute. All samples were washed in this manner for a period of 0.5 hour. Reagent blanks and unsprayed fruit blanks were run with all samples. All results, as reported, have been corrected for reagent and fruit blanks.

Soft Fruit

Although parathion has not been recommended to date for insect control on soft fruits, there are indications that some injurious insects may be controlled by applications of parathion. Experimental sprays of 1 pound of the 25% wettable powder per 100 gallons of water were applied to soft fruits to determine the amount of parathion residue that would remain on the surface at harvest time. The parathion residue at harvest time is presented in Table I.

Table I. Parathion Residue on Surface of Soft Fruits at Harvest Time

Variety	Days between Last Spray and Analysis	Surface Parathion, P.P.M.
Bing cherry	14	0.30
	25	0.08
	36	0.08
	43	0.08
Morpark apricot	75	0.00
	79	0.00
Tilton apricot	14	0.40
	20	0.21
	38	0.00
	47	0.01
	72	0.02
Yakamine	28	0.03
Italian prune	60	0.00
Elberta peach	7	1.63
	30	0.80
	55	0.03
	75	0.00
	97	0.01
Golden Jubilee peach	38	0.57
	38	0.76[a]
J. H. Hale peach	53	0.00

[a] Two sprays of 2 pounds of 25% wettable powder per 100 gallons.

Parathion, used for the control of injurious insects on soft fruits, would be applied more than 30 days prior to harvest. Infestation of the various potential insects is relatively early in the spring. Where sprays of 1 pound of 25% wettable powder per 100 gallons of water were applied more than 30 days before harvest, the parathion residue at harvest time on Bing cherries, Morpark and Tilton apricots, Yakamines, Italian prunes, Elberta and J. H. Hale peaches ranged from 0.00 to 0.08 p.p.m., as shown in Table I. When less than 30 days were allowed to elapse between spraying and analysis, the parathion residue on the above soft fruits ranged from 0.30 to 1.63 p.p.m. Increased concentrations of parathion applied to Golden Jubilee peaches resulted in a higher parathion residue.

The amount of parathion residue was roughly proportional to the length of time between date of application and date of harvest.

Table II. Parathion Residue on Pears and Apples

	Area Sampled and Parathion Residue, P.P.M.						
Variety	North side	South side	East side	West side	Top	Average	Range
Bartlett pear[a]	0.01	0.00	0.00	0.03	0.01	0.01	0.00 to 0.03
Bartlett pear[a]	0.02	0.04	0.04	0.00	0.02	0.02	0.00 to 0.04
D'Anjou pear	0.04	0.03	0.04	0.05	0.04	0.04	0.03 to 0.05
D'Anjou pear	0.02	0.00	0.00	0.04	0.00	0.01	0.00 to 0.04
Jonathan apple	0.02	0.02	0.01	0.01	0.03	0.02	0.01 to 0.03
Delicious apple	0.04	0.02	0.01	0.01	0.03	0.02	0.01 to 0.03
Winesap apple	0.02	0.03	0.04	0.04	0.04	0.03	0.02 to 0.04

[a] Plots sampled twice.

Apples and Pears

Single tree plots of Bartlett and D'Anjou pears and Jonathan, Delicious, and Winesap apples were sprayed on June 2 and 28 with 1 pound of 25% wettable para-

thion powder and 2 pounds of 50% 2,2-bis-*p*-chlorophenyl-1,1,1-trichloroethane (DDT). Samples of one full box (1 bushel) were selected from the north, south, east, and west sides and from the top of the tree. These samples were subsampled in the laboratory and analyzed by the same procedure as the samples in Table I. The results are presented in Table II.

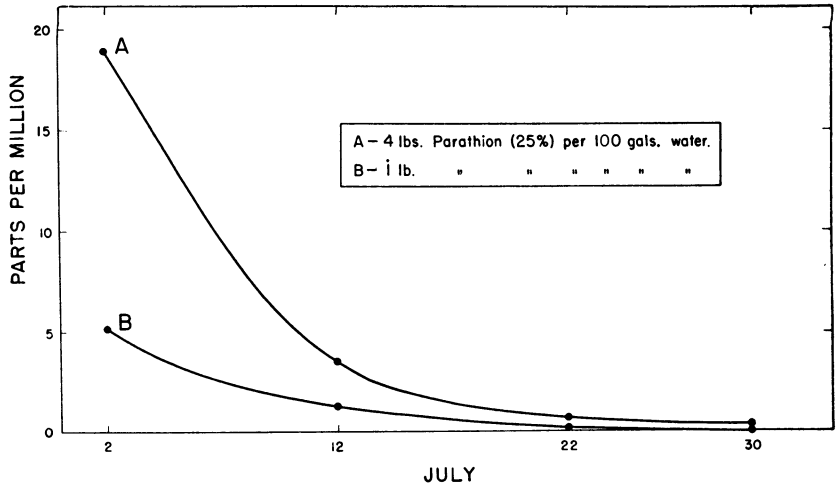

Figure 1. Loss of Parathion Residue under Field Conditions

Statistical analysis of the data in Table II shows no significant difference between varieties or between positions on the tree. The average parathion residue on all varieties is equal to or slightly less than the variation between samples.

Loss of Parathion Residue under Field Conditions

Spray programs of 1 and 4 pounds of 25% wettable parathion powder per 100 gallons of water were applied to Delicious apples on July 2 (plots 1 and 2). Samples were taken as soon as the fruit became dry and at 10- to 13-day intervals for a period of 32 days. These plots were sprayed again on August 3 with the same mixtures and resampled over a 30-day period (plots 3 and 4). The results and the percentage of loss of parathion are shown in Table III and Figure 1.

All plots lost from 80 to 85% of their parathion residue from the surface of the fruit in 12 to 13 days and from 93 to 100% in 30 to 32 days. Plots 3 and 4 were sprayed later in the season (August 3) than plots 1 and 2 (July 2), and the slightly smaller loss of parathion residue from the surface of the fruits at the later date may be due to a decrease in volatility of parathion because of lower day and night temperatures.

Relation of Concentration of Spray Mixture to Residue

Cover sprays, at concentrations ranging from 0.25 to 4 pounds of the 25% wettable parathion powder per 100 gallons of water, were applied to Delicious apples. These sprays were applied on June 7, July 2, and August 3. The residue samples were selected at harvest time and held in cold storage (32° F.) for a period of time and then analyzed. The elapsed period of time between spraying and analysis was 90 days. The results are presented in Table IV.

Under normal insect infestations (mites and aphids) encountered in the state of Washington, a spray program of not more than three cover sprays at a concentration of not more than 0.5 to 1 pound of the 25% wettable powder per 100 gallons of water should give

very excellent control of the insects. Normally, a period of 90 days between the last application and harvest would not elapse (45 to 60 days would be expected).

The parathion residue at harvest time resulting from a program of 0.5 to 1 pound of 25% wettable powder applied 45 to 60 days before harvest could be expected to be 0.10 p.p.m. or less. The application of sprays with a concentration in excess of 0.5 to 1 pound (25%) would result in no measurable increase in insect control, waste of materials, and higher parathion spray residues.

Table III. Parathion Residue and Rate of Loss under Field Conditions

Plot No.	Parathion Residue, P.P.M.	Days between Spraying and Analysis	Loss, %
1[a]	5.1[a]	0	0
	1.0	12	80
	0.1	20	98
	0.0	32	100
2[a]	19.1	0	0
	3.2	12	83
	1.1	20	94
	0.8	32	96
3[b]	4.7	0	0
	0.7	13	85
	0.4	21	91
	0.3	30	94
4[b]	18.9	0	0
	3.5	13	81
	2.0	21	89
	1.4	30	93

[a] Sprayed July 1.
[b] Sprayed August 3.

Table IV. Relation of Concentration of Spray Mixture to Residue

Plot No.	Concentration[a], Lb.	Parathion Residue, P.P.M.
1	4	0.60
2	3	0.19
3	2.5[b]	0.18
4	2	0.18
5	1	0.08
6	0.25	0.01

[a] Pounds of 25% wettable parathion powder per 100 gallons of water.
[b] Four cover sprays applied June 7 and 23, July 2, and August 3.

Parathion Spray Residue on Commercially Sprayed Apples

Fifty-five samples of apples—Jonathan, Delicious, Rome Beauty, and Winesap—were collected from commercially sprayed orchards that had received a single parathion spray and where the spray program was known. These samples were analyzed by the previously described method and the results are presented in Table V.

Table V. Parathion Spray Residue on Commercially Sprayed Apples

Spray Program	No. of Samples	Days between Spraying and Analysis	Parathion Residue, P.P.M.
1/2 Lb. 15% wettable powder/100 gallons water	29	32–81 (av. 55.5)	0.00–0.14 (av. 0.041)
1/3 Lb. 15% wettable powder/100 gallons water	5	33–79 (av. 54.0)	0.00–0.07 (av. 0.010)
1/4 Lb. 15% wettable powder/100 gallons water	21	29–78 (av. 51.9)	0.00–0.07 (av. 0.026)

Regression analysis comparing the number of days from spraying to analysis and the parathion spray residue show no significant relationship. The difference in parathion residue due to the 1/2-, 1/3-, and 1/4-pound applications was not significant. All residues were only a fraction of 1 p.p.m.

Acknowledgment

Appreciation is expressed to W. J. O'Neill and E. W. Anthon of the Division of Entomology, State College of Washington, for their kind assistance in the field.

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Absorption of DDT and Parathion by Fruits

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Commensurate with the need for information on the effectiveness of new organic insecticides has been the need for determining the magnitude and distribution of toxic residues on and in edible produce. Absorption of insecticide residues of DDT and parathion by fruit was investigated. Specific techniques for the physical separation of component fruit parts with minimization of sample contamination are described. DDT residues were analyzed by the dehydrohalogenation method. The magenta color reaction as modified by Gunther and Blinn was used for parathion residues. Results indicated the presence of DDT and parathion in the peel but not in the pulp of harvested citrus fruits. The relatively rapid penetration of the toxicants was followed by a slower loss with the retention of appreciable amounts over long periods of time. Neither DDT nor parathion was found in the pulp of apples, pears, or peaches following treatment with standard dosages in sequence applications. Spectrographic examination of transmission–wavelength curves of the benzene extractives of DDT-treated and parathion-treated navel oranges in comparison with curves for parent compounds indicated definite shifts in absorption bands.

Although various workers (*6*, *18*, *20*, *21*) have reported on the mammalian toxicity of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT) and *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate (parathion), more detailed studies with these toxicants are being conducted by numerous pharmacological groups because available information indicates that certain hazards may be associated with the use of these materials as economic poisons. In addition to the possible dangers from exposing personnel to these toxic materials in the course of handling or making insecticide applications, the retention of deposits on edible products or the accumulation of residues by translocation may be objectionable or hazardous. As a means of clarifying the problems incident to this aspect of food contamination, agricultural chemists and entomologists have recognized the desirability of projecting parallel studies to determine the magnitude and location of DDT and parathion residues on or in treated produce.

In many instances the problem of surface contamination has been principally studied (*3–5*, *7–10*, *16*, *17*, *19*, *22*). However, early in the DDT studies on citrus fruits, it became evident that appreciable amounts of this insecticide penetrated into certain components of the peel. Following this disclosure, suitable techniques were investigated and an evaluation of the penetration of DDT and later of parathion into various kinds of fruit was undertaken.

The studies reported herein consider for the most part the occurrence of penetration following the practical uses of these insecticides on specified crops and, to a limited extent, the ultimate fate of the penetrated compounds. They may additionally serve to indicate the nature of the problems which may be associated with other uses. The final interpretation of these results will be dependent on the fuller elaboration of pharmaco-

logical studies and on the subsequent clarification of the need for legal tolerances for residues of these specific toxicants or their degradation compounds on consumer products.

Materials and Methods

These studies were limited to work with navel and Valencia oranges, lemons, grapefruit, apples, pears, and peaches. Preliminary results have also been obtained with avocados, grapes, olives, plums, and certain vegetable crops.

Representative fruit samples were assembled from trees which had been treated with experimentally formulated technical grades of DDT and parathion or with commercially available formulations of these toxicants. All insecticide applications were made with power equipment typical of that used in commercial practice. As a means of making direct comparisons and of assuring greater control over those variables, inherent in field studies, most fruit samples were taken from test plots in experimentally treated grove areas. The experimental groves were located in Los Angeles, Orange, Riverside, San Bernardino, San Diego, Tulare, and Ventura counties of California.

Approximately eight pounds of fruit constituted each analytical sample (*11*, *14*); the number of fruits per sample varied from ten to thirty. Fruits were selected at random from within a peripheral band around the tree 3 to 6 feet above the ground. Individual samples were constituted with fruits from six to eight trees and replicate samples were taken from different groups of trees. In some cases, samples of deciduous fruits were collected from three trees and additionally involved a portion of fruit from the upper quarter of the tree. Duplicate, or more generally triplicate, samples were utilized for analyses. All fruits for penetration studies were collected in paper bags, which were immediately stapled to ensure sample integrity.

The sample preparations and quantitative estimations of residues were completed as soon after the fruits were sampled as proved practicable (*14*).

Standardized procedures were adopted with regard to sample preparation, recovery of toxicant, and chemical assay. In order to determine the nature and magnitude of penetrated residues, it was necessary to disassociate all extra-surface residues. The techniques originally developed to effect this separation and which were used in most of the DDT penetration studies have been described by Gunther (*11*). Certain modifications which have been developed subsequently in connection with the parathion studies are described in detail below since this phase of penetration studies assumes singular importance (see also *14*).

Citrus Fruit Types. The method previously described (*11*) consisted essentially of scrubbing the fruits with a warm 10% trisodium phosphate solution, rinsing with distilled water, halving each fruit, and reaming the juice and pulp from each half with a power juicer. Pieces of pulp adhering to the insides of the individual hemispheres of peel were carefully scraped free and combined with the remainder of the pulp and juice. Independent analyses were then completed on the discrete peel and pulp-juice samples. Whenever desirable the flavedo and albedo components of the peel were separated with peeling tools, and each was pooled and analyzed.

This method proved inadequate in the parathion studies because of trace contaminations and the following procedure of sample preparation was adopted:

Approximately 8 pounds of fruit were scrubbed manually in warm 10% trisodium phosphate solution. One hemisphere of peel was then removed from each fruit, using a household-type peeler. From the pooled segments of peel a 1-pound subsample was used for processing.

To obtain the pulp sample a circle of peel approximately 1.5 inches in diameter was lifted from the center of the remaining hemisphere of peel on each fruit and a decontaminated No. 15 cork borer with a serrated cutting edge was forced through the fruit antipodally in such a manner as to avoid all flavedo peel. The albedo-bearing ends of each extracted plug of fruit pulp were carefully removed with the aid of a clean razor blade. After being pooled, a 1-pound subsample of the pulp was utilized for processing.

The samples of citrus peel obtained by either method may have contained trace amounts of extra-surface DDT residues as differentiated from subsurface or penetrated

residues (*2*, *13*). Although difficult to establish experimentally the empirical evidence indicated that the washing of fruits with warm trisodium phosphate solution was nearly quantitative in removing or degrading DDT extra-surface residues. The extra-surface residues of parathion shortly after application appear to be nil; the amounts obtained by stripping procedures apparently were extracted from the waxlike cuticle of fruits (*2*).

In the preparation of citrus pulp samples by this revised method the likelihood of contamination appeared to be eliminated providing the tools and work area were free of contaminants and the manipulations were carefully executed. However, the samples so prepared do not contain any of the pulp from the area immediately adjacent to the peel and to that extent are not totally representative.

Pome Fruit Types. As with citrus fruit types, the method of sample preparation was modified for the parathion studies. In the earlier studies the DDT-treated apples and pears were scrubbed in a warm 10% solution of trisodium phosphate, and all the peel was removed from the water-rinsed fruit with a household-type potato peeler. The pooled samples of peel and pulp were then processed independently to recover the contained toxicant for subsequent estimation.

In the parathion studies and the more recent DDT studies, the fruits were split in half by cutting axially one third through the fruit with a broad-bladed knife and then twisting the blade to one side. The core and pulp, exclusive of the part touched by the knife blade, were carefully scraped out with sharpened melon ballers.

As much of the pulp as possible was removed but in the event the skin was punctured the fruit was discarded and the scraping tools decontaminated. The pulp taken from the individual fruits of the sample was pooled for subsequent processing. Separate samples of fruit were used for surface residue estimations (*12*).

Stone Fruit Types. Very preliminary studies of DDT or parathion penetration into peach fruits were completed. The pulp samples were prepared by immersing the intact fruits in boiling water for 1 minute, slipping the skins off, rinsing thoroughly with water and removing the seeds.

Subsequent processing of the fruit components and extraction of the contained toxicants have been described (*11*, *12*, *14*, *15*). Analyses for DDT residues have been made with the dehydrohalogenation method (*11*, *15*). The magenta color reaction of Averell and Norris (*1*) as modified by Gunther and Blinn (*14*) was used to analyze for parathion residues. Appropriate fruit blanks were run with each set of analyses.

The results of all determinations are expressed as parts per million based on fresh weight of analyzed substrate.

When dissection of fruits was involved in the preparation of the samples, the values reported represent the parts per million of toxicant based on fresh weight of the indicated component only and not of the weight of the whole fruit. As separated in these studies the peel of most citrus fruits constitutes approximately one sixth the weight of the whole fruits.

Results

The summarized data presented in Tables I to VII indicate the nature of the results which have been obtained in certain of these studies. It is not intended that these be interpreted as absolute values since cogent limitations exist not only in the techniques of

Table I. Immediate Posttreatment Residues of DDT in Peel of Citrus Fruits

(Sampled 48 hours after application)

Type Formulation	Technical Grade DDT/100 Gal., Lb.	P.P.M.[a], DDT		
		Navel oranges	Valencia oranges	Eureka lemons
Wettable powder	2.0	5.6	5.8	5.6
Kerosene solution	2.0	13.9	16.4	12.2
Kerosene solution	1.0	3.9	10.7	7.5
Light medium oil solution	1.2	17.1	6.3	13.1
Light medium oil solution	0.6	11.8	5.9	7.8

[a] Based on fresh weight of peel only.

sampling and sample preparation but also in the methods of recovery by extraction and in the quantitative procedures of estimation. For example, in these studies the over-all efficiency of the DDT dehydrohalogenation method from sampling through calculated results appears to be approximately 90% whereas that for parathion is probably not more than 60% (*14, 15*). On the other hand, the data may help to characterize the nature and extent of the problem imposed by the use of these or similar materials and to indicate the relative effects of some of the factors in penetration phenomena.

Consistent with the definition of terms adopted for the discussion in this series of papers of integral phases of the residue studies being conducted by the Division of Entomology, University of California Citrus Experiment Station (*2, 13–15*), the following distinctions are noted: Residues may be specified as pretreatment, posttreatment, harvest, or ultimate. The latter refers to the residue on or in foodstuffs, whether fresh or processed, at the time of consumption (*2, 13*). The location of residues with reference to fruit parts may be extra-surface (external to the cuticle) or subsurface. Subsurface residues may be differentiated with reference to actual location as cuticular residues or specified intracarp residues. Residues in the cuticular layers or in any of the cellular structures or matrices are herein indicated as subsurface (penetrated) residues (*2, 13*).

Citrus Fruits. The recovery of demonstrable amounts of DDT in the peel of oranges and of lemons indicated the necessity for detailed studies with regard to the effect of dosage, formulation, number of applications, method of application, fruit development at time of application, and other factors. Within the precision of the methods utilized DDT has never been recovered from the pulp portions of DDT-treated citrus fruits. Results typical of the amounts found as subsurface DDT in the composite peel of citrus fruits are collated in Tables I and II. The infiltration of DDT into the peel was rapid, and it persisted there over long periods during the development and maturing of the fruits. The magnitude and distribution of penetrated DDT residues were influenced by the specific nature of the formulation applied. Dissection of orange and lemon peel into subsamples of flavedo (outer peel) and albedo (inner peel) showed in subsequent analyses that in all cases most or all the DDT was present in the flavedo. In a limited number of instances relatively small amounts of DDT were recovered in the albedo tissues, particularly when the fruits were sprayed with DDT formulations involving large amounts of kerosene or heavier fractions of petroleum oils.

Table II. Harvest Residues of DDT in Peel of Citrus Fruits

(Averages for all samples)

Spray Schedule[a], No. Applications	Fruit Development at Time of Last Application: Post blossom	Intermediate	Approaching maturity
	P.P.M.[b] DDT		
Valencia oranges			
1	11.3	..	16.7
2	..	12.7	22.0
3	10.7	..	25.3
Navel oranges			
1	4.7	5.3	..
2	4.7	13.3	25.3
3	7.3	12.7	36.7

[a] All sprays were formulated by dissolving 2 pounds of technical grade DDT (8 grams DDT/100 ml. solvent) in 3 gallons of kerosene-Velsicol AR-60 (95-5) and emulsifying the solution with 4 ounces of blood albumin spreader in 100 gallons of finished spray.
[b] Based on fresh weight of peel only.

The use of DDT at application rates indicated in Table II represents the maximum dosage that has been considered for commercial use on citrus. The largest current uses of DDT on citrus involve single applications at the rate of 0.75 pound of technical grade compound per 100 gallons of spray mixture. The amount of DDT found in the peel of oranges harvested from 2 to 7 months after treatment with these dosages has ranged from 0.6 to 4.6 p.p.m., fresh weight of peel.

Varietal differences with regard to the amount of DDT present in the peel of harvested fruits sprayed at different times during the development of the fruit suggest the

possibility of determining in subsequent investigations certain of those factors limiting the infiltration of DDT into fruit tissues (Table II).

Studies with parathion-treated citrus fruits have also shown no parathion to be present in the endocarpal, or pulp segment, portions of the fruit (Tables III, IV, and VI). Since the magenta color reaction for parathion is extremely sensitive, it is believed that even trace quantities are not present in the pulp. The rapidity of the subsurface penetration of parathion into the peel of citrus fruits and the persistence of these residues in the peel is demonstrated in Tables III through VII.

Table III. Posttreatment Residues of Parathion in Citrus

(Sprayed with 4 pounds of 25% parathion wettable powder per 100 gallons water 4-26-48)

Sampled after, No. Days	P.P.M. Parathion			
	Valencia Oranges[a]		Eureka Lemons[a]	
	Peel[b]	Pulp[c]	Peel[b]	Pulp[c]
1	12.4	0.0	15.9	0.0
2	9.8	0.0	11.8	0.0
4	9.1	0.0	10.5	0.0
8	6.2	0.0	8.9	0.0
10	4.4	0.0	5.1	0.0
14	2.9	0.0	4.6	0.0
17	2.7	0.0	2.8	.
53	3.0	0.0	..	..

[a] Valencia oranges approaching maturity and lemons mature when sprayed.
[b] Based on fresh weight of peel only.
[c] Based on fresh weight of entire fruit.

Table IV. Harvest Residues of Parathion in Valencia Oranges

Technical Grade Parathion/ 100 Gal.[a], Lb.	Time after Application, Days			
	105	140	170	230[b]
	P.P.M. Parathion			
Peel[c]				
0.5	3.4	2.1	2.0	1.9
0.75	5.6	4.6	3.9	3.9
1.0	5.0	4.5	4.3	4.4
Pulp[d]				
0.5	0.0	0.0	0.0	0.0
0.75	0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.0	0.0

[a] Applied October 28, 1947, as 25% wettable powder.
[b] Fruit fully mature when sampled.
[c] Based on fresh weight of peel only.
[d] Based on fresh weight of entire fruit.

Table V. Harvest Residues of Parathion in Peel of Mature Citrus Fruit

(Average for all samples)

Variety	Technical Grade Parathion[a] per 100 Gal., Lb.						
	0.125	0.25	0.5	0.75	1.0	1.25	1.5
	P.P.M.[b] Parathion						
Navel oranges	0.1	1.7	2.5	4.2	5.2	..	..
Valencia oranges	0.1	0.7	1.9	3.5	4.3	6.4	8.1
Eureka lemons	..	..	2.1	..	2.6	..	..
Marsh grapefruit	..	..	..	..	4.9	..	..

[a] Applied as 25% wettable powder.
[b] Based on fresh weight of peel only.

Preliminary tests have shown that the amount of parathion penetrating into the peel of navel oranges is not markedly altered by drenching the tree with water sprays 1 day after treatment. Table III shows that much of the parathion initially present as subsurface residue is lost 10 to 20 days after treatment, possibly by reissuance and volatilization. Metabolism within the fruit tissues has not been indicated in preliminary studies (see subsequent section). The parathion residues persisting in the peel over long periods of time tend to remain at a somewhat constant level (Table IV). Thus the gradient of parathion residues in peel with increased dosages shown in Table V suggests that the initial depth and distribution of penetrated parathion may be dependent on the steepness of the concentration gradient which existed immediately after application (Tables III and IV). In some instances slight varietal differences are indicated (Table V), but the de-

velopment of the fruit of each variety at the time of treatment is probably a more limiting factor (Table VII).

Since generally higher temperatures prevailed in the field during the last week of August and the first week of September in 1948 than during the corresponding period in 1947 the somewhat higher penetration values reported in samples taken at that time in 1948 (Table VI) may be a consequence of generally higher fruit temperature. Other factors may be operative.

Table VI. Harvest Residues of Parathion in Mature Navel Oranges

(Sampled during January and February of year following time of application)

Parathion Formulations	Technical Grade Parathion/100 Gal., Lb.	Date of Application	P.P.M. Parathion Peel[a]	P.P.M. Parathion Pulp[b]
25% wettable powder	0.25	8-16-48	1.6	..
	0.25	9-30-48	2.1	..
	0.25	11-11-48	1.4	..
	0.50	8-13-48	2.0	..
	0.50	8-13-48	2.2	..
	0.50	8-20-47	1.0	0.0
	0.50	8-24-48	3.0	0.0
	0.50	8-26-48	3.7	..
	0.50	8-30-48	2.3	..
	0.50	9-10-48	2.5	0.0
	0.50	9-10-48	2.5	0.0
	0.50	9-30-48	3.3	..
	0.75	8-13-48	3.5	..
	0.75	8-19-48	3.7	..
	0.75	8-24-48	6.5	0.0
	0.75	8-29-47	2.0	0.0
	0.75	9- 2-48	5.8	0.0
	0.75	9- 3-48	5.8	..
	0.75	9-10-48	6.7	0.0
	0.75	9-16-48	4.5	..
	0.75	9-22-48	4.1	..
	0.75	9-30-48	3.3	..
	0.75	10-11-48	4.6	..
	0.75	10-22-48	4.1	..
	0.75	10-28-48	2.3	0.0
	0.75	11-11-48	4.2	..
	0.75	11-11-48	3.8	..
	1.0	6-15-48	1.1	0.0
	1.0	8-24-48	7.5	0.0
	1.0	8-26-47	4.0	0.0
	1.0	9- 3-48	6.1	..
	1.0	9- 3-48	6.4	..
	1.0	10- 9-47	2.7	0.0
	1.0	10-15-48	6.4	0.0
	1.0	10-22-48	4.7	0.0
In light medium oil 0.25%	0.05	9- 9-48	0.3	0.0
0.5%	0.09	9- 9-48	0.7	0.0
0.5%	0.09	9-11-48	0.8	0.0
1%	0.18	9- 9-48	1.3	0.0
Technical grade compound emulsified	0.98	9-14-48	10.5	0.0

[a] Based on fresh weight of peel only. [b] Based on fresh weight of entire fruit.

Table VII. Posttreatment and Harvest Residues of Parathion in Peel of Citrus Fruits—Effect of Seasonal and Multiple Applications

(All sprays with 4 pounds of 25% parathion wettable powder per 100 gallons water)

Application Dates				P.P.M. Parathion[b]		
6-14-48[a]	8-17-48	10-29-48	Date Sampled	Navel oranges	Valencia oranges	Eureka lemons
Sprayed	.....	.....	11-19-48	1.3	0.5	4.0
Sprayed	.....	.....	1-11-49	1.1	0.4	1.0
Sprayed	.....	.....	2-11-49	0.7	0.4	0.5
Sprayed	Sprayed	.....	11-19-48	11.7	8.3	7.5
Sprayed	Sprayed	.....	1-11-49	6.8	8.8	6.2
Sprayed	Sprayed	.....	2-11-49	4.9	9.8	5.2
Sprayed	Sprayed	Sprayed	11-19-48	13.5	20.4	13.1
Sprayed	Sprayed	Sprayed	1-11-49	17.7	18.8	9.0
Sprayed	Sprayed	Sprayed	2-11-49	10.6	17.0	6.4
.....	Sprayed	Sprayed	11-19-48	13.6	26.6	13.3
.....	Sprayed	Sprayed	1-11-49	13.8	..	6.9
.....	Sprayed	Sprayed	2-11-49	10.4	18.4	..
.....	.....	Sprayed	11-19-48	12.2	9.5	5.8
.....	.....	Sprayed	1-11-49	7.7	8.4	1.6
.....	.....	Sprayed	2-11-49	6.4	9.1	2.7

[a] Fruits small when sprayed. [b] Based on fresh weight of peel only.

The importance of formulation on the penetration of parathion into the peel of citrus fruit is indicated in Table VI, the emulsified technical grade compound entering in greatest amount. This suggests the possibility of being able to formulate parathion in a manner which might effectively prevent the penetration of the compound into citrus peel but an attendant difficulty might be that its insecticidal effectiveness would be lessened.

Pome and Stone Fruits. Following the application of DDT and of parathion as wettable powders in control schedules, neither compound has been recovered from the pulp portions of apples, pears, and peaches. A maximum of six spray applications of DDT wettable powder and four spray applications of parathion wettable powder were involved in these studies. As high as 0.4 p.p.m. of DDT and 1.7 p.p.m. of DDT were found in the pulp of apple and pear fruits, respectively, following seasonal treatments with five to six applications of DDT formulated in a petroleum oil fraction.

Nature of Persisting Subsurface Residues

An exploratory spectrophotometric examination of subsurface extractants obtained from fully ripe Rome Beauty apples, previously sprayed with a deliberate overdosage of a parathion wettable powder, revealed a shift in the absorption maximum of the dyed product to the right of that found for the dyed technical parathion.

Since the composition of both the dyed technical parathion and the dyed carefully purified parathion has not been elucidated, it was not possible to attempt an interpretation

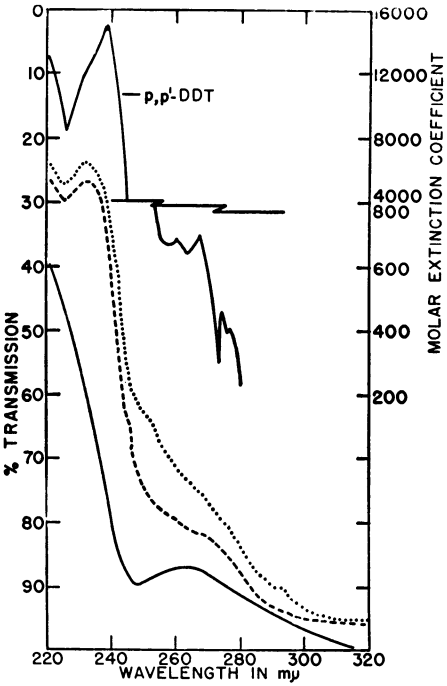

Figure 1. Transmission–Wave-Length Curves for Colorless Benzene Extractives from Peel of DDT-Treated Navel Oranges

Untreated ———; sample A · · · · · ·; sample B – – –. For comparison purposes ultraviolet absorption spectrum of carefully purified *p,p'*-DDT, in ethyl alcohol, has been plotted as upper solid line with different ordinate

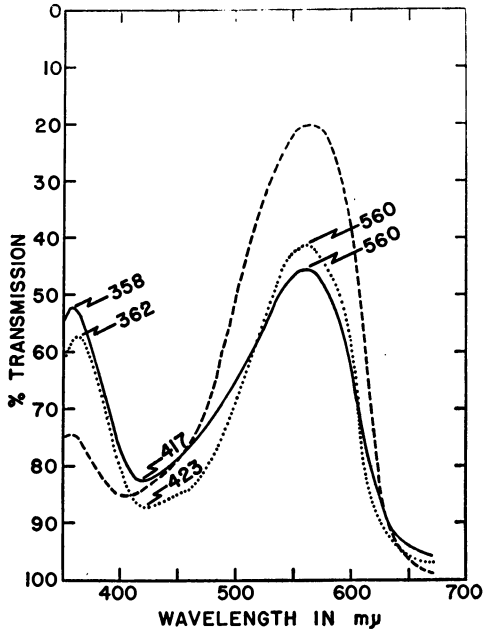

Figure 2. Transmission–Wave-Length Curves for Dyed Benzene Extractives from Peel of Parathion-Treated Navel Oranges

Sample A ———; sample B · · · · · ·; dyed parathion – – –; in 60% ethyl alcohol, pH 1.2. Instrument was set at 100% transmission prior to each transmission reading with untreated sample; numbers refer to λ_{max}.

of this displacement toward longer wave lengths. Present information indicates that there may exist at least three and possibly four colored components in each mixture.

Because of continued interest in the in situ degradation products of both DDT and parathion, preliminary spectrophotometric evidences, which are also indicative of structural alterations and/or changes in composition, have been secured with extracts of navel oranges previously treated with standard formulations of these compounds.

Approximately 5 months prior to sampling of fruits the trees were sprayed with the equivalent of 2 pounds of technical grade DDT per 100 gallons in an emulsified kerosene solution or with the equivalent of 0.75 pound of technical grade parathion per 100 gallons in a wettable powder preparation.

With the duplicate DDT-treated samples and one untreated sample, the benzene extracts were evaporated and the residue redissolved in ethyl alcohol and evaporated again. This procedure was repeated several times to remove the last traces of benzene. The final residue was dissolved in low-boiling (30° to 60° C.) petroleum ether and chromatographed on a column of activated alumina which afforded a colorless eluate. After removal of solvent the resulting residue was triturated with boiling ethyl alcohol, cooled, filtered, and spectrographed in a Beckman spectrophotometer. The resulting transmission–wave-length curves are shown in Figure 1. The duplicate samples show markedly different absorption characteristics from those of the untreated sample, particularly in the region 225 to 260 mμ. Further comparisons with the absorption spectrum of carefully purified *p,p'*-DDT in ethyl alcohol demonstrate complete absence of fine structure in the region 260 to 280 mμ for the DDT-treated samples. From the noncoincidence of the sample maxima with those of the DDT curve it may be concluded that very little if any DDT is left in the samples but that fragments of the original DDT molecule may be present.

With the duplicate parathion-treated samples and one untreated sample, the benzene extracts were processed in the usual manner and subjected to the dyeing procedure. Their transmission–wave-length curves are shown in Figure 2 with a companion curve for dyed parathion.

With reference to the curve for dyed parathion, shifts toward longer wave lengths in all maxima and minima are apparent with the greatest displacement in the region 400 to 440 mμ.

The intensities of absorption change proportions between sample and reference curves. The fact that the transmission–wave-length curve of dyed parathion is composite precludes the possibility of interpreting these observations at the present time.

These displacements were considered sufficiently interesting to warrant the development of more exact techniques to elucidate the nature of these persisting subsurface residues. Such studies are currently in progress.

Acknowledgment

The authors are indebted to the American Cyanamid Company and the Monsanto Chemical Company for supplying the parathion materials used in these studies.

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PAPER 625, University of California Citrus Experiment Station, Riverside, Calif.

Removal of DDT and Parathion Residues from Apples, Pears, Lemons, and Oranges

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Alkaline and halogen-carrier media, such as sodium silicate, trisodium phosphate, ferric chloride, sodium carbonate and bicarbonate, and alkaline soaps were used for chemical removal of DDT deposits. Mechanical removal was sought by using emulsifying agents, detergents, pressure sprays, and scrubbing brushes. Solvent removal attempts included the use of kerosene, mineral oil, xylene, and polymethylated naphthalenes. With apples and pears, sodium silicate frequently proved superior, removing as much as 90% of the residual surface DDT, and an alkaline soap effected significant removal from oranges. No experimental treatment has afforded significant removal of parathion from treated fruits.

The widespread commercial use of DDT and the anticipated use of parathion indicated the practical value of investigating the feasibility of removing their harvest residues without injury to the fruits.

A distinction has been drawn (*1*) between "harvest" and "ultimate" residues. The former term is being defined as the surface or penetrated residue of insecticide at the time of harvest, whereas the latter designation refers to the residues in or on the foodstuff, whether fresh or processed, at the time of consumption. Thus, there exist harvest surface and penetration or harvest total residues, as well as ultimate surface, penetration, and total residues. Where necessary, component parts of the foodstuff may be specified to delimit this concept further.

Begun in 1944 with DDT and in 1947 with parathion, the present report includes analytical data secured from certain chemical, mechanical, and solvent actions on apples, pears, lemons, and oranges. In the absence of established tolerances for these two insecticidal materials, it is hardly possible to interpret the significance of many of these data with respect to consumer hazard.

Although there are a number of published reports of the magnitude of [harvest] DDT residues, particularly on apples (*4, 5, 7, 8, 14, 15, 18, 19*), grapes (*9*), oranges (*10*), and pears (*2, 5, 18*), and of terminal residues in canned products (*15, 17*), only a few publications have been concerned either with the nature of the residual deposits (*6, 7, 11, 16*) or with their removal (*2, 14, 18*) prior to consumption. Hough (*14*), for example, reported that 1.3% hydrochloric acid solution, and dilute solutions of sodium silicate, trisodium phosphate, IN-181-P (a powder containing 50% sodium lauryl sulfate, as supplied by E. I. du Pont de Nemours & Company, Inc., Wilmington, Del.), and a Santomerse all failed to remove readily the DDT deposits on mature apples. (The Santomerses are the

salts of a homologous series of substituted aromatic sulfonic acids, supplied by the Monsanto Chemical Company, St. Louis, Mo.) Similarly, Borden *et al.* (*2*) recorded the conclusion that DDT applied with spray oils is not removed easily.

So far as the authors are aware, there have been no published results of attempts to remove parathion residues from harvested fruits.

Because DDT is readily dehydrohalogenatable, chemical expedition of deposit removal was sought through the use of alkaline and halogen-carrier media, such as sodium silicate, trisodium phosphate, ferric chloride, sodium carbonate and bicarbonate, and strong soaps. Mechanical removal was sought through the use of a variety of emulsifying agents, detergents, pressure sprays, and scrubbing brushes. Solvent removal attempts included the use of kerosene, mineral oil, xylene, and a mixture of polymethylated naphthalenes.

For purposes of coherence and clarity, methods and results with apples and pears are presented separately from those with oranges.

Methods with Apples and Pears

Apples. The Rome Beauty apples used in the wash tests were sampled from trees that had received varying amounts of DDT mixtures in as many as six cover sprays. Duplicate or triplicate samples of 30 apples each were taken at random for the residue analyses from the fruit passed through each experimental wash mixture. Additional lots of 30 washed apples each were placed in cold storage for subsequent examinations. Unless otherwise indicated, all washing tests were run in a flood-type washer of recent design (a BADD washer with a heated prewash tank unit, an unheated main tank unit, a water rinse tank unit, and a velour roller dryer unit, manufactured by the Bean-Cutler Division, Food Machinery Corporation, San Jose, Calif.). Surface deposits of DDT were determined as described (*10*, *12*) on samples taken just before and immediately after the washing treatments.

Pears. Triplicate samples of 20 Bartlett pears each were used and a fourth sample was placed in cold storage for subsequent examination, as with the apples. Washing trials were performed in a standard type washer (an Ideal fruit washer with an unheated main tank unit and a water rinse tank unit, Model WKA, manufactured by the Ideal Grader and Nursery Company, Hood River, Ore.).

Results with Apples and Pears

DDT on Apples. Exploratory investigations in 1944 with the flood-type tandem washer indicated that DDT surface residues of from 3.3 to 9.5 p.p.m. could readily be reduced 82 to 98% by a number of materials, both alone and in combination. These materials included sodium silicate solution (70 to 82 pounds of the 58.5° Baumé solution per 100 gallons), hydrochloric acid (1.25%), ferric chloride solution (1 ounce per 100 gallons), sodium hypochlorite solution (3 pints of 4% per 100 gallons), Mermaid Soap (a proprietary, buffered sodium salt of the fatty acids in cottonseed oil foots, as supplied by the Los Angeles Soap Company, Los Angeles, Calif.) (4 pounds per 100 gallons), trisodium phosphate (4 pounds per 100 gallons), and IN-181-P (1 ounce per 100 gallons). Solution temperatures ranged from 50° to 110° F. in a variety of wash sequences. Although combinations of IN-181-P washes and sodium silicate washes appeared to be the most efficient, all the washes reduced the residues appreciably. In some instances, slight visible deposits remained in the blossom and stem ends following the washing treatment. Washing tests subsequent to 1944 were designed to evaluate more precisely the efficiency of the sodium silicate solution, but other materials were considered sufficiently promising to be included. In Table I are summarized the data for the sodium silicate washes, at 80 pounds per 100 gallons, with water only in the main tank, for a variety of types of spray deposits.

In practically all cases the residual DDT was reduced to less than 1 p.p.m. fresh weight. However, some of the apples used in the 1944 tests carried as high as 9.5 p.p.m. of DDT as surface residue, whereas subsequent deposits prior to washing averaged about 2 p.p.m. From these and supplemental tests it would seem that such small harvest residues of DDT

can readily be reduced by at least 50% with a number of washing materials including sodium silicate (80 pounds per 100 gallons, 65° to 100° F.), trisodium phosphate (4 pounds per 100 gallons, 108° F.), and IN-181-P (1 ounce per 100 gallons, 65° F.).

Tennessee ball clay was the only extending material which when in composition with DDT appeared to interfere appreciably with the removal of DDT surface residues by sodium silicate washes.

None of these washing treatments occasioned apparent fruit injury or decreased storage life.

DDT on Pears. Early experiments indicated that the conventional hydrochloric acid bath, as used for the removal of lead arsenate residues, afforded partial DDT residue removal by virtue of mechanical action only—for example, a surface residue of 1.0 p.p.m. was reduced to 0.6 p.p.m. by such treatment, but the residue in the calyx only was untouched (15 p.p.m., fresh weight of calyx only). Supplemental wash tests with other materials afforded the residue data collected in Table II.

Table I. Removal of DDT Surface Residues from Rome Beauty Apples with Sodium Silicate Washes

	Spray Treatments	Dosage in 6 Cover Sprays, Lb.	DDT Surface Residues on Fruits, P.P.M. Wet Weight			% DDT Surface Residue Removed	
			Unwashed	Washed 80° F.	Washed 100° F.	80° F.	100° F.
1.	DDT (C.P.)–amorphous silica (35–65)	1.4	0.9	...	0.1	..	89
2.	DDT (aerosol)–amorphous silica (40–60)	1.4	2.0	0.5	0.4	75	80
3.	DDT (tech.)–amorphous silica (50–50)	1.4	1.7	0.7	0.6	59	65
4.	DDT (tech.)–amorphous silica (25–75)	2.8	1.8	...	0.2	..	89
5.	DDT (tech.)–amorphous silica (12½–87½)	5.6	2.0	0.3	0.6	85	70
6.	DDT (tech.)–amorphous silica (50–50), re-milled	1.4	2.0	0.7	0.2	65	90
7.	DDT (tech.)–calcium silicate (50–50)	1.4	3.1	0.4	0.2	87	94
8.	DDT (tech.)–ball clay (50–50)	1.4	1.6	1.0	1.2	38	25
9.	DDT (tech.)–kaolin clay (50–50)	1.4	2.7	1.1	0.6	59	78
10.	Gesarol AK-40[a]	1.4	1.7	0.4	0.6	76	65
	Av.	...	1.9	0.6	0.5	68	75

[a] Wettable powder with 40% DDT.

Table II. Wash Tests for Removal of DDT Surface Residues from Bartlett Pears

	Materials	Lb./100 Gal.[a]	DDT Surface Residues, P.P.M.[b] Unwashed	Washed	% Residue Removed
1.	Trisodium phosphate	16	7.2	1.9	74
2.	Trisodium phosphate	8	7.2	3.3	54
3.	Triton X-100[c]	1.67 ounces	7.2	2.3	68
4.	IN-181-P	1 ounce	7.2	2.4	67
5.	Ivory soap	2	7.2	2.4	67
6.	Mermaid soap	6	7.2	2.8	61
7.	Sodium silicate	80	7.2	3.2	56
8.	Ferric chloride C.P. Hydrochloric acid	4 ounces 5 quarts	7.2	3.0	58
9.	Mineral seal oil Triton X-100	1 gal. 0.5 ounce	7.2	2.7	62
10.	Treatment 9 followed by 7	....	7.2	2.7	62
11.	Xylene Triton X-100	2 quarts 0.5 ounce	7.2	2.6	64
12.	Treatment 11 followed by 7	....	7.2	2.8	61

[a] Fruits exposed to wash for approximately 20 seconds.
[b] Each figure represents average of 3 analyses, except 1, 5, and 9 where figure represents one analysis only.
[c] Liquid, anhydrous nonionic emulsifier, Rohm & Haas Co., Philadelphia, Pa.

The xylene emulsion wash caused injury in the lenticel areas of the fruits. Fruits washed in the mineral seal oil emulsion retained a considerable oil deposit even when washed subsequently with sodium silicate and exhibited considerable shrinkage while in storage.

Further wash tests with apples and pears have not been extensive because the magnitudes of typical DDT harvest residues suggest that no appreciable difficulty will be encountered in bringing fruits sprayed with the lower dosages under the provisional tolerance for DDT residues on these fruits.

Parathion on Apples and Pears. Although parathion deposits appear to be less

persistent than those from DDT, the ready sorption of parathion by certain fruit metabolites indicates that late seasonal application on early harvested varieties of apples and pears could result in measurable amounts of parathion as ultimate residues.

Washing tests for these trials were made in a flood-type washer with one wash and one rinse chamber. The results presented in Tables III and IV suggest that parathion residues (from wettable powders) are not readily removed with the materials and equipment employed; support is thus secured for the authors' contention that with many substrates parathion residues are actually subsurface and exist in situ in close association with the oily and waxy constituents of the plants.

Of these materials, kerosene and mineral oil decreased the storage life of the fruits.

Table III. Wash Tests for Removal of Parathion Residues from Rome Beauty Apples[a]

Materials	Gal./100 Gal.	Parathion in Total Fruit[b], P.P.M.		% Removed
		Unwashed	Washed	
Trisodium phosphate	6 lb.	2.5	1.5	40
Velsicol AR-60[c] (0.1% Triton X-100)	1	2.5	1.5	40
Mineral seal oil	1	2.5	1.7	32
Blood albumin spreader	1 ounce	1.0	0.8	20
Triton X-100	1 ounce	2.5	1.9	24
IN-181-P[d]	3 ounces	2.5	2.0	20
Kerosene	1	2.5	2.4	4
Blood albumin spreader	1 ounce			

[a] Temperature of wash water 50° F., wash 25 seconds, rinse 10 seconds.
[b] Entire fruits macerated for analysis representing total parathion.
[c] Mixture of polymethylated naphthalenes.
[d] Added in maximum amount without excessive foaming.

Table IV. Wash Tests for Removal of Parathion Residues from Bartlett Pears and Rome Beauty Apples[a,b]

Material	Lb./100 Gal.	Approximate pH	Parathion Surface Residues[c]					
			Series 1[d] Bartlett Pears		Series 2[e] Bartlett Pears		Series 3[f] Green Rome Beauty Apples	
			P.p.m.	Removed, %	P.p.m.	Removed, %	P.p.m.	Removed, %
No treatment (unwashed)	....	..	0.27	..	0.07	..	0.32	..
Trisodium phosphate	8	>10	0.23	15	0.03	57	0.30	6
Trisodium phosphate + Santomerse 1	8 + 2 ounces[g]	>10	0.25	7	0.04	43	0.28	12
Trisodium phosphate	16	>10	0.17	37	0.04	43	0.30	6
Trisodium phosphate + Santomerse 1	16 + 2 ounces	>10	0.21	22	0.03	57	0.33	..
Sodium triphosphate	8	9	0.23	15	0.03	57	0.30	6
Sodium acid pyrophosphate	8	4	0.20	26	0.03	57	..	..
Sodium acid pyrophosphate + Tetrasodium pyrophosphate	4 + 4	7	0.21	22	0.03	57	0.28	12
Tetrasodium pyrophosphate	4	9	0.25	7	0.04	43	0.25	22
Tetrasodium pyrophosphate + Santomerse 1	4 + 2 ounces	9	0.18	33	0.04	43	0.30	6
Trisodium phosphate + Triton X-100	8 + 36 ml.	>10	0.23	15	0.03	57	0.22	31
Tetrasodium pyrophosphate	8	10	0.22	19	0.04	43	0.24	25
Tetrasodium pyrophosphate + Santomerse 1	8 + 2 ounces	10	0.28	..	0.05	29	0.27	16
Sodium silicate	80	>10	0.20	26	0.03	57	0.21	34

[a] Temperature of wash water 68° F., wash 35 seconds, rinse 25 seconds.
[b] Surface residue values reported to second decimal largely because of close agreement between duplicate analyses and because amount of parathion in each sample was within range for maximum accuracy of analytical method. Therefore, ratios reported retain possible significance.
[c] Each figure represents average of 2 analyses.
[d] Fruits of this series sprayed with 4 ounces of actual parathion per 100 gallons specifically for this test.
[e] Fruits of this series sprayed with 2 ounces of actual parathion per 100 gallons July 18 and Aug. 10, sampled Aug. 26.
[f] Fruits of this series sampled after fourth cover of 4 ounces of actual parathion per 100 gallons.
[g] Maximum amount without excessive foaming.

Methods with Lemons and Oranges

DDT. With both the Eureka lemons and the Valencia oranges, trees were sprayed in a conventional manner with conventional high pressure equipment, and with the following mixtures:

I. Ten pounds of AK-20 (a wettable powder with 20% DDT) and 4 ounces of a casein spreader per 100 gallons of water.

II. A light-medium oil containing 4 grams of technical grade DDT per 100 ml. used at 1.67%, plus 4 ounces of a blood albumin spreader, emulsified in 100 gallons of water.

III. A kerosene containing 4 grams of technical grade DDT per 100 ml. used at 3%, plus 4 ounces of a blood albumin spreader, emulsified in 100 gallons of water.

Three field boxes of each fruit for each treatment were picked at random from the treated trees 2 days after spraying. One box of each sample was stripped with the wash-bottle technique and the DDT obtained thereby was estimated by the dehydrohalogenation method (*10*). The remaining boxes of treated fruit were subjected to the normal citrus packinghouse treatments, which include a thorough machine scrubbing with Mermaid soap (at Ontario, Calif., by-products plant of the California Fruit Growers Exchange). After having been so processed, these fruits were stripped and analyzed as before.

Parathion. The available evidence suggests that parathion is soluble in many plant oils and waxes. With citrus fruits, it is clear that topically applied parathion preparations quickly lose a part of their parathion essentially by a transfer into the surface waxes and then into the oil gland containing tissues of the fruits. For example, samples stripped either by the wash-bottle technique (*10*) or by a machine technique (*13*) 24 hours after treatment always exhibit little or no parathion, whereas total peel values for the same fruits may be relatively high in parathion content (see also *1*, *3*). Oil expressed from the peel of parathion-treated oranges may contain from 65 to 90% of the parathion originally present in that peel. Prescrubbing of treated citrus fruits with warm 10% trisodium phosphate solution does not alter appreciably the amount of parathion obtainable by surface stripping (*10*). It is concluded that there is no true measurable extrasurface residue of parathion on fruits such as citrus shortly after application, and that surface stripping, by either laving or brief steeping techniques, merely extracts some of the insecticide from subsurface regions. The fact that citrus waxes are completely miscible with benzene lends credence to the postulate that the parathion does not linger in the wax layers but migrates rapidly through the cuticle. This migration and attendant further redistribution of subsurface parathion are under investigation.

It would seem, therefore, that particularly with oil- and wax-soluble insecticides the older concepts of surface residues on plant tissues should be revised in terms of extrasurface—i.e., above the cuticle—and subsurface—i.e., within or below the cuticle—residues. The latter would in turn be subdivided into cuticular residues and various intracarp residues.

Results with Lemons and Oranges

DDT. In Table V are shown the results of attempts to remove DDT surface residues from treated lemons and oranges by the standard packinghouse processing. From these data it is apparent that such DDT surface residues are readily removable. The ultimate fates of penetrated DDT (*3*) or field-decomposed surface residues of DDT (*1*, *12*) have not been determined, but work along these lines is being continued.

Table V. Removal of DDT Surface Residues from Lemons and Oranges with Standard Packinghouse Processing

Fruit	Treatment[a]	DDT Surface Residue before Processing		DDT Surface Residue after Processing	
		γ[b]/sq. cm.	P.p.m.	γ[b]/sq. cm.	P.p.m.
Lemons	I	12.5	19.3	0.4	0.4
	II	1.0	1.4	0.5	0.5
	III	4.6	4.8	1.9	1.9
Oranges	I	9.1	8.4	0.0	0.0
	II	2.5	2.1	0.0	0.0
	III	2.3	2.0	0.3	0.3

[a] See text for explanation of field treatments.
[b] Each value represents average of duplicate determinations.

Parathion. At present it has not been found possible to remove parathion from the peel of citrus fruits.

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Summary

Because DDT is readily dehydrohalogenatable, chemical expedition of deposit removal was sought through the use of alkaline and halogen-carrier media, such as sodium silicate, trisodium phosphate, ferric chloride, sodium carbonate and bicarbonate, alkaline soaps, and others. Mechanical removal was sought by using a variety of emulsifying agents, detergents, pressure sprays, and scrubbing brushes. Solvent removal attempts included the use of kerosene, mineral oil, xylene, and polymethylated naphthalenes. With apples and pears, sodium silicate frequently proved superior, removing in some cases 90% of the residual surface DDT, and an alkaline soap (standard packinghouse treatment) effected significant removal from oranges.

None of the experimental treatments has afforded significant removal of parathion from treated fruits. A satisfactory chemical attack on parathion residues has not been achieved, possibly because these residues are actually subsurface.

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Synthesis and Development of Parathion and Related Compounds

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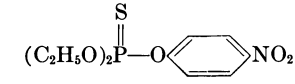
The investigations carried out and the many problems solved in the synthesis and development of parathion and related compounds are reviewed. Equations are given to illustrate the procedures used in this work.

Parathion is the generic name of a compound that was first prepared by German chemists and designated as E-605. In this country many trade names have been used. In 1946 a report by the British technical investigators Martin and Shaw (*6*) was released in the United States, disclosing among other insecticides a compound called *p*-nitrophenoxy thiophosphoric acid diethyl ester. As a result of a publication by Mastin, Norman, and Weilmuenster (*7*) in 1945, in which the name diethyl chlorothionophosphate was used, the compound as first prepared by United States chemists was designated as diethyl *p*-nitrophenyl thionophosphate.



(1)

Whether an arrow or a double bond is used between the phosphorus and sulfur is a much-discussed question. Both are probably correct.

In view of the large amount of field testing that was being carried out during 1947 and the possibilities of publications, it was desirable to have a common name as well as an approved chemical name for this product. As a result of meetings with members of the AMERICAN CHEMICAL SOCIETY'S Committee on Nomenclature, Spelling, and Pronunciation the chemical name *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate was adopted. By a series of similar conferences with members of the U. S. Department of Agriculture (in particular Haller and Rohwer), the name "parathion" was approved. Those participating in the selection of this common name proposed by Rohwer and concurring in its suitability included committees representing many scientific societies.

Physical Properties

Physical Properties of Parathion

Color	Pale yellow
Melting point, ° C.	6.1° ± 0.1° corrected
Boiling point, ° C.	157–162° at 0.6 mm.
$n_D^{25.2°}$	1.53668
$d^{27°}$	1.263

The technical material has a brown color, a refractive index that may differ slightly in the third place, and a density that is approximately the same as that of the pure material.

Parathion is soluble in water to the extent of about 20 to 25 p.p.m. It is completely

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miscible with many organic solvents, including esters, alcohols, ketones, ethers, aromatic and alkylated aromatic hydrocarbons, and animal and vegetable oils. It is very slightly soluble in paraffinic hydrocarbons such as petroleum ether, kerosene, and refined spray oils. The compound is very slowly hydrolyzed in water. Hydrolysis rates were reported in August 1948 by Peck and co-workers (*9*), who stated that 50% hydrolysis occurs at 25° C. after 120 days in saturated distilled water solutions or in 1 *N* sulfuric acid. The time required to reach 50% hydrolysis in a saturated lime solution is reduced to 8 hours. Hence parathion appears to be very stable to hydrolysis except under alkaline conditions.

Toxicology

Studies on the pharmacological effect of parathion in animals have been in progress for almost 2 years at the Hazleton Laboratory, Falls Church, Va. (*4*). Some phases of the work, such as long-range feeding tests, are continuing. Tests have been carried out on toxicity by dermal application, inhalation of dust, exposure to aerosol mists, exposure to vapors, and chronic toxicity. Test animals have included rabbits, guinea pigs, rats, mice, and dogs.

Acute Oral Toxicity of Parathion

	Form of Administration	Mean Lethal Dose, Mg./Kg.
Albino rats	Propylene glycol solution	5.0
	Wettable powder in water	12.5
Albino mice	Propylene glycol solution	6.0
	Wettable powder in water	21.0
Guinea pigs	Propylene glycol solution	9.3

Schrader (*12*) stated that in its pure form, Bladan is ten times as toxic to mammals as E-605 (parathion). "Bladan" is the name given by the Germans to a material which they thought to be hexaethyl tetraphosphate. Actually, this material is a mixture, the insecticidally active ingredient of which is tetraethyl pyrophosphate. Tests to determine the long-range effect on animals that ingest a sublethal dose of parathion have been in progress for more than 18 months. Although definite conclusions cannot be drawn at this time, average food consumption and weight gains are comparable to the controls. In all these tests the amount of material remaining on the food was over 50 times that anticipated in residual quantities on food for consumption by humans. Experimental and commercial experience with formulations containing parathion applied as sprays or dusts has covered two seasons. In a few cases where protective measures were not taken, temporary nausea, headache, or other effects were experienced when individuals were subjected to unusual exposure to vapors, dusts, or mists. It is believed that chronic toxicity hazards are greater from DDT than from parathion, even though the acute oral mean lethal dose of DDT is 250 mg. per kg.

Preparation of Parathion and Its Intermediates

$$PCl_3 + S \longrightarrow PSCl_3 \quad (2)$$

$$2C_2H_5ONa + PSCl_3 \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}—Cl + 2NaCl \quad (3)$$

$$(C_2H_5O)_2\overset{\overset{S}{\|}}{P}—Cl + NaOC_6H_4NO_2 \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}—OC_6H_4NO_2 + NaCl \quad (4)$$

Equations 2, 3, and 4 summarize the method proposed by the Germans for preparing parathion (compound E-605). Schrader (*13*) has reported that thiophosphoryl chloride was synthesized from phosphorus trichloride and sulfur by heating at 130° in a lead-lined autoclave. Woodstock and Adler (*14*) carried out a similar reaction at 150° to 160° C.

using alkali metal sulfides as catalysts, and were able to reduce the pressures that were normally necessary for the reaction. As shown in Equation 3, the alcoholic sodium ethoxide was combined with thiophosphoryl chloride at temperatures of $-5°$ to $-10°$ C., followed by removal of the sodium chloride by filtration. The final step was carried out in chlorobenzene during a 15-hour heating period. If this method of preparing diethyl chlorothiophosphate is used, other by-products may be formed which will enter into the final reaction unless the desired chloro ester is very carefully fractionated. Other methods of preparing thiophosphoryl chloride have been reported (*10*).

$$PCl_5 + H_2S \longrightarrow \underline{PSCl_3} + 2HCl \tag{5}$$

$$PCl_5.2FeCl_3 + 5S \longrightarrow 2FeCl_2 + 2S_2Cl_2 + \underline{PSCl_3} \tag{6}$$

$$Fe_2P + 7SCl \longrightarrow 2FeCl_2 + \underline{PSCl_3} + 6S \tag{7}$$

In Equation 6, a method is shown by which, Woodstock and McDonald (*15*) report, thiophosphoryl chloride can be obtained. Equation 7 shows a related method (*16*) for the preparation of this intermediate.

Glatzel (*8*) has reacted iron and antimony chlorides with phosphorus pentasulfide to obtain the desired compound.

$$\underline{6}FeCl_3 + 2P_2S_5 \longrightarrow 3FeCl_2 + \underline{4PSCl_3} + 3FeS_2 \tag{8}$$

$$SbCl_3 + P_2S_5 \longrightarrow SbPS_4 + \underline{PSCl_3} \tag{9}$$

$$3PCl_5 + P_2S_5 \longrightarrow \underline{5PSCl_3} \tag{10}$$

Weber (*8*) states that the intermediate thiophosphoryl chloride can be obtained from phosphorus pentachloride, as shown in Equation 10.

$$5POCl_3 + P_2S_5 \longrightarrow P_2O_5 + \underline{5PSCl_3} \tag{11}$$

$$6SOCl_2 + 2P_2S_5 \longrightarrow \underline{4PSCl_3} + 3SO_2 + 9S \tag{12}$$

$$3CCl_4 + 2P_2S_5 \longrightarrow \underline{4PSCl_3} + 3CS_2 \tag{13}$$

Carius (*8*) replaced the oxygen in phosphoryl chloride by sulfur. This may be somewhat analogous to the replacement of oxygen in a carbonyl group by sulfur using phosphorus pentasulfide. Prinz (*8*) used thionyl chloride in his reaction with phosphorus sulfide and De Fazi (*2*) used carbon tetrachloride in a very interesting preparation of this important intermediate.

Methods of Synthesizing Diethyl Chlorothiophosphate. In 1861 Carius (*1*) reported Equations 14 to 17.

$$O_3\!\left\langle\!\begin{matrix}PS\\(C_2H_5)_3\end{matrix}\right. + PCl_5 \longrightarrow Cl\!\left\langle\!\begin{matrix}O_2\\PS\\(C_2H_5)_2\end{matrix}\right. + Cl_3PO + ClC_2H_5 \tag{14}$$

$$(C_2H_5O)_3PS + PCl_5 \longrightarrow (C_2H_5O)_2\overset{S}{\overset{\|}{P}}\!-\!Cl + POCl_3 + C_2H_5Cl \tag{15}$$

$$O_3\!\left\langle\!\begin{matrix}PS\\(C_2H_5)_2Me\end{matrix}\right. + PCl_5 \longrightarrow Cl\!\left\langle\!\begin{matrix}O_2\\PS\\(C_2H_5)_2\end{matrix}\right. + ClMe + Cl_3PO \tag{16}$$

$$\left[(C_2H_5O)_2\overset{S}{P}\!-\!O\right]Me + PCl_5 \longrightarrow (C_2H_5O)_2\overset{S}{\overset{\|}{P}}\!-\!Cl + MeCl + POCl_3 \tag{17}$$

The authors' interpretation of the reactions which he carried out is given immediately under the equation taken from the original article. In 1945, Mastin, Norman, and Weilmuenster (*7*) reported the preparation of the compound as shown in Equation 18.

$$2C_2H_5OH + 2C_5H_5N + PSCl_3 \longrightarrow (C_2H_5O)_2\overset{S}{\overset{\|}{P}}\!-\!Cl + 2C_5H_5N.HCl \tag{18}$$

The yield obtained was approximately 24%. Much higher yields were probably obtained by Schrader's process (*12*) and by Equations 19 to 27.

$$2(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—SNa + 3Cl_2 \longrightarrow 2(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—Cl + 2NaCl + S_2Cl_2 \quad (19)$$

$$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—SNa + PCl_5 \longrightarrow (C_2H_5O)_2\overset{S}{\overset{\|}{P}}—Cl + PSCl_3(?) + NaCl \quad (20)$$

$$2(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—SH + 3Cl_2 \longrightarrow 2(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—Cl + 2HCl + S_2Cl_2 \quad (21)$$

When chlorine reacted with sodium diethyl dithiophosphate in the presence of an organic solvent, the desired compound was obtained in good yield. The reaction of phosphorus pentachloride on the sodium salt resulted in the same product. Thiophosphoryl chloride is a probable by-product of this reaction. It was later found that diethyl dithiophosphoric acid could be chlorinated in an organic solvent without the intermediate preparation of the sodium salt.

The purity and identity of diethyl chlorothiophosphate prepared by new methods were checked by reaction with phenylhydrazine according to the directions of Mastin, Norman, and Weilmuenster (*7*).

$$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—Cl + H_2NNH\langle\bigcirc\rangle \longrightarrow (C_2H_5O)_2\overset{S}{\overset{\|}{P}}—HNNH\langle\bigcirc\rangle + HCl \quad (22)$$

A general equation for the chlorination of certain dithiophosphoric acids and their salts can be written as follows:

$$2(RO)_2\overset{S}{\overset{\|}{P}}—SM + 3Cl_2 \longrightarrow 2(RO)_2\overset{S}{\overset{\|}{P}}—Cl + 2MCl + S_2Cl_2 \quad (23)$$

R = CH_3, C_2H_5, C_3H_7, iso-C_3H_7, C_4H_9, iso-C_4H_9, C_6H_5

M = H, K, Na

Using this general method, the chloro esters shown in Equation 23 were made. This method of preparation is a rather important discovery and the reaction is not a simple one.

There are numerous methods of preparing the desired intermediate, which can be combined with sodium nitrophenoxide.

$$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—Cl + NaOC_6H_4NO_2 \longrightarrow (C_2H_5O)_2\overset{S}{\overset{\|}{P}}—OC_6H_4NO_2 + NaCl \quad (24)$$

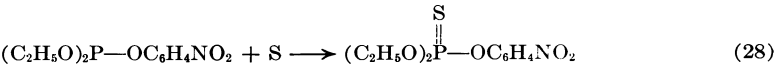
$$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}—Cl + HOC_6H_4NO_2 \xrightarrow{\text{Base}} (C_2H_5O)_2\overset{S}{\overset{\|}{P}}—OC_6H_4NO_2 + HCl \quad (25)$$

The reaction shown in Equation 24 gives good yields, using both aqueous and nonaqueous media, in a much shorter time than that reported by Schrader (*13*). The intermediate chloro ester can be combined directly with paranitrophenol using alkali metal salts in order to prepare sodium nitrophenoxide, which is used in situ.

Another method which has been proposed for the preparation of parathion is shown in Equations 26 to 28.

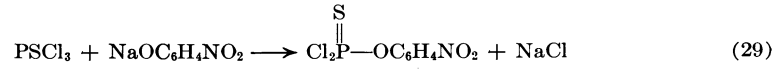
$$PCl_3 + NaOC_6H_4NO_2 \longrightarrow Cl_2P—OC_6H_4NO_2 + NaCl \quad (26)$$

$$Cl_2P—OC_6H_4NO_2 + 2NaOC_2H_5 \longrightarrow (C_2H_5O)_2P—OC_6H_4NO_2 + 2NaCl \quad (27)$$

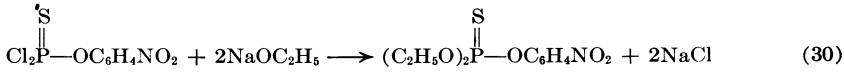


$$(C_2H_5O)_2P{-}OC_6H_4NO_2 + S \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2 \qquad (28)$$

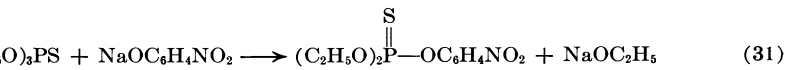
It has been reported that the compound resulting from Equation 26 may be hazardous to distill (*12*). However, the compound resulting from Equation 27 has been prepared and attempts have been made to add sulfur to this phosphite in order to obtain the desired thiophosphate. Similar reactions have been reported in the literature (*3*). A very vigorous reaction takes place. Another method is shown in Equations 29 and 30.



$$PSCl_3 + NaOC_6H_4NO_2 \longrightarrow Cl_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2 + NaCl \qquad (29)$$

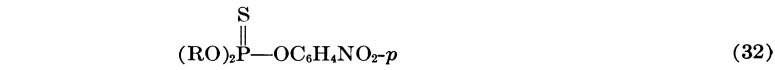


$$Cl_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2 + 2NaOC_2H_5 \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2 + 2NaCl \qquad (30)$$



$$(C_2H_5O)_3PS + NaOC_6H_4NO_2 \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2 + NaOC_2H_5 \qquad (31)$$

Still another method was reported by Schrader (*12*) (Equation 31). Compounds prepared by the general method of reaction of the chloro esters with the nitrophenol, either in the presence of base or by using a salt of the phenol, are shown in Formula 32.



$$(RO)_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2\text{-}p \qquad (32)$$

R = CH_3, C_2H_5, *n*-C_3H_7, iso-C_3H_7, *n*-C_4H_9, iso-C_4H_9, C_6H_5

All these compounds could be prepared by this method.

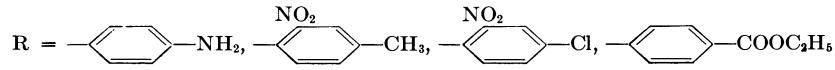
$$C_2H_5O\diagdown \quad \overset{\overset{S}{\|}}{P}{-}OC_6H_4NO_2\text{-}p \quad \diagup C_4H_9O \qquad (33)$$

$$(C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}OC_6H_5 \qquad (34)$$

$$(C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}OC_6H_4CH_3\text{-}p \qquad (35)$$

In Formula 33 is pictured a completely mixed ester of a thiophosphate in which all groups are different. The thiophosphoryl chloride method was used for preparing such compounds. It was of interest to see whether or not other compounds could be prepared by the condensation of a dialkyl chlorothiophosphate with a phenol to give products such as shown in Formulas 34 to 36.

$$(C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}OR \qquad (36)$$



R = —NH_2, —CH_3 (NO_2), —Cl (NO_2), —$COOC_2H_5$

The first compound of Equation 36 is not made by the condensation procedure, but by the chemical reduction of a nitro group in parathion. Some compounds are rather difficult to obtain by the general method. However, the next three compounds shown here were prepared by this method.

Oxygen Analogs of Parathion

Because the Germans had been interested in the oxygen analog of parathion, it was of interest to see whether or not their claims were correct and what other oxygen analogs might be made. In Equations 37 and 38, a convenient method is shown for preparing diethyl chlorophosphate. This method was reported by the British investigators McCombie, Saunders, and Stacey (*5*) in 1945.

$$3C_2H_5OH + PCl_3 \longrightarrow (C_2H_5O)_2POH + C_2H_5Cl + 2HCl \quad (37)$$

$$(C_2H_5O)_2POH + Cl_2 \longrightarrow (C_2H_5O)_2P(=O)—Cl + HCl \quad (38)$$

Diethyl chlorophosphate can also be prepared from ethyl alcohol and phosphoryl chloride in the presence of base, but the method shown is reported to give better yields. The intermediate diethyl chlorophosphate has been condensed with nitrophenol in a manner similar to that used for parathion (Equation 39).

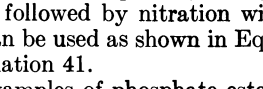
$$(C_2H_5O)_2P(=O)—Cl + HOC_6H_4NO_2 \xrightarrow{\text{Base}} (C_2H_5O)_2P(=O)—OC_6H_4NO_2 + HCl \quad (39)$$

$$(C_2H_5O)_2P(=O)—OC_6H_5 + HNO_3 \longrightarrow (C_2H_5O)_2P(=O)—OC_6H_4NO_2 + H_2O \quad (40)$$

$$(C_2H_5O)_2P(=S)—OC_6H_4NO_2 + HNO_3 \longrightarrow (C_2H_5O)_2P(=O)—OC_6H_4NO_2 + ? \quad (41)$$

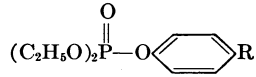
Because phenol is cheaper than nitrophenol, it might cost less to condense phenol with this chloro intermediate, followed by nitration with nitric acid. This method of preparing the oxygen analog can be used as shown in Equation 40. Still another method of preparation is shown in Equation 41.

Formulas 42 to 44 give examples of phosphate esters related to parathion and prepared by the above methods. In some of these compounds the ethyl group has been changed, whereas in others various substituents have been introduced into the benzene ring. A number of the structures shown have been reported by the Germans (*11–13*).



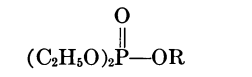
$$(RO)_2P(=O)—O—C_6H_4—NO_2 \quad (42)$$

R = C_2H_5, C_3H_7, *n*-C_4H_9

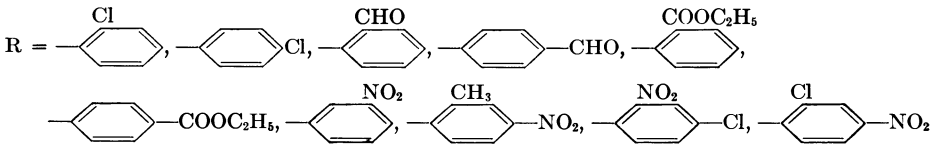


$$(C_2H_5O)_2P(=O)—O—C_6H_4—R \quad (43)$$

R = H, CH_3, SO_2NH_2, NO_2



$$(C_2H_5O)_2P(=O)—OR \quad (44)$$



A large number of problems were encountered and a great many scientists were involved in the development of parathion. Problems in synthesis, engineering, and analysis, as well as formulating and testing procedures were all solved. The compound is effective on a large number of crops and insects, and data sheets can be obtained giving recommended uses and precautions to be taken in handling the material.

In spite of the fact that parathion is such an amazing insecticide, it cannot replace all other insecticides. As a matter of fact, it can be used beneficially with a number of other insecticides and fungicides.

Excellent cooperation has been received from the United States Department of Agriculture and state agricultural experiment stations in carrying out field tests with this material. Without such cooperation this important economic poison would not be in general use at this time.

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Organic Phosphorus Insecticides

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In the field of organic phosphorus compounds there is a wealth of highly toxic compounds from which to pick a potential insecticide. The ultimate choice will be based not only on toxicity to a certain group of insect species, but on volatility, stability, safety in handling and applying, and freedom from plant injury, spray-residue and translocation hazards, and long-term toxicity to man and animals.

The new field of organic phosphorus insecticides was opened up during World War II by Gerhard Schrader, a German chemist employed at the Elberfeld laboratories of I. G. Farbenindustrie (*37*, *38*). Schrader, who had done earlier work on insecticides, was engaged primarily in the search for chemical warfare agents. In the preface to Schrader's report (*38*) Mumford and Perren make the following statement regarding the organic phosphorus insecticides described in the report:

> It is known that other substances of similar general type are still more toxic to higher animals, and it is quite possible that workers undertaking synthetic studies in this series may prepare a substance sufficiently toxic to constitute a real danger to themselves and others in the vicinity. It is therefore recommended that stringent safety precautions should be taken when preparing and testing any compound of this type, unless it has already been shown by appropriate tests to be comparatively harmless to higher animals.

From this statement it may be inferred that Schrader's insecticides merged into toxic warfare agents or vice versa, depending on his point of approach.

It is estimated from available reports (*32*, *38*, *40*, *44*, *45*) that Schrader synthesized well over 300 compounds containing organically bound phosphorus. Although the reports are notably lacking in completeness, it appears that most of Schrader's compounds were screened for biological activity against aphids (species not given). Approximately 150 compounds tested as a spray at 0.2% concentration gave 90 to 100% mortalities of the aphids. Apparently some of his data are missing, or else only a portion of the compounds active at 0.2% were tested at lower concentrations. However, it can be estimated that about 10% of the compounds active at 0.2% continued to give 100% mortalities when diluted to 0.002%. This will convey some idea of the extraordinary biological activity associated with this series of compounds. There is little evidence of any particular compound in Schrader's series possessing a selective toxicity to insects. In general (although there may be an exception), the compounds that are highly toxic to insects are also highly toxic to warm-blooded animals.

The toxic organic phosphorus compounds act as powerful inhibitors of cholinesterase, an enzyme found predominantly in the nervous tissue of animals, including insects. This enzyme hydrolyzes acetylcholine, which plays an essential role in the transmission of nerve impulses. The toxicity of compounds in this series can be largely accounted for on the basis of their anticholinesterase activity (*7*, *8*, *12*, *14*, *31*).

At about the time Schrader was synthesizing new organic phosphorus compounds of extraordinary toxicity, McCombie and Saunders (*29*) in England were intensively engaged in a similar endeavor. They investigated especially the dialkyl fluophosphates. Mackworth and Webb (*31*), who tested these compounds, found them to be highly potent inhibitors of horse serum cholinesterase. The most active ester, diisopropyl fluophosphate, which British investigators call diisopropyl fluorophosphonate (DFP), was 30 times as active as the alkaloid eserine. In their series of fluophosphates they found a correlation between the in vitro inhibitory power of cholinesterase and toxicity. Webb (*48*) also found that the inhibitory effects of the alkyl fluophosphates are not confined to the enzyme cholinesterase. They may poison a range of esterases, some of which have no activity at all toward acetylcholine as a substrate.

The inhibition by fluophosphates is similar to that reported earlier by Bloch and Hottinger (*5*) for tri-*o*-cresyl phosphate and tri-*o*-chlorophenyl phosphate. DuBois and Mangun (*14*) found that hexaethyl tetraphosphate exerts a strong inhibitory effect on mammalian and insect cholinesterase in vitro and in vivo. It is of interest that hexaethyl tetraphosphate and its principal active ingredient, tetraethyl pyrophosphate, were found by Jansen *et al.* (*25*) to inhibit the enzyme acetylesterase, which is of plant origin. Brauer (*7*) has studied the cholinesterase-inhibiting activities of a series of organophosphorus compounds. He has proposed a rule for the structural requirements of an organophosphorus inhibitor and also a mechanism for its inhibiting effect on the enzyme. DuBois and associates (*13*) found parathion to be a strong inhibitor of cholinesterase in rats.

Nachmanson and co-workers (*34*) have pointed out that the quantity of cholinesterase present in most organisms may vary considerably in different species and even in different tissues of the same species. Their studies indicate that an organism—an insect, for example—does not begin to show toxic effects until 66 to 95% of the enzyme has been inactivated. There also appears to be a very small differential between a nonlethal and a

Table I. Properties of Organic Phosphorus Insecticides

Compound	Boiling Point, ° C.	Refractive Index at 25° C.	Density at 25° C.	Solubility in Water	Effectiveness[a] Concn. of spray soln., %	Effectiveness[a] Kill of aphids, %	Remarks and References
Parathion	157–162[b] 0.6 mm.	1.5370[c]	1.2655[c]	Slight (15–20 p.p.m.)	0.001	100	Pale yellow, almost odorless oil; crystallizes in long needles, m.p. 6° C. Technical product may have garliclike odor (*2, 3, 17, 19, 46*)
Oxygen analog of parathion	148–151[d] 1 mm. 173/1 mm.[e]	1.5060[d]	1.269[d] (sp. gr.)	Slight (0.25%)	0.005 0.001	100 50	Practically odorless, reddish-yellow oil (*4, 10, 38*)
Tetraethyl pyrophosphate	104–110[f] 0.08 mm. 135–138[g] 1 mm.	1.4170[f] 1.4180[g]	1.1810[f] 1.1901[g] (sp. gr. at 24°)	Completely miscible	0.05	100	Odorless, colorless, hygroscopic oil. Hydrolyzes rapidly. Technical products called TEPP and HETP (*10, 15, 21, 23, 24, 37, 39, 47, 49, 50*)
Tetraethyl dithiopyrophosphate	110–113[d] 0.2 mm. 135/2 mm.[e]	1.4753[d]	1.196[d]	Slight	0.005	100	Yellow to colorless oil. Technical product may have unpleasant odor (*4, 22, 38*)
Tetraethyl monothiopyrophosphate	110–117[f] 0.3 mm.	1.4466[f]	1.1833[f]	Slight (0.06%)	0.005	100	Colorless oil of unpleasant odor (*10, 22, 38*)
Octamethyl pyrophosphoramide	118–122[f] 0.3 mm.	1.4612[f]	1.1343[f]	Completely miscible	0.05	100	Almost odorless, colorless, somewhat viscous oil. Systemic poison. Absorbed by living plant (*10, 11, 36, 38*)

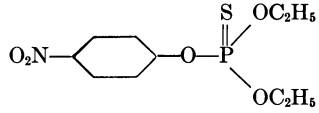
[a] Schrader's screening tests (*38*).
[b] Fletcher *et al.* (*18*).
[c] Edwards and Hall (*17*).
[d] Adler, Victor Chemical Works, Chicago, Ill.
[e] Schrader (*38*).
[f] Hall *et al.* (*22, 24*).
[g] Toy (*47*).

lethal dose. Their experiments with diisopropyl fluophosphate demonstrated that its toxicity must be attributed exclusively to the inactivation of cholinesterase.

Because toxicity to insects is only one criterion of a potential insecticide, no attempt is made here to examine all of Schrader's toxic compounds. Mumford and Perren (*38*) have indicated that some of these compounds may not be described for reasons of security. Only those compounds containing organically bound phosphorus which have already shown some promise of joining the family of insecticides are described here. All are high-boiling liquids which, in general, undergo thermal decomposition and hence must be distilled at reduced pressure. As a further generalization the P-O-P linkage of these compounds undergoes hydrolytic cleavage (more readily at alkaline pH), which results in loss of effectiveness of the compound. Some properties of the compounds are listed in Table I.

Parathion

Parathion is the accepted trivial name for *O,O*-diethyl *O*-*p*-nitrophenyl thiophos-



Parathion

phate, which at the present time is the most prominent of the organic phosphorus insecticides. In contrast to the tetraethyl pyrophosphate type of insecticide, which hydrolyzes completely in a day or two, parathion is reasonably stable. It is the first of Schrader's stable anticholinesterases to be developed as an insecticide on a commercial scale. Its popularity may be due to this fact or to the possibility that parathion may actually possess a certain combination of desirable properties not shared by any other compound in Schrader's series. It was first described as E-605 by Schrader (*32, 38, 44, 45*) who prepared it as follows:

$$PSCl_3 + 2C_2H_5ONa \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}Cl + 2NaCl \qquad (1)$$

$$(C_2H_5O)_2\overset{\overset{S}{\|}}{P}Cl + NaO{-}C_6H_4NO_2 \longrightarrow (C_2H_5O)_2\overset{\overset{S}{\|}}{P}{-}O{-}C_6H_4NO_2 + NaCl \qquad (2)$$

Chlorobenzene was the solvent preferred by the Germans for carrying out the reaction in step 2.

The foregoing reaction scheme had been described in a patent issued in 1934 to Clemmensen (*9*), who prepared certain organic esters of thiophosphoric acid embodying both aromatic and aliphatic radicals. These esters are claimed as new compositions of matter useful as fire retardants. Parathion itself is not specifically described in the patent.

Fletcher *et al.* (*18*) have given details of its laboratory preparation by Schrader's method, in which the yield in step 1 was 50%. In step 2, with ethyl alcohol used as the solvent, a yield of 75% of the vacuum-distilled ester was obtained. Water was also tried as the solvent in step 2, and the yield was 64% of crude undistilled parathion. Its preparation on a larger laboratory scale has been described in Australian patents (*2*) of the American Cyanamid Company. High yields of the crude ester were obtained in step 2, in which various solvents, especially ketones, were successfully employed. Also described is a modification of step 2 in which the diethyl chlorothiophosphate reacts with *p*-nitrophenol in the presence of anhydrous sodium carbonate or other suitable alkali. No directions are given in the patents for preparing the diethyl chlorothiophosphate or other dialkyl chlorothiophosphates used to prepare analogs of parathion. Thurston (*46*) has recently reviewed the methods for preparing dialkyl chlorothiophosphates.

The Australian patents also contain descriptions of analogs, which include nitrophenyl thiophosphates possessing more than one nitro group. Many of the analogs are biologically active, and several exhibit high activities comparable with parathion. The *o*-nitrophenyl isomer, which is present to some extent in crude parathion, is substantially less active than the *p*-nitrophenyl isomer (parathion itself). These patents disclose a synergistic effect of the ortho isomer with parathion.

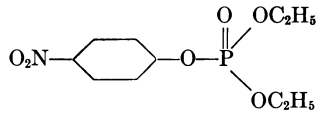
Pure parathion is a pale yellow, practically odorless oil, which crystallizes in long white needles melting at 6.0° C. (*17*). It is soluble in organic solvents, except kerosenes of low aromatic content, and is only slightly soluble in water (15 to 20 p.p.m. at 20° to 25° C.). Peck (*35*) measured its rate of hydrolysis to diethyl thiophosphate and nitrophenate ions in alkaline solutions. He found that the reaction kinetics are first order with respect to the ester and to hydroxyl ion. In normal sulfuric acid the rate of hydrolysis was the same as in distilled water. Peck concluded that hydrolysis takes place by two mechanisms—a reaction catalyzed by hydroxyl ions and an independent uncatalyzed reaction with water. He calculated that at a pH below 10 the time for 50% hydrolysis at 25° C. is 120 days; in the presence of saturated lime water the time is 8 hours. The over-all velocity constant at 25° C. is $k = 0.047\ [OH^-] + 4 \times 10^{-6}$ min.$^{-1}$

Averell and Norris (*3*) have developed an analytical method adapted to the determination of parathion in spray or dust residues, which is sensitive to about 20 micrograms. It is based upon the reduction of parathion with zinc to the amino compound, diazotization, and coupling with Bratton and Marshall's amine, which gives an intense magenta color with an absorption peak at 555 millimicrons. Bowen and Edwards (*6*) have used the polarograph to assay technical grades of parathion and its formulations.

Parathion is coming into extensive use. Applied at concentrations ranging from about 25 to 600 p.p.m., it has been found highly effective against an imposing list of insect species (*19*, *46*). The scope of safe application of this powerful insecticidal compound is not yet defined. If its high toxicity (*13*, *28*) to man and animals does not stand in the way of its use on food and fodder crops, it may indeed have a very wide field of application. The answer to the question of its ultimate usefulness must await the outcome of many large-scale field tests to cover all aspects of the spray-residue problem, including possible translocation (*20*) of the compound.

Oxygen Analog of Parathion

Diethyl *p*-nitrophenyl phosphate was designated by Schrader (*38*) as E-600, the



Oxygen analog of parathion

immediate forerunner of his E-605, parathion. In Germany, where the two esters were first compared for insecticidal use, parathion was preferred (*32*, *44*) because E-605 was less poisonous to man and not so readily absorbed through the skin. DuBois and associates (*13*) have found the oxygen analog more toxic than parathion to rats.

Schrader prepared the ester (*38*) in 60% yield by reaction of sodium *p*-nitrophenate with diethyl chlorophosphate, using xylene as solvent for the reaction. He made it, but in lower yields, from *p*-nitrophenol and diethyl chlorophosphate, using, respectively, pyridine and sodium cyanide as acceptors for hydrogen chloride. Schrader also prepared it in 96% yield by nitrating diethyl phenyl phosphate at 0° C. or below. Under the conditions he used, Schrader claims that the nitro group is directed to the para position. No yield is given for the diethyl phenyl phosphate, which he presumably made from sodium phenate and diethyl chlorophosphate. Diethyl chlorophosphate may be prepared in high yield (*30*) from diethyl phosphite and chlorine.

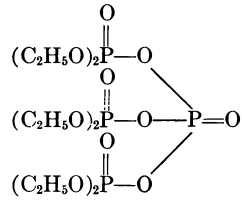
Diethyl *p*-nitrophenyl phosphate (E-600) is an odorless reddish-yellow oil which

possesses physical properties and solubility characteristics similar to parathion. Its solubility (*22*) in water is about 0.25% at 25° C. Schrader (*38*) dissolved 0.5 gram of the ester "by vigorous shaking in 10 liters of water." The solution, it is reported, was sprayed with complete effectiveness on a cineraria plant infested with aphids (species not given). The statement is made in one of the British BIOS reports (*32*) that E-600 but not E-605 is absorbed to a slight degree by the living plant. No data are given. Coates (*10*) has calculated that the ester is about 300 times more stable to hydrolysis than tetraethyl pyrophospate. His value for the over-all velocity constant is $k = 0.52\ [OH^-] + 1 \times 10^{-6}$ min.$^{-1}$ Ball and Allen (*4*) have found that diethyl *p*-nitrophenyl phosphate is the most effective of the organic phosphates against the housefly, milkweed bug, cockroach, and two species of aphids. The Bureau of Entomology and Plant Quarantine in preliminary tests has found it very effective against the European corn borer, armyworm, celery leaf tier, large milkweed bug, pea aphid, and two-spotted spider mite.

The colorimetric method of Averell and Norris (*3*) for estimation of parathion is also applicable to the oxygen analog, which gives a magenta color of identical absorption peak (*16*). The polarographic method of Bowen and Edwards (*6*) is also applicable to the analysis of this ester.

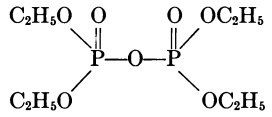
Tetraethyl Pyrophosphate Insecticides

The first of Schrader's organic phosphorus anticholinesterases to find rather large-scale application as an insecticide in Germany during World War II was his so-called hexaethyl tetraphosphate (*37*), which the Germans called Bladan. Schrader obtained German and United States patents (*39*) on this material, and ascribed to it a branched-chain structure. Hall and Jacobson (*24*) found that it is a mixture of ethyl polyphos-



Schrader's hexaethyl tetraphosphate
(structure hypothetical)

phates containing as its principal active ingredient the compound tetraethyl pyrophosphate. Hexaethyl tetraphosphate was first made (*39*) by reaction of 3 moles of triethyl



Tetraethyl pyrophosphate

phosphate with 1 mole of phosphorus oxychloride (Equation 3); the reaction was subsequently modified by increasing the molar ratio of triethyl phosphate to phosphorus oxychloride (Equation 4). Another process, patented by Woodstock (*49*), in which phosphoric anhydride was used instead of phosphorus oxychloride is illustrated in Equations 5 and 6.

$POCl_3$ Procedure.

$$3(C_2H_5)_3PO_4 + POCl_3 \longrightarrow (C_2H_5)_6P_4O_{13} + 3C_2H_5Cl \quad (3)$$

$$5(C_2H_5)_3PO_4 + POCl_3 \longrightarrow 3(C_2H_5)_4P_2O_7 + 3C_2H_5Cl \quad (4)$$

P_2O_5 Procedure.

$$2(C_2H_5)_3PO_4 + P_2O_5 \longrightarrow (C_2H_5)_6P_4O_{13} \quad (5)$$

$$4(C_2H_5)_3PO_4 + P_2O_5 \longrightarrow 3(C_2H_5)_4P_2O_7 \quad (6)$$

In all cases the reaction products are mixtures of ethyl polyphosphates, and, on the basis of elementary analysis, they approximate the empirical formulas given in the above equations. In Equations 3 and 5 the product has been arbitrarily called hexaethyl tetraphosphate, which may contain 8 to 20% of the active tetraethyl pyrophosphate. In Equations 4 and 6 the products have been called technical tetraethyl pyrophosphate, which may contain up to 40% of pure tetraethyl pyrophosphate. Hexaethyl tetraphosphate has also been made from phosphoric anhydride and diethyl ether by a process recently patented by Adler (*1*).

Kosolapoff (*26*, *27*) has proposed a mechanism for the reactions based upon addition of phosphoryl chloride or phosphorus pentoxide to the PO bond of triethyl phosphate to give intermediate phosphonium-type adducts which may form linear and cyclic polyesters by thermal decomposition. He has advanced the view that the actual tetraethyl pyrophosphate content of these complex mixtures may be very small and that the biologically active ester may be generated from the cyclic polyphosphates by partial hydrolysis. This theory may find some support in the entomological experiments of Smith, Fulton, and Lung (*43*) with tetraethyl pyrophosphate insecticides in methyl chloride applied as aerosols in greenhouses. They found that, although the kill of spider mites correlated well with the tetraethyl pyrophosphate content of their samples, such was not the case with aphid mortalities. The hexaethyl tetraphosphate manufactured by Schrader's original process was found more effective against aphids, suggesting that it contains toxic principles other than tetraethyl pyrophosphate. Smith and Fulton pointed out that with their mode of testing, the ethyl polyphosphate mixtures are in an anhydrous condition until they leave the nozzle at the time of application. The aerosols thus differ from spray solutions, in which at least some of the toxicity is lost by hydrolysis when the ethyl polyphosphates are dissolved in water before application. Because aerosol application in greenhouses is responsible for a large part of the production of this insecticide, there has recently been a trend back to the manufacture of hexaethyl tetraphosphate as originally produced about 3 years ago.

The purified tetraethyl pyrophosphate is a colorless, odorless, water-soluble, hygroscopic liquid (*24*, *47*). It possesses a very high acute toxicity (*28*), exceeding that of parathion, and is rapidly absorbed through the skin. There is no spray-residue problem, however, for tetraethyl pyrophosphate hydrolyzes even in the absence of alkali to nontoxic diethyl phosphoric acid. Hall and Jacobson (*24*) and Toy (*47*) have measured its rate of hydrolysis, which is a first-order reaction. Its half-life at 25° C. is 6.8 hours and at 38° C. is 3.3 hours. Coates (*10*) determined the over-all velocity constant at 25° C.: $k = 160\ [OH^-] + 1.6 \times 10^{-3}$ min.$^{-1}$ Toy (*47*) has described an elegant method for preparing this ester as well as other tetraalkyl pyrophosphates, based upon the controlled hydrolysis of 2 moles of dialkyl chlorophosphate:

$$2(C_2H_5O)_2POCl + H_2O \longrightarrow (C_2H_5O)_2\overset{\overset{O}{\|}}{P}—O—\overset{\overset{O}{\|}}{P}(OC_2H_5)_2 + 2HCl$$

The hydrogen chloride is removed either by reduced pressure or by salt formation with pyridine or sodium bicarbonate; the latter procedure gave high yields of the pure ester. Toy (*47*) also measured the hydrolysis rates and compared the toxicities of a series of tetraalkyl pyrophosphates. Of these tested, the tetraethyl ester was the most toxic to white mice.

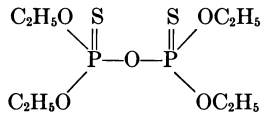
Several chemical-assay methods (*15*, *23*, *50*) for tetraethyl pyrophosphate were recently developed and applied by seven collaborating laboratories to samples of representative commercial products and to a sample of purified tetraethyl pyrophosphate which served as a common standard. Concordant results, which correlated well with bioassay results,

were obtained. The methods used (*21*) are based upon the selective hydrolysis of the sample followed by separation of the tetraethyl pyrophosphate from acidic constituents. The methods differ only in techniques of separation or hydrolysis.

The ultimate usefulness of technical tetraethyl pyrophosphate depends largely on its rapid killing action, its compatibility with sulfur, and its freedom from residual toxicity hazards. On the other hand, it is not profitably employed where a certain amount of residual toxicity is desirable or necessary to kill a particular insect species. Tetraethyl pyrophosphate has found wide use as an aerosol to control pests on greenhouse vegetables and flower crops. Because it leaves no residual toxic vapors, it offers a definite advantage from the standpoint of safety to the greenhouse operator. In addition, there is evidence that its use in greenhouses has a stimulatory action on plant growth, particularly on rose plants, which have produced "larger leaves with a deeper green color, longer and heavier stems, and more flowers" (*42*).

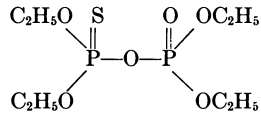
Thio Analogs of Tetraethyl Pyrophosphate

Tetraethyl dithiopyrophosphate has recently been made available in experimental



Tetraethyl dithiopyrophosphate

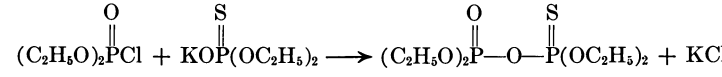
quantities and has been tested more widely than the tetraethyl monothiopyrophosphate.



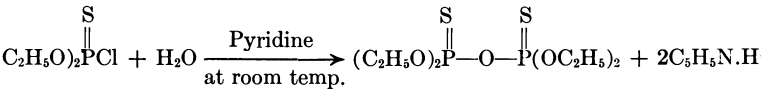
Tetraethyl monothiopyrophosphate

Schrader (*38*) in a brief description characterized tetraethyl pyrophosphate as "not completely water-stable," the monothio analog as "water-stable," and the dithio analog as "lime-stable."

The monothio analog was prepared (*22*) in 67% yield as follows:



As a solvent for this reaction, anhydrous methyl ethyl ketone was found satisfactory. Coates (*10*) determined the rate of hydrolysis of the monothio analog as approximately one fifth that of tetraethyl pyrophosphate under similar conditions. The dithio analog has been prepared (*22*) in 90% yield from diethyl chlorothiophosphate, water, and pyridine in a modification of the reaction Toy (*47*) used to make tetraethyl pyrophosphate:

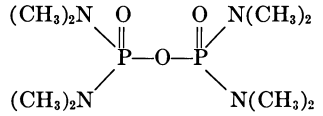


Ball and Allen (*4*) found that the dithio analog compared favorably with tetraethyl pyrophosphate. Other preliminary tests in the bureau have indicated that the dithio analog is very effective against the European corn borer, large milkweed bug, celery leaf tier, armyworm, and two-spotted spider mite. Smith (*41*) has tested both analogs as aerosols in greenhouses. It is too early to assess the value of these analogs, but they may fit into the insecticide picture as compounds of intermediate stability to hydrolysis, lying somewhere between tetraethyl pyrophosphate and parathion, in the region of tox-

icity desirable for the control of insects but not of sufficient duration to constitute a hazard to man.

Octamethyl Pyrophosphoramide

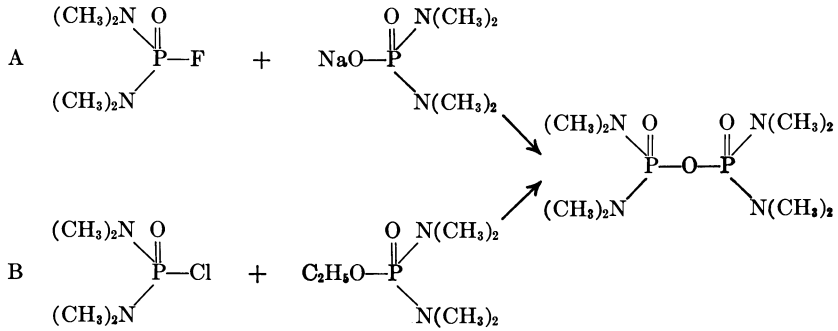
Toxic compounds that can be absorbed to a marked degree by a living plant through either its roots or its leaves have been called by British investigators systemic insecticides. Schrader (*38*) first found this peculiar property in certain acetals of 2-fluoroethanol and bis-(2-fluoroethoxy)methane, as well as in certain compounds of his organic phosphorus series, notably bis(dimethylamido)fluophosphate and octamethyl pyrophosphoramide.



Octamethyl pyrophosphoramide

The latter compound has the practical advantage over the former of not containing fluorine, which might on repeated application accumulate in the soil and poison it.

Schrader (*38*) prepared the pyrophosphoramide as follows:



No details are given for scheme A. Presumably one could use the phosphoryl chloride instead of the fluoride. Scheme B, in which ethyl chloride is formed, was run in boiling xylene using equimolar quantities of the reactants. Michaelis (*33*) has partially described the preparation of starting materials from secondary amines with phosphorus oxychloride and also ethyl dichlorophosphate. Schrader (*38*) obtained alkyl and amido fluophosphates by reaction of the corresponding chlorophosphates with sodium fluoride in aqueous or alcoholic solution.

Octamethyl pyrophosphoramide is a colorless oil, completely soluble in water, benzene, acetone, and many other common organic solvents except the paraffinic hydrocarbons. Its hydrolysis rate has not been measured, but it appears stable in the absence of alkali. In England, this systemic insecticide has been used to control aphids on hops. There it has been calculated that only a negligible quantity of the poison ultimately may find its way into the beer made from the hops. Despite calculations of this sort, the use of octamethyl pyrophosphoramide on food or fodder crops in this country is definitely not to be recommended. However, it may prove useful if properly applied to control certain insects, especially those attacking ornamental plants, such as rosebushes, and possibly on the cotton aphid and grape phylloxera. The compound has only recently been made available experimentally.

Recent Developments

Since the presentation of this paper in March 1949 at San Francisco there have been several new developments in the field of organophosphorus insecticides.

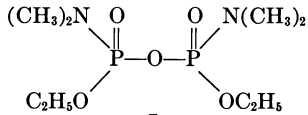
Methyl Homolog of Parathion. *O,O*-Dimethyl *O*-*p*-nitrophenyl thiophosphate

has been manufactured in Germany for insecticidal formulations to supplement or replace parathion. The methyl homolog (*22*) is a white crystalline compound melting at 35° C. Preliminary tests indicate that it is a somewhat less potent insecticide than parathion but may prove less hazardous to apply than parathion, which has caused a number of cases of acute poisoning and several deaths of individuals who handled and applied it without sufficient precautions.

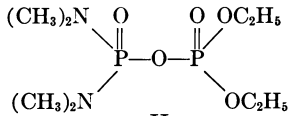
Tetraisopropyl Pyrophosphate. This ester, a water-white liquid, has been prepared by Toy (*47*) in 94% yield from diisopropyl chlorophosphate and water in the presence of pyridine. The ester is insecticidal and is about one tenth as toxic to white mice as tetraethyl pyrophosphate. An insecticidal dust of much greater stability than tetraethyl pyrophosphate dust can be formulated from tetraisopropyl pyrophosphate, inasmuch as the tetraisopropyl ester hydrolyzes at approximately $^1/_{50}$th the rate at which the tetraethyl ester breaks down in the presence of moisture.

Octamethyl Pyrophosphoramide and Analogs. David and Kilby (*11*) have recently published details of Schrader's synthesis, which gives a good over-all yield of octamethyl pyrophosphoramide. Also included in this paper are data on its absorption by the roots and leaves of a living plant, its phytotoxicity, and mode of action of insects infesting the plant. Ripper and associates (*36*) give entomological data on this insecticide and results of its action on plants and on warm-blooded animals. They point out that its systemic properties enable it to reach insects which are extremely difficult to kill by direct contact application and that its persistence enables it to be used early in the development of an aphis outbreak without fear of reinfestation.

Analogs of octamethyl pyrophosphoramide which act as systemic insecticides are the isomeric symmetrical (I) and unsymmetrical (II) diethyl bis(dimethylamido)pyrophosphates:



I



II

Conclusions

In the field of organic phosphorus compounds uncovered by Schrader there is a wealth of highly toxic compounds from which to pick a potential insecticide. The ultimate choice, however, will not be based solely on the toxicity of the compound to a certain group of insect species. Other important factors are volatility, stability, safety in handling and applying, freedom from plant injury, freedom from spray-residue and translocation hazards, and safety from the standpoint of its long-term chronic toxicity to man and animals. As a determining factor for its use, the cost per pound of an organic phosphorus insecticide may not be very important. To compare the cost per pound of parathion, for example, with that of DDT on a realistic basis, one must bear in mind that parathion is effectively applied at much lower concentrations (*2*, *19*, *46*). These potent new organic phosphorus insecticides may not be used, in general, to control insects affecting man and animals (household pests, cattle and sheep pests, etc.) because of their extreme toxicity to warm-blooded animals (*13*, *28*). However, because they are effective over a very wide range of insect species at concentrations so low as to be almost fantastic, the potential usefulness of this class of insecticides, if properly applied, needs no emphasis.

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Chemistry and Toxicity of Some Organofluorine Insecticides

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The chemistry, insecticidal, activity and toxicity of the major organofluorine insecticides are reviewed. In the ten years since the discovery of DDT opened up a new field of endeavor for the chemist, biologist, and toxicologist, activity in the field of fluorine-containing insecticides has been great.

The discovery of the phenomenal insecticidal activity of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT), which occurred less than 10 years ago, opened up a new field of endeavor for the chemist, biologist, and toxicologist. The activity in this field is considerable, and a portion of it has been directed toward efforts to locate useful insecticides among the fluorine-containing compounds. Some of the fluorine compounds known to be insecticidal were re-evaluated, other compounds were tested biologically for the first time, and new compounds were prepared to be subjected to such tests.

Elemental fluorine, inorganic combinations of fluorine, and organofluorine compounds are known to be general systemic poisons (*74*). Fluorine itself appears to be about as toxic as hydrogen cyanide to certain species of insects, with the effective insecticidal concentration lying between 100 and 1000 p.p.m. (*81*). A number of inorganic fluorine compounds—e.g., volatile fluorine compounds (*76*), metallic fluorides, fluosilicates, and fluoaluminates—have been employed for some 50 years mainly as stomach poisons for household and agricultural insects (*15*). Furthermore, the assertion has been made that any inorganic compound containing fluorine will mothproof wool (*16*). More recently improved methods for introducing fluorine into organic molecules (*1*, *73*) have provided samples of an increased number of fluorinated compounds for the determination of their toxicity toward insects. These developments would seem to justify a review of the pertinent information which has been published on the organofluorine insecticides, with special reference to the methods for their synthesis and to their biological activities. Such hybrids as the hydrofluorides (*108*), fluosilicates (*100*), and fluosulfonates (*98*) of aromatic amines and the fluoborates of some organic acids (*99*), although known to be active insecticides, are not included.

For the purpose of this review, the compounds considered are distributed among the following sections: 1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)-ethane, analogs of 1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)-ethane, and miscellaneous organofluorine compounds. The chemistry, insecticidal activity, and toxicity of these groups of compounds are considered in the order given. Much of the work in synthetic insecticides, especially the biological portion, is necessarily of an exploratory character, and hence will have to be supported before appraisals become final.

Chemistry

1,1,1-Trichloro-2,2-bis(*p*-fluorophenyl)-ethane. At present, the most important organofluorine insecticide is 1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)-ethane

(DFDT), the *p,p'*-difluorine analog of DDT. DFDT was not specifically mentioned in the original United States patent (*86*) issued to Paul Müller and assigned to J. R. Geigy, A.-G. However, it is reported to have been used in large quantities in Germany (under the name Fluorogesarol) as an all-purpose insecticide as early as 1944 (*64*). DFDT can be prepared by methods similar to those used to produce DDT—i.e., by condensing two molecular equivalents of fluorobenzene with chloral in the presence of concentrated sulfuric acid. The fluorobenzene required for the reaction was obtained in Germany by the fission of nitrogen from phenyldiazonium fluoride which resulted from diazotizing aniline in the presence of copper and hydrofluoric acid (*106*). The laboratory methods that have been used to prepare DFDT are given in outline form in Table I. The selected items of information were not uniformly available.

Table I. Synopsis of Laboratory Methods for Preparing 1,1,1-Trichloro-2,2-bis-(p-fluorophenyl)-ethane

CCl_3-CHO, Moles	PhF, Moles	Condensing Agent	Moles	Order of Mixing[a]	Conditions: Temp., ° C.	Conditions: Time, Hours	Yield, %	M.P., ° C.	Reference
1	2	H_2SO_4	...	...	...	..	..	...	(*20*)
1[b]	2	HSO_3Cl	...	...	...	..	..	b.8 160–80	(*39*)
0.1	0.2	$H_2SO_4.H_2O$	8	H_2SO_4	30–50	36	..	43–4	(*85*)
0.42[c]	0.84	H_2SO_4[d]	20	PhF	45	2	67	41–2	(*65*)
[c]	...	H_2SO_4	...	...	...	..	..	26	(*107*)
1.1	2.0	H_2SO_4	4	H_2SO_4	25	2	28	42–3	(*10*)
1.1	2.0	H_2SO_4	4	H_2SO_4	0	12	50	...	(*10*)
0.8	2.0	H_2SO_4[e]	3.4	Acid	10–20	2	81	...	(*10*)
					30	10			
0.23	0.72	HSO_3Cl	0.35	HSO_3Cl	Cool	..	76	...	(*10*)
0.10	0.20	HSO_3Cl	1.8	HSO_3Cl	5	2	68	40	(*109*)
					30	20			
0.53[c]	0.117	HSO_3Cl	0.11	HSO_3Cl	...	12	73	b.7–8 172–6	(*60*)
0.1[f]	0.25	H_2SO_4	1	H_2SO_4	Cooled	12	..	45–6	(*33*)

[a] Compound listed in this column added to mixture of other reactants.
[b] Chloral ethylate used in place of free chloral in this reaction.
[c] Chloral hydrate used in place of free chloral in this reaction.
[d] 60 ml. of 20% fuming H_2SO_4 added during course of reaction.
[e] 50 ml. of fuming H_2SO_4 added during course of reaction.
[f] 1,2,2,2-Tetrachloroethyl ether used in place of chloral.

TABLE I. It is probable that all the indicated yields can be improved. However, using these data as a criterion, it appears that the chloral can be replaced by its hydrate or alcoholate and that a moderate excess of fluorobenzene favors the reaction. There is one report that aluminum chloride brings about this condensation (*34*), but here, as with DDT, the choice condensing agents are concentrated sulfuric acid (with or without the addition of oleum), and chlorosulfonic acid (*84*). A moderate temperature and prolonged stirring, which must also be vigorous, increase the yield.

DFDT is usually obtained in the laboratory as an almost-white, gummy material with an odor resembling that of ripe apples. (A recent sample of technical grade DFDT supplied through the courtesy of W. M. Lee, Pennsylvania Salt Manufacturing Company, Philadelphia, Pa., met this description and melted at 35–37° C.) In some instances, refrigeration is necessary to bring about solidification. This product is a mixture of several isomers of 1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)-ethane from which the *p,p'*isomer, boiling point, 133–34° C., melting point 42–43° C., can be separated by distillation at reduced pressures (*10*). The recrystallization of crude DFDT is attended by considerable loss due to its high solubility in the common organic solvents. Dissolving the low-melting crude mixture in ethyl alcohol and adding water to the point of incipient precipitation followed by cooling raised the melting point to 40° (*109*). Recrystallization from ethyl alcohol produced needle-shaped crystals, melting point 44–45° (*79*), and from methanol (twice) crystals melting at 45° (*78*). (The oil from which these crystals were obtained had a boiling point at 1 mm. of 133–134° and a refractive index of 1.5707 at 20° C.) The vapor pressure of DFDT (0.5 mm. of mercury at 178°) (*79*) is reported to be some fifteen times that of DDT (*3*). In a recent study in this laboratory, Goette found that a 400 mg. per square foot deposit of DFDT on protected glass panels lost 95% of its weight in 9 weeks

as compared to a 15% loss for DDT (*36*). This has considerable influence on the activity of DFDT as a residual insecticide. Both DDT and DFDT are extremely insoluble in water, but DFDT (*79*) is approximately ten times as soluble as DDT (*61*) in such solvents as mineral oil, kerosene, dibutyl phthalate, Velsicol AR-60 (a mixture of polymethylnaphthalenes supplied by the Velsicol Corporation, Chicago, Ill.), carbon tetrachloride, xylene, *o*-dichlorobenzene, and cyclohexanone. These solvents are commonly used as vehicles for insecticidal formulations, thus giving DFDT an advantage over DDT in this respect.

Like other 2,2-diaryltrichloroethanes, DFDT undergoes dehydrohalogenation in the presence of a base to yield 1,1-dichloro-2,2-bis(*p*-fluorophenyl)-ethylene. The rate of this reaction has been found to be directly proportional to the temperature, and the rate constant for DFDT is approximately one seventh that for DDT at ordinary temperatures (*18*, *110*). This ethylene derivative has been oxidized by the use of chromic anhydride to *p,p'*-difluorobenzophenone, a sample of which did not depress the melting point of an authentic sample prepared by a different route (*10*).

DFDT has been subjected to the usual analysis for its halogen content, but no specific method has been worked out for its determination, nor has an investigation been made of its response to the known colorimetric reactions of DDT.

Analogs of 1,1,1-Trichloro-2,2-bis(*p*-fluorophenyl)-ethane. The broad and powerful insecticidal activity of DDT appears to a marked degree in several compounds related to it in structure. Because the activity of DFDT against certain species of insects compares very favorably with that of DDT, several analogs of DFDT have been prepared for biological testing. Some of these analogs were obtained by reactions corresponding to those used to prepare DFDT, while others were prepared directly from the DDT or DFDT molecules themselves. The reactions which have been used to prepare the DFDT analogs are outlined in Table II. All available information is included.

TABLE II. Several of the reactions outlined in Table II were discussed under the chemical reactions of DFDT. In general, the DFDT analogs listed here were prepared in the laboratory for the purpose of producing a sample of the material for biological testing rather than to study the several reactions involved or to improve the yields. The latter were usually omitted in the literature.

Samples of 1,1,1-trifluoro-2,2-bis(*p*-fluorophenyl)-ethane, and the corresponding bis-(*p*-chlorophenyl) derivative were obtained by applying Henne's fluorination method to DFDT and DDT, respectively. In both instances a mixture of the mono-, di-, and trifluoro compounds was obtained, but the desired trifluorinated material was separated by fractional recrystallization from methanol and cooling with a mixture of dry ice and acetone.

The reactions used to prepare 2,2-bis(*p*-fluorophenyl)-1,1-dichloroethane and 1,1,1-tribromo-2,2-bis(*p*-fluorophenyl)-ethane are reminiscent of those used for DDT.

Insecticidal Activity

It has been demonstrated that the organofluorine compounds are capable of killing insects as either stomach or contact poisons. Some of the more volatile members of this group have a degree of fumigating action. However, most of the investigations of this group have been directed toward a determination of their activity as contact poisons. With the possible exception of DFDT, too few of the organofluorines have been tested under controlled conditions to permit a definite evaluation of their usefulness even in this one field.

It is generally agreed that the contact-insecticidal activity of the DDT type of compound depends on at least one toxic component and the CCl_3 group or some other lipoid-soluble group for penetration. Beyond this point, there is a lack of agreement as to the exact mechanism by which the contact insecticides exert their action.

Although a lipoid-soluble group characterizes many contact insecticides, simple oil-solubility of a compound is not always a criterion of activity. Busvine (*14*) tested a series of DDT analogs and found that solubility in oil was not essential to activity. Kirkwood

Table II. Synopsis of Laboratory Methods Used to Prepare Analogs of 1,1,1-Trichloro-2,2-bis(p-fluorophenyl)-ethane Having Insecticidal Interest

Product	Reactant	Mole	Reactant	Mole
$(p\text{-}FC_6H_4)_2CHCF_3$	$(p\text{-}FC_6H_4)_2CHCCl_3$	0.94	HF^a	Excess
$(p\text{-}ClC_6H_4)_2CHCF_3$	$(p\text{-}ClC_6H_4)_2CHCCl_3$	0.11	HF^a	Excess
$(p\text{-}ClC_6H_4)_2CFCFCl_2$[b]	.....	....	.....	
$(p\text{-}ClC_6H_4)_2CHCF_2Cl$[c]	$(p\text{-}ClC_6H_4)_2CHCCl_3$	....	SbF_3	
$(p\text{-}FC_6H_4)_2CHCHCl_2$	PhF	(0.2)	$CHCl_2CH(OEt)_2$	(0.1)
$(p\text{-}FC_6H_4)_2C{=}CCl_2$	$(p\text{-}FC_6H_4)_2CHCCl_3$	....	KOH/EtOH	
$(p\text{-}FC_6H_4)_2CH{-}CCl{=}CCl_2$	PhF	....	$CHCl_2CHCl{=}CCl_2$	
$(p\text{-}FC_6H_4)_2CHCBr_3$	PhF	....	CBr_3CHO	
$(p\text{-}FC_6H_4)(p\text{-}ClC_6H_4)CHCCl_3$[d]	.....	....	.....	
$(p\text{-}FC_6H_4)_2CHOH$[e]	.....	....	.....	
$(p\text{-}FC_6H_4)_2CO$	$(p\text{-}FC_6H_4)_2C{=}CCl_2$	....	CrO_3	

Condensing Agent		Order of Mixing	Conditions		M. P., °C.	Reference
	Moles		Temp., °C.	Time, hours		
HgO	0.22	HF	20–30	...	78–80	(65)
HgO	0.25	HF	20–30	...	64–65	(65)
....	...	...	....	...	....	...
....	...	...	160+	...	89–90	(91)
$H_2SO_4.H_2O$	4.5	Acid	Cool	12	77	(85)
Oleum (26%)	18 ml.	...	20–30	...		
....	...	...	Reflux	3	42–42.5	(10)
$AlCl_3$	...	$AlCl_3$	5	15	$b_{0.2}$ 149–150	(85)
H_2SO_4	...	...	....	...	....	(34)
....	...	...	....	...	....	(95)
....	...	...	....	...	34–36	(79)
....	...	...	120	1.25	106–107	(10)

[a] Liquid hydrogen fluoride.
[b] Presumably prepared by addition of fluorine to dehydrochlorinated product of DDT.
[c] In *Chemical Abstracts* and in English summary of article (91). Body of paper refers to compound as p,p'-dichlorodiphenyldifluorodichloroethane, but gives no analytical data.
[d] Synthesis of this compound not given in abstracts, but similar hybrids have been prepared via 1-trichloro-2-chlorophenylethanol (103).
[e] Reported as prepared by R. Picard, University of Illinois.

and Phillips (66) showed that both DDT and 1,1,1-trifluoro-2,2-bis(p-chlorophenyl)-ethane are very soluble in fats, but only DDT is insecticidal. When fed under the same condition to rats, the DDT accumulated in the perirenal fat of rats, while the noninsecticidal analog did not. The approximate tenfold increased solubility in fixed oils of DFDT over DDT fails to increase its insecticidal activity in anywhere near this ratio. These findings support the earlier postulation that the oil-water distribution coefficient of a compound is more important in this respect than its solubility in oil (70).

There are several theories concerning the mechanism by which the toxic component of a contact insecticide exerts its action. The toxicity has been credited to the condensed chlorobenzene system, which is also lipophilic in character (70).

If this is the explanation, DFDT and the other compounds depending on the fluoroaryl system for toxicity would be expected to be less toxic than the corresponding chloro compounds, for fluorobenzene is generally less toxic than chlorobenzene. A second theory advanced by Martin and Wain (77) is based on the observation that DDT and certain other related insecticides easily lose hydrogen chloride, which presumably affects vital centers of the insect. The results of some recent work of Cristol with numerous halogenated insecticides detract from this theory (17). DDT and DFDT, having similar insecticidal activities, differ considerably in the rate at which they undergo dehydrohalogenation. Finally, a study of DDT on several enzyme systems disclosed that it significantly inhibited phosphatidase, possibly through an affinity for the cholesterol of the lipoid membrane of the cell (71). The comparative effect of DFDT on phosphatidase has not been reported.

Efforts have been and are being made to standardize testing procedures for evaluating insecticides (*41*, *83*), but considerable variations still exist in the methods, and results therefrom are not always uniform.

A description of the various methods requires too much space to be given here. In general, the test insects are placed on a surface or held in an atmosphere with known concentrations of the insecticide. In other procedures, the insects are treated directly with a prepared spray or dust of a known strength of the compound under investigations. Larvicidal activity is determined by placing samples of the larvae in water or aqueous preparations containing serial dilutions of the product to be tested. After the various types of exposure, the test insects are usually placed under optimum conditions for recovery and so held for stated observation periods to determine the knockdown and/or mortality percentages in a given group.

Busvine, using adult lice and bedbugs, obtained more precise results from the spray method (followed by forced contact on a filter paper surface) than from an exposure to the same insecticide in the form of a prepared dust (*14*). The surfaces used for the contact tests range from those peculiar to the insects' habitat to those easily provided in the laboratory—e.g., glass and filter paper. Fresh deposits of several contact insecticides gave higher kills of fruit flies on glass than on filter paper (*93*). Contrarily, a DDT deposit on dry bamboo, bark, rusty metal screen, and pine plywood remained toxic to adult female mosquitoes longer than did a similar deposit on new sheet metal, glass, tile, palmetto thatch, and new metal screen (*28*).

The variations in testing apparatus, insect species, dosages, exposures, and criteria of activity render difficult the appraisal of individual or groups of insecticides.

Tables III to XI have been arranged in an effort to show the comparative activity of the organofluorine insecticides, principally DFDT, against a variety of organisms by including a reference standard compound, usually DDT. It is recognized that it would be advantageous to compare the chlorinated and fluorinated pairs, and this is done in so far as the sketchy data permit.

Table III. Comparative Insecticidal Activity of DDT and DFDT against Insects of Diptera Order

Insects	% Mortality DDT	% Mortality DFDT	Dosage	Experimental Period, Hours	Reference
Adults					
Fruit fly	35	23	0.04 γ/sq. cm.	24	(*79*)
Fruit fly	52	36	0.08 γ/sq. cm.	24	(*79*)
Fruit fly	78	63	0.16 γ/sq. cm.	24	(*79*)
Housefly	38	99	0.25% DDT, 1% DFDT[a]	0.5	(*92*)
Fruit fly	65	11	2.5 mg. %	18	(*11*)
Fruit fly	71	100	25 mg. %	18	(*11*)
Fruit fly	89	100	100 mg. %	18	(*11*)
Housefly[b]	60[c]	100[c]	200 mg./sq. ft.[d]	0.1	(*27*)
Fruit fly	49	50.5	0.6 mg./10 ml.[e]	..	(*93*)
Fruit fly	53.5	81.2	0.85 mg./10 ml.[e]	..	(*93*)
Fruit fly	56.8	96.8	1.20 mg./10 ml.[e]	..	(*93*)
Fly (*C. vomitoria*)	3+	4+	.....	..	(*23*)
Larvae			P.P.M.		
A. quadrimaculatus	60	5	0.005	24[f]	(*21*)
A. quadrimaculatus	100	85	0.005 DDT 0.01 DFDT	48	(*40*)
A. quadrimaculatus	100	100	0.06	24	(*30*)
C. quinquefasciatus	50	50	0.01 to 0.05	..	(*92*)
C. apicalis	100	100	0.025	72	(*89*)
Pupae					
C. apicalis	100	100	0.025	72	(*89*)

[a] With 0.025% of added pyrethrins to each, DDT (0.1%) gave 73% kill and DFDT (0.2%) gave 91% kill.
[b] When same dosages were tested against female flies having some degree of DDT resistance, mortality percentages were 44 and 86 for DDT and DFDT, respectively, with 7-day-old deposits, but with 18-day deposits, mortality percentages were approximately the same with both insecticides.
[c] Female flies; kill was 100 and 95%, respectively, for DFDT and DDT against male flies.
[d] Deposit 7 days old when tested; with 18-day-old deposits, mortality for DFDT remained at 100% and that for DDT rose to 93%, both percentages based on female flies.
[e] Concentration of solution drained from vials in which mortalities were determined.
[f] At 48 hours, same dosage of DDT gave 95% mortality and DFDT gave 10% mortality.

TABLE III. It is obvious from the data in Table III that the housefly and the mosquito, in both the adult and larval stage, are susceptible to insecticides of the DDT type. However, the extravagant claims that DFDT is far superior to DDT as a contact insecticide against flies are not borne out by the results of controlled laboratory tests. The Peet-Grady testing technique used by Prill (*92*) would indicate that in the presence of added pyrethrins DDT is definitely superior to DFDT when applied as a spray. On the other hand, DFDT gave higher percentage kills than DDT when flies were placed under a Petri dish and held in contact with deposits of the compounds on glass surfaces. A comparison of the activity of these compounds against adult mosquitoes has not been reported.

The testing techniques used with mosquito larvae, usually the addition of calculated quantities of the larvicide previously dissolved in a water-miscible solvent, are somewhat more comparable. However, the results here are conflicting. The *A. quadrimaculatus* larvae are killed within 24 hours by either DDT or DFDT at concentrations somewhat

Table IV. Comparative Insecticidal Activity of DDT and DFDT against Insects Classified by Order

Insect	% Mortality DDT	% Mortality DFDT	Dosage	Experimental Period, Hours	Reference
Anoplura					
Body lice	50	50	0.3% for DDT[a] 1.4% for DFDT[a]	...	(*14*)
Coleoptera					
Vegetable weevils	70	100	85 γ/sq. cm.	96	(*79*)
Flour beetles	81	91	15 γ/sq. cm.	120	(*79*)
Soldier beetles	100	100	10 mg./63.3 sq. cm.	20	(*78*)
Grain weevil	78	63	2.5 mg. % DDT 12.5 mg. % DFDT	120–168	(*12*)
Flour beetle	29	24	2.5 mg. % DDT 12.5 mg. % DFDT	120–168	(*12*)
Lepidoptera					
Cabbage looper	80	100	10 mg./sq. cm.	20	(*78*)
Arctiid caterpillar	65	30	Ad lib.	96	(*79*)
Oakworm (larvae)	75	35	85 γ/sq. cm.	96	(*79*)
Flour moth	62	66	2.5 mg. % DDT 12.5 mg. % DFDT	40	(*12*)
Hemiptera					
Bedbugs	50	50	0.53% DDT[a] 5.0% DFDT[a]	...	(*14*)
Milkweed bugs	60	100	2500 γ/sq. cm.	96	(*79*)
Large milkweed bugs	55	52	2.5 mg. % DDT 12.5 mg. % DFDT	40	(*12*)
Orthoptera					
German cockroach	0	100	2500 γ/sq. cm.	48	(*79*)
German cockroach	37	80	2.5 mg. % DDT 12.5 mg. % DFDT	48	(*12*)
Homoptera					
Red scale crawlers	100	55	1–1000	504	(*79*)
Hymenoptera					
Red ants	100	100	100 γ/sq. cm.	[b]	(*79*)
Honey bees	100	100	1000 γ/sq. cm.	[c]	(*79*)
Thysanoptera					
Greenhouse thrips	50	50	0.001% DDT 0.006% DFDT	...	(*80*)
Greenhouse thrips	100	37	0.005%	24	(*79*)
Dermoptera					
Forficula	12	86	0.125 mg./sq. cm.	69	(*78*)
Acarina					
Citrus mites	50	50	<10% DDT < 1% DFDT	...	(*80*)
Citrus mites	0	0	1–100	24	(*79*)
Rat mites	76	99	0.1 mg./sq. cm.	0.5	(*87*)
Rat mites	90	93	200 mg./sq. foot	0.5	(*29*)
Isopoda					
Pillbug	60[d]	100[d]	0.25%	58	(*78*)

[a] Values obtained with oil spray. As dusts, DDT and DFDT gave slightly better results; this preparation of DFDT was outstandingly active against bedbugs.
[b] DDT produced 100% mortality in 48 hours and DFDT in 16 hours.
[c] DDT produced 100% mortality in 20 hours and DFDT in 5 hours.
[d] Values determined on basis of direct contact insecticidal activity. DFDT showed superiority to DDT against other species of Isopoda.

less than those used in field larviciding. Concentrations lower than these gave erratic results. It is reported that the culicine larvae are more resistant than the anopheline larvae to DDT (*26*), but it is not yet possible to draw a distinction here between DDT and DFDT.

TABLE IV. Riemschneider (*95*) reported that a dust with 1% DFDT was equal to 10% DDT against lice, flies, and bedbugs, but apparently these ratios do not hold when the compounds are compared in preparations other than dusts (*14*). Busvine was forced to use a higher concentration of DFDT to obtain the same degree of kill against lice and bedbugs. Regardless of the concentration required, it is known that DFDT acts more rapidly against these insects (*12*).

Among the Coleoptera, the soldier beetles were most susceptible to DDT and DFDT, with small dosages producing comparatively rapid kills. In general, DFDT was superior to DDT against all insects of this order.

DFDT closely resembles DDT in its activity against the two adult members of the Lepidoptera where they were compared, but DDT is somewhat more active against the larvae of two other insects in this order.

Based on the restricted number of species involved, it appears that DFDT is superior to DDT against the German cockroach, European earwig, citrus mites, and rat mites with an outstanding advantage in the case of the cockroach. The marked susceptibility of the German cockroach to DFDT has been noted by several workers. DFDT's effectiveness against the cockroach may depend on a combination of its high toxicity and increased vapor pressure which permits contact with this particular insect.

Both compounds show about the same degree of activity toward red ants and honey bees, but DDT is superior against red scale crawlers and greenhouse thrips.

Table V. Approximate Insecticidal Activity of DFDT against Insects

Insect	Degree of Activity	Reference
Diptera		
Flies	7 times as effective as DDT	(*106*)
Flies	1% equal to 10% DDT	(*95*)
Fly	Superior to DDT	(*85*)
Anoplura		
Lice	Moderate	(*107*)
Lice	1% equal to 10% DDT	(*95*)
Lice	Equal to DDT	(*23*)
Coleoptera		
Grain weevil	Superior to DDT	(*85*)
Potato beetles	Approaches DDT	(*94*)
Weevil	Much superior to DDT	(*23*)
Lepidoptera		
Flour moth	Equal to DDT	(*23*)
Clothes moth[a]	Superior to DDT	(*85*)
Hemiptera		
Bedbugs	1% equal to 10% DDT	(*95*)
Bugs	Moderate	(*19*)
Hymenoptera		
Ants	Better than DDT	(*23*)
Ants	Better than DDT	(*85*)
Corrodentia		
Book lice	Superior to DDT	(*85*)
Acarina		
Mite larvae	Equal to DDT	(*23*)

[a] *Tineola biselliella.*

TABLE V. The expressions used in the second column of Table V to indicate the relative potency of DDT and DFDT are exact quotations from the respective reports.

The sevenfold activity against houseflies credited to DFDT by the Germans actually represents the comparative activity of Gix, the active constituent of which is DFDT. It is possible that the other constituents of Gix acted as a synergist for the DFDT. Alessandrini (*2*) reports the isolation from Gix of a compound melting at 99° to 100° C., which obviously is not DFDT. A crude sample of DFDT, a sample of recrystallized DFDT, and a sample of the impurities separated in the recrystallization of DFDT were found by Fay

and Buckner (*27*) to have the same contact toxicity toward houseflies. Proverbs and Morrison (*93*) report DFDT to be a better poison than DDT for fruit flies, which is in opposition to the claim by Kirkwood and Dacey (*65*) that DFDT is two fifths as effective here.

Although most of the values in Table V are only approximate, they are in general agreement with the more precise values obtained with the same species of insects. Table V provides values for three insects not appearing in earlier tables—namely, potato beetles, cnethocampidae, and book lice. The potato beetle is more susceptible to DDT, and the cnethocampidae and book lice are more susceptible to DFDT.

Table VI. Comparative Knockdown Rate and Power of a Series of Organofluorine Compounds

Compound	Insect	Dosage	Knockdown, Hours	% Knock down	Reference
$(p\text{-}FC_6H_4)_2CHCCl_3$[a]	Housefly	0.2%	0.17	91	(*92*)
	Housefly[b]	200 mg./sq. foot[c]	0.10	16[d]	(*27*)
	Housefly	....	Slower than DDT	..	(*5*)
	Blow fly	1 mg./sq. cm.	2[e]	100	(*79*)
		γ/Sq. Cm.			
	Fruit fly	15	1[f]	50	(*79*)
	Soldier beetle	150	1.5[g]	100	(*79*)
$(p\text{-}FC_6H_4)_2CHCHCl_2$	Fruit fly	15	3.5	50	(*79*)
$(p\text{-}FC_6H_4)_2C{=}CCl_2$[h]	Fruit fly	15	6	50	(*79*)
$(p\text{-}FC_6H_4)_2CHCCl{=}CCl_2$[h]	Fruit fly	15	24	50	(*79*)
$(p\text{-}FC_6H_4)_2CHCF_3$	Fruit fly	15	5	50	(*79*)
$(p\text{-}ClC_6H_4)_2CHCF_3$	Fruit fly	15	6	50	(*79*)
$(p\text{-}FC_6H_4)CHOH$[h]	Fruit fly	15	24	50	(*79*)
$(p\text{-}FC_6H_4)_2CO$[h]	Fruit fly	15	24	50	(*79*)

[a] Pyrethrins, 0.025 gram/100 ml., were added. Under identical conditions, DDT at 0.1% gave 85% knockdown. A 1% spray of DFDT without added pyrethrins gave 99% knockdown in 10 minutes.
[b] Mixed sample of male and female flies.
[c] This deposit was 7 days old, but an 18-day-old deposit gave approximately same results.
[d] DDT gave no knockdown under the same conditions.
[e] DDT required 18 hours to give same knockdown.
[f] DDT required 8 hours to give same knockdown.
[g] DDT required 24 hours to give same knockdown.
[h] Chlorine analogs required 24 hours to give 50% knockdown.

TABLE VI. The knockdown power of the organofluorine insecticides has been determined principally against members of the Diptera order and by a limited number of workers. There is general agreement that DFDT acts more rapidly than DDT, at least against those species with which they have been compared. Prill (*92*) found that twice the amount of DFDT compared to DDT was required to give the same knockdown against houseflies when tested by a space spray technique with added pyrethrins. The forced contact method of Fay and Buckner (*27*) revealed that without added pyrethrins DFDT was a more powerful knockdown agent than DDT.

The dehydrohalogenation of DFDT materially reduced its knockdown rate, but the resulting *p,p'*-difluorodiphenyldichloroethylene derivative is more rapid in action than the analogous trichloropropylene compound. The fact that the propylene compound maintained some activity suggests that its increased weight might explain the difference in activity.

Substitution of the chlorines on the alkane bridge of DDT by fluorine gave a slower-acting knockdown, and the rate was not restored by the further substitution of fluorine for chlorine in the ring.

TABLE VII. There was no significant difference between the potency of *o*-chlorofluorobenzene and *p*-bromofluorobenzene toward codling moth larvae, but *o*-bromofluorobenzene and *p*-chlorofluorobenzene must be tested to determine whether the detectable difference is due to the difference in halogen substitution or to position isomerism.

The complete replacement of chlorine in the DDT molecule by fluorine gives $(p\text{-}FC_6H_4)_2CHCF_3$, a compound with greatly decreased toxicity toward fruit flies and thrips and probably other species. This reduced activity is matched by the low activity of $(p\text{-}ClC_6H_4)_2CHCF_3$ which, when contrasted to the potency of DDT and DFDT, shows that the attenuation is caused by the replacement of the three chlorine atoms on the alkane bridge. The corresponding substitution of only one chlorine atom by fluorine gave a compound having as much activity as DDT against the chafer beetle.

Table VII. Insecticidal Activity of Some Miscellaneous Organofluorine Compounds

Compounds	Physical Constants, ° C.	Chem. Ref.	Insect	Order	Degree of Activity	Biol. Ref.
o-FC_6H_4Cl	B.p. 138–140	(*97*)	Codling moth larvae	Lepidoptera	91% toxic to larvae	(*22*)
p-FC_6H_4Br	B.p. 152	(*113*)	Codling moth larvae	Lepidoptera	100% toxic to larvae	(*22*)
$(p\text{-}FC_6H_4)_2CHCF_3$[a]	M.p. 79–80	(*65*)	Greenhouse thrips	Thysanoptera	52% mort. from 0.1% in 24 hours	(*79*)
			Fruit flies	Diptera	1/100 that of DDT	(*11*)
$(p\text{-}ClC_6H_4)_2CHCF_3$[a]	M.p. 64–65	(*65*)	Greenhouse thrips	Thysanoptera	34% mort. from 0.1% in 24 hours	(*79*)
			Fruit flies	Diptera	1/100 that of DDT	(*11*)
$(p\text{-}FC_6H_4)_2CHCHCl_2$[b]	M.p. 77	(*85*)	Flour beetles	Coleoptera	15 γ/sq. cm. gave 2% mort. in 120 hours	(*79*)
			Flies	Diptera	Equal to DDT	(*85*)
			Grain weevils	Coleoptera	Inferior to DDT	(*85*)
			Cnethocamidae	Lepidoptera	Inferior to DDT	(*85*)
			Greenhouse thrips	Thysanoptera	59% mort. from 0.01% in 24 hours	(*79*)
			Ants	Hymenoptera	Superior to DDT	(*85*)
			Book lice	Corrodentia	Superior to DDT	(*85*)
$(p\text{-}ClC_6H_4)_2CFCFCl_2$	.....	(*11*)	Fruit flies	Diptera	Not active	(*11*)
$(p\text{-}ClC_6H_4)_2CHCF_2Cl$	M.p. 89–90	(*91*)	Chafer beetle	Coleoptera	Equal to DDT[c]	(*91*)
$(p\text{-}FC_6H_4)(p\text{-}ClC_6H_4)\text{-}CHCCl_3$	.....	(*95*)	Flies	Diptera	1% equal to 10% DDT	(*95*)
			Bedbugs	Hemiptera	1% equal to 10% DDT	(*95*)
			Lice	Anoplura	1% equal to 10% DDT	(*95*)
$(p\text{-}FC_6H_4)_2C{=}CCl_2$[a]	M.p. 42–42.5	(*10*)	Greenhouse thrips	Thysanoptera	100% mort. from 1% in 24 hours	(*79*)
			Woodlice	Isopoda	10 mg./63.3 sq. cm. affected 50%[d]	(*78*)
$(p\text{-}FC_6H_4)_2CHCCl{=}CCl_2$	B.p., 0.2 mm., 149–150	(*85*)	Flies	Diptera	Inferior to DDT	(*85*)
			Grain weevils	Coleoptera	Equal to DDT	(*85*)
			Cnethocamidae	Lepidoptera	Inferior to DDT	(*85*)
			Greenhouse thrips	Thysanoptera	31% mort. from 1% in 24 hours	(*79*)
			Ants	Hymenoptera	Equal to DDT	(*85*)
			Book lice	Corrodentia	Inferior to DDT	(*85*)
$(p\text{-}FC_6H_4)_2CHOH$[a]	M.p. 34–36	(*79*)	Book lice	.....	67% mort. from 1.0% in 24 hours	(*79*)
$(p\text{-}FC_6H_4)CH(OH)\text{-}CCl_3$	.....	(*11*)	Fruit flies	Diptera	1/8 to 2/8 as active as DDT	(*11*)
$CH_2(FCH_2CH_2O)_2$[e]	.....	(*75*)	Bean aphis larvae[f]	Homoptera	100% mort. from 2 g. after 5 days[g]	(*4*)
			Lepidopterous larvae[f]	Lepidoptera	100% mort. from 0.5 g. after 21 days[g]	(*4*)
			Weevils[f]	Coleoptera	0% mort. from 1.0 g. after 28 days[g]	(*4*)
$(p\text{-}FC_6H_4)_2CO$[a]	M.p. 106–107	(*10*)	Greenhouse thrips	Thysanoptera	17% mort. from 0.1% in 24 hours	(*79*)
FCH_2CO_2H[h,i]	M.p. 33	(*111*)	*A. quad.* larvae	Diptera	0.1 p.p.m. gave 10% mort. 24 hours	(*22*)
					0.1 p.p.m. gave 65% mort. 48 hours	(*22*)
$FCH_2CO_2CH_2CHEt\text{-}(CH_2)_3CH_3$[j]	B.p., 2 mm., 65–68	(*47*)	Aphids	Homoptera	100% mort. from 0.01% soln.	(*47*)
p-$FC_6H_4NH_2$	B.p. 185–189	(*112*)	Codling moth larvae	Lepidoptera	90% toxic to larvae	(*104*)
$(p\text{-}FC_6H_4)_2S_2$[k]	B.p., 7.5 mm., 165–167	(*67*)	Body lice	Anoplura	1% gave 100% knockdown in 1 hour	(*24*)
			Body louse eggs	Anoplura	0.1% gave 88% mort.[l]	(*25*)
			A. quad. larvae	Diptera	50% mort. in 48 hours from <1 p.p.m.	(*22*)
p-$CH_3C_6H_4SO_2F$	M.p. 43–44	(*43*)	Body louse eggs	Anoplura	0.1% gave 62% mort.	(*25*)

[a] Moderately superior to corresponding chlorine analogs in this particular test.
[b] Moderately inferior to corresponding chlorine analog in this particular test.
[c] Paralyzing effect decreased, but toxicity remained unchanged.
[d] Exposure time 68 hours; under same conditions 16% were affected by DDT.
[e] Systemic poison, applied in soil supporting plants.
[f] Killed on tick beans, cauliflower, and wheat in order given.
[g] Degree of control proportional to dosage; smaller dosage gave less control.
[h] Prepared by hydrolyzing methyl fluoroacetate (from corresponding iodoacetate and AgF at 170°) with barium hydroxide.
[i] Tested as sodium salt.
[j] Prepared by heating ethyl fluoroacetate with 2-ethylhexanol at 160° in presence of *p*-toluenesulfonic acid monohydrate.
[k] Prepared by reducing *p*-fluorobenzenesulfonyl chloride with HI (60% yield).
[l] Same concentration in pyrophyllite gave 95% mortality.

The replacement of the one remaining hydrogen atom in the bridge of $(p\text{-}ClC_6H_4)_2CHCFCl_2$ gave an inactive compound against the fruit fly, the only insect tested. This is precedented by the known inactivity of $(p\text{-}ClC_6H_4)_2CClCCl_3$.

The following halogenated hydrocarbons, $(p\text{-}FC_6H_4)_2CHCHCl_2$ and $(p\text{-}FC_6H_4)(p\text{-}ClC_6H_4)CHCCl_3$, have a close structural relationship to DDD, DDT, and DFDT and presumably as a consequence share the high insecticidal activity of these compounds.

1,1-Difluorodiphenyl-2,2-dichloroethylene, produced by the dehydrochlorination of DFDT, is almost devoid of activity, at least against thrips and woodlice. The activity of the analogous 1,1-dichloro-2-chloro-3,3-difluorodiphenylpropene is even less against thrips, but the latter compound is said to be as active as DDT against grain weevils and ants.

The fluorinated carbinols, $(p\text{-}FC_6H_4)CH(OH)CCl_3$ and $(p\text{-}FC_6H_4)_2CHOH$, and the ketone corresponding to the latter have not been tested very widely. The carbinol with the trichlorinated carbon atom is more promising structurally, and its activity against fruit flies warrants additional testing.

The absorption of the ether bis(2-fluoroethoxy)methane from the soil and its translocation in plants in a concentration sufficient to kill insects is a recent discovery heretofore observed only in some selenium compounds and in the insecticidal esters of some polyphosphoric acids (*4*). Whether this property is shared by other organofluorine compounds will doubtless be determined for the purpose of studying plant metabolism and of evaluating any practical application.

Monofluoroacetic acid offers little promise as a mosquito larvicide, but 2-ethylhexyl monofluoroacetate is a very powerful aphicide. The high toxicity of the fluorinated lower aliphatic acids and their esters to vertebrates probably precludes their use as insecticides except under highly controlled conditions. The toxicity of the fluorinated acids to the vertebrates is attributed to their interference with an enzyme system, but the possibility of finding one with a high specificity for insects is not excluded.

Difluorodiphenyl disulfide, when tested on woolen swatches, is an outstanding knockdown agent and toxicant for body lice. Several compounds with *p*-fluorinated phenyl radicals have exhibited the same order of activity against lice, but too few compounds with the disulfide linkage have been tested to warrant an appraisal of its effect here.

Table VIII. Miscellaneous Organofluorine Compounds Credited with a Degree of Activity against Some Specific Insect

Compound	Physical Constants, ° C.	Chem. Ref.	Insect	Order	Biol. Ref.
CHF_3[a]	B.p. −82.2	(*44*)	Clothes moths	Lepidoptera	(*50*)
$CH_3(CH_2)_{10}CH_2F$	.....	(*8*)	Housefly	Diptera	(*9*)
o-$CH_3C_6H_4F$[b]	B.p. 114	(*46*)	Red scale	Homoptera	(*19*)
1,2,4-$(CH_3)_3C_6H_2F$	M.p. 26	(*35*)	Moths	Lepidoptera	(*50*)
4,4′-$FC_6H_4.C_6H_4F$[c,d]	M.p. 88–89	(*6*)	Codling moth	Lepidoptera	(*50*)
p-FC_6H_4OH	B.p. 186–188	(*113*)	Moths	Lepidoptera	(*51*)
FCH_2CO_2H	M.p. 33	(*111*)	Moths	Lepidoptera	(*50*)
3,4-$NO_2FC_6H_3CO_2H$	M.p. 121–122	(*105*)	Moths	Lepidoptera	(*50*)
p-$CH_3C_6H_4SO_2F$	M.p. 43–44	(*45*)	Moths	Lepidoptera	(*59*)
1,5-$C_{10}H_6(SO_2F)_2$	M.p. 203	(*43*), p. 6	Moths	Lepidoptera	(*59*)
$(3,4\text{-}Cl_2C_6H_3)SO_2(4\text{-}FC_6H_4NH)$	.....	(*48*)	Moths	Lepidoptera	(*48*)

[a] Prepared in 80% yields by heating bromoform with mixture of antimony chloride and mercuric chloride at 100°.
[b] Prepared by treating *o*-toluenediazonium sulfate with hydrofluoric acid.
[c] Prepared by eliminating 2 atoms of bromine from 2 molecules of *p*-bromofluorobenzene by means of metallic sodium.
[d] This compound, used as 1% aqueous solution of sodium salt, was toxic to moth and highly toxic to codling moth.

TABLE VIII. It is difficult to conceive of fluoroform, which is chemically and physiologically inert, having toxicity to moths unless it acts as a mechanical asphyxiant.

The activity of monofluorododecane against houseflies is probably explained on the basis of the alteration in the physical properties of the hydrocarbon molecule which already possesses a degree of toxicity toward this species.

Of a series of fluorinated benzenes and fluorinated toluenes tested with and without

added hydrocyanic acid against red scale, only *o*-fluorotoluene could be rated as moderately toxic.

The balance of the compounds in Table VIII support the earlier statement that any compound containing fluorine is toxic to moths. These fluorinated hydrocarbons, phenols, acids, sulfonic acids, and sulfones probably act against moths as stomach poisons. The fluorosulfonic acid derivatives and the sulfone with a fluorinated substituent were key compounds whose toxicity to moths laid the groundwork for the deductions which led to the synthesis and testing of DDT as an insecticide.

TABLES IX AND X. A description of the methods used to determine the insecticidal activity of the compounds listed in Tables IX and X is not available. The compounds are classified in both tables on the basis of their chemical structures, with a view to providing a guide for future searches for insecticides among organofluorine compounds.

Table IX. Fluorinated Hydrocarbons, Acids, and Acid Derivatives Patented as General Insecticides

	Reference
Fluorinated hydrocarbons	
Monofluorobutane	(*53*)
Difluorobutane	(*55*)
α,α,α-Trifluorotoluene[a]	(*37*)
1,2-Difluoro-1,1-diphenylethane	(*69*)
o-Trifluoromethylbenzal fluoride	(*101*)
1,1,2,2-Tetrafluoro-1,2-dinitroethane	(*42*)
1-Chloro-1,2,2-trifluoro-1,2-dinitroethane	(*42*)
1,2-Dichloro-1,2-difluoro-1,2-dinitroethane	(*42*)
Fluorinated acids and acid derivatives	
Fluorostearic acid[b,c]	(*102*)
9,10-Difluorostearic acid[d]	(*102*)
Monofluoroundecanic acid[e]	(*53*)
Sodium monofluoroleate	(*53*)
Methyl fluoroundecanate[b]	(*102*)

[a] Nontoxic to housefly.
[b] Prepared by treating corresponding unsaturated compound, in carbon tetrachloride, with hydrogen fluoride at 0–10°.
[c] Metallic salts of esters of this acid with hydroxyethanesulfonic acid are described as insecticides (*53*).
[d] Calcium salt of difluorostearic acid of unspecified composition is described in (*53*).
[e] Used in form of salt.

Table X. Miscellaneous Organofluorine Compounds Patented as General Insecticides

Organofluorine Compounds	Reference
Fluoro-octadecyl alcohol	(*53*)
Trifluoromethylbenzaldehyde[a]	(*54*)
Dichloroethylfluorosilane	(*90*)
Chloroethyldifluorosilane	(*90*)
Ethyltrifluorosilane	(*90*)
Dichlorofluorophenylsilane	(*90*)
Chlorodifluorophenylsilane	(*90*)
Trifluorophenylsilane	(*90*)
Diethoxyfluorophenylsilane	(*90*)
Difluoromethylphenylsilane	(*90*)
Chlorofluorodiphenylsilane	(*90*)
Difluorodiphenylsilane	(*90*)
Methyl *N*-fluorobutyryl-*N*-methylaminoacetate	(*53*)
N-Monofluorostearoylglycine	(*55*)[b]
N-Difluorostearoyltaurine	(*55*)
Diethylaminomonofluorophosphoric acid[c]	(*58*)
Diethylaminodifluorophosphoric acid[c]	(*58*)
Diethylaminomonofluorothiophosphoric acid[c]	(*58*)
Methanesulfonyl fluoride	(*57*)
1-Piperidinesulfonyl fluoride	(*52*)
N,N-Bis(2-chloroethyl)sulfamyl fluoride	(*52*)
N-Hexyl-*N*-methylsulfamyl fluoride	(*52*)
N-Dodecyl-*N*-methylsulfamyl fluoride	(*52*)
N-Carbomethoxymethyl-*N*-methylsulfamyl fluoride	(*52*)
N,N-Bis(2-cyanoethyl)sulfamyl fluoride	(*52*)
Phenyltrifluoromethyl sulfone	(*56*)
p-Tolyltrifluoromethyl sulfone	(*56*)
p-Chlorophenyltrifluoromethyl sulfone	(*56*)
p-Nitrophenyltrifluoromethyl sulfone	(*56*)
m-Bis(trifluoromethylsulfonyl)-benzene	(*56*)
N-Methyl-*N*-phenethylsulfamyl fluoride	(*52*)
4-Morpholinesulfonyl fluoride	(*52*)

[a] Prepared by treating trifluoromethylbenzal fluoride with sulfuric acid.
[b] This patent described monofluorostearyl alcohol-sulfone as having insecticidal activity.
[c] Prepared by reacting diethylaminophosphoric compounds, containing one halogen replaceable by F, with potassium fluoride or antimony fluoride.

Toxicity

The physiological effects of the fluoride ion were reviewed by McClure (*74*) in 1933. Lehmann (*72*) published an article on the toxicity of aromatic fluorine compounds in 1928. However, it has been impossible to investigate the toxicity of the organofluorine compounds as thoroughly as that of the organochlorine compounds.

Some of the fluorinated hydrocarbons—e.g., trifluoromethane (*44*), 1,1-difluoroethane (*45*), and difluorodichloromethane (*82*)—are known to be chemically inactive and physiologically inert. Stepwise replacement of the chlorine atoms in chloroform by fluorine yields the dichlorofluoromethane and chlorodifluoromethane, neither of which is as toxic as the original substance (*7*).

In a few instances, the introduction of fluorine into certain compounds containing oxygen, nitrogen, or phosphorus increases the toxicity of such compounds to a spectacular degree.

The rodenticide monofluoroacetic acid has an LD_{50} in certain species of warm-blooded animals of less than 1 mg. per kg. of body weight (*49*). The esters of certain fluorinated aliphatic acids (*75*) are likewise very toxic, as are the alkyl esters of fluophosphoric acid (*63*, *68*). The toxicities of these compounds greatly exceed that of the fluoride ion, and they are thought to function through their interference with an enzyme system. The substitution of chlorine or fluorine in the methyl radical of *m*-toluidine increases the toxicity of the parent compound to frogs, but fluorine increases it about 50% over chlorine. *m*-Trifluorotoluidine is a quick-acting and lasting narcotic for frogs (*72*).

In contrast to the toxicity of these fluorinated oxygen and nitrogen-containing compounds, the products from trifluorinated methyl ketones and amines are reported to have low acute toxicities (*62*). The fluorocarbons are insoluble in water, alcohol, and hydrocarbons (*38*).

The toxicity of insecticides to vertebrates is frequently a deciding factor in determining where and under what conditions, if any, they can be used. Therefore, the tox-

Table XI. Comparative Toxicity of DDT and DFDT and Some Miscellaneous Organofluorine Insecticides to Vertebrates

Compound	Animal	Dosage, Mg./Kg.	Route Adm.	No. of Animals	% Mortality	Reference
$(p\text{-}ClC_6H_4)_2CHCCl_3$	Mice	200	Oral	15	47	(*110*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Mice	900	Oral	10	40	(*110*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	Mice	250	Oral	20	60	(*32*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Mice	1000	Oral	20	45	(*32*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	Mice[a]	150–500	Oral	7	60	(*88*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Mice[a]	250–1000	Oral	4	0	(*88*)
$(p\text{-}ClC_6H_4)_2CHCCl_4$	Mice	400	*per os*	..	50	(*23*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Mice	440	*per os*	..	50	(*23*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	Mice	....	...	..	Equal	(*96*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Mice	....	...	..	....	(*96*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	White rats	250	Oral	10	20	(*32*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	White rats	250	Oral	10	0	(*32*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	White rats	500	Oral	10	80	(*32*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	White rats	500	Oral	10	0	(*32*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	White rats	1000	Oral	10	90	(*32*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	White rats	1000	Oral	10	30	(*32*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	White rats	1500	Oral	10	100	(*32*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	White rats	1500	Oral	10	70	(*32*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	Mice	2200[b]	Oral	4	75–100	(*11*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Mice	2200[b]	Oral	4	75–100	(*11*)
$(p\text{-}FC_6H_4)_2CHCF_3$	Mice	2200	Oral	4	0	(*11*)
FCH_2CO_2H	White rats	2.5	Oral	24	58	(*114*)
$FCH_2CO_2CH_2CHEt(CH_2)_3CH_3$	Rabbit	10	Ear	1	100	(*47*)
		P.P.M.				
$(p\text{-}ClC_6H_4)_2CHCCl_3$	Goldfish	0.1	...	10	100	(*89*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	Goldfish	1.0	...	10	100	(*89*)
$(p\text{-}ClC_6H_4)_2CHCCl_3$	*Gambusia*	0.01	...	10	100	(*89*)
$(p\text{-}FC_6H_4)_2CHCCl_3$	*Gambusia*	0.125	...	10	100	(*89*)

[a] White-footed mouse, *Peromyscus leucopus*.
[b] Dosage given over a period of 6 days.

icity of organofluorine insecticides is not only of academic interest, but also of extreme practical importance. The acute oral toxicity of DDT and DFDT for several species of laboratory animals is compared in Table XI.

TABLE XI. The substitution of fluorine for chlorine on the two rings in DDT reduces the acute oral toxicity to a significant degree. The acute toxicity of DFDT appears to be of the order of one fourth to one third that of DDT to mice and white rats. At the dose levels at which symptoms were seen with both compounds, DDT appeared to act more rapidly. Replacement of the phenyl chlorine atoms of DDT by fluorine reduces the toxicity to a greater degree than by bromine or iodine, but to a lesser degree than by *tert*-butyl, methoxy, butoxy, amyloxy, and acetoxy groups (*110*). Failure to relate the toxicity of a series of DDT analogs to their solubility in oil and their dehydrohalogenation rates led von Oettingen and Sharpless (*110*) to investigate the metabolic fate of this series of compounds. DFDT, the least toxic of the *p,p'*-dihalogen analogs of DDT, represents the strongest halogen-carbon bond.

DDT and DFDT showed the expected high toxicity toward fish, but on the basis of the preliminary results DFDT appears to be one fifth to one tenth as toxic as DDT. Goldfish were more resistant to both compounds than was the wild gambusia.

Riemschneider (*96*) purposely swallowed both DDT and DFDT, and reports that no essential pharmacological differences could be detected in the two compounds.

There has been an increased exploration in the organofluorine compounds since 1940, when an authoritative and comprehensive article giving the toxicity of 551 miscellaneous organic compounds to young screwworms did not include a single compound containing fluorine (*13*).

Acknowledgment

A large percentage of the data on miscellaneous organofluorine compounds which are listed in Tables IX and X was taken from (*31*). A paper by Metcalf (*79*) provided a good measure of the data on the comparative activity of these compounds listed in Tables III, V, VI, and VII. The writer is indebted to his associates, R. W. Fay, H. P. Nicholson, and Thomas B. Gaines for the activity and toxicity data credited to them in Tables III, IV, and XI, and to Mary B. Goette for some physical measurements on DFDT. Eugene Odum of the University of Georgia supplied the information on the relative toxicity of DDT and DFDT to the white-footed mouse.

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Alkali-Stable Polychloro Organic Insect Toxicants, Aldrin and Dieldrin

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The chemistry and general properties of two new alkali-stable insect toxicants, aldrin and dieldrin, are discussed, and the general properties of these materials are given. Entomological data serving to illustrate the magnitude of the insect toxicity of these compounds are included. Aldrin is an alkali-stable, relatively nonresidual insect toxicant with activity equal to or greater than the activity of γ-hexachlorocyclohexane. Dieldrin is an alkali-stable insect toxicant with high activity and high persistence. Its period of residual activity is equal to or greater than that of DDT. In general, its activity level is somewhat higher than that of aldrin.

With the advent of DDT it became possible to think in terms of the eradication of insect pests instead of their control only. Although very lethal organic toxicants such as the pyrethrins and rotenone had been previously employed, their instability under normal conditions of use limited their utility.

DDT is only the first of a new group of toxicants, members of which were discovered or invented independently almost simultaneously in various parts of the world. Of these, the group of halogenated hydrocarbons, which includes DDT, toxaphene, γ-hexachlorocyclohexane, and chlordan, is the best known. Although some members of the group possess undesirable properties which severely limit their practical importance, others, like chlordan, are many times more toxic than DDT toward many insect species and possess no disadvantage not common also to DDT and other members of the group. Until now, however, no compound has been available which combined high potency with the long period residual activity characteristic of DDT.

One of the greatest practical disadvantages possessed by the hitherto commonly used halogenated hydrocarbon insect toxicants has been the extreme ease with which they are dehydrohalogenated by alkaline reagents and by many metal halides. This of itself is serious, but even more serious is the fact that simultaneously these compounds lose all or almost all of their insecticidal activity. In fact, so marked is this reaction pattern that some investigators have attempted to devise theories of insecticidal activity based on the ability of these compounds to lose hydrogen chloride and to correlate the degree of activity with the ease, under various experimental conditions, with which dehydrohalogenation could be induced.

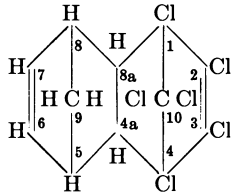
Chemical and Physical Properties

During the past 30 months two new compounds, aldrin and dieldrin, possessing great usefulness as insect toxicants, have been devised, synthesized, and studied in the laboratories of Julius Hyman & Company. Both compounds are completely impervious

to the action of alkaline reagents, in either aqueous or alcoholic media. Under conditions of practical use they likewise appear unaffected by acidic reagents. Despite this fact, they possess insecticidal activity equal to or greater than that of the best halogenated insect toxicants known or used.

At the time of original presentation of this paper Compounds 118 and 497 had not yet been named. Since then the Interdepartmental Committee on Pest Control of the U. S. Department of Agriculture has adopted the names aldrin and dieldrin, respectively, for (*a*) "an insecticidal product having not less than 95% of its principal constituent, the chemical 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene. . ." and (*b*) "an insecticidal product having not less than 85% of its principal constituent, the chemical 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,-8-dimethanonaphthalene. . ." "Aldrin (recrystallized)" and "dieldrin (recrystallized)" are defined to refer to products containing not less than 99% of the above-named chemicals. Because continued use of the names 118 and 497 can only lead to confusion, the names aldrin and dieldrin have been adopted for use in the nonentomological portions of this paper to refer to the pure chemical compounds, with the recognition that such usage does not exactly correspond with that officially adopted.

Chemically, aldrin is 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene and possesses, in planar representation, the structure:



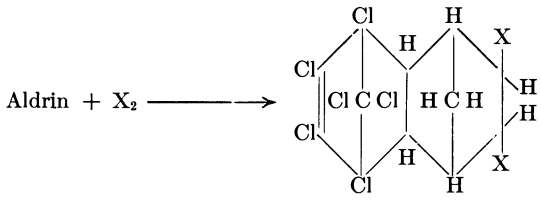
The planar representation shown serves, in part, to obscure somewhat the complexity of the structural problem involved. Actually, four stereoisomers are represented by the simple planar representation shown; it is not yet known with certainty which is the one corresponding to aldrin. Physically pure aldrin is a white crystalline solid with the properties set forth in Table I.

Table I. Physical Properties of Aldrin

Melting point. 104–104.5° C.
Odor. Substantially odorless at room temperature. Mild pinelike odor when warm

Solvent	Solubility of Aldrin at 30° C., Grams/100 Ml.
Methanol	9
Acetone	159
Benzene	350
Hexane	98
Base oil (deobase)	89
Water	Insoluble

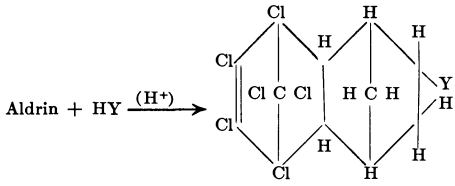
Aldrin exhibits in many instances the chemical behavior expected on the basis of its structure. Thus, it is readily attacked by halogens to yield the expected halides.



Chlorine adds to form the *trans*-dichloride; bromine adds, in carbon tetrachloride solution, to give a mixture of *cis*- and *trans*-dibromides; this addition can be directed wholly to the trans derivative by conducting the bromination in a mixed phase reaction in aqueous suspension.

The double bond in the unchlorinated ring, in the presence of acidic catalysts, adds a variety of reagents of the type HY, illustrated in Table II.

Table II. Reaction of Aldrin with Reagents HY

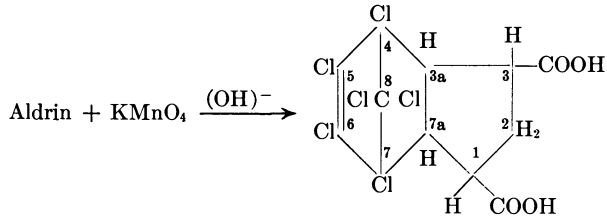


Y	Product
Halide ion	Halide
R—C(=O)—O—	Ester
RO—	Ether

Many other additions, similar in type, are possible. Although acidic reagents can bring about alterations in aldrin, such reactions proceed only in the presence of strong acids or strongly acidic catalysts in the homogeneous phase and hence are without significance for the conditions under which insecticides are normally utilized.

Because aldrin contains the bicyclo-(2.2.1)-heptene ring structure, it reacts typically with phenyl azide to form a phenyldihydrotriazole derivative. This reaction is of importance in that it provides the basis for an analytical method for determining aldrin (discussed more fully in *2*).

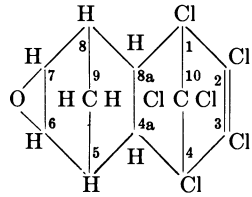
The double bond in the unhalogenated ring is readily attacked by oxidizing agents. Chromic acid in acetic acid, and potassium permanganate in alkaline solution, oxidize the compound to the expected dicarboxylic acid, 4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-1,3-dicarboxy-4,7-methanoindane.



Potassium permanganate in neutral solution and lead tetracetate give rise, respectively, to the anticipated glycol and to its diacetate.

By far the most interesting oxidation of aldrin, at least from the present viewpoint, is the oxidation with per acids. This oxidative process, which occurs normally, produces 6,7-epoxy-6,7-dihydroaldrin, the compound now called dieldrin.

Dieldrin is then 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,-8-dimethanonaphthalene:



Dieldrin, which, when pure, is a white crystalline solid, possesses the physical properties shown in Table III.

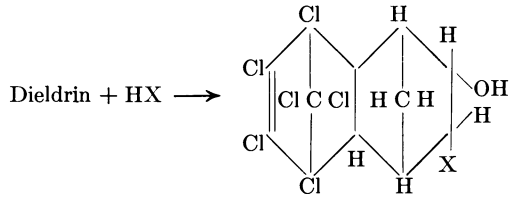
Table III. Physical Properties of Dieldrin

Melting point. 175–176° C. Odor. Odorless

Solvent	Solubility of Dieldrin, Grams/100 Grams	
	26° C.	0° C.
Methanol	4.9	3.4
Acetone	54.0	35.4
Benzene	75.0	36.9
Hexane	7.7	2.5
Base Oil (Std. oil 10)	4.3	1.3
Water	Insoluble	

While dieldrin will, under suitable conditions, exhibit many of the expected reactions of epoxy compounds, it is a remarkably stable oxide. Thus, in its preparation the presence in the oxidizing solution of 1% or more of sulfuric acid in no way affects it.

However, when dissolved in butyl ether and refluxed with concentrated solutions of the halogen acids, dieldrin reacts typically to yield the halohydrin.



X = Cl, Br, I

The somewhat unexpected inertness of dieldrin toward mineral acids has great practical significance; despite its structure it remains, under practical conditions of use, insecticidally active under both acidic and alkaline conditions.

Insect Toxicity

The data of Table IV give a concise general summary of the relative toxicity of these two compounds to a number of common insect pests. In each case technical chlordan is used as the standard of comparison and accordingly is assigned a base value of 10. In some instances similar data for other of the common toxicants are included in order better to enable visualization of the relative activity levels. (In Table IV and the tables which follow γ-$C_6H_6Cl_6$ represents the gamma isomer of hexachlorocyclohexane.) As the data in this table are presented the figures given represent relative activity; as activity increases the relative activity figure also increases. Thus, the first line of the table indicates that aldrin is approximately four times as effective against the housefly as is chlordan and that DDT is only about one third as effective as is chlordan.

Table IV. Relative Toxicity of Aldrin and Dieldrin to Insects

	Chlordan	Aldrin	Dieldrin	DDT	Toxaphene	γ-$C_6H_6Cl_6$
Housefly	10	40	120	3	2	40
German roach	10	60	300	...	...	30
American roach	10	60	60	...	...	40
Black carpet beetle	10	70	...	...	...	..
Milkweed bug	10	140	96	...	...	45
Squash bug	10	160	...	...	...	..
Confused flour beetle	10	68	200	...	...	30
Differential grasshopper (adults)	10	40–50	70	...	...	..
Fall webworm	10	100	...	12.5	...	..
Imported cabbage worm	10	40	...	6–7	3–4	..
Chinch bug	10	80	80	...	...	40
Plum curculio	10	60	...	...	...	..
Red spider mite	–	–	+	–	–	–
Mexican bean beetle	–	–	+	–	–	+

Kearns, Weinman, and Decker rate the more common halogenated insect toxicants in the following order of decreasing toxicity (*7*): dieldrin, aldrin, heptachlor, γ-hexachlorocyclohexane, chlordan, toxaphene, and DDT. This rating follows as the result of rather extensive tests on ten species of insects and is believed to represent, in general, the order of their relative activity.

In order to evaluate the actual magnitude of the insect toxicity of aldrin and dieldrin and the utility of these new compounds, additional data must be considered. (Many of the data herein presented have been obtained from letters and other unpublished communications. The details of the entomological investigations thus represented will, in most instances, be published in appropriate journals by their authors.)

Kearns, Weinman, and Decker determined the dosages of a number of the chlorinated compounds, dissolved in 95% ethyl alcohol, required to produce a 50% mortality of the housefly (*7*). The values obtained, expressed as micrograms of toxicant per gram of fly weight, are listed in Table V.

Table V. LD_{50} Values on Houseflies

(Based on topical application of some chlorinated compounds dissolved in 95% ethyl alcohol. Values expressed as micrograms of toxicant per gram of fly weight[a])

Compound	LD_{50}, γ
DDT	20.5
Chlordan	4.0
γ-$C_6H_6Cl_6$	2.9
Aldrin	1.6
Dieldrin	1.1

[a] Data from results of tests made by W. N. Bruce, assistant entomologist, Illinois Natural History Survey.

The Dacus fly (*Dacus oleae*, olive fly), a pest producing serious damage to olives and one related to other flies that attack a variety of fruits, is also highly susceptible to aldrin (*6*).

Weinman and Decker list the LD_{50} values against adult grasshoppers (*M. differentialis*) for a number of the more common toxicants, both as contact poisons and as stomach poisons, giving the values shown in Table VI (*14*).

Table VI. LD_{50} Values for Single Compounds Tested for Contact and Stomach-Poison Effect against *M. differentialis* Adults

(Expressed as micrograms of toxicant per gram of grasshopper weight)

Compound	Contact	Stomach Poisons
DDT	9380	2579
Toxaphene	61	91.5
Chlordan	9.8	12.0
γ-$C_6H_6Cl_6$	3.4	6.7
Aldrin	1.8	2.3
Dieldrin	1.4	3.7

Weinman and Decker studied the toxicity of aldrin to young grasshoppers on $^1/_{40}$th acre plots surrounded on all sides by untreated check plots. The small size of the test plots and the "high pressure" of grasshoppers from the surrounding untreated areas made it impossible to secure control under these circumstances. Nonetheless, a significant reduction in the number of grasshoppers in the control plots was obtained even after 5 days with application of aldrin at the very low dosage rate of 0.05 pound per acre (0.75 mg. per square foot).

For the control of locusts, grasshoppers, and crickets, 2 to 4 ounces of aldrin per acre, applied as a spray to host crops, yield highly effective results. The 4-ounce dosage is necessary under adverse control conditions such as prevail where vegetation is dry and most of the insects are in advanced stages of growth (*9*).

The effectiveness of the insect toxicant aldrin to a wide variety of cotton pests has been determined through a large number of field tests. As a result of these tests, recommendations have been published suggesting the use of a mixture of 2.5% aldrin and 5%

DDT at 10 pounds per acre to control the bollworm, boll weevil, cotton fleahopper, tarnished plant bug, rapid plant bug, and some species of cutworms and thrips. (On cotton just up it was found that only 0.07 pound per acre of aldrin, applied as an emulsion, was necessary for the control of cutworms and thrips.) Significantly, no increase in aphid population was noted following the use of the aldrin-DDT mixture (*4*).

Tests of dieldrin against cotton pests have not been as extensive, but the Mississippi 1950 Cotton Insect Control Recommendations state that "it kills a larger proportion of immature weevils in squares than any other insecticide tested thus far and is considered very promising for cotton insect control" (*4*).

Aldrin and dieldrin have been found similarly highly effective against a wide variety of other economically important insects.

In Georgia, Savage and Cowart determined the relative effectiveness of several commercial insecticides for eradication of plum curculio on peaches (*10*). Their data are summarized in Table VII. A similar order of effectiveness was found for the adult curculio.

Table VII. Plum Curculio Larvae Emergence

Toxicant	Pounds per 100 Gal.	% Infestation
Acid lead arsenate	2	41.59
Chlordan	1.5	4.08
Parathion	0.45	1.87
Aldrin	0.75	0.30

Studies on the control of various species of ants show conclusively that aldrin consistently gives control when applied to infested turf at the rate of 1 ounce per 1000 square feet (4 ounces of 25% wettable powder in 200 gallons of water). This dosage is only half of that required when chlordan is the toxicant employed (*11*, *12*).

Several reports on the use of aldrin to combat other soil-infesting pests indicate its high order of effectiveness for this purpose. In a series of field tests on Japanese beetle grubs, chinch bugs (*12*), white grubs (*16*), and tropical earthworms (*15*), aldrin has been found to be the most effective material tested. Against wireworms, preliminary work indicates aldrin to be effective when applied to the soil as a side dressing at the rate of 1.5 pounds per acre (*3*). One series of experiments also indicates aldrin to be at least twice as effective as chlordan in combatting the cabbage maggot (*13*).

The foregoing comments and data, although far from complete, serve to indicate the high degree to which aldrin and dieldrin possess the property of insect toxicity. These materials have been tested as toxicants for more than one hundred insect genera. Although broad generalizations are unsatisfactory and inadequate, it is clear that to a large number of insect species these two compounds are the most toxic halogenated hydrocarbons yet available.

Residual Activity

Aldrin and dieldrin show high toxicity to insect life, but differ greatly with respect to the length of time during which they exhibit residual activity.

Aldrin, like chlordan, exhibits residual effectiveness under field conditions for somewhat less than 3 weeks. Even when aldrin is applied at the uneconomical and unnecessary rate of 5 pounds per acre, leafy material so treated exhibits only slight insect toxicity after 3 weeks. Aldrin, therefore, falls into that class of materials which exhibit pronounced initial toxicity but relatively short residual action.

The situation with respect to dieldrin is altogether different. No insect toxicant hitherto available, with the exception of DDT, has been characterized by the possession of insect toxicity which continued for long periods after its application. In this respect, dieldrin is unique in that, in addition to its high order of insect toxicity, it possesses a span of residual activity comparable to that of DDT.

The comparative residual activity of deposits of DDT and dieldrin, using the common housefly as the test insect, is illustrated in Table VIII. For purposes of comparison, both chlordan and aldrin are included in the tabulations. This material is taken from the paper by Kearns, Weinman, and Decker (*7*).

The residual effectiveness against flies of a number of formulations of insect toxicants was studied by investigators of the U. S. Public Health Service. Over a 2-month period the formulations containing dieldrin were found to give the best results (*5*).

Roaches have also been used in studying the length of time for which deposits of dieldrin are active (*7*). Table IX gives some of the data obtained using the German roach as the test insect. In this case, the toxicants were applied at the rate of 1 mg. per 1000 sq. cm. of surface; the table lists the mortalities obtained when the insects were left on surfaces of various ages for periods of 24 and 48 hours. DDT is not included in this tabulation, because it is inactive at the dosage rates tested.

Table VIII. % Mortality of Houseflies

(24 hours after a 30-minute exposure to a deposit of 50 mg. per sq. foot of some chlorinated insecticides at various intervals after application. Each figure based on three replicates of 125 flies each. Compounds deposited on glass plates[a])

	Age of Residues, Days					
	5	14	21	28	35	51
Compound	Per Cent Mortality					
Chlordan	93.4	58.9	1.8	...	...	...
Aldrin	83.8	30.0	17.9	4.6	...	...
DDT	73.7	41.1	19.1	11.5	...	4.3
Dieldrin	100.0	100.0	...	95.7	71.1	64.3

[a] Data from results of tests made by W. N. Bruce, assistant entomologist, Illinois Natural History Survey, and G. F. Ludvik, special research assistant, Illinois Natural History Survey and Illinois Agricultural Experiment Station.

Table IX. Residual Toxicity of Chlorinated Insecticides to Adult Male German Roaches

(1 mg./1000 sq. cm. deposits at various intervals after application. Percentage dead and moribund after exposure periods of 24 and 48 hours)

	Age Deposit, Days											
	1		7		21		28		42		49	
	Exposure Period, Hours											
	24	48	24	48	24	48	24	48	24	48	24	48
Compound	Per Cent Dead and Moribund											
Chlordan	100	100	24	92	0	0	0	0	..	..	..	..
γ-$C_6H_6Cl_6$	100	100	14	18	0	0	0	0	..	..	..	..
Aldrin	100	100	22	100	0	0	0	0	..	..	..	..
Dieldrin	100	100	100	100	100	100	100	100	46	100	24	90

The formulation of aldrin and dieldrin can be readily accomplished in normal fashion; no difficulty has been encountered in incorporating these materials into dusts, wettable powders, or emulsifiable concentrates.

Phytotoxicity

The evaluation of new materials intended for use as insect toxicants requires consideration of the toxicity of such materials to other forms of life. The question of phytotoxicity can be disposed of very rapidly. The evidence available indicates that even when applied in gross overdosage, neither aldrin nor dieldrin is harmful to plants. Thus, for example, the application of aldrin to the soil at the rate of 100 pounds per acre led to no apparent inhibiting effect on the germination or growth of corn or of cucumbers (*16*). Other more extensive investigations conducted by Bauer and Dahm, using in some experiments soil treatment at the rate of 100 pounds per acre and in others up to seven applications of aldrin to growing plants, at the rate of 0.5 pound per acre per application, on thirteen varieties of plants showed no abnormal effects, except that lettuce seed germination was slightly reduced and bean plant emergence was delayed 2 days; tobacco plants on the treated plots developed more rapidly and bloomed earlier than did the tobacco plants grown on the untreated plots (*1*). Bauer and Dahm report further that the vegetables grown under these conditions possess no foreign taste or objectionable odor. These work-

ers further demonstrate the complete absence of aldrin in most of the plants so raised and the presence of only trace quantities of aldrin in soybeans and tomatoes similarly grown. Bauer and Dahm state that "the application of Compound 118 to the soil at the rate of 100 pounds per acre represents a dosage that is probably from 20 to 50 times that required for practical insect control."

Mammalian Toxicity

Much work remains to be done before all aspects of mammalian toxicity of aldrin and dieldrin are completely known. However, the work already completed has established many important facts. When administered in edible oil to albino rats, aldrin demonstrates an acute LD_{50} ranging from 40 to 50 mg. per kg. of body weight. Much chronic toxicity work is in progress. Albino rats on a daily diet containing 75 p.p.m. of aldrin incorporated in an edible oil continued normal in all respects after 6 months. Cattle, sheep (pregnant ewes), and lactating cows with suckling calves were wintered on alfalfa hay sprayed with 0.5 pound of aldrin 8 days before harvest. The hay in these tests was baled for storage. None of the animals fed on this hay developed abnormal symptoms, nor was evidence of damage found on autopsy of sacrificed individuals. Furthermore, aldrin could not be detected in the milk from the lactating cows (*8*).

Dieldrin administered in edible oil to albino rats demonstrates an acute LD_{50} toxicity of 50 to 55 mg. per kg. The chronic toxicity of dieldrin has not been established, although tests are now in progress to determine its possible hazard to animals sprayed or dipped in formulations containing varying percentages. It has been established experimentally that rats are considerably more resistant to the action of dieldrin than rabbits. Among farm animals exposed to spray formulations containing dieldrin, swine proved to be the most resistant and very young calves proved to be the most susceptible. Preliminary results of work in this field indicate that dieldrin is less toxic to young calves than are some other comparably effective chemicals when applied in a similar manner, although dieldrin is definitely more residual in action against the insect parasites affecting farm animals.

Recommended Applications

Aldrin exhibits only moderate persistence and evaporates completely under field conditions in somewhat less than 3 weeks. Consequently, if the simple precaution of applying aldrin not later than 3 weeks before crop harvest is observed, the possibility of undesired toxicant residue on harvested crops should be very slight.

It is recommended that aldrin be considered, on an experimental basis, both as a crop insecticide and for the control of subterranean pests. On crops intended for human and animal food no application should be made later than 3 weeks before the harvest of these crops is planned.

Although dieldrin has been found effective against all the insects susceptible to the action of aldrin, it should not at present be recommended for use against any pests in situations where its residue might constitute a hazard on edible foods or on forage crops. It is suggested that dieldrin be employed experimentally, especially for the control of flies, mosquitoes, cotton insects, forestry pests, termites, pests in soil, pests of lumber products, cloth-eating insects, and industrial pests not actually infesting food products. Dieldrin should be considered wherever extended residual effectiveness is advantageous.

Summary

Two new insect toxicants, aldrin and dieldrin, provide new halogenated insect toxicants with an extremely high order of toxicity toward insects, combined, for the first time, with complete stability to alkalies. Under all the usual conditions of use these new toxicants are also stable to acids. Data illustrate the order of magnitude of the insecticidal activity of these materials and their utility. Aldrin is a relatively nonresidual material, in contrast to dieldrin which, because of its high persistence, exhibits prolonged residual activity.

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Insecticidal Activity and Dehydrochlorination Rates of Some Polychloro Insecticides

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The hypothesis that the insecticidal activity of various polychloro insecticides is due in large measure to the ability of the compound to liberate hydrogen chloride at the site of action of the insecticide is examined. Reaction-rate constants for elimination of hydrogen chloride with ethanolic alkali were compared with insecticidal activity against *Anopheles quadrimaculatus* larvae and with Hammett substituent constants for eighteen analogs of DDT; the data lend no support to any hypothesis in which significant correlation between dehydrochlorination rate and insecticidal activity is required. Considerations of toxicity toward other insects also show no correlation, as do similar comparisons with isomers of benzene hexachloride and polycyclic compounds related to chlordan.

The problem of the relationship between constitution and insecticidal activity in organic compounds is one of great fascination to chemists and biologists, and any correlation that might be found to exist between chemical reactivity and biological activity would be of outstanding importance. For this reason, the hypothesis of Martin and Wain (*16*, *17*) that the insecticidal activity of various polychloro insecticides is due in large measure to the ability of these materials to liberate hydrogen chloride at the site of action of the insecticide requires careful consideration. This hypothesis was based on early investigations on DDT analogs, where it was observed that the relative biological activities of DDT [$(p\text{-}ClC_6H_4)_2CHCCl_3$], its related olefin [$(p\text{-}ClC_6H_4)_2C{=}CCl_2$], DDD (or TDE) [$(p\text{-}ClC_6H_4)_2CHCHCl_2$], and the hexachloro compound [$(p\text{-}ClC_6H_4)_2CClCCl_3$] seemed to bear a relationship to the abilities of these compounds to lose hydrogen chloride to alkali.

This hypothesis has been criticized by Busvine (*2*, *3*), Domenjoz (*10*), Müller (*18*), and Cahn (*4*). Domenjoz and Müller have shown that there is no direct correlation between activity toward a variety of insects in a number of compounds of the type Ar_2CHCCl_3 and the amount of hydrogen chloride liberated under standard conditions. Busvine attempted a correlation for similar compounds between activity toward lice and bedbugs and this author's reaction-rate constants (*2*, *5*) for second-order elimination with ethanolic alkali and found that no statistically significant correlation exists.

It appeared to the author some years ago that, irrespective of the mechanism of the toxic action of DDT, there might be a correlation of structure and toxicity in analogous compounds. Hammett (*13*) has shown that the rate and equilibrium constants of over 50 side-chain reactions of meta and para substituted aromatic compounds may be correlated with the so-called substituent constant σ, according to the equation $\log k - \log k_0 = \rho\sigma$, where k and k_0 are rate (or equilibrium) constants for substituted and unsubstituted compounds, respectively, ρ is the reaction constant giving the slope of the linear relationship, and σ is the substituent constant, which is determined by the nature and

position of the substituent and is independent of the reaction studied, being defined as the difference between the logarithms of the ionization constants of the substituted and unsubstituted benzoic acids. This relationship is effective whenever the meta or para substituents affect the reactivity of the compounds by virtue of their electronic effects, and satisfactory relationships are not found in cases where steric interactions are involved, as, for example, in the reactions of ortho substituted compounds. Thus the observation of such a relationship is good evidence of the relative unimportance of steric factors in reactivity.

Accordingly, it was felt that such a correlation might be observed with reactions of the trichloroethyl group of DDT and its analogs. Elimination of hydrogen chloride with ethanolic alkali is such a reaction, and a satisfactory correlation was found for the second-order reaction-rate constants for elimination with ethanolic alkali (*5*). On the assumption that the toxic reaction of DDT also involved a reaction of the trichloroethyl group without steric involvement of the ring substituents, a similar correlation was attempted between the insecticidal activity and the substituent constants. Results of insect mortality were available from the work of the entomologists and chemists of the Orlando laboratory of the Bureau of Entolomogy and Plant Quarantine for larvae of the common malaria mosquito (*Anopheles quadrimaculatus* Say) and have since been published (*9*).

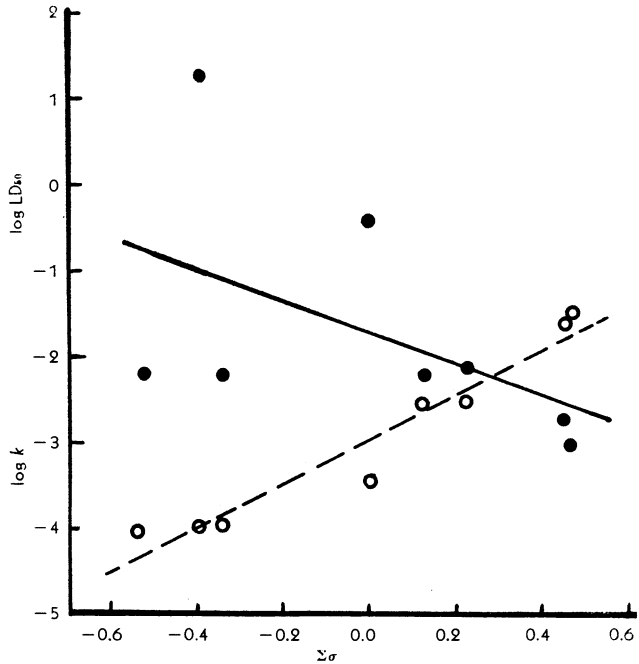


Figure 1. Relation among Log *k* of Dehydrochlorination Reaction, Median Lethal Dosage to Mosquito Larvae, and Substituent Constant for Trichloroethyl Compounds

● Median lethal dosages
○ Rate constants

The data used are given in Table I. The elimination rate constants included were determined at 20° C. (*5*). The toxicity to mosquito larvae, given as median lethal dosages (concentration in parts per million of water required to cause 50% mortality in 48 hours), was estimated from the data of Deonier *et al.* (*9*) and is probably reproducible to within 30%.

The logarithms of the elimination rate constants were plotted against substituent constants to give the lower line of Figure 1, and the logarithms of the median lethal dosages were treated similarly to give the upper line. The straight lines were constructed by the method of least squares. The reaction-rate data fit a line with the equation

$$\log k = 2.661\sigma - 2.938$$

A statistical test of these data gave a correlation coefficient of 0.968 (*19*), with only 0.834 required for 1% significance. Thus the elimination rates are functions dependent

Table I. Dehydrochlorination-Rate Constants and Insecticidal Activity of DDT Analogs

Para Substituents	Dehydrochlorination Rate Constant, 10^5 k, L./Sec./Mole	Toxicity to *A. quad.* Larvae LD_{50}, P.P.M.	Hammett Substituent Constant, σ
	A. Diaryltrichloroethanes		
Di-Br	3470	0.001[a]	0.464
Di-Cl	2480	0.002[a]	0.454
H, Cl	301	0.008[a]	0.227
Di-F	303	0.007[a]	0.124
Di-H	36.9	0.4[a]	0.000
Di-CH_3	10.9	0.007[a]	−0.340
Di-*tert*-butyl	10.7	20[a]	−0.394
Di-CH_3O	9.18	0.007[a]	−0.536
	B. Diaryldichloroethanes		
Di-Br	776	0.001[b]	0.464
Di-Cl	568	0.001[a]	0.454
Di-F	71.0	0.1[b]	0.124
Di-H	14.3	0.4[b]	0.000
Di-CH_3	4.33	0.025[b]	−0.340
Di-CH_3O	3.36	1 (est.)[b]	−0.536
	C. Diarylmonochloroethanes		
Di-Br	127	0.07[b]	.....
Di-Cl	91.0	0.06[a]	.....
Di-CH_3	1.0 (est.)	0.3[b]	.....

[a] (*9*).
[b] (*8*).

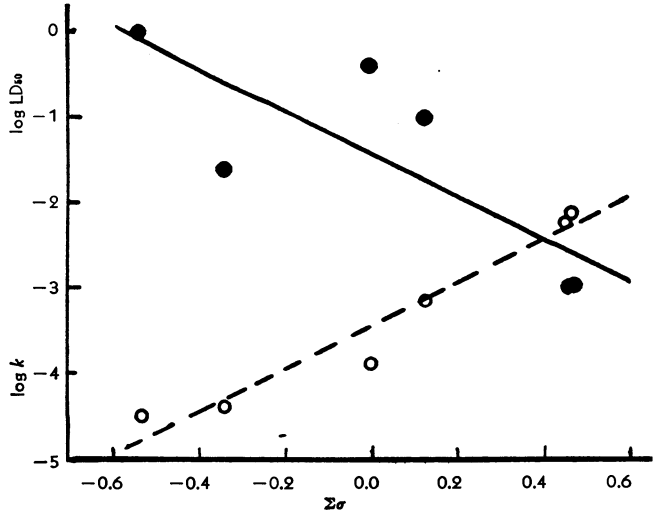


Figure 2. Relation among Log *k* of Dehydrochlorination Reaction, Median Lethal Dosage to Mosquito Larvae, and Substituent Constant for Dichloroethyl Compounds

● Median lethal dosages
○ Rate constants

upon side-chain reactions and relatively independent of steric factors in the para substituents. The best line relating insect mortality and substituent constant has the equation

$$\log LD_{50} = -1.87\sigma - 1.69$$

and the poorness of fit can be noted from Figure 1. These data have a correlation coefficient of only 0.505 with 0.707 required for 5% significance. It is apparent from the data in Figure 1 that fit to a curve seems no more valid than the linear relationship. Attempts to find statistically significant relationships of the biological data were not successful when correlation was made with dehydrochlorination rates directly or with dosages in molar units rather than weight units. Hence, it appears unlikely that any relationship exists between dehydrochlorination rate or any other reaction of the trichloroethyl groups without consideration of the steric involvement of the para substituents in the DDT analogs.

In the course of work on the mechanism of elimination reactions, the author and his co-workers have measured reaction-rate constants for the second-order elimination of hydrogen chloride from six dichloroethyl compounds of type $Ar_2CHCHCl_2$ and three monochloroethyl compounds of type Ar_2CHCH_2Cl (*7*). Samples of each of these materials were furnished to the Bureau of Entomology and Plant Quarantine for insecticidal testing, and the author is indebted to C. C. Deonier and I. H. Gilbert for permission to use certain of their data in this paper. The rate constants and larvicidal results are given in Table I.

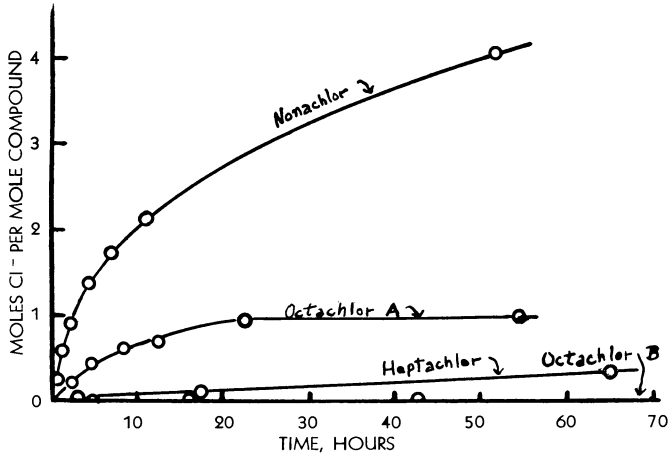


Figure 3. Rate of Chloride Production from Chlordan Constituents

Solvent 95% ethyl alcohol, 0.04 M sodium hydroxide, 0.005 M halide, 45.7° C.

Treatment of the data on the dichloroethyl compounds is indicated in Figure 2. Here again there is excellent correlation between substituent constants, σ, and the reaction-rate constants, fitting the line

$$\log k = 2.456\sigma - 3.430$$

and no statistically significant correlation between substituent constant and insecticidal activity, the best straight line having the form

$$\log LD_{50} = -2.50\sigma - 1.43$$

These data lead to the conclusion that, contrary to the hypothesis of Martin and Wain, there is no statistically significant relationship between dehydrochlorination rate and insecticidal activity in DDT analogs, at least as regards activity against *A. quadrimaculatus* larvae. Similar comparisons of insecticidal data against adult houseflies (*11*, *12*),

adult yellow fever mosquitoes (*Aedes aegypti*) (*11*, *12*), codling moth larvae (*12*), corn borer larvae (*12*), and screw-worm larvae (*12*) do not fit the dehydrochlorination rate hypothesis.

The isomers of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) also eliminate the elements of hydrogen chloride to alkali, and comparative data regarding the proposed hypothesis are available. Table II gives reaction-rate constants for dehydrochlorination (*6*) and comparative toxicities to larvae of *A. quadrimaculatus* (*12*). Here, of course, the compounds differ only in stereochemistry—that is, in spatial arrangement of the atoms—and there is no apparent relationship between reactivity and toxicity.

Martin (*16*) has also suggested such a relationship in the chlorinated polycyclic compounds such as chlordan. Accordingly the author has measured the reactivities of the

Table II. Dehydrochlorination-Rate Constants and Toxicity to *Anopheles quadrimaculatus* Larvae of Isomers of Benzene Hexachloride

Isomer	Rate Constant, (20°)[a], L./Sec./Mole	Dosage, P.P.M.	% Mortality[b], 48 Hours
Alpha	0.170	2.5	92
Beta	3×10^{-4}	100	40
Gamma	0.0446	0.01	100
Delta	Fast	2.5	62

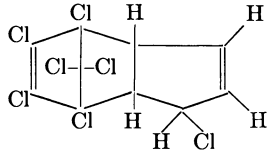
[a] (*6*).
[b] (*12*).

Table III. Relative Effective Dosages of Polycyclic Compounds toward Houseflies[a]

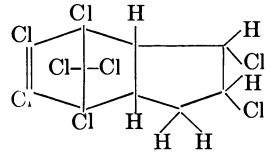
Compound	Relative Effective Dosages (Technical Chlordan = 0.01)
Heptachlor (I)	0.0043
Octachlor (IIA) active isomer	0.0056
(IIB) inactive isomer	0.017
Nonachlor (III)	0.020
118 (IV)	0.0040
497 (V)	0.0028

[a] Data from R. E. Lidov, Julius Hyman and Co.

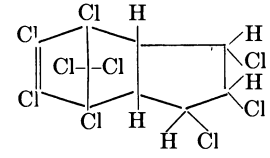
various constituents of technical chlordan (*14*) toward ethanolic sodium hydroxide (Figure 3). The compounds referred to in the figure are: heptachlor, 1,4,5,6,7,8,8-heptachloro-3a,4,6,6a-tetrahydro-4,7-methanoindene (I); octachlor, 1,2,4,5,6,7,8,8-octachloro-2,3,3a,-4,7,7a-hexahydro-4,7-methanoindene, of which two isomers are known (*15*), one (IIA) more active insecticidally than the other (IIB); and nonachlor, 1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (III) (the stereochemistry of the compounds has not been reported).



I



II

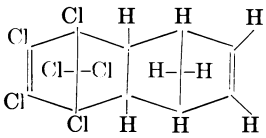


III

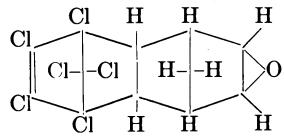
In reactions with 0.04 *M* ethanolic sodium hydroxide at 46° C. the insecticidally more active octachloro isomer, IIA, and the nonachloro compound, III, reacted much more rapidly than the heptachloro compound, I, and the less active octachloro compound, IIB, showed no measurable reaction in 43 hours. In refluxing 1 *M* ethanolic alkali, the octachloro compound, IIB, is reactive. The relative toxicities of these compounds toward houseflies (*14*) are given in Table III. Here again there is no correlation between chlo-

ride ion formation rate and relative toxicity, heptachloro compound I being the most effective insecticidally, yet of intermediate reactivity.

The recent announcements of the insect toxicants Compound 118, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene (IV) (*1*, *15*), and Compound 497, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene (V) (*15*) are of further interest. The official name aldrin has been designated for material containing at least 95% of IV, and dieldrin for material containing at least 85% of V.



IV



V

These compounds are much more toxic than chlordan (Table III), and yet are stable toward alkaline reagents (*15*), being unable to eliminate hydrogen chloride without the formation of a double bond at a bridgehead carbon atom. Thus in this type of compound the conclusion must again be reached that dehydrochlorination with alkali and insecticidal activity have no systematic relationship.

Biological systems are, in general, specialized as regards steric factors. Although the biological chemistry involved in the action of the chlorinated insecticides is still unknown, the data of this paper suggest the relative importance of steric factors in the action.

Acknowledgment

The author wishes to acknowledge the aid of the Office of Naval Research, U. S. Navy Department, for support of the work on elimination rates; of H. L. Haller, E. F. Knipling, W. V. King, C. C. Deonier, and I. H. Gilbert of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for the use of unpublished insecticidal data; and of Rex E. Lidov of Julius Hyman & Co. for information regarding the polycyclic insecticides and for research samples.

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Colorimetric Method for Estimating Small Amounts of Aldrin (Compound 118)

A. A. DANISH and REX E. LIDOV

Julius Hyman & Company, Denver, Colo.

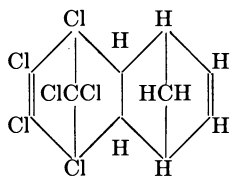
A colorimetric procedure is described for the determination of small amounts of Compound 118 (1,2,3,4,10,10-hexachloro - 1,4,4a,5,8,8a - hexahydro - 1,4,5,8 - dimethanonaphthalene). Reaction with phenyl azide to form a dihydrotriazole derivative and subsequent treatment with diazotized dinitroaniline in strongly acid medium produce an intense red color. Amounts of the insect toxicant of 10 to 40 micrograms in the final 10-ml. aliquot are readily determined with a spectrophotometer. Commonly used insect toxicants do not interfere.

The synthesis of an alkali-stable highly potent insect toxicant, tentatively called Compound 118, has been announced recently (*2*). Structurally Compound 118 is 1,2,3,4,10,-10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene (I). The chemistry and applications of this material have been discussed by Lidov, Bluestone, Soloway, and Kearns (*3*).

The availability of Compound 118 for wide scale experimentation in the field of agricultural, household, and public health uses makes necessary a method for determining minute amounts such as would be present in spray or dust residues on plants and in biological fluids and tissues.

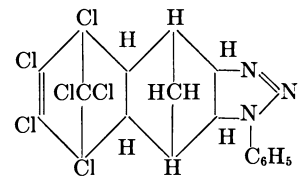
An analytical procedure based on the bicycloheptene structure of Compound 118 offers a more or less specific method, and is the subject of this paper.

Compound 118 — I



+ $C_6H_5N_3$ ⟶

Compound 118-phenyldihydrotriazole — II



II + dinitrophenyl diazonium salt ⟶ colored compound (III)

Phenyl azide reacts at the double bond of the halogen-free bicycloheptene ring of Compound 118 to form a phenyldihydrotriazole derivative (118-phenyldihydrotriazole, II). This type of reaction has been discussed by Alder and Stein (*1*).

The triazole derivative, in turn, produces an intensely colored substance, III, with a diazonium compound under the experimental conditions devised for this analysis. The reactions leading to the formation of the colored substance have not yet been fully elucidated, but it appears that opening of the triazole ring as well as coupling is involved. The chemistry of the color formation will be discussed in another publication.

The aforementioned series of reactions provides a basis for a colorimetric analytical method for Compound 118 in which the commonly used agricultural chemicals do not interfere. The procedure described herein permits the estimation of as little as 10 micrograms of Compound 118, and has been successfully applied to the analysis of this insect toxicant in insecticidal dusts, in film residues on glass and paper, in human and animal urine, and in mixture with other insecticides. Application of this procedure to the determination of Compound 118 in milk and in spray and dust residues on plants appears promising.

Method

The analytical procedure for Compound 118 consists of the following basic steps:

Quantitative formation of 118-phenyldihydrotriazole (II) by heating Compound 118 with an excess of phenyl azide liquid, in absence of solvent, and then removing unreacted azide with a mechanical vacuum pump. (Because of the small amounts of 118 and phenyl azide reacting, the presence of conveniently measured amounts of solvent makes the rate of dihydrotriazole formation too slow to be practicable.)

Treating the triazole residue in alcoholic hydrochloric acid with an excess of diazotized 2,4-dinitroaniline, and then acidifying strongly to produce an intensely red colored compound (III). (The presence of hydrochloric acid is essential. Other strong acids, in the absence of hydrochloric acid, do not lead to the desired color formation.)

Evaluation of the developed red color, using a spectrophotometer and a standard transmittance-concentration curve from data on known amounts of purified 118-phenyldihydrotriazole.

Preparation of Standard Optical Transmittance Curve of 118-Phenyldihydrotriazole

Purified 118-phenyldihydrotriazole, prepared from Compound 118 and phenyl azide, was used for plotting a standard optical transmittance curve.

A stock solution of the triazole in hexane was made up and diluted to various strengths, and 1.0-ml. aliquots of the diluted solutions were carried through the procedure described below. The transmittance of the colored solutions obtained from 10 to 50 micrograms of the 118-phenyldihydrotriazole was plotted against concentration to make a standard curve. In subsequent analyses, the amount of Compound 118 is readily calculated from the amount of dihydrotriazole formed.

Absorption curves of the colored solutions were run on a Beckman spectrophotometer using 1.00-cm. quartz cells. A typical curve, shown in Figure 1, has an absorption maximum at about 515 millimicrons. The Coleman Junior spectrophotometer was used for routine determinations of Compound 118 throughout this study.

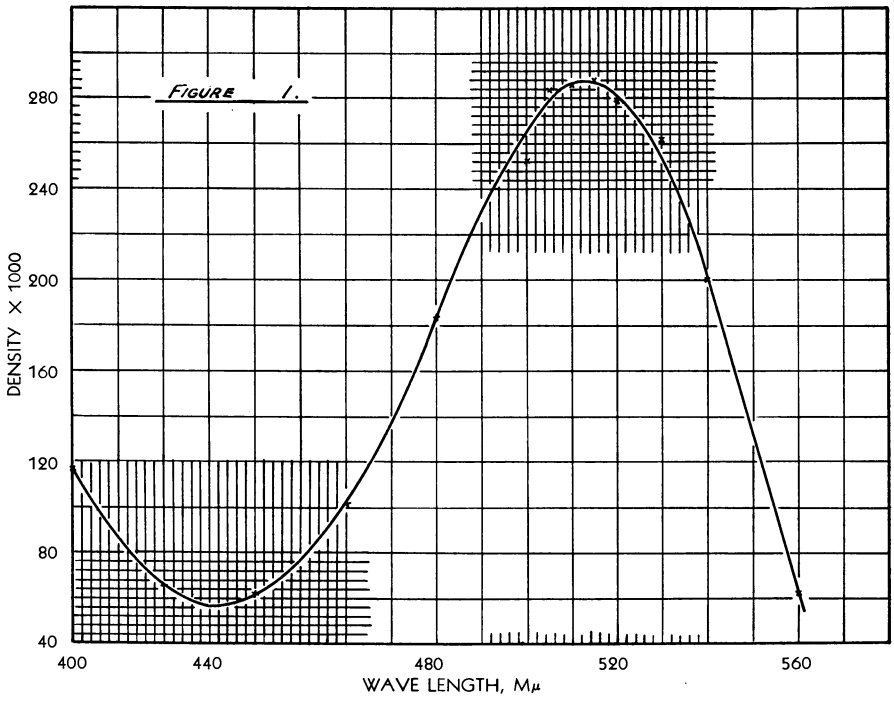
Preparation of Standard 118-Phenyldihydrotriazole Curve

Reagents. PHENYL AZIDE (freshly distilled), boiling point 49–50° C., at 5 mm. The phenyl azide was prepared according to the method of Lindsay and Allen (*4*).

COMPOUND 118. A material recrystallized from methanol and melting at 100–102° is satisfactory. A sample of crystalline Compound 118 may be obtained from Julius Hyman & Company, Rocky Mountain Arsenal, Denver, Colo.

CRYSTALLINE 118-PHENYLDIHYDROTRIAZOLE. The dihydrotriazole is prepared by refluxing a solution containing 10.0 grams of Compound 118 and 4.0 grams of phenyl azide in 30 ml. of hexane for 40 minutes. The dihydrotriazole begins to precipitate out during the refluxing period. The white crystalline solid is collected by suction filtration, recrystallized once from 1 to 5 benzene–ethyl alcohol, and dried in a vacuum desiccator. Melting point is 174° C. (decomposes). (The melting point is determined by plunging the capillary tube containing the triazole in the melting point bath at 164° and heating at the rate causing a 2° rise in temperature per minute.) Purity may be determined by total nitrogen analysis (theory is 8.67%).

Other reagents used are: 2,4-dinitroaniline, Eastman; sodium nitrite, finely powdered, reagent grade; concentrated hydrochloric acid; acetone, C.P.; phosphoric acid,



85%, C.P.; absolute ethyl alcohol; concentrated sulfuric acid, C.P.; hexane, redistilled (Skellysolve B, boiling point 60 to 70°); and sulfuric acid, 2 to 1, made by mixing 2 volumes of concentrated sulfuric acid with 1 volume of distilled water.

COLOR DILUTING SOLUTION. This is prepared by mixing 5 volumes of absolute ethyl alcohol, 1 volume of concentrated hydrochloric acid, and 4 volumes of 2 to 1 sulfuric acid. It is made up just before use. (In the equation for calculation of results, this solution is referred to as Solution B.)

DIAZOTIZED 2,4-DINITROANILINE. A slight modification of Schoutissen's procedure is employed (*5*). A solution of 1.5 grams of 2,4-dinitroaniline in 30.0 ml. of concentrated sulfuric acid is prepared by heating to 90°. The solution is cooled to 0° and then 0.7 gram of finely powdered sodium nitrite is sifted onto the sulfuric acid solution at 0°. After standing for 1 to 2 hours, at a temperature below 15°, the sodium nitrite dissolves. To the resultant solution, 40.0 ml. of 85% phosphoric acid are added with stirring. The temperature should not exceed 40° during the phosphoric acid addition. After standing at room temperature for an additional 2 hours, the preparation is ready for use. The final solution is pale yellow and is stable at room temperature for at least a week. On prolonged standing the reagent solution darkens progressively to a deep orange, and should be discarded.

STOCK STANDARD SOLUTION OF COMPOUND 118-PHENYLDIHYDROTRIAZOLE. Exactly 0.1000 gram of 118-phenyldihydrotriazole is dissolved in 5 ml. of acetone and enough hexane is added to make 1000 ml. One milliliter contains 100 micrograms.

STOCK STANDARD SOLUTION OF COMPOUND 118. Exactly 0.1000 gram of Compound 118 is dissolved in hexane to make 1000 ml. of solution. Each milliliter contains 100 micrograms of Compound 118.

Apparatus. A Coleman Junior Model 6A spectrophotometer, with tubular cells 9 mm. in diameter, is used (Catalog No. 6-302).

Beakers; 10 to 1000-ml. volumetric flasks; 1-ml. volumetric pipet, serological pipet; steam bath; vacuum desiccator; vacuum pump; and water aspirator are also required.

Procedure. Five aliquots of the triazole stock solution of 5, 10, 15, 20, and 25 ml. are introduced into 50-ml. volumetric flasks. Each aliquot is diluted to 50 ml. with hexane, making working standards equivalent to 10, 20, 30, 40, and 50 micrograms of triazole per ml., respectively. Using a volumetric pipet, 1 ml. of each working standard is

quantitatively transferred to a cylindrical spectrophotometer cell and 1.0 ml. of hexane, which serves as a blank, is placed in another cell. Two drops of phenyl azide (30 to 40 mg.) are added to each cell, care being taken that none of the azide liquid strikes the glass walls.

The hexane is evaporated completely at room temperature by placing the cells in a vacuum desiccator attached to a water aspirator. The evaporation requires 10 to 20 minutes. A drop of oily residue remains. This evaporation is handled conveniently by placing the cells in a beaker containing a 2.5-cm. level of mineral oil at room temperature and transferring the whole to the desiccator. The oil bath prevents excessive cooling of the hexane solution during the evaporation.

After complete removal of solvent, the vacuum is broken gently and the colorimeter cells are then heated in an oven at 75° to 80° C. for exactly 30 minutes. It is during this heating period that, in an actual analysis, any Compound 118 is quantitatively converted to the triazole.

The excess phenyl azide is then removed in vacuo at 1 to 2 mm. by almost completely immersing each cell in a beaker of water at 45° to 50° and attaching each cell directly to the vacuum pump for 3 minutes. The nearly colorless film residue at the bottom of the cell is now ready for coupling with diazotized dinitroaniline.

The film residue is dissolved in 5.0 ml. of absolute ethyl alcohol and then 1.0 ml. of concentrated hydrochloric acid and 0.3 ml. of diazotized dinitroaniline solution are added. A serological 1.0-ml. pipet, graduated in tenths, is used to measure out the diazotized dinitroaniline solution. The solutions are mixed well and allowed to stand for 20 minutes, during which an orange color develops. Finally, 3.7 ml. of 2 to 1 sulfuric acid are added slowly to each solution to make a final volume of 10 ml. The solutions are mixed well and allowed to stand for at least 3 minutes. An intense red color is produced at this point. In the equations for calculation of results this solution is called Solution A. The blank solution is used to set the galvanometer index line at 100% transmittance at 515 millimicrons, and the transmittance of each red solution is measured. The standard curve is prepared by plotting per cent transmittance against concentration expressed as micrograms of 118-phenyldihydrotriazole per 10 ml.

Determination of Compound 118 in Hexane Solutions

Reagents and apparatus are the same as those used in the preparation of the standard curve.

Procedure. A hexane solution of Compound 118 is diluted or concentrated so as to bring the 118 content within a range of 15 to 150 micrograms per ml. In cases where the hexane solution requires concentration, the evaporation is carried out in a beaker on a steam bath with a gentle stream of air passing over the surface. The concentrated or diluted solution of 118 is washed with hexane into a volumetric flask and made up to volume with the hexane washings. One milliliter of the adjusted Compound 118 solution is precisely measured into a spectrophotometer cell, 2 drops of phenyl azide are added, and the dihydrotriazole is quantitatively formed and then treated with diazotized dinitroaniline to produce the red color as in the preparation of the standard curve. A blank, starting with 1.0 ml. of hexane and 2 drops of azide, is run at the same time.

The final blank solution is set at 100% transmittance and the transmittance of the test solution is then measured. Reading from the standard curve, one obtains the number of micrograms of triazole.

Calculation of Results

The percentage of 118 present in the samples analyzed can be obtained by means of the following equations.

$$\% \text{ Compound 118} = \frac{\text{micrograms of dihydrotriazole read from standard curve} \times \left(\frac{\text{volume of Solution A} + \text{volume of Solution B}}{\text{volume of Solution A}}\right) \times \text{aliquot ratio} \times 0.754}{\text{grams of test sample} \times 10{,}000}$$

The 0.754 factor represents the ratio of the molecular weight of Compound 118 to that of the 118-phenyldihydrotriazole. Thus:

$$\text{Micrograms of Compound 118} = \text{micrograms of triazole} \times 0.754$$

Using this conversion factor, the standard 118-phenyldihydrotriazole curve may be replotted, if desired, to read directly in micrograms of Compound 118. In that case the calculation becomes:

$$\%\ \text{Compound 118} = \frac{\text{micrograms of Compound 118 read from standard curve} \times \left(\frac{\text{volume of Solution A} + \text{volume of Solution B}}{\text{volume of Solution A}}\right) \times \text{aliquot ratio}}{\text{grams of test sample} \times 10{,}000}$$

The factor in parentheses is omitted when Solution A is not diluted with Solution B.

The aliquot ratio is the ratio of the total volume of the solution containing Compound 118 to the volume of that solution taken for analysis.

The conversion of micrograms to grams requires a factor of 10^{-6}. The conversion of the analytical result to a percental basis requires multiplication by 10^2. The figure 10,000 present in the denominators of the equations above accounts for these two factors.

Notes on Procedure

On heating, phenyl azide decomposes partially to give nonvolatile products which form colors with diazonium compounds. To minimize the presence of these impurities, the quantity of phenyl azide is limited to 2 drops in the triazole formation step.

If the color intensity of the final solution corresponds to more than 50 micrograms of triazole (15% transmittance), dilution of the sample and blank with color diluting solution will give a transmittance value within a more desirable range for color intensity measurement. In equations for calculation of results the color diluting solution is called Solution B. Should it be found that more than 250 micrograms of dihydrotriazole had been present before dilution, the entire analysis should be repeated, after readjusting the concentration of Compound 118 test solution to 20 to 40 micrograms per ml., using hexane as the diluent. This step is necessary because the amount of phenyl azide in the first determination may have been insufficient for quantitative conversion of all the Compound 118 to the dihydrotriazole. On the other hand, should the transmittance be equivalent to less than 20 micrograms of dihydrotriazole, the analysis should also be repeated, starting with 2 or 3 ml. of the hexane solution of 118 and 2 drops of phenyl azide, evaporating the solvent in a vacuum desiccator, and continuing the determination as in the preparation of the standard curve.

The transmittance readings should be taken within 5 to 10 minutes after red color development. The developed red color is stable on standing up to at least 2 hours when working with crystalline dihydrotriazole only; however, when phenyl azide is used, as it must be in an actual analysis for Compound 118, some of its nonvolatile thermal decomposition products develop colors on standing.

It appears that the decomposition of phenyl azide is significantly accelerated by direct exposure to light. Consequently, during all steps of the procedure in which phenyl azide is present, including that of its removal in vacuo, exposure of the reaction mixtures to direct light must be avoided.

The phenyl azide reagent should be stored in the cold and distilled on the day it is used in order to minimize extraneous color formation.

Complete removal of excess phenyl azide after triazole formation is necessary to ensure reproducible results.

From the manipulative standpoint, the critical step lies in the vacuum evaporation of the solvent from the solution of Compound 118 and the two drops of phenyl azide. Care must be observed that no foaming or undue chilling occurs during the evaporation; undue chilling may cause some Compound 118 to crystallize out of contact with the phenyl azide and prevent quantitative formation of the dihydrotriazole.

The application of vacuum and its release must at all times be gradual, so that none of the crystalline Compound 118 or its dihydrotriazole is swept out by a surging air stream. A two-way stopcock may be conveniently used for this purpose.

The aliquots of Compound 118 solution should be pipetted at the same temperature as that at which it was made up to volume.

Effect of Evaporative Concentration of Hexane Solutions of Compound 118

Hexane was found to be a convenient extracting solvent for insecticidal dusts containing Compound 118.

To determine whether any loss of Compound 118 occurs on evaporation, a stock solution of Compound 118 in hexane was made up, and aliquots containing 20 to 250 micrograms of Compound 118 were measured into tall-form beakers and diluted to 250 ml. with hexane. The solutions were evaporated to 10 ml. on a steam bath with a jet of air passing gently over the liquid surface. Following the same procedure, solutions of Compound 118 of varying concentrations were reduced to convenient final volumes. After concentration, in the manner described, the Compound 118 content of the final concentrates was determined.

The data of Table I show a 2 to 6% loss of Compound 118, depending on the volume of hexane evaporated. The loss of Compound 118 is roughly 2 to 3% for each 250 ml. of hexane evaporated. Where a 94 to 98% recovery is not sufficiently accurate for the objectives sought, a correction can be applied to compensate for evaporative loss.

Table I. Recovery of Compound 118 after Evaporative Concentration of Hexane

Hexane Solution Concentrated, from	Compound 118, γ		Recovery, %
	Added	Recovered	
250 to 10 ml.	20	19	95
	50	49	98
	100	97	97
	125	120	96
	150	142	95
	200	188	94
	250	241	96
500 to 50 ml.	1000	920	92
500 to 25 ml.	500	460	92
100 to 10 ml.	100	97.5	97.5

The complete procedure was tested by analyzing hexane solutions containing known amounts of Compound 118. The recoveries, shown in Table II, average about 96.5%.

Table II. Analyses of Known Amounts of Compound 118

Taken for Analysis, γ	Found Present, γ	Recovery, %
10	8.8	88.0
15	14.5	96.5
20	19.3	96.5
25	24.2	96.0
30	28.8	96.0
35	35.4	101.0
40	40.0	100.0

To test further the accuracy of the method, synthetic mixtures of Compound 118 with its insecticidally active epoxide derivative, Compound 497, were prepared (*2*). The results, shown in Table III, indicate that Compound 118 can accurately be determined in the presence of gross amounts of structurally related material.

Table III. Analyses of Known Amount of Compound 118 in Compound 497

Compound 118 Added to Compound 497, %	Compound 118 Found, %
3.8	4.5
16.0	16.6
25.3	25.8

This colorimetric procedure is applicable to the estimation of Compound 118 in cow's urine.

To 100-gram batches of 1-day-old samples of cow's urine were added 0.05, 0.1, and 0.5 mg. of Compound 118 in acetone to give 0.5, 1.0, and 5.0 p.p.m., respectively. The urines were then extracted with two 50-ml. batches of hexane. Occasional emulsions were broken by centrifuging. The hexane extracts were dried with anhydrous sodium sulfate, filtered, evaporatively concentrated, and analyzed for Compound 118 as described under Procedure. The results of these analyses are shown in Table IV. Similar experiments with human urine gave slightly better recoveries.

Table IV. Recovery of Compound 118 Added to Cow's Urine

Compound 118 Added, %	Compound 118 Found, P.P.M.		Recovery Corrected for Blank, %
	Uncorrected for blank	Corrected for blank	
0	0.20[a]	...	..
0	0.35[a]	...	..
0.5	0.62	0.40	80
0.5	0.60	0.41	82
1.0	1.10	0.90	90
1.0	1.00	0.93	93
5.0	5.45	4.91	98
5.0	5.36	4.84	97

[a] Calculated as Compound 118.

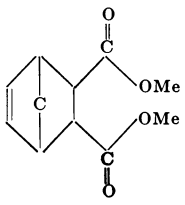
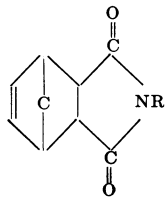
For analysis of Compound 118 in commercial dusts or wettable powders, a preliminary extraction with hexane is necessary. A Soxhlet apparatus containing a sample large enough to be representative is satisfactory. A minimum of 30 minutes' extraction time has been used, and 1 hour is recommended.

Interferences

The commonly used organic insect toxicants do not interfere in the analysis of Compound 118 by this new procedure. Hexane solutions of chlordan, DDT, methoxychlor, hexachlorocyclohexane (BHC), and toxaphene treated according to the procedure for determining Compound 118 gave a pale yellow color similar to that of the blank.

Dimalone [bicyclo-(2.2.1)-5-heptene-2,3-dicarboxylic acid dimethyl ester] and Octacide 264 [the *N*-octyl imide of bicyclo-(2.2.1)-5-heptene-2,3-dicarboxylic acid] do produce a red color with an absorption maximum in the same region as that obtained in the analysis of Compound 118. However, because Dimalone is an insect repellent and Octacide 264 is a pyrethrum synergist, neither of these products is likely to be encountered in commercial mixtures of Compound 118. The response to the colorimetric test for Compound 118 of some chemicals commonly used for insect control is listed in Table V.

Table V. Response of Some Substances Used for Insect Control to Color Test for Compound 118

Interfering Substances		Noninterfering Substances
Repellent	Pyrethrum synergist	
		Chlordan DDT Methoxychlor Hexachlorocyclohexane (BHC) Toxaphene Compound 497
Dimalone	Octacide 264	

Acknowledgment

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Polarographic Determination of *O,O*-Diethyl *O-p*-Nitrophenyl Thiophosphate (Parathion)

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Parathion may be determined quantitatively by means of the polarograph. The electrolysis is carried out in an acetone-water solution with 0.05 *N* potassium chloride as the electrolyte, and 0.01% gelatin as the suppressor at 25° ± 0.5° C. An accuracy of ±1% is obtained. Several commercial products were analyzed.

The only reported method for the estimation of *O,O*-diethyl *O-p*-nitrophenyl thiophosphate (parathion) is a colorimetric procedure (*1*) based upon the reduction of the nitro group to an amino group with subsequent diazotization and coupling with *N*-(1-naphthyl)-ethylenediamine to produce a color that may be measured. This procedure has had application in the determination of spray and dust residues where 90% recovery is satisfactory, but is not suitable for use in the assay of technical materials. Consequently, reliable and sensitive methods of analysis are greatly needed for this new and highly toxic material in insecticidal formulations. Because the reduction occurs so readily with zinc in the above-mentioned procedure and nitrobenzene (*6*) was the first organic compound to be investigated with the polarograph, it was considered probable that parathion would be easily reduced at the dropping mercury electrode and thus be determined by this means.

Apparatus

A Sargent Model XXI polarograph was used in this investigation. The reduction was carried out in an H-type electrolysis cell with a saturated calomel reference cell in one arm (*5*). A thermostatically controlled water bath maintained the cell at 25° ± 0.5° C. During the recording of the polarogram the air stirrer was stopped in order to eliminate vibration and the heating system was disconnected at the bench outlet to remove the possibility of stray current effect (*3*). It was observed that other operating electrical appliances, such as hot plates on the same bench, had a stray current effect on the polarograph.

Preparation of Standard Curves

The *O,O*-diethyl *O-p*-nitrophenyl thiophosphate used in the preparation of the standard curves was obtained by isolation from a high-purity technical parathion according to the method devised by Edwards and Hall (*2*). It was a crystalline material that melted sharply at 6° C. The physical constants were in agreement with those published by Fletcher *et al.* (*4*).

A sample of 0.4863 gram of this purified *O,O*-diethyl *O-p*-nitrophenyl thiophosphate was dissolved in acetone to make 1 liter of standard solution. A 20-ml. aliquot, containing 9.73 mg., was placed in a 100-ml. volumetric flask and 30 ml. of acetone were added. Then 0.35 gram of potassium chloride and 0.6 gram of acetic acid were dissolved in about 25 ml. of water and added to the acetone solution; 0.01 gram of gelatin was dissolved in a few milliliters of water by warming, cooled, and added to the above, and the

solution was brought to the mark with water. (This solution is 0.05 N with respect to potassium chloride, and 0.1 N with respect to acetic acid, and contains 0.01% of gelatin and 50% of acetone in water.) The acetic acid was added to prevent any hydrolysis of the ester during the electrolysis.

The sample side of the H cell was emptied and rinsed by means of suction without being removed from the thermostat bath. The used mercury was retained in the suction flask. The cell was rinsed well with acetone and then with a portion of the sample solution before being filled with the sample solution. Prior to the electrolysis oil-pumped nitrogen was bubbled through the sample solution for 10 minutes to remove dissolved oxygen. The nitrogen was passed through a 1 to 1 acetone-water solution before it reached the sample solution. For electrolysis the dropping mercury electrode was placed firmly in the cell and the polarograph set to record the wave at 0 to −1.5 volts at a sensitivity of 0.020 microampere with maximum damping. Waves were recorded in duplicate for 0.020-, 0.030-, and 0.040-microampere sensitivity to allow for considerable leeway in the size of the sample.

The sensitivities of the polarograph refer to the microamperes corresponding to 1-mm. deflection of the recorder. Polarographic waves were obtained in the same manner for 7.29-, 4.86-, and 2.43-mg. samples of parathion. Figure 1 shows the average wave height for from 2 to 10 determinations at each concentration plotted against the concentration to give the standard curves.

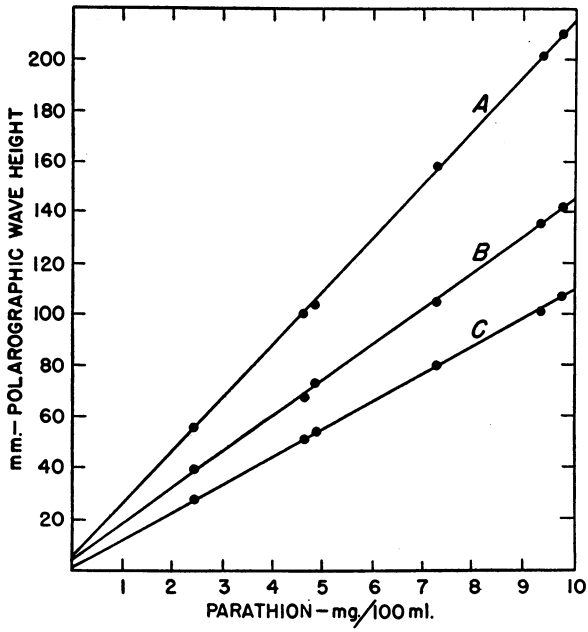

Figure 1. Standard Curves for Parathion Determination

Sensitivity, microampere per millimeter, A, 0.020; B, 0.030; C, 0.040

After some of the standardization polarograms had been obtained, it was decided that considerable time could be saved by using an aqueous stock solution of twice the normality of potassium chloride and acetic acid desired in the sample to be analyzed instead of weighing these materials for each determination. The gelatin was weighed fresh each day.

Analysis of Commercial Products

Technical parathion samples were analyzed in the same manner as the standard samples (Table I). For dust formulations at least 1 gram was taken for a sample and

made to volume with acetone to obtain an extract containing approximately 1 mg. per ml. of parathion based on the manufacturer's claims. The sample was shaken intermittently for 1 hour, and allowed to stand for 10 minutes, and a portion was centrifuged in a glass-stoppered tube until clear. An aliquot of this clear solution to contain approximately 10 mg. was taken and the procedure described was followed. The recovery of parathion from dusts by this procedure was checked by extracting two of these commercial dusts in a Soxhlet apparatus. The results were found to be within the limits of accuracy of the method, as shown in Table II. Dusts of known parathion content prepared in this laboratory were analyzed after Soxhlet extraction, and the recovery as shown in Table II was found to give results also within the limits of accuracy of the method.

Table I. Analysis of Commercial Samples of Technical Parathion

Sample No.	Parathion Found, %
1	84.7
	83.9
	85.3
	Av. 84.6
2	92.3
	91.5
	92.0
	Av. 91.9
3	98.1
	97.5
	97.7
	Av. 97.8
4	93.1
	92.0
	93.2
	Av. 92.8
5	94.4
	93.5
	95.3
	Av. 94.4

Table II. Analysis of Parathion Formulations

Material	Parathion Present, %	Parathion Found	
		Soxhlet extraction, %	Flask extraction, %
Commercial dusts			
Sample 1 (25%)	...	...	24.0
			24.0
			24.1
			Av. 24.0
Sample 2 (25%)	...	24.4	24.0
		24.1	24.1
		24.4	24.0
		Av. 24.3	Av. 24.0
Sample 3 (1%)	...	...	0.97
			0.92
			0.94
			Av. 0.94
Wettable powders			
Sample 1 (25%)	...	...	24.0
			23.7
			23.9
			Av. 23.9
Sample 2 (25%)	...	23.3	23.9
		23.3	23.7
		23.6	23.8
		Av. 23.4	Av. 23.8
Synthetic dusts			
Sample 1	10.0	...	9.7
			9.7
			9.8
			Av. 9.7
Sample 2	12.2	12.2	
		12.0	
		12.1	
		Av. 12.1	
Sample 3	29.1	29.2	
		29.3	
		29.3	
		Av. 29.3	

Discussion

Normal curves for the polarograms were obtained with the technical materials as well as with the purified sample. The decomposition potential of −0.30 volt and a half-wave potential of −0.39 volt were obtained against the saturated calomel electrode.

p-Nitrophenol, which is a major contaminant of the technical parathion, does not reduce at the dropping mercury electrode until after the parathion has been completely reduced, and consequently does not interfere with the curve obtained in the analysis. The decomposition and half-wave potentials for *p*-nitrophenol under the conditions for the determination of parathion were found to be −0.45 and −0.68 volt, respectively.

Diethyl *p*-nitrophenyl phosphate, the oxygen analog of parathion, was investigated to ascertain whether it would interfere, if present, in the determination of parathion. It was found, however, under the conditions used in this method to have a decomposition potential of −0.37 volt and a half-wave potential of −0.47 volt.

A mixture consisting of one-third parathion and two-thirds oxygen analog, instead of giving the anticipated broken wave beginning at the decomposition voltage for parathion, gave a normal curve with a decomposition potential of −0.34 volt. This indicates an interference if small amounts of the oxygen analog should be present, a situation not likely to occur with present methods of synthesis (*4*).

The polarographic method of analysis of parathion as described here has an accuracy of ±1%, and 2 mg. of *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate per 100 ml. of solution are apparently a minimum concentration for the sensitivities investigated. However, the polarograph used is equipped with resistors, so that a sensitivity of 0.003 microampere per millimeter may be used. At this sensitivity it would be possible to obtain a sufficient wave height to determine parathion at a concentration of less than 1 p.p.m.

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Effect of Acetic Acid on Recovery of Ethylene Dibromide from a California Soil

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In the evaluation of ethylene dibromide as a soil fumigant, a sensitive procedure was needed for determining concentrations lower than 0.2 mg. per cubic inch in soil. When the method of Brenner and Poland was used, addition of a small quantity of acetic acid considerably increased the percentage of ethylene dibromide recovered.

In the evaluation and development of ethylene dibromide (1,2-dibromoethane) as a soil fumigant or disinfectant for the sugar beet wireworm *Limonius californicus* (Mann) and various nematodes, it was desirable to study its behavior in the soil. This chemical is usually ejected into the soil at the rate of 2 to 4 gallons per acre from shanks 12 inches apart and 6 inches deep. If 2 gallons of ethylene dibromide were evenly distributed, without loss, in the top foot of soil, the amount in each cubic inch would be 0.217 mg. However, it is unlikely that the distribution would be uniform. Accordingly, it was necessary to develop a procedure sensitive enough to determine concentrations of ethylene dibromide lower than 0.2 mg. per cubic inch before studying the manner in which it distributes itself in soil.

Dillon, Young, and Lucas (*3*) described a method for determining dibromobutanes in 99% methanol. Dillon (*2*) expanded this work to include a procedure for determining ethylene dibromide. Brenner and Poland (*1*) described a procedure in which they determined 1.000 mg. of ethylene dibromide with a recovery of 65.8%. Equations 1 and 2 show the main reactions that occur.

$$C_2H_4Br_2 + 3I^- \longrightarrow C_2H_4 + 2Br^- + I_3^- \quad (1)$$

$$I_3^- + 2S_2O_3^{--} \longrightarrow 3I^- + S_4O_6^{--} \quad (2)$$

When 0.50 mg. of ethylene dibromide was added to 70 grams of air-dried soil and subsequently removed by steam distillation, it was not possible to detect it by their procedure.

Inasmuch as the soil used was slightly alkaline (pH 7.70) it was believed that some of the triiodide produced in the soil distillate by Reaction 1 would be lost. The addition of a small quantity of acetic acid to this distillate considerably increased the percentage of ethylene dibromide recovered in the analysis (Table I). However, the addition of acid aids the oxidation of iodide ion to triiodide ion by oxygen of the air according to Equation 3.

$$4H^+ + 6I^- + O_2 \longrightarrow 2I_3^- + 2H_2O \quad (3)$$

By adjusting the acid concentration it was possible to minimize both Reaction 3 and the loss of triiodide ion and thus modify the procedure of Brenner and Poland (*1*) sufficiently to obtain a 59.6% recovery when 0.125 mg. of ethylene dibromide had been added to 70 grams of air-dried soil. Larger quantities could be recovered more completely.

Table I. Effect of Adding Acid in Analysis of Ethylene Dibromide in Soil

Ethylene Dibromide Added, Mg.	Acetic Acid Added, Ml.	Ethylene Dibromide Recovered (Corrected for Blank), Mg.	Ethylene Dibromide Equivalent to Blank, Mg.	Recovery, %
0.125	0.15	0.073	0.055	58.4
	0.15	0.076	0.060	60.8
0.250	0.15	0.163	0.047	65.2
	0.15	0.160	0.055	64.0
0.500	0.00	...	0.068	0.0
	0.00	...	0.055	0.0
0.500	0.15	0.332	0.049	66.4
	0.15	0.330	0.066	66.0
0.750	0.00	0.123	0.066	16.4
	0.00	0.149	0.049	19.9
0.750	0.15	0.507	0.047	67.6
	0.15	0.517	0.060	68.9
1.00	0.00	0.328	0.068	32.8
	0.00	0.394	0.049	39.4
0.975	0.15	0.699	0.047	71.7
	0.15	0.719	0.066	73.7
1.23	0.10	0.910	0.068	74.0
	0.20	0.910	0.088	74.0
	0.30	0.913	0.088	74.2
	0.40	0.912	0.088	74.1
1.50	0.15	1.18	0.074	78.7
	0.15	1.17	0.066	78.0

Reagents

Ethylene Dibromide. Technical material was washed three times with concentrated sulfuric acid, rinsed with water, and dried over calcium chloride. It was then distilled through 1 foot of glass beads. The fraction boiling between 130.2° and 131.2° C. was used. Every third day an aqueous solution was prepared by weighing approximately 0.5000 gram of ethylene dibromide into 2000.0 grams of water. It was stored in a dark bottle to minimize light decomposition.

Acetic Acid, glacial, U.S.P., 99.5%.

Potassium Iodide. Iodate-free reagent grade was screened so that all crystals used were between 2 and 5 mm. in diameter.

Starch Indicator. Five grams of soluble starch were dissolved in 1 liter of distilled water. A few drops of toluene were placed on top of the solution as a preservative.

Potassium Iodate. Analytical reagent grade was dried for 4 hours at 110° C. in the oven. Approximately 0.00300 *N* solution was made by making 100.0 ml. of 0.1500 *N* solution and diluting a 10.00-ml. aliquot to 500.0 ml.

Sodium Thiosulfate. A 0.00250 *N* solution was made by dissolving 0.63 gram of sodium thiosulfate and 0.10 gram of sodium carbonate per liter of freshly boiled water. It was standardized each day against approximately 0.00300 *N* potassium iodate solution.

Ethyl Alcohol. Formula 3A, 95% ethyl alcohol denatured with 5% commercially pure methyl alcohol.

Soil. Approximately 5 pounds of air-dried Hanford fine sandy loam soil were selected from a lima bean field in an area where all plants had been killed by wireworms. The pH of a water slurry of this soil was 7.70. It was screened through a 20-mesh sieve, blended, and stored in a closed can.

Procedure

In some of this work soil samples were desired from fields that had been treated with ethylene dibromide.

Samples were taken with a thin-walled, cylindrical, sampling tube which took cores 1.0 inch in diameter and 1.0 inch long. Three cores were placed in each 250-ml. wide-mouthed Erlenmeyer flask. In the laboratory the rubber-stoppered flask was shaken to break up the cores and 5 ml. of water were added immediately before analysis.

However, in this preliminary work to standardize a procedure, a 70.0-gram portion of air-dried soil was weighed into each flask. With a 50-ml. buret the quantities of aqueous ethylene dibromide solution necessary to give 0.125 to 1.500 mg. of this chemical

were measured into each sample of soil. Enough water was added to bring the total volume of solution and water added to the soil to 10 ml.

The ethylene dibromide and water were distilled from the soil by heating it for 1.5 hours in an oil bath maintained between 125° and 130° C. A blank containing no ethylene dibromide was run simultaneously with every three samples, all of which were heated in a common oil bath. Each water-cooled condenser was modified by bending the inlet down and fitting it with a rubber stopper which accommodated the wide-mouthed flasks. The outlet of each condenser was bent so that it dipped below the surface of 50 ml. of ethyl alcohol contained in a 250-ml. receiving flask with a standard-taper neck. It was necessary to avoid drafts and to maintain the temperature of the bath as constant as possible; otherwise, alcohol would climb up the condenser from the receiving flask. When this began to happen it was usually necessary to lower the receiving flask just long enough to allow air to enter the condenser.

At the end of the distillation the condenser was rinsed by forcing 15 ml. of 3A ethyl alcohol through it and into the distillate by connecting a separatory funnel to the upper condenser opening with a rubber tubing and blowing the alcohol through. The receiving flask was removed and 0.15 ml. of glacial acetic acid was added with a 0.200-ml. serological pipet. After addition of a boiling chip, the flask was fitted to a standard-taper condenser and placed on an electric hot plate, and the distillate was boiled several minutes before 5 grams of potassium iodide crystals were introduced by dropping them down the condenser. Twenty-five milliliters of ethyl alcohol were added to wash down any potassium iodide crystals that stuck in the condenser. The solution was refluxed gently for 2.5 hours.

At the end of this period the solution was removed from the condenser while still hot and titrated immediately with 0.002500 *N* sodium thiosulfate before any appreciable oxygen could be absorbed and oxidize iodide ion to triiodide ion. The disappearance of the yellow color of triiodide ion against a white background was used for the end point. These solutions usually had a slight brown tint at the end point, which was assumed to be organic matter distilled over from the soil. Accordingly, the blank was usually titrated first and its final color was used as a standard end point color for the other three solutions run with it.

The results were corrected by subtracting the amount of thiosulfate used for the blank run parallel with each determination.

Calculations. Ml. of thiosulfate $\times$ N $\times$ 93.94 = mg. of ethylene dibromide.

Results and Discussion

It was necessary to analyze the soil samples as soon as possible to avoid loss of ethylene dibromide.

The results shown in Table I demonstrate the value of adding acetic acid in this procedure. Nothing was gained by adding more than 0.15 ml. to each reaction medium.

The error introduced by Reaction 3 was further diminished by flushing out much of the oxygen from the refluxing system by boiling the contents before adding the potassium iodide. Crystals between 2 and 5 mm. were used because smaller ones had a tendency to stick to the walls of the wet condenser. Early in this work it was noticed that crystals of potassium iodide which were not washed down were often partly oxidized to iodine, which worked down into the flask and caused high results. Thus it was important to wash all the potassium iodide down the condenser with water. Crystals of potassium iodide were used rather than a solution because this avoided the possibility of such a solution being oxidized to contain triiodide ion.

The disappearance of the faint yellow color of triiodide ion was used to determine the end point of the titration with sodium thiosulfate. This gave better results than were obtained when the solution was diluted with sufficient water to use the starch triiodide end point in the presence of ethyl alcohol.

In a method subject to both oxidizing and reducing side reactions it was necessary to run a blank containing everything but ethylene dibromide with each set of three analyses run in parallel. Possible inaccuracies resulting from impurities in the ethyl alcohol or other reagents were also corrected by the blank. However, blanks were not always foolproof. Carelessness in treating any flask differently from those run parallel with it resulted in errors. Accordingly, all runs were at least duplicated.

Acknowledgment

The author wishes to express his gratitude to Robert P. Huston for his assistance in the experimental work and to Joseph M. Rule for helpful suggestions.

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Multiple Responsibilities of the Insecticide Chemist

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The chemist should recognize the requirements for a new agricultural chemical—effectiveness, economy, specificity, and safety. His responsibilities are to effect cooperation with all contributing researchers, increase specificity of action, make available the most practical materials we have today, and develop new chemicals which better fulfill the over-all requirements.

Times change. The transition from one basic way of thinking to another takes place gradually, but the realization that we have gone through such a metamorphosis sometimes comes rather suddenly. We realize today that we are in a new era of insecticide chemistry. It might be called the DDT era as compared with the former lead arsenate era; it would be better perhaps to call it the era of specific organic insecticides as compared with the past era of shotgun inorganic insecticides.

From a different point of view, we also recognize a parallel change in thinking. Perhaps this new era in insecticide chemistry should be dubbed the era of conscious responsibility. If we are to recognize our responsibilities in introducing a new chemical substance with potential usefulness in the agricultural industry, we must find some way of classifying its basic requirements and reducing them to the simplest terms. When we have these basic requirements well in hand, we can more readily recognize our responsibilities.

There are many difficulties in attempting to classify all the complex requirements of an insecticide according to a simple scheme, but they may be arbitrarily placed under four simple headings: that it be effective, economic, specific, and safe. These terms cover a multitude of detailed problems.

Effectiveness

This requirement is obvious, but one point should be recognized. The term "effective" refers to practical control and not necessarily to kill. The aim is the maximum of quality of product and maximum productivity of the host plant through the prevention of damage by the pest. If we can accomplish this by interference with the activities of the pest, such as prevention of multiplication or prevention of invasion, it is as satisfactory as the kill of the individual insect.

Economy

This subject is more complicated than it at first seems. This requirement may be defined as the lowest cost to society per unit of high quality agricultural product over several years. By requiring a period of several years, we take into account the possibility that the chemical may affect the potential productivity or quality through a sustained effect on the host plant or its environment. It must be calculated on the basis of the

quality and cost to society per unit of the final agricultural product. The cost per pound of the insecticidal chemical is obviously not the answer, nor is the cost per acre.

Other factors in the economy of production of an insecticide material are often overlooked. Each product represents a major, capital investment in productive capacity. Although it is necessary to take advantage of scientific developments, so that our agricultural industry will have the best possible protection of its product, we should not jump into a new development without taking into account all the factors involved in its use and in so doing place a capital investment in manufacturing capacity which must be discarded in a short time to be replaced by another large capital investment. The end result would be an over-all increase in cost of agricultural chemicals, and an over-all increase in cost of the final agricultural product. The long-term economy represented by the introduction of a new substance into the agricultural industry is not always recognized in all its ramifications by the chemist.

Specificity

This is the most difficult and varied requirement for a new insecticide. It is desirable to control a pest insect with the least possible undesirable effect on any other factor in the complex biological system within which we are operating. The practical agricultural field man will inform us immediately that it is necessary to control a wide variety of organisms. From a practical point of view, it is desirable to have a chemical substance which in one application will control a great many different pest organisms. As we broaden the spectrum of effectiveness against pest organisms, we are at the same time broadening the spectrum of activity against a wide range of other organisms, many of which are beneficial and necessary to the biological balance. Among the beneficial organisms is always the host plant and there are other plants of desirable nature in the immediate area. There are desirable insects, which are necessary for the pollination of the host plant or of other plants in the area, and there are organisms which are highly beneficial as predators or parasites in controlling undesirable pest insects. The effect of DDT on the predators and parasites of the red mite is well known. The effect of insecticides on pollinating insects did not begin with DDT but has long been with us. The need for greater specificity must always be qualified by economic and practical usability.

Safety

Although the requirement for safety to the operator in handling an agricultural chemical and safety to the final consumer of the agricultural product is too obvious to require argument, the matter of attaining this safety is not so obvious. In handling and applying agricultural chemicals, the problem is not solely the toxicity of the substance to man. The practical question is—can it be handled by reasonable operating procedures without encountering difficulties due to health hazards? A few materials are so low in toxic effect to man and animals that an individual is not likely to get into trouble with them under ordinary conditions of use. Piperonyl butoxide, methoxychloro, and the di(4-chlorophenoxy)methane (Neotran) would probably fall in this category in the insecticide field. This is the direction in which we should attempt to move with new insecticidal materials; however, we must always balance off this requirement with the requirements of effectiveness and economy. It seems unreasonable to limit the use of a material that has the effectiveness, economy, and specificity desired simply because it has a fairly high toxicity to man, if it can be handled without hazard to the operator. Our decision then should be based on the practicability of handling a material in a way that will not represent a hazard to the operator.

We must consider the effect on the quality of the agricultural product and particularly the existence of any residue. Such residue, if any, must present no hazard to the final consumer of the agricultural product, whether man or animal.

The Chemist's Responsibilities

What responsibilities do these factors place upon us in developing a future program? The chemist today recognizes more than ever the necessity for taking into account all these

requirements when designing a molecule which he wishes to introduce into the agricultural industry. If, however, the chemist is attending to his job of synthesizing a new organic chemical in the chemical laboratory, he does not have time to become thoroughly acquainted with the many biological problems introduced by a new chemical substance in a complex, biological system such as the agricultural industry. He must depend to a great degree upon the biologists who are working in that field for this information. This implies a much greater cooperation between the chemist and the biologist in future developments. Our first responsibility in designing our future program is to effect the greatest possible cooperation between all the groups that are in a position to contribute to this very important field—the chemist, the biologist, and the practical man in the agricultural field.

Present demands require moving in the direction of greater specificity for the purpose of improving effectiveness and at the same time reducing as far as practicable the side effects on other desirable living organisms in the system. From other biological fields, we can glean the fact that it is possible to control a particular species of organism with high specificity. Each species of organism has within itself certain biological mechanisms which are unique to that species of organism. We know enough about specific antagonists for certain biological systems to know that with the proper knowledge of these systems, we could control an individual species of organism in the presence of a great variety of other organisms. We can look forward to such developments in the very near future.

One of the best illustrations of high specificity is di(4-chlorophenoxy)methane (Neotran), which has been used for some time commercially in the field, principally for the control of the citrus red mite. So far there has been no indication of undesirable effect upon pollinating insects or on desirable predators or parasites.

There are advantages in moving in the direction of greater specificity for the action of agricultural chemicals, but this desirable characteristic must be balanced with the effectiveness and practical economy of today. Our second responsibility is to recognize the possible advantages of increased specificity for our insecticidal chemicals, qualifying this with the best balance of the practical needs of the agricultural industry.

Because of the requirements of circumstances, it is impossible to wait until the ideal combination which should be met in the development of a new insecticide is attained. We are faced today even more than in the immediate past with the necessity of protecting our food and fiber crops from depredations. Our third responsibility is to make available the best material we have today, which will aid in the production of our most critically needed food and fiber crops.

At the same time we must be looking for new materials. Year by year there is an increase in crop concentration. This is a natural "enrichment culture" for increasing pests. There seems also to be some increase in resistance of the insects to older insecticides. This means running hard to stay in the same place, but we must not stay in the same place. We must drive ahead. Therefore, our fourth and last responsibility is to develop much better materials in the future, materials with the best balance of the four major requirements: effectiveness, economy, specificity, and safety.

Responsibilities of the Chemist in the Development of Insecticides

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The chemist must work with the entomologist, the toxicologist, and others concerned in the formulation of new and better insecticides, or in the improvement of old ones. All formulations must be tested to determine their insecticidal efficiency, as well as their toxicity to warm-blooded animals, before manufacture on a large scale is begun.

The successful development of insecticides requires a broad and thorough knowledge of the ever-changing and unfilled needs for better products, and of the fields of chemistry, physics, engineering, and biology that pertain to the development of better insecticides.

The research chemist must understand and appreciate teamwork. The development of insecticides depends on close cooperation of the research, manufacturing, and analytical chemists with the entomologists, toxicologists, and others who may be directly or indirectly interested in the program. It is especially important that the research chemist have a thorough knowledge of organic chemistry and of the methods of organic synthesis. He should also have a sufficient knowledge of biology to enable him to understand the biochemical structures and functions of insects and their plant and animal hosts on which the insecticides are to be used. Such information is helpful in understanding the action of insecticides and may also point the way to the discovery of more effective and safer chemicals for insecticidal use. The chemist should have a general knowledge of chemical research in other laboratories, from which products of insecticidal value may be obtained.

Needs for Better Insecticides

More effective and safer insecticides are needed, in spite of the extensive progress made in recent years. The chemist should familiarize himself with the needs in the agricultural, storage, household, livestock, and industrial fields. The weak and strong points of the products in use should be understood. Some of the commonly used products need better formulations, or better methods of application with the use of more effective supplements. Better products should replace some of those now in use. Statistical evidence of ample potential should be available before work on a problem is begun.

Several factors may govern the fitness of a product for a given use. Changes in biological factors may render the insecticide unsuited for use. A new variety of the host, or crop plant, may be injured by the insecticide, or a strain of the insect that is resistant to the chemical may evolve as a result of continued use. In such cases a modification of the insecticide to render it more effective, or a replacement, must be found. Certain insecticides, including the arsenicals and some of the newer chlorinated compounds, although they are safe when applied to foliage, accumulate in the soil to the extent of eventually causing injury to crops.

A new insecticide must be compatible with other insecticides, fungicides, and supple-

ments with which it may be used. This involves the need for a knowledge of all such compounds that come into extensive use.

For some important insect pests there are still no satisfactory chemical controls. Such problems should be given due consideration in the development program. Many of these problems appeared to be solved with the discovery of DDT, benzene hexachloride (hexachlorocyclohexane), and some of the more recent insecticides. Further studies of the toxicity of some of these products to warm-blooded animals have raised the important question of the advisability of continuing their use where food and feed products are concerned. Considerable attention is being centered on finding safer analogs, such as TDE and methoxychlor, and new and better insecticides.

Synthetic Organics

Synthetic organics dominate the field of new insecticides. Much time and money have been spent in attempting to determine the chemical structures of rotenone, pyrethrum, and other natural insecticides, and to reconstruct them through synthesis. A great deal has been learned about the chemical structures of these compounds, but little success has been attained toward synthesis. Nicotine and, recently, a pyrethrumlike compound are exceptions, but the synthesis of nicotine is too expensive to be practical. The basic information obtained has possibly been helpful in directing the thoughts of the chemist to the synthesis of entirely new compounds.

All available information that offers possibilities must be used. However, what appears to be logical theory may not prove up in the selecting of chemicals for insecticidal purposes. The chemical structure that, according to such theory as can be drawn from available knowledge, should be effective often proves ineffective, whereas structures considered ineffective under the same system of theorizing may prove to be effective. Much still depends on the "cut and try" method.

The chemist should explore the fields of chemicals with structures related to compounds known to be effective. He should also study new classes of structures. Safety appears to lie in numbers. The failure of several members of a new class of compounds does not prove that there are no effective members in the group. Only certain of the analogs of DDT are good insecticides. Even DDT is not effective on some species of insects. Only the gamma isomer of benzene hexachloride is highly effective.

The possibility of finding an insecticide that is safe to plants and warm-blooded animals and universally effective appears remote. However, investigations have uncovered a few very promising products. An outstanding example is methoxychlor, one of the analogs of DDT, which is far less toxic to warm-blooded animals than DDT. Poisonous insecticides are being used extensively. However, safety to plants and warm-blooded animals, including man, should be one of the leading goals of the development program. The statement that "it is hard to find a chemical that will kill protoplasm and not kill protoplasm" overlooks the fact that some chemicals have properties that enable them to reach the living protoplasm of one organism and not that of another. Many factors may be involved. The chemist should be prepared to take full advantage of such differences. A thorough understanding of the chemical and biological actions involved is valuable.

Formulation

The effective use of insecticides depends to a large extent on formulations prepared for or adjusted to specific uses. Seldom is an insecticide used in its pure or technical form. In order to do an efficient job of formulating insecticides, the chemist should know how they are to be used and by what type of machine they are to be applied. Is the insecticide to be used as a spray, mist, fog, dust, aerosol, or dip? Will it be applied to plants, valuable animals, or humans? Will it come in contact with humans or products that may be used for food by humans or valuable animals? With this information at hand, suitable carriers, solvents, and wetting, emulsifying, spreading, penetrating, stabilizing, and sticking agents may be selected for trial. An extensive knowledge of possible supplements is desirable. The supplements must be compatible with the active chemical and with other

supplements used. The use of synergists may be considered. The finished formulations must be stable in storage and compatible with other chemicals, including other insecticides and fungicides with which they may be used. This involves a knowledge of the chemistry of fungicides and other chemicals that may be used in conjunction with the insecticide.

The preparation of formulations may involve the principles of physics and mechanics. The preparation of emulsions, fine particle materials, etc., constitutes an important part of insecticide development. A knowledge of the kinds of equipment used in the preparation of formulations enables the chemist to handle the job with greater facility.

Formulation is not confined to the preparation of suitable mixtures of new chemicals. Many old or established insecticides have been improved and in some cases have been adapted to new uses through the development of new and suitable formulations. To see and take advantage of these opportunities, the chemist must be familiar with the weak and strong points of the products in use. Close cooperation with entomologists is necessary. All formulations should be thoroughly tested in the laboratory and/or greenhouse to determine their insecticidal efficiency. Products that survive reasonably severe preliminary tests are ready for field trials. Extensive chemical work often is required to readjust the formulations as the laboratory and field trials are in process. It may take months or even years to arrive at a suitable formulation for some uses after the chemical is known to have outstanding merit. Even when a formulation has been proved satisfactory for a certain use, much remains to be done in the development of suitable formulations for other uses and in following through with the effective product. It may be desirable to develop means of removing residues of the product from treated surfaces.

Toxicology

Among the most important characteristics of an insecticide is its safety to humans. In recent years the United States Government, state authorities, and commercial organizations have given special and extensive attention to toxicity problems and to the safe use of toxic products. New insecticides are being thoroughly studied by competent toxicologists. Unfortunately, some of the outstanding new chlorinated compounds are not as safe as they were once thought to be. It has been necessary to modify or even withdraw claims concerning their safety to humans and specify methods of safe use, but advantage is still being taken of the original claims of safety in some Latin-American countries, for instance, where poisonous products are recommended for use on stored grain and other food products.

In order to avoid hazards to the chemist and his associates as well as to the user and consumer of treated products, a chemical showing sufficient promise should be subjected to acute toxicity tests by a competent authority in its early stages of development. Further experimental work should be governed by the outcome of these tests. If toxic, it should be handled with care. If symptoms of poisoning do not occur, the product should still be handled with reasonable care until chronic toxicity studies can be made. Such studies are expensive, and, as a rule, elaborate chronic studies are not considered necessary until it is reasonably certain that the product will be put into use. At this stage the question of public health becomes an important factor. Further toxicological studies, including those of chronic nature, may be required, so that effective directions for safe use may be issued. Attention should be given to the safety of any new formulations of the product once it is in use. New or modified formulations of old poisonous insecticides should be considered and the necessary directions for safe use be given. This may or may not involve the need for toxicological investigations.

Manufacture

After a product has passed the critical tests as an insecticide and a preferred process of manufacture has been decided upon, full information concerning use, potential raw materials, and process is the basis for the preparation of firm cost estimates. These estimates may cover production on a small or pilot plant scale, or on a large scale. If estimates are favorable, the process information is passed along to the manufacturing unit which, as a

rule, prepares for manufacture on a pilot plant basis. The research chemist should maintain close cooperation with the manufacturing unit to ensure getting the process properly established for uniform production of a high-grade material. The analytical chemist should make regular checks on quality of production.

When this pilot plant product goes into limited commercial use, the entomologists and chemists concerned should follow the results closely, to be sure that the product is up to standard in all respects under different environmental conditions. Its stability under different storage conditions should be studied. Compatibility under conditions of use with other products with which it may be used is important. Everything possible should be done to assure its success at this point and to be sure that it merits large scale production.

If the product survives the pilot plant stage of development and sufficient potential use is indicated, it passes to large scale production, where the responsibility shifts largely to the manufacturing and analytical chemists to produce a uniform, high-grade product. Interest of the research chemist should continue through cooperation with the manufacturing chemist, the entomologist, and the technical sales service men with the purpose of maintaining high efficiency and correcting any unforeseen weaknesses in the product.

Pest-Control Chemicals in the Production of Food

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The use of agricultural chemicals makes possible higher yields and food products of higher quality and contributes more than any one other factor to lower production costs. At the same time the farm has been made a virtual factory in a rural setting.

Today the agricultural chemical industry has made available, through chemical research, the largest selection in history of agricultural chemicals for the control of pests. These agricultural chemicals are making possible higher yields and products of higher quality. In this day of potential surplus they are contributing more than any one single factor to lower production costs.

Effect of Agricultural Chemicals on Crops

Potato yields, on a national basis, have been increased one third, largely because of more effective pest control.

After 10 years of research on wireworm control in California, the application of recommended agricultural chemicals on lima beans to control this pest added $7,500,000 in income to growers in 1948. Only in recent months has it been known that this same pest reduces the production of sugar cane in some areas by nearly half; consequently it is now possible to double the yield in those areas by controlling this one insect.

The production of livestock and livestock products has been greatly increased through the effective application of agricultural chemicals, as evidenced in the control of insects and diseases on and around dairy cattle, which has resulted in many instances of increased milk production ranging between 15 and 20%. It has been reliably estimated that the treatment of beef cattle for insect pests has resulted in additional gains of 50 pounds per head of beef animals treated. The dollar value of these increases in both beef and milk has been estimated at $54,000,000 per year.

Several examples demonstrate the effectiveness of agricultural chemicals in the production of fruit. The use of a fungicide in the production of Anjou pears has increased production 300,000 bushels a year.

The apple crop of the nation, representing an investment by commercial growers of about $750,000,000 in land and trees alone, and with a crop averaging $240,000,000 to $250,000,000 a year during the past 5 years, is entirely dependent on the use of agricultural chemicals to control insects and diseases.

In the states of Montana and Wyoming, some 2700 acres of grassland were treated in 1949 as a part of the grasshopper control program. Competent estimates show that this program resulted in saving half of the grass grown on the treated lands from destruction by grasshoppers. The grass saved could produce 11,000,000 pounds of beef on the hoof. The soil conservation value is also very evident, because were the land to be laid bare, it would be subject to damage during the period required to re-establish grass cover.

Stored grain losses caused by insects throughout the world total approximately 6,000,000 bushels a year. The development of new chemical methods for determining weevil infestation and the increased application of chemicals for the control of other pests are rapidly reducing these staggering losses.

Grain and corn production in the United States has been tremendously increased in many areas through the application of agricultural chemicals—for grain, increases have been recorded ranging from 25 to 100% through the use of weed control chemicals. Corn production has been increased from 200 to 300 pounds of green ear corn per acre through the control of the corn borer and corn earworm.

The 1948 farm value of agricultural products in New Jersey amounted to $320,750,000. Included in this production are the following:

Vegetable products	$ 51,200,000
White potatoes	19,800,000
Nursery and greenhouse	17,500,000
Tree fruits	7,000,000
Berries	6,000,000
Total	$101,500,000

None of these crops, the value of which is one third of all New Jersey agricultural production, could be produced without agricultural chemicals. Another phase of New Jersey agriculture involving the use of agricultural chemicals can be added to this total, including the production of grains valued at $21,900,000, and poultry, eggs, and dairy products valued at $65,400,000. Hence, the importance to agriculture in New Jersey of agricultural chemicals to control insects and diseases is abundantly evident. The analysis of other sections of the country produces evidence which parallels that reported for New Jersey.

Another side to this picture, however, deserves careful consideration by chemists. At the present time many phases of pest control are being severely criticized by the American Medical Association, segments of the food industry, and consumer groups. The agricultural chemical industry fully appreciates its responsibility for proper labeling and sound education to promote the intelligent selection and proper use of various chemicals, and to guard the health of workers and of those using the materials as well as the consumer. In this educational program the industry is working in cooperation with the U. S. Department of Agriculture, the state land grant colleges, the U. S. Public Health Service, and many other governmental and private agencies.

Never before has a grower had such a wide range of materials from which to make his selection, ranging from those which create no residue problem, to products which, because of their chemistry, require various degrees of precaution in handling and usage. J. G. Townsend, chief, Division of Industrial Hygiene, U. S. Public Health Service, speaking in September 1949 before the annual meeting of the National Agricultural Chemicals Association, said:

> Until a decade ago, the farm may have been considered outside the pale of industrial hygiene. Today, however, the industrialization of agriculture and the advent of new insecticides and other chemicals have made the farm a virtual factory in a rural setting. On the farm, as well as in the factory, dangerous chemicals may be used safely with proper precautions. If manufacturers, processors, and distributors had to relinquish certain substances because they are toxic, American industry would be in a primitive stage. Likewise, the farmer need not bow to economic pests, but can use to the fullest advantage the potent new insecticides if he is scrupulous in protecting himself.

The establishment of a permanent section on agricultural chemicals in the AMERICAN CHEMICAL SOCIETY can contribute much in the public interest. The more discussion and intelligent consideration given to problems arising from the usage of pest-control chemicals, the better able industry will be to provide agriculture with effective pest-control chemicals which increase the efficiency of production and make possible the continuance of an abundant supply of wholesome food.

Military Characteristics of Insecticides

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A high degree of insecticidal activity per unit weight, effectiveness against many species of insects, prolonged residual effect combined with rapid knockdown, compatibility with various vehicles, and availability under wartime conditions are among the most important military characteristics of insecticides. No present insecticide is entirely satisfactory.

The properties of insecticides are, in military parlance, called characteristics. Many of these characteristics are of importance in both military and civilian usage, but because of the vast difference between military and civilian operations, certain properties of insecticides become extremely important to the armed services, and in many instances are more important to the military economy than to the civilian economy.

Possibly the most important single military characteristic is a high degree of insecticidal activity per unit weight. The necessity for this requirement may be more fully appreciated when it is realized that approximately 3 tons of equipment and supplies per individual soldier are necessary during the assault stages of an invasion, and 0.75 ton of supplies and equipment per man is necessary to maintain one combat soldier for one month in the field. With this vast amount of equipment and supplies for each individual soldier, the weight of each specific item needed for his support must be critically scrutinized.

Prior to the discovery of DDT, with its high degree of insecticidal activity, it was necessary to include far larger weights of insecticides than at present. Although further improvements may be made in the direction of increasing the toxicity of insecticides per unit weight of the pure chemical, it is not believed that this requirement can be changed appreciably. Future research should be directed, not toward finding insecticides of higher insecticidal activity per unit weight, but toward finding insecticides that fulfill more adequately the other military characteristics discussed below.

Effectiveness

Another extremely important military characteristic is effectiveness against many different species of insects without the development of resistant strains. Every insecticide that must be added to the military list of supplies geometrically increases the difficulties of procurement and distribution. At the present time, nineteen different insecticides and insect repellents and four different rodenticides are issued by the Army Quartermaster. These figures do not include the different formulations of insect repellents issued under the same stock number. The three basic insect repellents are dimethyl phthlate, Indalone, and Rutgers 612. These repellents are issued either alone or in various combinations, further complicating the supply situation because of the variation in efficiency of these substances against different species of mosquitoes in different parts of the world.

Although all insecticide containers are adequately marked when they are filled at the factory, extreme difficulty has been encountered in identifying many insecticides following

long storage in the field under adverse conditions. Even under the best conditions the supply problem may become very complicated in overseas areas because of the necessity of utilizing unskilled foreign labor in supply dumps. In many instances this may result in the storage in the same stockpile of items that differ from each other only in percentage composition.

The results of this situation can be readily foreseen. A requisition for a specific item is occasionally filled with an item which, at first glance, appears to be the one requested but, actually, was formulated for a different purpose and is either inadequate or dangerous to use for the intended purpose. A specific example of the hazards inherent in this situation may be found in the case of insecticide space spray composed of 1% DDT, 0.1% pyrethrins, or 2.5% thiocyanate in deodorized kerosene and 5% residual-effect DDT, both of which are issued in 5-gallon steel drums. Obviously, if a requisition for residual-effect DDT were to be filled with space spray, the application of the solution as a residual-effect compound would be of little or no value. Under some conditions, when stocks have been exposed to such adverse weather conditions that all gross identifying marks have been removed from the containers, the assumption has been made by the untrained native laborers that all unidentifiable cans of the same size contained the same material. Were it possible to have just one insecticide for all military purposes, such a situation could easily be avoided.

It is also necessary, from the military point of view, that the insecticides supplied to troops in the field be readily convertible into end-use items. It should not be necessary to go through an involved procedure in preparing insecticides for field utilization. For example, the earliest lots of DDT that were received in the North African theater were of the consistency of beeswax, and were extremely difficult to get into solution. It was necessary to process all this material through meat grinders, of the hamburg variety, requisitioned from the civilian economy before this material could be satisfactorily dissolved in Diesel oil. Such a situation complicates field operations unnecessarily, and should be avoided in the development of future insecticides.

Residual Effect and Knockdown

Another extremely important military characteristic is prolonged residual effect combined with rapid "knockdown." Although, at the present time, we have some chemicals which give a comparatively long residual effect, and other chemicals which give a relatively quick knockdown, the military still require more prolonged residual effect and more rapid knockdown in their insecticides.

Although DDT has a prolonged residual effect, and is extremely valuable in the control of such insects as mosquitoes and sand flies of the *Phlebotomus* type, improvements are needed in the speed of knockdown, particularly of mosquitoes. There is an appreciable time interval after the mosquito first comes in contact with DDT before its biting reflexes are no longer inhibited. This factor is not so important with insects such as the *Phlebotomus*-type sand fly, because this group of insects makes extremely short flights of 2 to 3 yards and then stops to rest. Residual DDT applied to breeding areas and buildings will adequately prevent sand flies from entering buildings and biting personnel. Mosquitoes, insects capable of sustained flight, may conceivably enter a building, come in contact once or twice with DDT, and still bite and infect a person with malaria before they are killed by the DDT. At the present time we have no chemical that combines both quick knockdown and prolonged residual toxicity for use against all insects of medical importance.

Still another requirement is that the concentrated form of the insecticide readily combines with various types of vehicles. At the present time, pure DDT can be dissolved only in organic solvents, and it has been necessary to provide a 50% water-dispersible DDT powder when water was to be used as the vehicle. With the advent of 90% water-dispersible DDT, it may be possible to utilize this material in the preparation of both water suspensions and organic solutions of the chemical. The ideal chemical in this respect would be one that could be shipped as 100% dust, and could be readily diluted with inert dust, water, and organic solvents in the field.

Another extremely important military requirement that is not usually associated with civilian requirements is stability in prolonged storage under adverse conditions. For example, the first lots of fly spray that were shipped to North Africa, in 1942, contained pyrethrum as the principal active ingredient. It was not possible to store this material under cover and much of it remained under constant exposure to the intense tropical sun, undoubtedly deteriorating very rapidly. Covered storage facilities are the exception rather than the rule in a combat theater of operations, and all insecticide compounds furnished to the military forces should withstand this type of storage.

Availability

The last, but by no means the least, important of the military characteristics is availability under wartime conditions. Materials are classified as strategic, critical, and noncritical in this respect: A strategic material is one that must be imported into the United States; a critical material is one that is available in the United States but, because of either limited plant facilities or excessive demands, becomes nonavailable; a noncritical material is one that is readily available in sufficient amounts under wartime conditions. An insecticide to be of value to the military forces must be readily available under wartime conditions, must not be dependent upon the importation of certain essential materials, and must not impose a burden upon plant facilities in the United States.

The only military characteristic which has been met adequately at the present time is that of a high degree of insecticidal activity per unit weight. The other requirements are not being met adequately by the insecticides in commercial production at this time and a vast amount of research is necessary before the armed forces can be furnished with an insecticide which is entirely satisfactory for military needs.

Development and Use of Synthetic Organic Insecticides

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The synthetic organic insecticides offer great advances in insect control. Their high degree of toxicity and specificity to certain species are noticeable features. Hazards to health in manufacture, formulation, and application are primary considerations. New methods of application are being developed. Official guidance on residue hazards will aid entomologists and farmers in developing application programs. Resistance to insecticides is of growing importance. The effect of pesticides on naturally occurring beneficial insects is causing heretofore unimportant forms to become pests of major concern. The search for the solution to the insecticide problem is a specialized and cooperative venture among a team of scientists trained in many fields.

Frequently it helps us to understand a current problem if we review what has taken place in previous years. This is particularly true with the present situation in the field of synthetic organic insecticides.

The use of plant extracts for insect control dates into antiquity; the use of Paris green as an insecticide for control of the Colorado potato beetle in 1867 probably marks the beginning of the modern era of chemical control of injurious insects. The development of lead arsenate followed later in the nineteenth century for gypsy moth control. The commercial production of nicotine insecticides, the production of calcium arsenate at the time of the first world war, and the use of fluorine, arsenical, and cyanide compounds, as well as other inorganic chemicals for insect control, were important steps in pest control. These chemicals were applied largely by dilute high pressure sprays or dusts.

The concern over fluorine, lead, and arsenical residues in the 1920's and 1930's seems to have tied in with greater interest in the development and use of the botanical insecticides, pyrethrum, rotenone, and nicotine. Every effort was made to supplement the effectiveness of these materials through formulation, combinations, or, as in the case of lead arsenate, by adding deposit builders, spreaders, and other similar materials. Progress with application equipment continued along established lines with streamlining of equipment, increased pressures, and similar measures. Farmers developed a background of experience in the use of these insecticides through the years and knew fairly well what to expect in terms of hazards of use and performance. The developing problem of harmful residues at harvest time alerted growers, entomologists, and the consuming public to the need for research in the production of safer insecticides.

Recent Developments

All of us have witnessed developments of the past decade when a second world war engulfed most of the civilized world. Insect control was of prime importance to the protection of the armed forces against insect vectors of disease organisms as well as to the pro-

duction and protection of food and fiber. The American farmer was called upon to increase production beyond all previous limits. Shortages existed in the supply of almost all insecticides and application equipment. The loss of the Dutch East Indies to Japan early in the war shut off the principal supply of rotenone to the Allies. In spite of every conceivable handicap, industry and government teamed up to aid the farmer to make the excellent production record which is so well known to everyone.

The advent of DDT brought about revolutionary practices in economic entomology. Its effectiveness for many pests was nothing short of miraculous. The uncertainty of use and residue hazards brought about precautions for grower application. Control of many insects became feasible for the first time because of the effectiveness of DDT as well as the small amounts of the insecticide needed. It is still amazing to watch a plane applying one gallon of insecticide solution per acre of forest, at the rate of 100 acres per minute, with results of near eradication of a pest like the gypsy moth. Technology in the field of synthetic organic insecticides and application equipment has recently made unbelievable strides.

To the uninitiated, it seemed certain that DDT was almost the final answer to all our insect problems. Following DDT, other chlorinated hydrocarbons were introduced, including benzene hexachloride (hexachlorocyclohexane), chlordan, toxaphene, and others. More recently, there have been the organic phosphates including parathion, tetraethyl pyrophosphate, and hexaethyl tetraphosphate, several of the dinitro compounds, and many others still in the research and development stages. The rapid development of the chemical industry in producing new insecticides left all, including the farmer, without a background of use experience with these materials, which for the most part were more potent in killing insects than anything that had ever been used. Is it any wonder then, that the average farmer and entomologist became and are still confused?

Close working relationships have of necessity developed with this rapid introduction of new insecticides. No longer can an insecticide be recommended for use on the basis of its insect-killing properties alone. The chemists, toxicologists, pharmacologists, physiologists, manufacturers, and others are all contributing to the story.

Problems with New Materials

What are some of the problems that exist today relating to the development and use of new insecticides? Obviously there are hazards in manufacturing, formulating, and applying these chemicals. Doubtless the first two processes are under more constant supervision than the last. Many farmers and farm workers are careless about reading precaution labels before handling and applying insecticides. The use of a mask or respirator, rubber gloves, and other protective clothing with some of the newer materials is not, unfortunately, general practice in the field. Part of the negligence has been blamed on DDT, because few if any of the possible difficulties in the use of DDT insecticides ever materialized. It is now difficult to make many farm workers realize that all the new chemicals are not in the same safety category, particularly if they or their neighbors have applied one of the phosphate compounds, for example, without suffering ill effects. The three deaths reported from use of parathion in the field in the summer of 1949 are creating more respect for the material among growers than all the words of warning could ever do. In brief, the problem of educating the farmer on safety precautions to be observed in applying insecticides is not solved.

The very nature of the over-all problem has made it impossible for an agency of government like the Food and Drug Administration to establish official guidance in terms of the residue hazards and degree of contamination that might be permitted if a given chemical must be used for pest control in a production program. Through its research, the FDA has been extremely helpful in giving guidance during this critical period. New York farmers, like others throughout the nation, are anxious to know what levels of contamination will be considered safe on fruits, vegetables, and other commodities, in order to guide their choice and use of insecticides. Certainly, the entomologist will be glad to receive this guidance in making recommendations. The hearings by the Food and Drug Administration to deal with residue hazards in the use of insecticides deserve the sup-

port of all who have data bearing on the problem and an interest in it. It is a very tangible illustration of the important role of toxicologists and pharmacologists on the team.

Variation in Insect Species

Insects, like other living organisms, show variation within species. This has long been recognized in so far as structure, size, and color are concerned. It has been demonstrated from a physiological viewpoint where two strains, seemingly identical structurally, behave differently in their habits and seasonal history. Insect toxicologists have consistently demonstrated the variation in individual susceptibility of a population of an insect species to a uniform dosage of an insecticide. Variation among living organisms is a basic concept of biology. It is not startling, then, that we should find the development of resistant or tolerant strains of a species after continued use of an insecticide. Much is being said about the development of resistance to insecticides, and in a number of instances data substantiate the claim. It is also likely that resistance will be blamed in many cases of faulty timing, poor application, or poor materials.

Insect Resistance to Insecticides

The development of resistant strains of an insect to a given insecticide is not new. Melander (*7*) in 1914 pointed out that the San Jose scale in Washington had developed a resistance to lime-sulfur sprays. Recently Babers (*1*) of the Bureau of Entomology and Plant Quarantine brought together an excellent evaluation and summary of the literature dealing with the development of insect resistance to insecticides; he lists 111 references to work on this phenomenon.

Quayle (*11*, *12*), working in California, noted resistance of the California red scale, black scale, and citricola scale to hydrocyanic acid gas. Hough (*5*) first called attention to the developing resistance of the codling moth to arsenicals. In South Africa a blue tick, *Boophilus decoloratus*, was found by Du Toit (*3*) and others to be resistant to arsenic. Boyce and Persing (*2*) reported resistance among the citrus thrips, *Scirtothrips citri*, to tartar emetic sprays. Knipling (*6*) found that the larvae of the screw worm fly, *Cochliomyia americana*, could acquire resistance to phenothiazine. Mosna (*9*) in Italy reported a variety of mosquito resistant to DDT. Missiroli (*8*) also working in Italy reported on the failure of DDT to control houseflies in 1945 and 1946, owing to resistance to DDT. Wilson and Gahan (*13*) developed a laboratory strain of houseflies resistant to DDT and several other insecticides.

These examples from the literature compiled by Babers indicate that resistance to different materials is developing among different species and in several localities. It may become more extensive with organic insecticides because of their more widespread use as well as the greater number of chemicals that will be applied in the field.

New Pest Species and Resistance in New York

Several examples of resistance have been observed in New York. The codling moth has long been considered the No. 1 pest of commercial apple production. In the Hudson River Valley, Chapman of the New York Agricultural Experiment Station staff reported one orchard in 1930 where codling moth could not be controlled with the usual lead arsenate schedule. Since that time practically the entire orchard area of the Hudson Valley has developed a codling moth problem which lead arsenate will not handle satisfactorily. The same experience was noted in western New York, except that it began a few years earlier. Harman (*4*), reporting to the Horticultural Society in 1945, indicated that in spite of everything growers could do in areas of western New York, inability to control codling moth made apple growing unprofitable except for the very high prices being received for fruit at that time. The number of cover sprays increased to as many as six in a season. Deposit builders, stickers, spreaders, oil added as an ovicide, nicotine, and other materials supplementing lead arsenate comprised the only weapons until DDT proved to be so tremendously effective in codling moth control. Thus far there has been no sign

of a reduction in efficiency of DDT for control of the codling moth. Spray practices of applying the minimum effective dosages of lead arsenate for economy and residue considerations may have favored the development of resistant strains.

The greenhouse red spider, more properly called the two-spotted spider mite, has developed a resistance in New York to two chemicals, an ammonium potassium selenosulfide compound, marketed as Selocide, and parathion. With both Selocide and parathion excellent initial kills were obtained. The experience with parathion is still in progress. Blauvelt of the Cornell University staff reports some cases where parathion aerosols no longer will effectively kill spider mites in greenhouses. There are strong indications that the rapid development of parathion-resistant populations in some ranges was associated with the use of a marginal program of treatments. This allowed a considerable build-up of populations between treatments and thus may have afforded favorable conditions for the selective survival and increase of resistant individuals or strains originally present in small numbers. On certain ranges where cooperative trial programs with parathion aerosols have been carried on for over two years, no significant increase in resistance to parathion has yet developed where applications were made often enough to keep the mite population at a very low level from the start. The short life cycle and more or less continuous breeding of the spider mites in greenhouses favor a more rapid development of resistance than is possible with a slower breeding species like the codling moth.

Another interesting case is the growing feeling among farmers that a 0.75% rotenone dust is no longer effective against Mexican bean beetle—dosage of 1% seems to be required. There are also complaints that DDT wettable powders are not as effective against the potato flea beetle as in former years. Such circumstantial evidence cannot be accepted as proving resistance to insecticides, but it bears watching and investigation.

New York has experienced a rather widespread breakdown in housefly control with the use of DDT. Schwardt of the Cornell University staff first noticed this failure of DDT in 1948. In 1949 the fly problem was very bad. Farmers, remembering the exceptional control of the past few years with DDT residual sprays, were greatly disturbed when DDT was first withdrawn from use in dairy barns because of the danger of DDT contamination in milk. Methoxychlor under conditions in 1949 did not measure up to the performance of DDT in other years; neither did DDT. Lindane (gamma isomer of hexachlorocyclohexane) has been hailed by many dairymen as the successor to DDT, and by some farmers the question is raised—"What is the successor of lindane to be?"

Along with development of resistance has come the destruction of important natural enemies of pest species, which has been attributed to field use of insecticides and certain fungicides. Pickett (*10*) recently presented evidence to show that the development of the oyster shell scale problem in Nova Scotia orchards is related to the use of sulfur fungicides. The scale was present as an occasional pest of apples for over half a century, but beginning in 1930 it built up to dangerous levels. Sulfur was shown to destroy the parasitic wasp and the predatory mite responsible under natural conditions for keeping the pest under control. Prior to 1930 lime sulfur was used as a fungicide and it served also as an insecticide for the scale. With the change to mild elemental sulfurs about that time, the oyster shell scale population built up to injurious levels, because the milder sulfurs continued to kill the parasites and predators but did not kill the scales. Pickett further recorded a high build-up of the European red mite on apples when mild sulfurs were used as a fungicide as opposed to copper fungicides. Again sulfur is charged with destruction of parasites and predators of the red mite. The same correlation between copper and sulfur fungicides and the degree of codling moth infestation is presented by Pickett over a 5-year period in Nova Scotia. He thinks possibly sulfur has reduced the natural enemies of the codling moth to a greater extent than copper fungicides.

Upsetting Balance in Nature

DDT has been blamed by some for upsetting the balance in nature. Certainly the balance in nature was upset by many factors long before DDT, not the least offender being man. We recognize specific problems, however, where the use of DDT for the control of one pest has been associated with the rise to prominence of other pests. One example

from experience in New York is the red-banded leafroller. The incidence of this pest is closely correlated with the use of DDT as a codling moth insecticide. It is possible that the natural enemies of the leafroller which were not disturbed by lead arsenate were killed by DDT. The red-banded leafroller is now a major pest capable of destroying an entire apple crop. Similarly, the two-spotted spider mite became an economic problem in many New York orchards following the use of DDT for codling moth control. This species was never considered as an orchard pest prior to the use of DDT. Although the reasons for this development are not completely explained, it again is probable that parasites and predators of the mite have been reduced. The European red mite on apples has increased in importance in New York with the use of DDT. Yet research workers have not been able to say that the rise in populations of this species is directly attributable to the use of DDT.

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Toxicological Investigations by Industry

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Consideration of the many factors involved in the collection and use of toxicological data on chemicals and the types of problems posed in manufacturing, marketing, transportation or usage suggests that, in general, information required by manufacturers to conform to statutes is best gathered in laboratories established by industrial companies, individually or cooperatively. The final correlation and interpretation of data in terms of human hazard are best made by individuals or committees qualified in medicine as well as in pharmacology and toxicology.

The rapid accumulation of statutes, regulations, and ordinances controlling the manufacture, transportation, labeling, and use of chemicals is making acute the problem of obtaining adequate information on the health hazards which these chemicals might afford under various situations. This growth in the mass of legislative regulation is due to a trend in public thought, which has occurred simultaneously with the increase in industrialization of the United States. Before the chemical industry in this country started its mushroomlike expansion, the country was already fairly highly industrialized. The inevitable problems of employer-employee relationships and fair compensation for injuries to workmen led to dissatisfaction with the ponderous, expensive methods of the common law by which each particular case was compared with a pyramid of precedent. In many fields, statutory law replaced common law and by 1909 several states already had passed occupational injury acts governing the compensation of workers for injuries resulting from accidents within their plants. As more and more workers were exposed to new and untried chemicals in manufacture or in usage, it was realized that in addition to the possibility of an acute injury, there was possibility of chronic disease; the time of onset of the disease could not be determined usually and, therefore, could not be used to designate the date of a specific accident. The compensation laws concerning accidents were, therefore, either extended by clauses covering occupational diseases, or other laws were passed specifically concerning occupational diseases. This trend has been particularly important since 1920. The obligation of a manufacturer or marketer of hazardous chemicals to warn his customer of the hazard was still subject to decisions under common law, except where the introduction of chemicals into foods was governed by the Food and Drug Act of 1906, and the interstate transportation of certain hazardous chemicals was regulated, particularly with respect to containers, by the Interstate Commerce Commission regulations. Probably the greatest impetus to statutory regulation has come from the necessity for an industrial population to conserve its food supply. The use of chemicals as insecticides quite often demands a degree of exposure to the user which would not be tolerated in the manufacture of the same chemical. The Federal Insecticide, Fungicide, and Rodenticide Act of 1947, plus state acts regulating and registering insecticides, emphasize this situation.

At the present time, the list of statutes and regulations governing the manufacture, transportation, and use of new chemical products includes the following: Twenty-four

states have occupational disease laws, which either mention specific chemicals as the cause of disease or which give blanket coverage against injury from chemicals; thirty-nine states and the Federal Government have laws regulating the sale of insecticides—of these, the Federal Act, twenty-nine states, and the Territory of Hawaii require registration of the insecticide and control labeling, including literature used in sales; three states have laws governing labeling of industrial chemicals; Massachusetts has special laws on benzol and carbon tetrachloride; Interstate Commerce Commission regulations govern the transportation of hazardous chemicals, and many states and municipalities have more specific laws and regulations concerning the use of certain materials in residential areas or the storage of hazardous materials; Maryland has this year passed a law requiring the labeling of paints and finishes on children's toys or furniture, contact with which might be injurious to the child. The Federal Food, Drug, and Cosmetic Act regulates not merely chemicals introduced deliberately into foods, or used in drugs or cosmetics, but also those employed in food containers. Nor is this the complete picture. The agitation for control of stream pollution and the smog incidents at Donora and Los Angeles have resulted in the consideration during the last year of some two hundred state regulations concerning stream and atmospheric pollution.

Lest this total picture of accumulated regulation of almost all phases of the chemical industry should lead the general public to believe that chemicals as such are an important cause of death or injury in the United States, a few facts are of value. Table I shows data rearranged from reports in "Accident Facts," 1949 edition, National Safety Council. Actually, acute death due to chemicals occurred in some 3500 cases, but of these a great proportion were either the result of exposure to motor vehicle exhaust, sewer gas, or illuminating gas, or were due to injudicious usage of barbiturate medicaments. Eliminating these causes, which are obviously not attributable to lack of warning by manufacturers or to any factors which could in any way be controlled by chemical manufacturers, there were about 1000 deaths in the United States in 1947 due to chemicals. The favorite national weapon, the automobile, which is relatively uncontrolled by legislation, would accumulate an equal "bag" in less than two national holiday week ends.

Table I. Comparative Data on Causes of Death in 1947 (1)

Heart disease	460,580		
Cancer	189,811		
Cerebral hemorrhage	111,725		
		Automobile	32,697
		Home	34,500
		Occupational	17,000
Accidents	99,579	Others	11,940
Nephritis	80,288		
Pneumonia	54,172		
Tuberculosis	48,064		
Premature birth	41,053		
Diabetes	37,515		
Congenital malformations	20,315		
		Absorption of poisonous gas	1938
		Motor vehicle exhaust	285
		Utility gas	1009
		Other sources of carbon monoxide	354
		Other poisonous gases	290
		Acute poisoning by liquid or solid[a]	1504

[a] More than 25% due to barbituric acid and derivatives.

Data on nonfatal injuries or chronic diseases caused by chemicals are very difficult to collect. Far too often, it is gathered from reports of compensation commissions or court decisions in which legal rather than scientific "proof" prevails. One might venture the guess that at least as many injuries and chronic conditions are caused by synthetic medicaments used improperly, or without adequate medical control, as are caused by the ordinary products of the chemical manufacturing industry.

It might be debatable whether this really good record of chemicals is due to the efficiency of regulatory laws. Certainly in the field of industrial health, the industrial disease acts have led to a very great improvement in the picture, but whether regulations—for example, on labeling, however adequate they may appear to be on paper—have actually served to protect the general user of hazardous chemicals is questionable. Far too often, the practice of the user is to employ the product first and then read the label if he gets into trouble.

Despite the possibility of dispute over the reason for the good record of chemicals as a

source of death or injury, it is quite probable that there will be a continual increase in regulation. Changes in policy—for example, of the Food and Drug Administration—have already led to the possibility that a chemical incorporated in a food container and having, coincidentally, antimold or bactericidal properties, but not used in the container for those properties, can, if it should enter the food in the container, be subject to regulation. Arbitrary changes of so-called "maximum allowable concentrations" of atmospheric contaminants may completely alter the effect of state occupational disease laws.

If both common law and statutory obligations incurred by a manufacturer of new chemicals are considered, there is a "cradle to grave" coverage with respect to health hazards, for obligations now arise with the birth of the chemical in the research laboratory and continue through its life in mass production, packaging, transportation, on to its final conversion into a new substance or final distribution or dissipation by the ultimate user.

Much confusion exists—and much vague legislation has resulted—from careless use of the term "poison." To the uninitiated, it would seem that a simple classification of new materials into "poisonous" and "nonpoisonous" would satisfy all needs, and further that such classification is a simple matter of laboratory tests. This idea is erroneous, because there is not only the question of whether the materials are inherently poisonous (whatever definition is used for poison), but also whether, under the conditions of human contact—contact of respiratory or digestive tracts, or skin, or mucous membrane—to be expected in any phase of their use, there is a significant health hazard. A particular chemical may be intrinsically highly toxic, but because of its manner of manufacture and use, the hazard may be minimal. The defining of hazard must be based on an assessment of all factors involved in a particular situation—the intrinsic acute or chronic physiological action of the chemical, its physical properties, and the conditions of its use. In the interests of clarity, the term "poison" should be discarded for the term "hazardous chemical."

In tracing the life of a new chemical, a definition of the intensity and nature of hazard is necessary at the general stages of development shown in Table II.

Table II. Evaluation of Hazardous Chemicals

Stage	Purpose of Information
Research: small scale production of crude chemical and its purification; study of properties and possible uses	Protection of research staff; decision as to suitability for desired use or selection of least toxic chemical from a number with equal technical possibilities
Semiworks: moderately large scale	Decision as to possible health hazards in mass production; preliminary information on type of hazard which might exist in mass manufacture
Design of new plant	Safety of equipment; necessity for ventilation, safe atmospheric concentration; selection of suitable physical protective devices for workers (respirators, protective clothing, showers); procedure for medical treatment in emergency if this requires special equipment
Before operation of new plant	Decision as to health services needed at plant; plan of preventive medicine (including selection of workers according to expected degree of exposure and methods of periodic check on health)
During operation	Continual check on efficiency of protection; treatment in emergencies
Transportation	Classification of product under I.C.C. regulations
Use by industrial customer	Adequate labeling information to protect customers' workers; advice when mode of use involves new form or degree of hazard
Final user (general public)	Adequate labeling and use information either to conform to statute or to fulfill common law obligations; special requirements of federal and state insecticide or pure food laws
At all stages	Fundamental research on new techniques for study of hazards to animals and men

To this outline must be added the need for information on the safe disposal of industrial wastes containing commercially insignificant, but perhaps physiologically important, residues of the compound. If such disposal involves production of new substances—for example, when wastes are burned—the hazard from these substances needs definition.

The information needed at each of these stages in the life history of the compound relates, first, to hazards involved in a specific use and, second, to the hazard to humans, not to laboratory animals. The needed information, therefore, can be gained adequately only by designing laboratory experiments with animals to cover the pertinent conditions of the specific use. Finally, this information can only be interpreted adequately by those versed in normal and abnormal human physiology and pathology. The interpretation of

the degree of hazard which the chemical affords to industrial workers selected by a rigid pre-employment examination and followed thereafter by periodic studies under a preventive medical program may be quite different from the interpretation in terms of the hazard to a customer's workers, who are not selected or studied medically, or to the general public, who may be suffering from a variety of diseases which could be complicated by exposure to a chemical which is harmless to those in good health. The final interpretation, therefore, can seldom be made adequately by those not qualified in medicine.

Collection of Data on Health Hazards

To collect data for the assessment of health hazards at the several stages of development and use described here, physical chemistry, biochemistry, physiology, toxicology, pathology, and clinical medicine are all employed, plus an intimate knowledge of manufacturing procedures and specific methods of use. But these are not used efficiently if isolated in separate departments. The whole program of investigation must be planned and timed with the closest possible correlation of all fields of knowledge. Development of chemical and physical methods of identification or measurement of a chemical contaminating the atmosphere must be based on knowledge of the range of concentrations which may be of significance in the health hazard. The biochemical studies of the fate of the chemical in the body must be designed to be directly applicable, especially in details of technique, to humans. The medical knowledge applied in the assessment of the entire picture must not stop short at an understanding of diagnosis and treatment, but must include a clear concept of health as an entity with degrees varying from optimal to those bordering on disease. Briefly, the research organization must be a closely knit, cooperative unit in which each field of science functions as a dynamic part of the whole and not as an isolated entity. Such an organization was assembled in the medical division of the Army Chemical Corps at Edgewood Arsenal, Md., during the recent war and operated, in the writer's opinion, as one of the most valuable medical research groups ever assembled.

The type of organization defines the training required of the senior members at least. Among them should be represented the disciplines of physics, biochemistry, physiology, pharmacology and toxicology, and pathology and clinical medicine. They should constitute (*2*) "a team of scientists, each a specialist in his own field, but each possessing a thoroughly sound and trained acquaintance with the fields of his neighbors, all in the habit of working together, or knowing one another's intellectual customs, and of recognizing the significance of a colleague's new suggestion before it has taken on a full formal expression. The mathematician need not have the skill to conduct a physiological experiment, but he must have the skill to understand one, to criticize one, and to suggest one. The physiologist need not be able to prove a certain mathematical theorem, but he must be able to grasp its physiological significance and to tell the mathematician for what he should look." Above all, they should never forget that their ultimate decisions must be made in terms of human health.

The junior staff of such research organizations should consist of the technical assistants and untrained laboratory helpers and animal caretakers needed to facilitate the functioning of the whole organization.

Currently, toxicological problems for the chemical manufacturing industry are occasionally studied in special laboratories established by particular corporations but more frequently either in departments of biochemistry or pharmacology attached to university medical schools or by commercial laboratories, but seldom is the whole pattern of investigation, needed to cover all phases of manufacture and use of a chemical, set up in one place. Seldom, also, do those undertaking investigations have the required close contact with the technical or medical problems of industry. In wartime, it is possible to establish such research organizations; in peacetime, the scientists needed for similar problems are scattered widely among medical schools and isolated in separate departments of those schools. Therefore, the responsibility of the manufacturer of chemicals for obtaining adequate information on the materials which he uses and sells can best be fulfilled by toxicological research laboratories within industry. These could be established either by individual corporations or, when financial considerations make that difficult, by groups of

corporations having similar interests. Since, in the final analysis, the decision as to hazard from a given compound is a medical one, the toxicological laboratory should be a part of, or closely associated with, the medical organization of the company or group. Further, there should be freedom of exchange of scientific knowledge between such research laboratories so that the industry as a whole may benefit from information gained by each and duplication of effort may be eliminated.

Twenty years ago this program would have been regarded as the dream of an idealist; it is no longer an ideal but a very real, immediate need. Manufacturers of chemicals cannot inform the public adequately on the health hazards from the materials which they make and sell unless they, themselves, have adequate information. In the absence of proper education of the public, hysterical, ignorant, or malicious gossip will lead to legislative regulation, which may be harmful to progress. Two examples of such legislation, or attempts at legislation, have appeared within the last 12 months: Section 323 of Article 27 of the Annotated Code of Maryland, 1939 edition, states:

It shall be unlawful for any person to manufacture, sell, or offer to sell any toy or plaything, including children's furniture, decorated or covered with paint or any other material containing lead or other substance of a poisonous nature, from contact with which children may be injuriously affected, unless such article shall contain a label affixed thereto stating in clear and unambiguous language that the said paint, or other material, contains lead or other substance of a poisonous nature.

This law, which is a criminal law, is so loosely worded that it is at the moment impossible to establish criteria for paints, varnishes, or lacquers to be placed on children's furniture or toys to be sold in Maryland. Further, the Maryland law has little protective value, since anyone can repaint children's furniture or toys with any material regardless of its suitability for the purpose and its inherent toxicity. This law, as it stands, places a heavy and complex burden on the manufacturer and marketer of children's toys and furniture—and necessarily on the manufacturer of paints, lacquers, and varnishes. The Maryland law has greater significance as a piece of troublesome legislation in that in the courts of the state of Maryland, the jury is the judge of the law as well as the facts under the law, and since under criminal law each case would be argued on its own merits, two different juries might reach contradictory decisions on the same set of circumstances.

Again, during the last 12 months, there has been strong agitation for an increase of severity in the labeling of carbon tetrachloride in the state of California. Two deaths were caused in the city of San Francisco by grossly improper use of carbon tetrachloride, which had been removed from its original container. The original container apparently had been properly labeled according to the opinion of competent authorities. Public agitation in the city of San Francisco demanded that carbon tetrachloride be labeled "poison," and that, therefore, it carry an antidote. It is a principle of good labeling that the word "poison" should not be used indiscriminately lest its frequent sound in the ears of the public lead to a dangerous dilution of its value. It is a standard principle of toxicology that one cannot prescribe an antidote for materials which are hazardous by inhalation. Both in the Maryland and the San Francisco situations, adequate and proper public knowledge of the problem would have had great value.

Undoubtedly the establishment of toxicological research organizations will be costly, but the cost will be much less than the cost of not establishing them. There are many difficulties to be faced, but none is greater than the practical difficulties which manufacturers of modern synthetic chemicals continually face in their visions of new products or in their attempts at more economic production of well known products.

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Toxicological Action and Metabolic Fate of Chlordan

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In rabbits under light amytal anesthesia, chlordan has no direct effect on the blood pressure, but produces a type of respiration having many characteristics in common with Cheyne-Stokes type. The generalized tremors, opisthotonus, tonic and clonic convulsions, produced by chlordan were decreased or abolished and respiration restored to normal by suitable injections of the sodium salts of amytal, phenobarbital, and pentothal. The LD_{50} of chlordan, which was about 20 mg. per kg. on intravenous administration to intact rabbits, was increased to about 60 mg. per kg. through the antidotal action of the barbiturates. An unidentified chlorine-containing degradation product with acidic properties was recovered from the urine of rabbits treated with chlordan. Approximately one third of its chlorine content was set free on hydrolysis at 100° C. with sodium hydroxide in either absolute alcohol or in water.

It was previously shown (*4*) that the oral LD_{50} of chlordan in rats was 200 to 250 mg. per kg., in rabbits about 300 mg. per kg., and on intravenous injection in rabbits in Tween 20 about 20 mg. per kg. It was also shown (*4*) that the chronic toxicity of chlordan in both rats and rabbits was greater than that of DDT under comparable conditions.

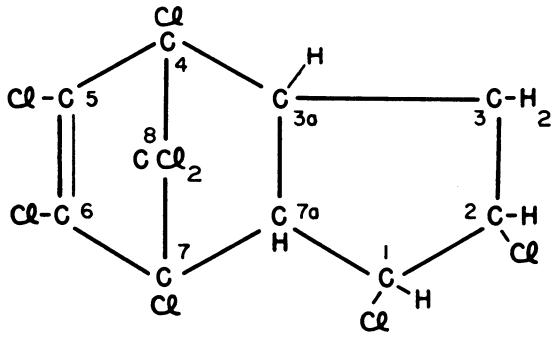
The purpose of this paper is to give some of the results obtained in a study of the antidotal action of barbiturates in chlordan poisoning, and of the metabolic fate of chlordan.

Experimental

In this work the chlordan used was taken from the same lot employed in previous work (*4*). The effects of this compound on blood pressure and respiration were studied in rabbits under barbiturate anesthesia of light to moderate depth. Forty milligrams per kg. were the usual dose of barbiturate, administered intravenously. Chlordan was administered 25 to 40 minutes later intravenously as a 40% solution in Tween 20. At this time animals having received sodium amytal were under slightly deeper anesthesia than those having received equivalent amounts of either sodium phenobarbital or sodium pentothal. Additional amounts of the barbiturates were given, when the animals developed severe general tremors and respiratory symptoms, in order to determine their antagonistic action against chlordan.

The antidotal action of the barbiturates was also investigated in intact rabbits by the intravenous administration of both chlordan and the barbiturate. Sixty milligrams per kg. of chlordan were injected as a 40% solution in Tween 20 at a uniform rate over a period of 3 minutes. The sodium salts of the barbiturates were injected slowly as a 2.5% solution in distilled water in two doses. The first dose was given 2 to 5 minutes preceding

the chlordan administration and the second, 5 to 15 minutes following it. All animals were observed a minimum of 30 days.



1,2,4,5,6,7,8,8-OCTACHLORO-2,3,3a,4,7,7a-HEXAHYDRO-4,7 METHANO-INDENE

Structural Formula of Chlordan, $C_{10}H_6Cl_8$

The metabolic fate of chlordan was studied in rabbits by analysis of the relative chlorine content of chlordan added to normal rabbit's urine and of the chlorine content of the urinary excretory product. The method of analysis was similar to the one previously used (*2*, *4*). In addition, hydrolysis of chlordan and of the urinary excretory products was carried out by adding solid sodium hydroxide to saturation to a 10-ml. solution of these substances in hot absolute ethyl alcohol. The mixture was refluxed for 3 hours in a round-bottomed flask immersed in boiling water and the amount of inorganic chlorine determined. Hydrolysis was similarly carried out with solutions of the respective substances in aqueous sodium hydroxide.

Results

The effects of chlordan on the blood pressure and respiration of rabbits under sodium pentothal are illustrated in Figure 1. The injection of 60 mg. per kg. of chlordan produced a gradual drop in blood pressure of about 20 mm. of mercury, starting during the injection and continuing for about 3 minutes after the injection (Figure 1, *A*). It then rapidly returned to the preinjection level. Since a similar drop in blood pressure was produced by the injection of an equivalent amount of the solvent, Tween 20, alone, it appears that chlordan has little if any direct effect on the blood pressure. Initially there was an increase in respiratory rate with a slight decrease in amplitude. This was followed by a marked increase in respiratory rate and amplitude with a simultaneous onset of generalized tremors. Cheyne-Stokes type of respiration followed (Figure 1, *B*). This consisted of alternating cycles of rapid and deep respirations with short periods of apnea, during which slight temporary elevations in blood pressure may be seen. These cycles lasted from several minutes to 1.5 hours in different experiments. Mild to moderate tremors occurred more or less continuously, whereas the more severe tremors occurred in cycles corresponding to the periods of apnea in the Cheyne-Stokes cycle.

The antagonistic effects of sodium pentothal on the blood pressure and the respiratory effects of chlordan are illustrated in Figure 1, *C*. The intermittent severe tremors with concomitant Cheyne-Stokes type of respiration produced by the injection of chlordan were decreased or entirely abolished, and both the respiratory rate and depth were restored to near normal. A dose of barbiturate sufficient to diminish the tremors appreciably and to restore the respiration to normal also produced a temporary drop of 10 to 40 mm. of mercury in blood pressure.

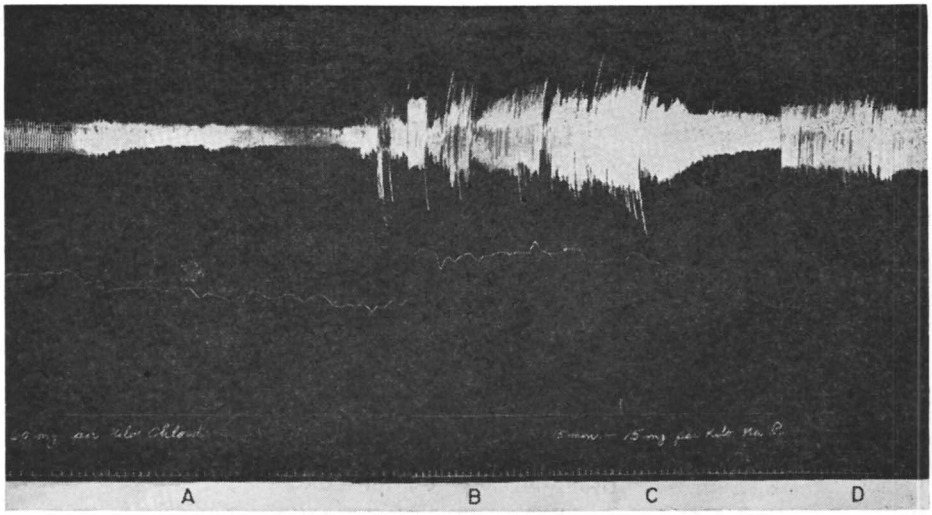


Figure 1. Kymographic Tracing Showing Effects of Chlordan on Blood Pressure and Respiration of Rabbit under Light Pentothal Anesthesia and Antagonistic Action of Sodium Pentothal

From top down: respiration, blood pressure, injection signal, and base line, time in 5 seconds; *A* = 60 mg./kg. chlordan, intravenously; *B* = 6 minutes later, Cheyne-Stokes respiration; *C* = 15 mg./kg. sodium pentothal 15 minutes later (respiration restored to normal and lowered blood pressure due to temporary action of pentothal); *D* = 20 minutes after pentothal injection

The effects of chlordan on the blood pressure and respiration of rabbits under sodium pentothal, in general, were characteristic of those obtained under sodium amytal and sodium phenobarbital, with some variation in intensity and length of duration.

Table I. Antidotal Action of Barbiturates in Chlordan Poisoning in Rabbits

Barbiturate	Barbiturate Used. Mg./Kg.[a] Before chlordan	After chlordan	Chlordan, Mg./Kg.[a]	No. Died/ No. Used
Amytal Na	20	30	60	1/13
Pentothal Na	25–30	15	40	0/3
	20–30	15–50	60	8/14
Phenobarbital Na	20–30	30–40	60	5/14
Controls	0	0	30	10/10
Controls	0	0	20	17/23

[a] Administered intravenously.

The antidotal action of the barbiturates in intact animals is shown in Table I. All three barbiturates in the amounts given were effective in decreasing the severity of the intermittent general body tremors, opisthotonus, extensor rigidity, salivation, and in the prevention of convulsions. In some cases the symptoms were entirely abolished throughout the critical period—the first 2 hours following chlordan administration. When deaths did occur they were generally delayed beyond the time of occurrence of death in the nontreated groups (*4*). The most effective antidotal action, however, was obtained with sodium amytal. The shorter acting sodium pentothal had considerably less antidotal effect under the conditions of these experiments. This may have been due to its relatively shorter duration of action, as a result of which the effects of chlordan were not antagonized over a sufficiently long period of time. The intermediate antidotal effect obtained with the long-acting phenobarbital is probably due in part to its relatively more shallow depth of anesthesia and slower action. The preliminary dose of the barbiturate was given in order to make the administration of the full dose of chlordan possible. Without this the severity of the tremors and convulsions developing during the injection made the complete

Table II. Fractionation of Organically Bound Chlorine Excreted in Urine of Chlordan-Poisoned Rabbits

	Extracted, Mg.			Chlorine Split Off on Hydrolysis at 100° C., % of Total	
	With ether from acid urine, pH 3.8	From ether with dilute alkali, pH 8.6	With ether from aqueous alkali after acidification to pH 3.8	Alcoholic NaOH	Aqueous NaOH
Experimental	65.6	55.0 (83.8%)	55.0 (83.8%)	38.2	34.5
Control plus 228 mg. chlordan	194.5 (85.3%)	0	0	44.3	6.9

injection of the relatively large doses of chlordan difficult or impossible. As a result of the antagonistic action of these barbiturates, rabbits tolerated up to approximately three times the LD_{50} dose of chlordan.

The analysis of urinary excretion of organically bound chlorine by chlordan-poisoned rabbits is shown in Table II. Apparently all the organic chlorine excreted in the urine of chlordan-poisoned rabbits is extractable with ether from urines acidified to pH 4.0 or lower. Only part of it was extracted from urines acidified to pH 6.0, and relatively little was extracted from alkaline urines. Nearly all of the organic chlorine-containing constituents (83.8%) were extracted from ether with dilute alkali, and in turn were again re-extracted from aqueous alkali with ether after acidification. In contrast, chlordan added to normal rabbit urine could be extracted from either an acid or an alkaline urine with ether, to the extent of about 80% of the amount added. Moreover, this was not extractable with dilute alkali from the ether phase. About one third of the total organic chlorine in the urinary excretory product was split off with either alcoholic or aqueous sodium hydroxide, whereas only about 7% of the total chlorine was removed from chlordan by aqueous sodium hydroxide under comparable conditions. These data indicate that the urinary excretory product probably consists of one or more acidic chlorine-containing degradation products of chlordan, which are water soluble at pH 8.6 or higher. It seems possible that the labile chlorines in positions 5 and 6 may be split off in the body, followed by the oxidation of the corresponding carbons.

Discussion

The barbiturates are effective against convulsions induced experimentally from all central locations, the cerebrum, medulla, or spinal cord. They may be used clinically as well as experimentally to suppress most, if not all, varieties of convulsions of central origin (*3*). Since they are effective in the prevention of the tremors, tonic and clonic convulsions, and in the restoration of normal respiration from the Cheyne-Stokes type, produced by chlordan, it appears that these symptoms may have their origin in the central nervous system.

The antidotal action of the barbiturates is probably limited to the effects of chlordan on the nervous system. They most likely have no beneficial antagonistic action against the delayed parenchymatous degenerative changes produced by chlordan (*4*). Therefore, they are primarily only of possible value in acute poisoning in which severe stimulation of the central nervous system may be the primary cause of death.

The conversion in the animal body of at least some of the water-insoluble chlordan to a water-soluble degradation product must facilitate the elimination of the poison through its excretion into the urine by the kidneys. Moreover, the degradation of chlordan as shown in the present experiments may be a mechanism for its detoxification, as in the case of DDT (*1*). Only the isolation of the degradation product, its identification, and a study of its toxicity can determine this point.

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DDT in Eggs and Tissues of Chickens Fed Varying Levels of DDT

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The concentration of DDT in eggs and tissues of chickens fed a mash made with DDT-treated alfalfa hay is reported. The amount of DDT in the fat, muscle, and eggs was correlated with the DDT intake.

The use of DDT to control lygus bugs (*Lygus elisus* Van D; *Lygus hesperus* Knight) and alfalfa weevil (*Hypera postica* Gyll.) in the production of seed alfalfa has been demonstrated by Lieberman (*10*), Smith and Michelbacher (*17*), and Lieberman and Hare (*11*). Alfalfa hay is also markedly improved by the use of this insecticide (*7*). This has resulted in widespread use of the compound. With the dissemination of DDT dusts and sprays over large forage crop areas, it is important to know the effect of the insecticide on livestock and poultry. A series of studies has been conducted in the authors' laboratories to gain information on this problem.

When DDT is fed to animals, even in small quantities, there is an accumulation of the compound in the tissues, particularly the fat. Telford and Guthrie (*18*), Orr and Mott (*13*), Woodward *et al.* (*20, 21*), and Laug and Fitzhugh (*9*) have demonstrated that DDT will accumulate in certain tissues and in milk fat of domestic and laboratory animals. Marsden and Bird (*12*) found that DDT was toxic to turkeys in concentrations above 0.075% of the diet, and that turkeys fed the insecticide for 7 to 8 weeks stored DDT in their fat at concentrations ranging from 4 to 8 times that in the diet. Rubin *et al.* (*14*) reported that hens fed 0.062% DDT in their diet for 12 weeks showed reduced egg production with lowered hatchability. At one half this concentration there was a detrimental effect on egg production, but hatchability was not seriously affected. The hens were killed by doses of 0.125% DDT. The insecticide was found in the eggs in quantities much smaller than in the body fat. Harris *et al.* (*8*) have shown that DDT will accumulate in considerable quantities in the fat of lambs fed DDT-treated hay. Small amounts of the insecticide were found in other tissues.

Carter and Fitzhugh (*2, 5*) have summarized the results of experiments on domestic

Table I. Basal Mash

	%
Ground wheat	25
Bran	24.25
Ground barley	25
Fish meal	10
Soybean oil meal	10
Salt	1
Ground limestone	1.75
Bone meal	2.25
Fish liver oil	0.25
Vitamin concentrate	0.5

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and laboratory animals in which DDT was found to have accumulated in small amounts in the tissues. Carter *et al.* have shown (*3*) that cooking meat from DDT-treated animals did not seriously change its DDT content and (*4*) that the insecticide accumulated in the fat of pigs fed beef that contained DDT.

The purpose of this work was to determine the extent of accumulation of DDT in eggs and the tissues of chickens during and after a period of feeding a mash made with DDT-treated alfalfa hay.

Design of the Experiment

The alfalfa hay used in making the mashes in this experiment was from the same field as that described by Harris *et al.* (*1*, *8*). This alfalfa was dusted with 0, 1, 2, and 4 pounds of technical DDT per acre, which was approximately the same amount used by hay producers. The harvested hay was ground into meal and a mash was made for laying hens, containing 15% alfalfa meal. DDT was also added to mash made from untreated alfalfa meal at levels of 0, 50, 100, and 200 p.p.m. to determine a possible margin of safety for feeding DDT-treated alfalfa.

A total of 48 White Leghorn pullets approximately 6 months of age were used in the experiment. Thirty-two pullets were selected at random from the original 48 birds, and divided into four groups of 8 birds each. One group was then assigned to each mash made with the hay dusted with 0, 1, 2, and 4 pounds of technical DDT per acre.

The remaining 16 pullets were divided into four groups of 4 birds each, and were assigned to mashes containing 0, 50, 100, and 200 p.p.m. of DDT. This mash was made by thoroughly mixing an ether solution of DDT with 1 pound of mash and then mixing this with 19 pounds of mash (see Table I).

The mash fed was made by mixing 15% alfalfa meal with 85% of the basal mash. This was available to the hens at all times. In addition, the hens were fed a grain mixture once daily. This was made up of 50% wheat, 40% barley, and 10% oats.

All hens were in excellent laying condition at the start of the experiment. Each hen was assigned at random to a compartment of a laying battery.

The eggs were collected daily and were kept under refrigeration until they were analyzed for their DDT content. Feed consumption and production records were kept for each hen. The data on weight gains, food consumption, and egg production will be published at the end of a 3-year feeding period.

At the end of one year of feeding DDT-treated mash, 16 of the hens were killed and their leg and breast muscle and fatty tissues were analyzed for DDT. During this time 8 hens had died of leukemia complex, and their death was not attributable to the DDT. At the beginning of the second year 24 of the original 48 hens were still on the experiment. In order to replace those hens that were killed and those that had died, 24 more 6-month-old pullets were assigned at random to the eight groups of the experiment, making a total of 48 hens at the beginning of the second year.

The DDT residue of the hay was determined by the total chloride method of Umhoefer (*19*) as described by Harris *et al.* (*8*). The eggs and tissues were analyzed by the method of Schechter *et al.* (*16*) with the modifications described by Harris *et al.* (*7*). The eggs were sampled by mixing the yolks and the whites of all the eggs from one hen for one month in a Waring Blendor. A 50-gram sample of the eggs was placed in a 250-ml. centrifuge bottle and 75 ml. of 95% ethyl alcohol and 50 ml. of Skellysolve B were added. The

Table II. DDT Content of Eggs of Chickens Consuming Mash Containing DDT-Treated Hay (1947–1948)[a]

DDT Applied per Acre, Lb.	DDT Residue, P.P.M.		Months after Beginning DDT Feeding		
	On hay	In mash[b]	8	9	10
			DDT in Eggs, P.P.M.		
0	0	0	1.7 ±2.0	1.7 ±2.0	0.9 ±1.2
1	15	2.3	2.0 ±2.0	2.1 ±1.6	1.8 ±0.8
2	22	3.3	1.6 ±1.3	3.7 ±4.9	2.4 ±2.5
4	42	6.3	3.3 ±1.6	4.1 ±1.0	2.5 ±1.2

[a] Each value is average ± S.D. of 6 to 8 hens.
[b] DDT content of mash by calculation.

addition of the ethyl alcohol served to precipitate the protein and to prevent the formation of emulsions with the Skellysolve B. The DDT was extracted from the egg mixture by the Skellysolve B. After centrifuging, the Skellysolve B was easily separated from the mixture. From this point the analysis was carried out as with milk in the method of Schechter *et al.* (*15*).

All colorimetric readings were made on the Beckman quartz spectrophotometer.

Results and Discussions

None of the chickens showed ill effects from the ingestion of DDT. The mortality of the 48 hens used was 15% over a period of a year, which is below the average mortality for laying hens in this area. The deaths were not associated with DDT feeding. There was no significant difference in live weight gain between any of the hens on any of the DDT treatments or between any of the DDT levels and the control chickens.

The amount of DDT present on the treated hay and in the eggs for the first year of the experiment is shown in Table II. The DDT residue on the alfalfa hay varied from 0 to 42 p.p.m. The undusted hay contained no DDT and the dusted hay contained amounts that increased progressively with the quantity of DDT applied. Small amounts of DDT that appeared in the eggs of the control chickens might be explained by the hens scattering the mash during feeding to other compartments in the laying battery. The presence of DDT in small amounts does not detract from the significance of the other data. With an increase of DDT in the feed there is generally an increase of the compound in the eggs.

The DDT content of the eggs from chickens fed 0, 50, 100, and 200 p.p.m. in the mash during the first year is presented in Table III. The eggs of the 12 control chickens were analyzed as one group; therefore, the results in both Tables II and III are the same for the control chickens. The amount of DDT found in the eggs of the chickens fed mash containing DDT in most cases is roughly proportional to the amount of DDT ingested.

Table III. DDT Content of Eggs of Chickens Consuming Mash Containing DDT (1947–1948)[a]

DDT Applied to Mash, P.P.M.	Months after Beginning DDT Feeding		
	8	9	10
	DDT in Eggs, P.P.M.		
0	1.7 ±2.0	1.7 ±2.0	0.9 ±0.8
50	7.5 ±8.7	7.7 ±6.9	23.3 ±0[b]
100	13.3 ±5.8	18.7 ±8.4	18.3 ±10.6
200	65.6 ±25.2	57.1 ±5.9	43.5 ±12.8

[a] Each value is average ± S.D. of 2 to 4 hens.
[b] Value of one hen consistently high during 3-month period.

In Table IV are tabulated the concentrations of DDT found in the tissues of the chickens killed after one year of feeding. The concentration of DDT was markedly higher in the fat than in the diet. Only small amounts of the insecticide were found in the breast and leg muscle tissues. In each case the fatty tissues contained DDT at markedly higher levels than the eggs.

Table IV. DDT Content of Chickens Killed at End of 12 Months of Feeding DDT (1947–1948)

DDT Applied per Acre, Lb.	DDT Residue, P.P.M.		No. of Chickens	Leg, P.P.M.	Breast, P.P.M.	Fat, P.P.M.
	On hay	In mash				
0	0	0	3	0.3	0.2	...
1	15	2.3	2	0.5	0.1	30.0
2	22	3.3	3	0.7	0.8	29.8
4	42	6.3	4	1.0	0.4	44.1
DDT Added to Mash, P.p.m.	50		1	..	0.2	....
	100		1	1.8	0.5	103.6
	200		2	11.6	4.0	502.6

The concentration of DDT in the eggs during the second year is shown in Tables V and VI. During the first month of the second year DDT was not fed. However, 24 of the hens that had been on the experiment during the first year still had DDT present in their

eggs. The 24 new pullets did not have DDT in their eggs during the first month, but thereafter the level of DDT was about the same as that in the eggs during the first year.

Table V. DDT Content of Eggs of Chickens Consuming Mash Containing DDT-Treated Hay (1948–1949)[a]

DDT Applied per Acre, Lb.	DDT[b] Residue in Mash, P.P.M.	Months after Beginning DDT Feeding								
		1[c]	2	3	4	5	6	7	8	9
		DDT in Eggs, P.P.M.								
0	0	0.3 ±0.6	0.3 ±0.5	0.3 ±0.6	0.2 ±0.4	0.7 ±0.8	0.2 ±0.3	0.3 ±0.8	0.8 ±0.7	1.2 ±0.4
1	2.3	0.4 ±0.5	0.1 ±0.4	2.3 ±0.6	2.2 ±0.6	3.6 ±1.0	2.6 ±0.8	2.9 ±0.8	2.6 ±0.8	3.1 ±0.9
2	3.3	....	1.0 ±0.4	2.1 ±0.6	0.8 ±0.3	3.4 ±0.5	3.4 ±0.7	3.1 ±0.9	2.7 ±0.9	3.1 ±1.3
4	6.3	0.2 ±0.3	0.5 ±0.4	4.3 ±1.5	2.7 ±1.5	8.2 ±1.9	7.9 ±1.9	8.3 ±2.8	7.6 ±2.0	6.9 ±2.2

[a] Each value is average ± S.D. of 6 to 8 hens.
[b] DDT content of mash by calculation.
[c] DDT not fed during first month. 16 hens were from experiment the preceding year and still contained DDT in their eggs. 16 new pullets did not have DDT in their eggs during first month.

Table VI. DDT Content of Eggs of Chickens Consuming Mash Containing DDT (1948–1949)[a]

DDT in Mash, P.P.M.	Months after Beginning DDT Feeding								
	1[b]	2	3	4	5	6	7	8	9
	DDT in Eggs, P.P.M.								
0	0.3 ±0.6	0.3 ±0.5	0.3 ±0.6	0.2 ±0.4	0.7 ±0.8	0.2 ±0.3	0.3 ±0.8	0.8 ±0.7	1.2 ±0.4
50	....	1.1 ±1.1	15.9 ±3.3	10.5 ±8.8	23.0 ±5.6	19.8 ±0.8	22.6 ±1.8	18.5 ±2.1	21.0 ±0.9
100	3.2 ±5.5	11.2 ±8.9	29.0 ±1.0	29.3 ±4.7	56.1 ±2.2	50.5 ±6.8	46.7 ±6.9	32.2 ±2.9	47.3 ±3.3
200	6.6 ±9.3	7.7 ±3.0	28.9 ±4.5	48.4 ±16.4	66.5 ±2.7	64.1 ±8.1	51.9 ±7.6	42.9 ±3.7	58.2 ±4.1

[a] Each value is average ± S.D. of 3 to 4 hens.
[b] DDT was not fed during first month. 8 hens were from experiment of preceding year and still contained DDT in their eggs. 8 new pullets did not have DDT in their eggs during first month.

Rubin *et al.* (*14*) have shown that 1250 p.p.m. of DDT in the diet of laying hens for 12 weeks produced toxic symptoms and death. In the work reported here the highest level of DDT used was 200 p.p.m. The authors did not observe toxic symptoms at this or lower levels.

The results of this study indicate that when alfalfa is dusted with DDT and the harvested hay is used in making a mash for chickens, DDT will appear in considerable quantities in the tissues and eggs of chickens consuming such mash. The daily intake of DDT in this study was not sufficient to produce toxic symptoms. However, if the same level of DDT is consumed for a long period of time, DDT may accumulate in sufficient concentration to produce harmful effects in the chickens, or in a human consuming the meat or eggs. Fitzhugh and Nelson (*6*) have shown that the withdrawal of food from animals fed a high level of DDT produced characteristic DDT tremors; DDT-treated animals made sick by infection also showed characteristic DDT tremors. They attributed these effects to the fact that the animals were metabolizing their body fat, which contained large amounts of DDT, at an increased rate during starvation or infection. This effect could be important in cases of human infection or starvation if there was a large storage of DDT in the fat tissue.

Summary

Alfalfa was dusted with 0, 1, 2, and 4 pounds of technical DDT per acre as an insecticide and the harvested hay was ground into meal. A mash was made for laying hens containing 15% alfalfa meal. DDT was also added to mash made with untreated alfalfa hay at 0, 50, 100, and 200 p.p.m. Four groups of 8 hens each were fed the mash made with the field-dusted hay and four groups of 4 hens each were fed the mash containing 0, 50, 100, and 200 p.p.m. of DDT.

The DDT residue of the field-treated hay was 0.0, 15, 22, and 42 p.p.m. for the 0, 1, 2, and 4 pounds of DDT per acre treatment, respectively.

During the first year of feeding the mash, DDT was present in the eggs at a maximum concentration of 1.7, 2.1, 3.7, and 4.1 p.p.m. from the hens consuming the mashes

containing alfalfa treated with 0, 1, 2, and 4 pounds of DDT per acre, respectively. The eggs from the hens consuming mashes containing 0, 50, 100, and 200 p.p.m. of DDT contained maximum amounts of 1.7, 23.3, 18.7, and 65.6 p.p.m. of DDT, respectively.

The highest concentration of DDT was found in the fat of the chickens killed after being fed DDT for 1 year. The average amount present was 30.0, 29.8, 44.1, 103.6, and 502.6 p.p.m. when hay treated with 1, 2, and 4 pounds of DDT per acre and 100 and 200 p.p.m. of DDT, respectively, was fed. Muscle tissues varied from 0.1 to 11.6 p.p.m.

The concentrations of DDT in the eggs during the second year of feeding the DDT-treated mash were generally slightly higher than the first year.

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DDT in Milk and Tissues of Dairy Cows Fed DDT-Dusted Alfalfa Hay

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Untreated alfalfa hay and hay dusted with technical DDT were fed to 16 Holstein cows during 2 years DDT appeared in the milk within 4 days after the feeding of DDT-treated hay and the amount gradually increased until a fairly constant level was reached. More DDT was found in milk and tissues of cows that consumed hay treated with higher levels of DDT. DDT persisted in the milk for 4 months and disappeared approximately 6.5 months after feeding of DDT-treated hay was discontinued.

Previous studies have shown that DDT appears in the milk and body fat of dogs (*13*), in the milk of rats and goats (*11*), in the tissues, especially fatty tissues, of rats (*5*, *6*) and rabbits (*4*), in the milk and tissues of dairy cows (*1*, *10*), and in the tissues (*2*) and milk (*7*) of sheep following the ingestion of DDT or of feeds containing DDT residues. DDT persists in the milk of cows for relatively long periods of time after they have been sprayed with DDT for the control of flies (*3*).

Inasmuch as these studies established the accumulation of DDT in the fatty tissues and its appearance in the milk following the ingestion of relatively large doses of DDT, the question arose as to whether the milk of dairy cows consuming alfalfa hay with low DDT residues contained sufficient DDT to be hazardous to animals and humans consuming the milk. The problem assumed greater importance because of the practice in this and other areas of dusting forage alfalfa with DDT for the control of alfalfa weevil and other insects and the feeding of such alfalfa hay to dairy cows.

This paper reports data obtained following feeding of DDT-dusted alfalfa hay to Holstein dairy cows. Milk, blood, and tissues were analyzed for their DDT content during two different years of feeding of the DDT-dusted hay.

Materials and Methods

Alfalfa hay was obtained from a 44-acre unirrigated field at Petersboro, Cache County, Utah. The field was divided into sixteen 1-acre plots with intervening untreated buffer strips of sufficient width (150 feet) to prevent drift of the DDT dust from one plot to another at the time of application. A block of four 1-acre plots received the following treatments during the summer of 1947: no DDT, 1 pound, 2 pounds, and 4 pounds of technical DDT per acre, and no DDT, 0.5 pound, 1 pound, and 2 pounds of technical DDT per acre during the summer of 1948. These treatments were repeated on the remaining three blocks of plots, so that a total of four 1-acre plots received each treatment. The treatments within each block were assigned at random.

The DDT dust in a pyrophyllite carrier was applied with a power duster when a sub-

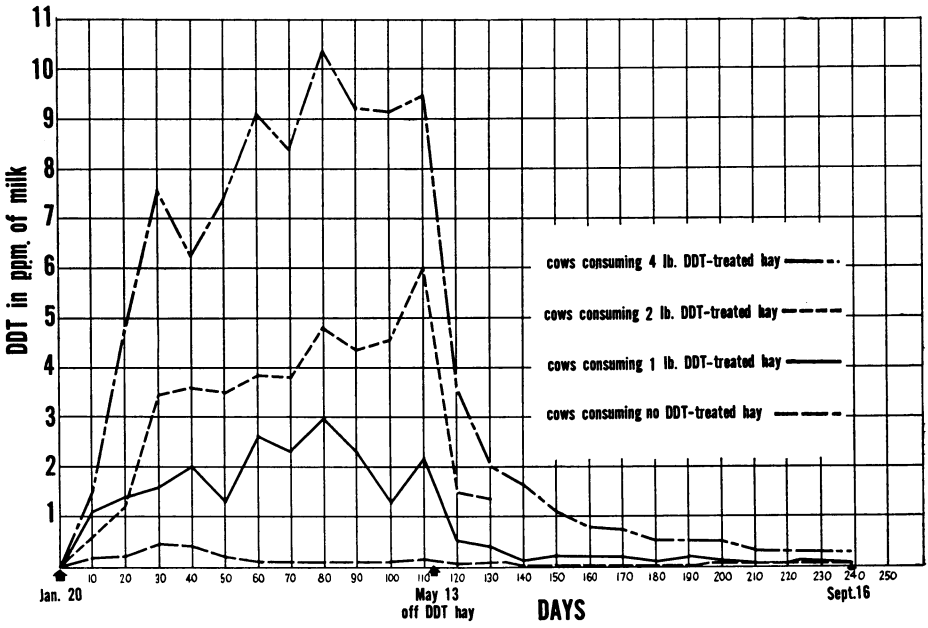

Figure 1. DDT in Milk of Cows Fed Alfalfa Hay Dusted with Technical DDT (1948)

stantial number of alfalfa weevil (*Hypera postica* Gyll.) larvae were present on the alfalfa. The number was determined by sweeping each of the plots with a hand net and counting the larvae obtained from each plot. These data will be published elsewhere.

The alfalfa used was second crop in 1947 and first crop in 1948. It was cut when the majority of the plots dusted with DDT were judged to be in the tenth-bloom stage. The alfalfa within each plot was allowed to sun-cure, and was then baled and hauled to the college barns for storage.

Eight cows were chosen from the college experimental herd each year, two cows being assigned to hay from each of the field treatments. Milk and blood samples were obtained from each cow before the experiment started (January 21, 1948, and November 24, 1948), and these samples were analyzed for DDT. On the day after the cows began eating the control and experimental hays (November 1948), and on the fourth day after (January 1948), milk samples were obtained and analyzed for DDT. Samples were then collected and analyzed at 3-day intervals until the concentration of DDT in the milk had reached a relatively constant level, after which samples were collected at weekly or longer intervals until the experiments were terminated several months later (Tables I and II and Figures 1 and 2). Feeding of alfalfa hay was discontinued on May 13, after 113 days, the first year of the experiment, at which time the cows went to pasture. Sampling and analysis of the milk for DDT continued, however, until September 16. During the second year, the feeding of the alfalfa hay commenced on November 24 and continued until February 13, a period of 81 days. Between this time and the time the cows went to pasture (May), they were fed untreated hay from the college fields. Blood and milk samples were collected the second year before, during, and following the period of feeding the treated alfalfa hay.

The DDT content of the tissues and milk was determined by the colorimetric method of Schechter *et al.* (*8*, *9*) as modified by the authors (*2*). The amount of DDT residue on the hay was determined by the total chloride method of Umhoefer (*12*).

Cow Hu 29 (fed hay treated with 2 pounds of DDT per acre) was slaughtered on May 20, 1948, one week after experimental feeding was discontinued. Kidney and mesenteric

fat, muscle, and liver were analyzed for DDT content. In 1949 cows Hu 36 (fed hay treated with 1 pound of DDT per acre) and Hu 329 (fed untreated hay) were slaughtered at the close of the feeding period and the tissues were analyzed for their DDT content as before. Cow E 139 (fed hay treated with 1 pound of DDT per acre) was slaughtered on June 29, 1949, approximately 4 months after the feeding of the DDT-treated hay was discontinued, and the tissues were likewise analyzed for their DDT content.

In addition to the alfalfa hay, the cows received a grain ration which consisted of a mixture of 80% barley and 20% molasses dried beet pulp, to which were added 2% steam bone meal and 1% fine hay salt (sodium chloride). This ration was fed at the rate of 0.75 pound of grain per day for each pound of butterfat produced during the previous week. If the butterfat production dropped below 0.8 pound per day, the grain ration was routinely discontinued.

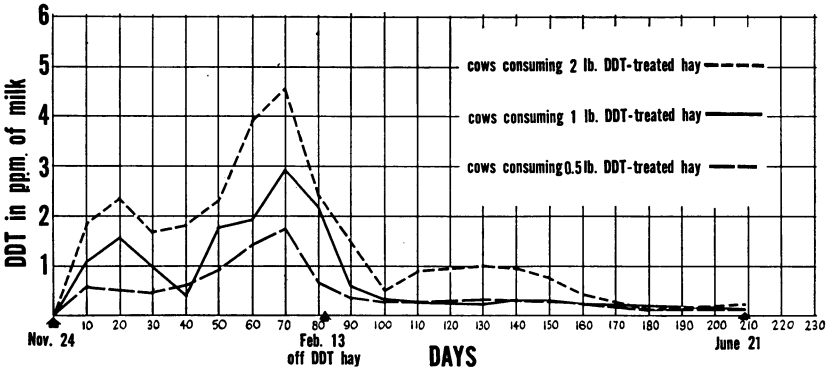

Figure 2. DDT in Milk of Cows Fed Alfalfa Hay Dusted with Technical DDT (1948–49)

In order to determine whether there was sufficient DDT in the milk to produce effects in rats, milk from the cows was separated and the cream was incorporated into a synthetic diet which was fed to a group of rats for a period of 4 months. Butter was churned from some of the cream and this was likewise incorporated into a diet and was fed to another group of rats. These data will be published elsewhere.

Results and Discussion

The amount of DDT present in the milk of the cows during the winter of 1948 is presented in Table I and Figure 1. No DDT was present in the milk of any of the cows at the beginning of the experiment. However, within 4 days after the feeding of DDT-treated hay was begun, DDT appeared in the milk. The amount gradually increased until a fairly constant level was reached before the feeding of DDT-treated hay was discontinued. Calculation of the average amount of DDT present in the milk throughout the period of feeding of the DDT-treated hay shows that the amount in the milk was dependent upon the amount applied to the hay (1 pound of DDT applied, 1.7 p.p.m. in the milk; 2 pounds of DDT applied, 3.3 p.p.m. in the milk; and 4 pounds applied, 7.1 p.p.m. in the milk).

One of the control cows (A 133) had a trace of DDT in her milk shortly after the feeding of the alfalfa hay was begun. Inspection of the manger of this cow revealed an opening through which it was possible for her to obtain a small amount of hay from the adjoining manger, and the attendant observed this cow obtaining such hay (treated with 2 pounds of DDT per acre). After the manger was repaired, the amount of DDT in the milk of this cow decreased, but a trace of DDT persisted even after the cow freshened several months later (see Table I).

The other cows, E 139 and Hu 106, came to the end of their lactation period after 3 to 3.5 months on experiment. After freshening there was still a trace of DDT present in

Table I. DDT in Milk of Cows Fed Alfalfa Hay Dusted with DDT (1948)

(P.p.m. of DDT)

Day	No DDT			1 Lb. DDT per Acre			2 Lb. DDT per Acre			4 Lb. DDT per Acre		
	A 133	Hu 88	Av.	Hu 39	E 139	Av.	Hu 29	Hu 106	Av.	E 180	E 199	Av.
0 (Jan. 21)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	1.4	0.7	1.0	2.2	1.7	1.9	2.2	2.2	2.2
7	0.0	0.0	0.0	0.3	0.4	0.3	0.6	0.4	0.5	1.3	0.7	1.0
11	0.0	0.0	0.0	0.5	1.8	1.1	0.7	0.6	0.6	1.6	1.5	1.5
13	0.5	0.0	0.2	2.0	1.7	1.8	2.6	3.0	2.8	8.0	5.1	6.5
16	0.6	0.0	0.3	2.2	1.5	1.8	3.8	2.9	3.3	8.1	4.4	6.2
19	0.4	0.0	0.2	1.3	1.6	1.4	1.1	1.0	1.0	5.4	4.3	4.8
22	0.3	0.0	0.1	0.5	1.1	0.8	1.1	2.2	1.6	6.1	2.8	4.4
25	0.6	0.0	0.3	2.5	0.8	1.6	..	3.0	3.0	8.7	5.2	6.9
28	0.9	0.0	0.4	1.3	2.1	1.7	3.7	2.2	2.9	9.0	6.0	7.5
31	1.1	0.0	0.5	0.8	2.4	1.6	3.9	3.3	3.6	8.7	6.5	7.6
34	1.4	0.0	0.7	2.6	2.7	2.6	4.9	3.8	4.3	12.5	7.3	9.9
37	1.2	0.0	0.6	2.2	1.8	2.0	4.4	3.6	4.0	..	7.8	7.8
40	1.2	0.0	0.6	1.9	2.2	2.0	3.8	3.5	3.6	8.1	4.4	6.2
43	0.5	0.0	0.2	2.3	2.2	2.2	2.4	2.9	2.6	6.8	5.0	5.9
50	0.5	0.0	0.2	0.6	2.1	1.3	3.4	3.3	3.3	9.8	5.0	7.4
57	0.3	0.0	0.1	2.6	1.8	2.2	3.4	2.4	2.9	10.0	7.6	8.8
64	0.3	0.0	0.1	..	2.9	2.9	4.5	..	4.5	11.5	7.3	9.4
71	0.4	0.0	0.2	1.6	3.0	2.3	4.1	3.6	3.8	9.7	7.2	8.4
78	0.3	0.0	0.1	2.7	3.8	3.2	5.5	4.5	5.0	12.5	9.0	10.7
85	0.2	0.0	0.1	1.6	2.6	2.1	4.9	3.6	4.2	11.5	8.3	9.9
92	0.2	0.0	0.1	1.9	2.7	2.3	4.5	4.5	4.5	11.3	7.1	9.2
99	0.2	0.0	0.1	1.3	Dry	1.3	4.8	4.1	4.4	11.9	6.7	9.3
106	0.2	0.0	0.1	2.6	Dry	2.6	6.3	4.3	5.3	9.6	8.0	8.8
113[a]	0.4	0.0	0.2	1.8	Dry	1.8	7.2	5.4	6.3	12.3	7.5	9.9
120	0.1	0.0	0.05	0.5	Dry	0.5	Killed	1.5	1.5	4.2	3.1	3.6
127	0.2	0.0	0.1	0.5	Dry	0.5		1.7	1.7	2.8	1.6	2.2
134	0.2	0.0	0.1	0.3	Dry	0.3		1.1	1.1	1.9	1.9	1.9
141	Dry	0.0	0.0	0.1	Dry	0.1		Dry	..	2.0	1.5	1.7
148	Dry	0.0	0.0	0.2	Dry	0.2		Dry	..	1.1	1.3	1.2
155	Dry	0.0	0.0	0.2	Dry	0.2		Dry	..	1.0	1.1	1.0
162	Dry	0.0	0.0	0.1	Dry	0.1		Dry	..	0.8	0.9	0.8
169	Dry	0.0	0.0	0.2	Dry	0.2		Dry	..	0.5	0.9	0.7
176	Dry	0.0	0.0	0.1	Dry	0.1		Dry	..	0.6	0.6	0.6
183	Dry	0.0	0.0	0.1	Dry	0.1		Dry	..	0.5	0.5	0.5
190	Fresh	0.0	0.0	0.2	Dry	0.2		Fresh	..	0.4	0.6	0.5
197	0.1	0.0	0.05	0.2	Fresh	0.2		0.4	0.4	0.4	0.6	0.5
204	0.1	0.0	0.05	0.1	0.4	0.3		0.3	0.3	0.3	0.5	0.4
211	0.1	Killed	0.1	0.05	0.5	0.3		0.2	0.2	0.4	0.2	0.3
225	0.1		0.1	0.1	0.3	0.2		0.2	0.2	0.3	..	0.3
232	0.1		0.1	0.05	0.3	0.2		0.2	0.2	0.3	0.4	0.3
239 (Sept. 16)	0.1		0.1	0.05	0.2	0.1		0.3	0.3	0.3	0.4	0.3

[a] May 13, cows off hay, to pasture.

Table II. DDT in Milk of Cows Fed Alfalfa Hay Dusted with DDT (1948–49)

(P.p.m. of DDT)

Day	No DDT			0.5 Lb. DDT per Acre			1 Lb. DDT per Acre			2 Lb. DDT per Acre		
	Hu 85	Hu 329	Av.	E 181	Hu 83	Av.	Hu 36	E 139[a]	Av.	Hu 106[b]	A 133[c]	Av.
0 (Nov. 24)	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0.1	0.05	0.2	0.1	0.2	0.3	0.2	0.3
6	0	0	0	0.7	0.8	0.8	1.1	1.5	1.3	1.9	1.6	1.8
10	0	0	0	0.3	0.8	0.6	0.8	1.3	1.1	1.0	2.6	1.8
13	0	0	0	0.4	0.5	0.5	0.5	0.7	0.6	1.3	1.0	1.2
16	0	0	0	0.8	1.1	1.0	1.6	1.6	1.6	2.1	2.6	2.4
19	0	0	0	0.6	0.6	0.6	1.5	1.3	1.4	2.1	2.7	2.4
22	0	0	0	0.3	0.4	0.4	0.9	0.7	0.8	1.1	1.2	1.2
25	0	0	0	0.3	0.4	0.4	0.4	1.2	0.8	1.5	2.1	1.8
33	0	0	0	0.6	0.3	0.5	1.1	1.1	1.1	1.7	1.5	1.6
40	0	0	0	0.6	0.6	0.6	0.4	0.4	0.4	1.7	1.9	1.8
47	0	0	0	0.7	0.6	0.7	1.3	1.2	1.3	1.5	2.9	2.2
54	0	0	0	0.8	1.3	1.1	2.1	2.3	2.2	2.0	2.7	2.4
61	0	0	0	1.3	1.5	1.4	1.9	..	1.9	4.0	3.7	3.9
69	0	0	0	1.4	2.2	1.8	2.6	3.1	2.9	3.5	5.6	4.6
75	0	0	0	1.4	0.9	1.2	2.4	1.6	2.0	2.5	5.4	4.0
81 (Feb. 13)	Last day that DDT-treated hay was fed											
82	0	0	0	0.5	..	0.5	2.0	2.3	2.2	2.1	2.8	2.4
91	0	0	0	0.3	..	0.3	0.6	0.5	0.6	1.4	1.5	1.5
96	0	0	0	0.2	..	0.2	0.8	0.3	0.6	0.2	0.6	0.4
103	0	0	0	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.9	0.6
110	0	0	0	0.1	0.2	0.2	0.1	0.2	0.2	0.5	1.2	0.9
131	0	0	0	0.2	0.2	0.2	0.3	0.2	0.3	0.5	1.4	1.0
145	0	0	0	Dry	0.4	0.4	0.4	0.2	0.2	0.5	1.2	0.9
161	0	0	0	Dry	0.2	0.2	Killed	0.0	0.0	0.2	0.5	0.4
180	0	Killed	0	Dry	0.1	0.1		0.1	0.1	0.1	Dry	0.1
209 (June 21)	0		0	Dry	0.1	0.1		0.1	0.1	0.2	Dry	0.2

[a] Cow received hay dusted with 1 lb. DDT per acre during 1948 (Table I).
[b] Cow received hay dusted with 2 lb. DDT per acre during 1948 (Table I).
[c] Cow received hay dusted with no DDT during 1948 (Table I).

the milk of each 4 to 4.5 months later. This would seem to indicate that the DDT is released into the blood stream from the storage sites at a slow rate. This is further indicated by the presence of traces of DDT in the milk of all the cows that consumed DDT-treated hay for more than 4 months after the ingestion of the hay was discontinued. Cows A 133, Hu 106, and E 139 were all used during the second year's feeding trials. Reference to Table II shows that at the beginning of the feeding period (November 24, 1948) there was no DDT present in the milk of any of these. Thus, the DDT had disappeared completely from the milk within approximately 6.5 months after the feeding of the DDT-treated hay was discontinued.

The amount of DDT present in the milk of the cows during the second winter (1948–49) is given in Table II and Figure 2. Three of the cows used the previous winter (A 133, Hu 106, and E 139) were used again; the remaining cows were new to this project. The results obtained are essentially like those of the previous winter. During the second winter hay treated with 0.5 pound of DDT per acre was substituted for hay treated with 4 pounds. Otherwise the experimental procedure was the same as during the first winter.

As before, a greater quantity of DDT was found in the milk of the cows that consumed the hay treated with the higher levels of DDT. Calculation of the average amount of DDT present in the milk during the feeding period shows a close agreement in the amount of DDT present during the two years, where it is possible to make such a comparison. For example, the average amount of DDT present in the milk of the cows consuming hay treated with 1 pound of DDT per acre during 1948 was 1.7 p.p.m.; in 1948–49, 1.3 p.p.m. Following the 2-pound treatment in 1948, 3.3 p.p.m. of DDT were present in the milk, whereas in 1948–49 2.2 p.p.m. of DDT were present. The average amount of DDT present in the milk of the cows consuming hay treated with 0.5 pound of DDT per acre during the feeding period was 0.7 p.p.m. Considering all the possible sources of variation in the experiments, the agreement in the data from the two years seems fairly good.

The amount of DDT residue present on the alfalfa hay fed to the dairy cows during the two years is given in Table III. A greater DDT residue was present on the hay the second year than during the first. A possible explanation of this difference is that during the harvest in the summer of 1947 the hay was rained on several times after it was cut and before it was dry enough to bale, whereas in the summer of 1948 the hay was not rained on between cutting and baling. Whether this difference in the curing and handling of the hay is the only reason for the difference in DDT residue is not known. The method of application and calibration of the power duster and calculation of the dosage used each year was the same. The details of these procedures will be published elsewhere.

Table III. Average DDT Residue on Alfalfa Hay Fed to Dairy Cows and Average DDT Appearing in Milk during Period of Feeding

DDT Treatment, Lb./Acre	1947–48		1948–49	
	DDT residue, p.p.m.	DDT in milk, p.p.m.	DDT residue, p.p.m.	DDT in milk, p.p.m.
None	0	0.05[a]	0	0
0.5	..	..	9.0	0.7
1	9.6	1.7	19.2	1.3
2	12.1	3.3	30.0	2.2
4	36.0	7.1	..	..

[a] DDT-treated hay obtained from adjoining manger.

The hay used during the first winter was analyzed by both the colorimetric and total chloride methods (*8*, *9*, *12*). The results obtained with the total chloride method were less variable than those obtained with the colorimetric; consequently, the former method was adopted. In one third of the samples, the values obtained with the two methods were the same. In the remaining two thirds of the samples, the values with the colorimetric method were approximately 20% below those of the total chloride. However, analysis of the control samples by both methods revealed the absence of DDT in the untreated hay, thus indicating that organic compounds containing chlorine were absent in the untreated hay.

Analysis of the blood of the cows for DDT before the feeding of DDT-treated hay was

begun both years showed that no DDT was present. After the DDT-treated hay had been fed for 113 and 81 days, analysis showed an average of 0.1, 0.1, and 0.2 p.p.m. of DDT in the blood of cows consuming hay treated with 1, 2, and 4 pounds of DDT per acre, respectively. These trace amounts of DDT in the blood were found at the same time the DDT concentration was highest in the milk.

Three of the cows used were slaughtered at the end of the feeding period, and a fourth was killed 4 months later. The amount of DDT present in various tissues at these times is presented in Table IV. It is evident that large amounts of DDT are present in the fatty tissues, whereas there is little present in muscle, liver, or kidney. Apparently its presence in the fat, and the slow release into the blood stream from these storage sites, account for the persistence of the DDT in the milk for long periods of time after the feeding of the DDT-treated hay was discontinued. Cow E 139 still had 3 p.p.m. of DDT in her fat 4 months after the feeding of the DDT-treated hay was discontinued. It was not possible to obtain a milk sample at this time, for the cow had ceased lactating some time before she was slaughtered.

Table IV. DDT in Tissues of Cows at End of Feeding Period

Cow	DDT Added to Hay Consumed, Lb./Acre	No. of Days Fed Hay	DDT, P.P.M. Mesenteric fat	Kidney fat	Muscle	Liver	Kidney
Hu 329	0	81	0	0	0	0	0
Hu 36	1	81	21.4	19.3	0.4	0.2	0.1
Hu 29	2	113	89	90	1.3	1.1	..
E 139[a]	1	81	3	3	0.1	0	0

[a] Slaughtered approximately 4 months after feeding of DDT-treated hay was discontinued.

Data showing daily feed consumption and milk and butterfat production of the cows for the two years are given in Table V. During the period of feeding, there was no evidence that the cows receiving DDT-treated hay were injured in any way, or that their milk production was affected. Apparently the amount of DDT ingested daily and the release of DDT from the fatty tissues did not provide sufficient DDT in the blood stream to affect feed consumption or milk production.

Table V. Average Daily Feed Consumption and Milk and Butterfat Production of Dairy Cows

DDT Added to Hay, Lb./Acre	Cows	No. of Days Fed	Hay Consumed, Lb.	Grain Consumed, Lb.	Milk Produced, Lb.	Butterfat Produced Lb.	Butterfat Produced %
			1947–48				
0	A 133	113	32.2	4.9	25.3	0.86	3.4
	Hu 88	113	33.2	5.5	31.7	1.04	3.3
1	Hu 39	113	33.4	7.0	33.2	1.25	3.7
	E 139	113	29.6	0.6	11.6	0.39	3.4
2	Hu 29	113	32.9	4.5	20.2	0.87	4.3
	Hu 106	113	30.9	5.0	26.0	0.96	3.7
4	E 180	113	23.2	5.8	26.4	0.79	3.0
	E 199	113	36.2	7.7	37.1	1.37	3.7
			1948–49				
0	Hu 329	81	44.8	4.8	25.2	0.84	3.3
	Hu 85	81	40.7	4.6	24.3	0.81	3.3
0.5	Hu 83	81	40.1	4.1	19.2	0.72	3.8
	E 181	81	41.0	4.7	22.5	0.95	4.2
1	Hu 36	81	40.6	5.3	29.5	0.96	3.3
	E 139	81	45.4	7.3	42.6	0.36	3.2
2	Hu 106	81	41.2	6.9	36.6	1.27	3.5
	A 133	81	43.9	7.4	35.6	1.22	3.4

Three cows calved after the feeding of the hay was discontinued (Table I). The pregnancy of the cows was apparently normal and there were no abnormalities in any of the calves. This indicated that the level of DDT was not sufficiently high to affect the cows or calves during the period of gestation.

The results of these experiments show that DDT accumulates in the fatty tissues of cows, and that it is present in the milk in varying amounts, the amount being dependent

upon the quantity of DDT ingested. Furthermore, sufficient residue remains on alfalfa hay after it has been dusted with the amount of DDT recommended to control insects, to cause substantial quantities of DDT to appear in the milk and fat. Smaller amounts of DDT persist in the fat and milk for at least 4.5 months after the ingestion of the DDT-treated hay has been discontinued. The possible toxicity of such milk and fat to humans would seem to be dependent upon a number of factors, among which are the amount of DDT applied to the forage, the frequency and total length of time during which the treated hay is ingested by the cow, the amount and frequency of consumption of such milk and fat by humans, and possibly the age of the humans consuming these products.

Summary

Alfalfa hay that had been dusted with varying amounts of technical DDT for insect control and similar untreated hay were harvested and fed to 16 Holstein dairy cows during two years.

DDT appeared in the milk promptly after the cows consumed the DDT-treated hay. The concentration gradually increased until maximum amounts of 2.2, 3.8, 7.2, and 12.5 p.p.m. of DDT were obtained in the milk of cows consuming hay dusted with 0.5, 1, 2, and 4 pounds, respectively, of DDT per acre. The average DDT residue on the hay varied from 9 to 36 p.p.m., there being a greater residue on the hay for a given treatment the second year than there was the first.

DDT persisted in the milk for 4 months after the feeding of the DDT-treated hay was discontinued. It disappeared approximately 6.5 months after discontinuance of the feeding of treated hay. Four cows had DDT in their milk at the end of their lactation period, and after calving traces of DDT were still present in their milk.

The blood was analyzed for DDT before the cows were placed on the DDT-treated hay and again before the hay was discontinued. No DDT was found before and a maximum of 0.2 p.p.m. at the close of the feeding period.

Four cows were slaughtered and the tissues were analyzed for DDT. The analysis showed a maximum of 89 p.p.m. of DDT in mesenteric fat, 90 p.p.m. in kidney fat, 1.1 p.p.m. in the liver, 1.3 p.p.m. in muscle, and 0.1 p.p.m. in kidney tissues. The greatest amount of DDT was present in the tissues of cows consuming the hay dusted with the higher levels of DDT.

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2,4-Dichlorophenoxyacetic Acid (2,4-D) as a Selective Herbicide

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In view of the great agricultural importance today of the synthetic plant growth substances in controlling the growth of plants of economic importance, details of the history of this development of investigations on plant hormones are presented.

During 1941 and 1942, two groups of workers in England independently investigated the phytocidal effects of a number of plant growth substances, including 2,4-dichlorophenoxyacetic acid. Nutman, Thornton, and Quastel (*27*), working at the Rothamsted Experimental Station on the problem of whether root hair deformation can be induced by 2-indolylacetic acid and 1-naphthaleneacetic acid, found that these substances not only produced marked root hair deformation in agar cultures of red clover but were highly toxic at a dilution of 1 in 10^7. The high toxicities of these substances suggested the possibility that such compounds might be used to control plant growth. Accordingly, experiments were made to determine whether toxicity would take place under soil conditions. It was found that the toxicity of 2-indolylacetic acid was retained in sterile soil but was lost in normal soil, the growth substance obviously being broken down by soil microorganisms.

It had been known for some time that 2-naphthoxyacetic acid is a plant growth regulator producing abnormal formative effects on leaves, stems, flowers, and fruit (*40*). It had already been mentioned as an active substance in this respect in 1938 (*16*) and later investigations (*6*, *7*, *41*, *44*) provided further details of its activity. Further studies by Zimmerman and Hitchock (*42*) showed that halogenated phenoxyacetic acids have marked physiological activity in plant growth, inducing formative effects. They noted that 2,4-dichlorophenoxyacetic acid was among the most active in influencing plant growth, inducing cell elongation of the tomato with concentrations as low as 0.0007% in lanolin.

In view of these activities of 2-naphthoxyacetic acid and of the halogenated phenoxyacetic acids, and the likelihood that the chlorinated molecules would be relatively resistant to attack by bacteria in the soil, investigations (*27*) were made of the toxicity of 2,4-dichlorophenoxyacetic acid and other substances to plant growth in agar culture and in normal soil. Tests made in October 1942 with red clover showed that 2,4-dichlorophenoxyacetic acid was much the most toxic of the compounds tested and, unlike the other substances, retained its toxicity to a satisfactory extent in a normal soil. It was then found that the lethal effect of 2,4-dichlorophenoxyacetic acid was selective—for example, in Woburn soil, the substance was strongly toxic to clover and especially to sugar beets, even at a concentration of 1 p.p.m. Wheat, however, was not appreciably affected at this concentration, though marked toxicity appeared at 10 p.p.m. In a Rothamsted soil, toxicity toward clover and wheat was less marked, although the action on sugar beets was again very striking. Investigations were made of the persistence of the toxicity of 2,4-dichlorophenoxyacetic acid in unsterilized soils, both on storage and after leaching. It was found that there is persistence in normal soils, sufficient to produce marked toxic

effects, at a concentration of 1 p.p.m. of soil solution, equivalent to 1 part in 4,000,000 of soil at 25% moisture content. This represents, on a field scale, about 0.5 pound of 2,4-D per acre of soil to 6 inches depth. The results showed that 2,4-dichlorophenoxyacetic acid has the properties of a differential plant poison under soil conditions, and indicated its possible importance as a controller of plant growth in agricultural practice. A report of the differential toxicity of 2,4-dichlorophenoxyacetic acid was first made to the secretary of the Agricultural Research Council on November 17, 1942. Publication (*27*) was not allowed until 1945.

A second group of investigators, Slade, Templeman, and Sexton, working at the Jealott's Hill Research Station, commenced their studies of the effects of growth substances on plants in 1936. Templeman (*31*) had observed that high concentrations of the growth substances depressed seedling growth. Templeman and Marmoy (*33*) found in 1940 that application of 1-naphthylacetic acid at the rate of 25 pounds per acre to oats weedy with yellow charlock killed the charlock. The oats received only a slight setback and recovered fully. The effects of known plant growth substances were then surveyed. Substituted phenoxyacetic acids and naphthoxyacetic acids were found to be outstanding as plant inhibitors and certain of them were found to be fifty times as effective as 1-naphthaleneacetic acid. Further experiments showed that 4-chloro-2-methylphenoxyacetic acid was one of the most active compounds tested and that it readily depressed the germination and early seedling growth of the corn buttercup, corn marigold, corn spurrey, and field poppy at concentrations that were without effect on cereals. 2,4-Dichlorophenoxyacetic acid was among the compounds found to be more active than 1-naphthylacetic acid. Slade *et al.* communicated the substance of their results in November 1942 to the secretary of the Agricultural Research Council, but publication was not allowed until 1945 (*7*). After November 1942, joint experiments in the field were carried out by the two groups of workers mentioned, in cooperation with G. E. Blackman. In 1943, a field trial showed that sodium 4-chloro-2-methylphenoxyacetate applied at the rate of 1 pound per acre gave 100% eradication of yellow charlock in spring oats without damaging the cereal and equally good results were obtained if application was made when the weeds were small or in full flower (*30*). Later field experiments in 1944 confirmed and extended these results. Blackman (*8*), working with a research team at the Imperial College of Science, London, subsequently found that the phenoxyacetic acid derivatives, among a variety of substances, alone showed promise for the control of perennial weeds.

Herbicidal Action

Herbicidal effects of 1-naphthylacetic acid (reduction of seed germination) were apparently noted by Templeman and Sexton (*34*) in October 1940, when a differential effect on the germination of yellow charlock and oats was observed. Field experiments on the herbicidal action of 2,4-dichlorophenoxyacetic acid were carried out in England in April 1943 (*8*), though its differential effects on plant growth were known to the English investigators in 1942.

So far as the author is aware, the first statement of the herbicidal action of 2,4-dichlorophenoxyacetic acid, made in the United States, was in a publication by Hamner and Tukey (*14*) in 1944. They sprayed 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on bindweed and obtained a complete kill down to the root tips. At about the same time Hamner and Tukey (*13*) and Marth and Mitchell (*24*) demonstrated the effect of 2,4-dichlorophenoxyacetic acid as a differential herbicide on lawns. All plants growing on the lawns (except the grasses) were destroyed with apparently no noticeable ill effect on the grasses.

Templeman and Sexton, besides describing the effects of substances of the type aryl-OCH_2COOH on seed germination and growth (*34*), have pointed out the remarkable inhibitive effects of arylurethanes—e.g., isopropyl phenylcarbamate—and related compounds in very low concentrations on the germination of seedling growth of cereals (*35*). Lefevre (*23*) in 1939 had already described the physiological effects of phenylurethane on wheat seedlings and considered the effects to be similar to those described for colchicine.

The latter substance, as is well known, interferes with nuclear division in plants. Templeman and Sexton (*35*) showed that, contrary to the effects of the phenoxyacetic acids, the arylurethanes destroy cereals more readily than the dicotyledonous plants.

In America, early in 1944, a project centered at Camp Detrick, Md., was initiated for the study of the inhibitory activities of synthetic growth-regulating substances (*36*). By August 1945 nearly 1100 substances had been tested. At the research center at Camp Detrick 1060 organic compounds were examined for their powers of selective plant inhibition and more than 100 of the substances were found to have high growth-regulating activities. Reference may be made (*11*) to the series of papers from Camp Detrick for much valuable information on the effects of growth-regulating substances, especially 2,4-dichlorophenoxyacetic acid, on plant growth, on the persistence of these substances in soil, on the remarkable specific toxicity of 2,4,5-trichlorophenoxyacetic acid toward Irish potatoes, and on the action of isopropylurethane on plants.

Kraus and Mitchell have published an interesting paper (*20*) on the effects of a variety of substances including 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on plant growth; their experiments were carried out in 1943.

A very large literature on the herbicidal effects of 2,4-dichlorophenoxyacetic acid has now developed and excellent reviews have appeared. Allard, De Rose, and Swanson (*1*), for example, have found 2,4-dichlorophenoxyacetic acid to inhibit germination of a number of species including kidney beans, wheat, and oats. Klingman (*18*) reports eradication of dandelion by the same substance without harm to bluegrass. Kephart (*17*) describes the use of 2,4-dichlorophenoxyacetic acid in killing dandelions, plantains, and other weeds in turf and indicates the promise shown for destruction of perennial weeds such as bindweed and Canadian thistle (see *9*, *25*, *29*, *32*, *37*, for further information on the weed-killing properties of the dichloro- and chloromethylphenoxyacetic acids).

Effects of Soil Conditions

Whether or not a given plant growth substance having toxic effects on plant growth will withstand soil conditions for 3 or 4 weeks is of decisive importance in determining its importance in agricultural practice. The toxicity of 2,4-dichlorophenoxyacetic acid on the growth of a variety of plants is not appreciably greater than that of 2-indolylacetic acid; yet the former is of great agricultural value and the latter of none. In fact, resistance to attack by microorganisms in the field must be regarded as an indispensable property of a herbicide which is to be used in agricultural practice. The results of experiments carried out in water culture, sand, or sterile soils, while of great theoretical value, are of little practical importance until they have been assessed in the field. Nutman, Thornton, and Quastel realized this in 1942 and their choice of 2,4-dichlorophenoxyacetic acid as a selective plant growth regulator was largely dependent on their observation of the relative resistance of 2,4-dichlorophenoxyacetic acid to attack in the soil. On the other hand, it is important that the toxicity of the herbicide in the field should not be lasting or cumulative; otherwise a general poisoning of the soil would ensue which would be detrimental to crop yield.

Much work has been carried out recently on the velocity of detoxication of 2,4-dichlorophenoxyacetic acid under a variety of soil conditions—e.g., high soil moisture content, high temperatures, high pH, and high organic matter content (*10*, *12*, *15*, *21*, *26*).

Lees and Quastel (*22*) in 1946, using their soil perfusion technique, found that when soil is exposed to a compound, which is gradually metabolized, a process of enrichment (of microorganisms) in the soil takes place, whereby the soil becomes more and more active in breaking down the compound in question. Ultimately a point of saturation occurs and at this point the rate of breakdown of the substance becomes maximal and is constant. Lees and Quastel and more recently Quastel and Scholefield (*28*) showed that in a biological process in soil, the time progress curve of the process follows a characteristic pattern, showing an initial lag period succeeded by an exponential rise, both significant of the rate of proliferation of microorganisms. After the initial stages a steady maximum rate of breakdown is reached, this being due to "saturation" of the soil with the organisms involved.

Audus (*3*) has followed the disappearance of 2,4-dichlorophenoxyacetic acid in soil using the soil perfusion technique. He found, with initial concentrations of 10 p.p.m., little or no alteration of the concentration of 2,4-dichlorophenoxyacetic acid in the soil at 70° F. for at least 10 days. Then there commences a rapid rate of breakdown of 2,4-dichlorophenoxyacetic acid typical of bacterial development, and in the 4 subsequent days all the 2,4-dichlorophenoxyacetic acid disappears. The soil at this point is enriched with organisms attacking the 2,4-dichlorophenoxyacetic acid, for if it is now perfused with another lot of 2,4-dichlorophenoxyacetic acid at the same concentration, this is completely broken down in 2 days. Moreover, if this is further perfused with a concentration of 2,4-dichlorophenoxyacetic acid ten times higher than the original perfusing concentration, all the 2,4-dichlorophenoxyacetic acid disappears within 3 or 4 days. Higher initial concentrations of 2,4-dichlorophenoxyacetic acid give longer initial lags but ultimately the substance is fully metabolized. The soil becomes highly enriched with organisms capable of rapid destruction of the herbicide.

These results show that the 2,4-dichlorophenoxyacetic acid can remain stable, and exert its herbicidal effects, only during the initial lag phase before the rapid logarithmic phase of destruction takes place. This initial lag phase depends upon the previous treatment of the soil, particularly on whether it has already been exposed to 2,4-dichlorophenoxyacetic acid. Clearly, once a soil is enriched with 2,4-dichlorophenoxyacetic acid destroying organisms, the herbicide, if added to the soil, will have little or no value in the control of plants growing there.

Reversibility of Action of 2,4-Dichlorophenoxyacetic Acid

It is essential for any understanding of the mechanism of action of the plant growth substances to know if their inhibitive effects can be reversed. If these compounds have toxic effects because they combine irreversibly with cell constituents, their effects will be irreversible, and washing the plant tissue to remove the toxic agent will not result in renewed growth of the plant. On the other hand, if these agents act by forming easily dissociated compounds with tissue constituents, whether they are enzymes or metabolites, washing the plant tissue to remove the toxic agent should result in renewed growth of the plant, so long as autolytic processes have not advanced too far during the period in which growth has not taken place.

Audus and Quastel (*2*, *5*) have shown that with both 2,4-dichlorophenoxyacetic acid and another selective herbicide, coumarin, reversibility does take place. When a seedling, for example, has ceased to grow in coumarin solution for periods up to 2 or even 3 days, recovery can take place when the plant is transferred to a fresh nutrient solution. It is scarcely to be expected that the recovery will be more than partial, first, because it is unlikely that washing, however good, will remove all the toxic agent, and second, because, during the period in which growth is not occurring, autolytic processes must be taking place to the detriment of the subsequent growth. It has been found (*2*, *5*) that recoveries from the inhibitive effects of coumarin and 2,4-dichlorophenoxyacetic acid are more complete with certain plants than with others. The inhibitions of both germination and root growth of cress seeds treated with coumarin may be reversed by washing, or transference to fresh nutrient, so long as exposure is not too great.

The facts indicate that the plant growth substances must effect their inhibitions by forming loose combinations, or easily dissociated compounds, with enzymes or metabolites in the plant cell.

Action of *p*-Aminobenzoic Acid

Audus and Quastel (*4*) have found that the presence of *p*-aminobenzoic acid exerts an antagonism, though a relatively feeble one, to the root growth inhibitory actions of 2,4-dichlorophenoxyacetic acid, 2-chloro-4-methylphenoxyacetic acid, 2-naphthoxyacetic acid, and 2-indoleacetic acid. For any marked degree of antagonism the concentration ratio of *p*-aminobenzoic acid to the growth substance must be of the order of 10,000. This relatively feeble action is in marked contrast to the effect of *p*-aminobenzoic acid on the

sulfonamide inhibition of bacterial growth. The physical conditions in the plant, however—e.g., pH, cation concentrations, permeability factors, etc.—may be so very different from those in the bacteria that the observed effect of aminobenzoic acid may be of real significance and helpful in elucidating the mechanism of action of 2,4-dichlorophenoxyacetic acid. It is felt, nowadays, so far as bacteria are concerned, that the function of *p*-aminobenzoic acid lies in its acting as a precursor of folic acid and that the sulfonamides act by inhibiting this synthesis. It is possible also that 2,4-dichlorophenoxyacetic acid may act by inhibiting the formation in the root cells of a metabolite essential for normal development.

Chemical Constitution and Herbicidal Effects

The results of the investigation of the effects on plant growth of a large number of organic substances, by Koepfli, Thimann, and Went (*19*) and Zimmerman and his colleagues (*39*, *43*, *44*), seem to indicate that certain minimum requirements must be met to produce an active compound. It (19, *38*) is suggested that they are as follows: (1) a ring system or nucleus; (2) in this ring a double bond; (3) a side chain; (4) in the side chain a carboxyl group (or group that can be readily converted into a carboxlyl group); and (5) a particular space relationship between the ring systems and the carboxyl group.

Veldstra (*38*) has made a very useful summary of the physiological properties of a large variety of organic substances and develops the view that "the action of the growth substance takes place in a boundary and that the ring system determines the degree of adsorption to that boundary, the carboxyl group performing the physiological function proper (influencing of the boundary potential) for which a definitely peripheral position of the group is required."

Conclusions

Although requirements such as those mentioned may be desirable or even essential for an active plant growth regulator, the mechanism of action of the substance in the cell is still unknown. The suggestion that certain of these active molecules may exercise their effects by combination with important thiol enzymes may be most fruitful.

However, such requirements in constitution, while important for the production of a plant-growth regulator, may be inadequate for the formation of a herbicide working under field conditions. Indoleacetic acid and coumarin are both plant metabolites of high potency as plant growth regulators, but they are of little value as herbicides. The substances must be so constituted as to withstand immediate attack by common soil organisms and yet so made up that they will gradually undergo breakdown either spontaneously or by stimulating the formation in soil of organisms that can attack them. The possession of chlorine atoms in the nucleus of 2,4-dichlorophenoxyacetic acid makes this substance relatively resistant to bacterial attack and it is this property that helps to make it so valuable. Investigations of the manner in which molecules, easily broken down in the soil, can be made more resistant to attack by soil organisms are of the greatest importance in the study of herbicides. The employment of a soil perfusion technique is of considerable value in work of this description. Further work on these lines should help greatly to classify and extend our knowledge of the relations between chemical constitution and herbicidal effects in the field.

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Metabolic Products of Elemental Sulfur 35 Applied to Lemons as an Insecticide

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Interference with conduction or radiation of heat from leaves or fruit during insolation increases the temperature of the plant part; linear temperature increases increase the rate of volatilization of elemental sulfur logarithmically; hydrogen sulfide production of sulfur-dusted lemons increases nearly linearly; and sulfur dioxide production of sulfur-dusted lemons increases linearly with increase in temperature. The sulfur content of the cell solution of the peel of sulfur-dusted lemons when determined as sulfate was shown to correlate with the increase in sulfur of "sulfur-burned" sides of peel of lemons injured on the tree. Using radioactive elemental sulfur, hydrogen sulfide, sulfur dioxide, and sulfuric acid to treat lemons incubated at insolation temperatures (41° to 44° C.) it was shown that radioactive hydrogen sulfide and/or sulfur dioxide was produced, that the percentage of sulfur determined as sulfate in lemon peel increased, and that the pH of water extracts of the peel decreased. The specific activities of the acid-soluble peel proteins were relatively high, whereas the specific activities of the alkali-soluble proteins were relatively low. There appeared to be a destruction of proteins in some of the treatments.

The drastic effect of high temperature and elemental sulfur on higher animals has been appreciated since biblical times. But the lethal effect of this combination on higher plants does not seem to have been recorded until the nineteenth century. Little work appears to have been done on the physiology of the injury to higher plants though the low cost of sulfur, its effectiveness in killing certain pests, and the increased toxicity of compatible pesticides in which it is used as a diluent still make its use desirable in spite of newer insecticides. The principal shortcoming of sulfur—injury to the plant during hot weather—perhaps may be overcome when the mechanism involved is understood. This paper discusses experiments that elucidate the mechanism in some respects.

The groundwork on the toxicity of sulfur to lower plants was laid in this country by McCallan and Wilcoxon, Liming, and Young and Williams. These studies and those of European investigators have been reviewed well by Horsfall (*4*) and by Frear (*2*).

In 1875 Pollacci (*7*) reported grape leaves treated with sulfur produced hydrogen sulfide gas. Recently it has been reported that hydrogen sulfide gas is produced by sulfur-dusted lemons and oranges (*22*). This gas which emanates from sulfured plants is also produced by the reactions of ingredients in the widely used lime-sulfur sprays. St. John and Groves (*9*) have excellently reviewed the chemistry of these mixtures.

Thomas and Hendricks (*13*) found that sulfur dioxide was produced by mild alkaline hydrolysis of plant tissues, and it has been subsequently reported that sulfur dioxide is produced by lemons treated with elemental sulfur (*19*).

Atmospheric oxygen appears to accelerate hydrogen sulfide and sulfur dioxide formation by sulfur-dusted lemons. An experiment in which lemons were incubated in a bottle which was opened hourly during the day yielded more hydrogen sulfide and more sulfur dioxide than when the bottle was kept closed at night, though the entire experiment was conducted in the dark (*18*).

Thomas and Hill (*16*) showed that sulfur dioxide fumigation of alfalfa increased the sulfate content of the leaves. In later work Thomas *et al.* (*14*) reported that the pH of alfalfa leaf tissue fluids was lowered 0.3 pH unit after fumigation with sulfur dioxide. Similar responses occur when lemons are incubated in sulfur dioxide gas, or when they are treated with elemental sulfur or with hydrogen sulfide (*18*). Likewise lemons treated with dilute sulfuric acid gave similar responses.

If a section cut through a small sulfur-burned area of a lemon injured on the tree is examined microscopically, coagulation of protoplasm and cell collapse are apparent. Also, the injured tissue stains abnormally dark with safranin indicating the protoplasm has become more acidic than in normal tissue (*18*). Sides of the peel of lemons burned by sulfur on the tree were found to be higher in total sulfate than were uninjured sides of the same peel. The high total sulfate content of the peel was subsequently found to be due in part to soluble sulfate, as shown by analyses of the expressed cell solution (*18*).

Small increases in temperature cause the volatility of elemental ground sulfur to increase greatly. One report shows that volatility increased from 0.15, at 24° C., to 10.00 mg. per square cm. per 24 hours, at 48° C., or the amount of sulfur volatilized increased 66.6 times though the temperature only doubled (*20*). Thus it may be surmised that the first effect of high temperature in sulfur injury is the increased amount of sulfur vapor available to the fruit. Such a view appears to be fortified by the observation that hydrogen sulfide and sulfur dioxide gases were produced in increasing amounts when sulfur-dusted fruit were incubated at increasing temperatures (*18*).

Under natural growing conditions, only lemon fruit and leaves that are exposed to direct sunlight are injured by sulfur dust. The question then arises as to what factors raise the temperature of the plant parts sufficiently to cause volatilization of sulfur and injury at one time and not at another (*18*).

In desert areas of southern California fruit are often injured but leaves are seldom injured by sulfur dust. In coastal areas fruit burn is less marked but "leaf burn" may be acute. Where the air-vapor density is high, leaf temperatures in the sun may sometimes become higher than fruit temperatures. The leaf, a better absorber of radiation and a better radiator than the fruit, has a higher surface-mass ratio and appears to be very sensitive to the "heat trap" effect of high vapor density; its temperature changes with great rapidity, but fruit temperature may lag until the danger period is passed (*18*).

Effect of Temperature

Air temperature and vapor density are two factors which influence the rate of dissipation of radiant energy received by plants from the sun, and they determine in a large measure the temperature of the plant part and consequently sunburn and sulfur burn (*18*), other factors, such as particle size of the sulfur, being constant.

The temperature of that portion of the peel of a lemon in direct sunlight is always warmer than the air, and usually warmer than leaves, providing that the vapor density of the air is moderate to low. Peel temperatures measured in direct sunlight reached 127.7° F. when the air temperature registered 104.7° F., a difference of 23° F. This suggests that during a warm summer day when air temperatures in the shade reach 118° F., peel temperatures of 141° F. might be expected. Such a temperature would probably result in sunburn even though the fruit were not dusted with sulfur, but if dusted it would certainly result in sulfur burn (*18*); sulfur burn occurs at about 10° F. lower temperature than sunburn (*18*).

The work described here was undertaken to determine what happens to the sulfur that is volatilized, and what is the source of the hydrogen sulfide, sulfur dioxide, and sulfate. The products formed by lemons treated with elemental sulfur were employed in radioactive form for the treatment of other lemons.

Methods

Mature yellow lemons were picked freshly from trees growing at the Citrus Experiment Station at Riverside prior to a given treatment, which resulted in pickings at various times over a year's period. Eureka lemons were used in the treatments with elemental sulfur* and with sulfuric* acid. (Asterisks are employed to indicate radioactive atoms.) Lisbon lemons were used in the treatment with hydrogen sulfide* and a mixture of both was used in the treatment with sulfur* dioxide. Pickings were made on April 27, 1948, and January 1, April 7, and May 10, 1949, for the various treatments.

Twenty-eight whole fruit, after dusting with elemental radioactive sulfur or after immersion in 0.1 *N* (pH 1.14) radioactive $H_2S^*O_4$ for 3 hours, were placed in large glass bottles connected to two gas absorption trains consisting of two smaller glass bottles on either side. Ground-glass joints were used throughout the apparatus. The first small bottle in each train contained a phosphate-borate buffer to absorb sulfur dioxide, and the second small bottle in each train contained 2% zinc acetate solution for absorbing hydrogen sulfide (*13*). The gases were drawn off as formed, by applying a vacuum on one side of the gas absorption train. When the radioactive gases (S^*O_2 and H_2S^*) were applied, a closed system was employed in which one gas train was closed off, while the other gas train was replaced by a mercury manometer to allow for the accumulation and expansion of vapors during incubation of the fruit. At the end of this period the manometer was replaced by a gas absorption train and the gases were drawn off. Constant incubation temperature was maintained by placing the large bottles in a water bath kept at temperatures similar to the more moderate temperatures that the fruit would have attained outdoors in the sun on the tree. Long incubation periods were employed (40 to 80 hours) in order to permit the reactions to reach as near completion as possible. Controls consisted of 28 nontreated fruit which were incubated only, and 28 nontreated, nonincubated fruit.

Table I. Constants in Treatments of Lemons with Sulfur^{35} and Sulfur^{35} Acids

Starting Compound	Sp. A. of Starting Compounds, Counts/Sec./Mg.	Temp. of Experiments, ° F	Duration of Experiments, Hours	Fruit Weight, Grams	Fruit Surface, Sq. Cm.	Gas Pressure, Cm. Hg
S^*	0.1107	106	68	2322	2492	1.0×10^{-5}[a]
H_2S^*	0.458	111	37	2841	2884	6.0×10^{-1}
S^*O_2	1.784	111	89	2978	3168	8.0×10^{-1}
$H_2S^*O_4$	64.7	111	43	2352	2698	1.8×10^{2}[b]

[a] Extrapolated from data given in Hodgman's Handbook of Physics and Chemistry.
[b] Calculated osmotic pressure.

All gaseous sulfur products obtained as a result of incubation of sulfur-treated fruit were oxidized with alkaline hydrogen peroxide, precipitated as barium sulfate, and counted with a thin window Geiger counter. The peel and peel proteins were oxidized with magnesium nitrate, the sulfur was precipitated as barium sulfate according to standard methods, and counted as in the case of the gaseous products. Counting data, as reported, are fully corrected.

The proteins were isolated as acid-soluble and alkali-soluble, using methods outlined by Sinclair *et al.* (*11*) and Tupper-Carey and Priestly (*17*), and were purified by three precipitations.

Measurements of pH were made with a glass electrode on 25 grams of lemon peel macerated in 100 ml. of distilled water in a Waring Blendor, and cleared by centrifugation.

The pH's of muskmelon leaf extracts were determined on mature leaves of United States Department of Agriculture strains Nos. 5 and 11353, grown at Riverside. The

pH values were obtained by the method used for lemon peel except that 25 grams of leaves were macerated with 75 ml. of distilled water. Buffer curve titrations were made with 0.01027 *N* hydrochloric acid on 25-ml. aliquots.

The constants for the various treatments with radioactive elemental sulfur and with the radioactive sulfur acids are shown in Table I.

Results

The radioactivity of the hydrogen sulfide produced by 28 lemons treated with radioactive elemental sulfur dust, 28 lemons treated with radioactive sulfur dioxide gas, and 28 lemons treated with a radioactive sulfuric acid solution is expressed as per cent specific activity (Table II), which for the purpose of this report is defined as:

$$\% \text{ specific activity (Sp. A.)} = \frac{\text{No. of counts/second of product/Mg. } BaSO_4 \text{ of product}}{\text{No. of counts/second of treating material/Mg. } BaSO_4 \text{ of treating material}} \times 100$$

Table II. H_2S Production and Activity of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated at 44° C.

Treatment	Sp. A. H_2S/Sp. A. Treatment, % (Treated and Incubated)	Total H_2S, γ/28 Fruit: Treated and incubated	Incubated only	Nonincubated
S*[a]	102.5	2,525	0	0
H_2S*	...	...	..	..
S*O_2	11.1	1,460	0	0
H_2S*O_4	4.9	93,000	0	0

[a] Incubated at 41° C.

Table II shows that 100% of the hydrogen sulfide* produced by sulfur*-dusted fruit was derived from the sulfur* applied, 11% from the sulfur* dioxide treated fruit, and 5% from the sulfuric* acid treated fruit. Thus, the higher the state of oxidation of the sulfur* applied, the more limited the production of radioactive hydrogen sulfide. No hydrogen sulfide was obtained from the controls.

The specific activity of the sulfur* dioxide was 14, 21, and 13% for the elemental sulfur*, hydrogen sulfide*, and sulfuric* acid treated fruit, respectively (Table III). Thus about the same proportion (within experimental error) of sulfur* dioxide is derived from the radioactive elemental sulfur and sulfur acids applied in the treatments.

Table III. SO_2 Production and Activity of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated at 44° C.

Treatment	Sp. A. SO_2/Sp. A. Treatment, % (Treated and Incubated)	Total SO_2, γ/28 Fruit: Treated and incubated	Incubated only	Nonincubated
S*[a]	14.4	266	0	0
H_2S*	21.0	247	0	0
S*O_2	..	..	..	..
H_2S*O_4	13.0	184	0	0

[a] Incubated at 41° C.

Table IV. SO_4 Content and Activity in Peel of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated at 44° C.

Treatment	Sp. A. SO_4 (Peel)/ Sp. A. Treatment, % (Treated and Incubated)	S Determined as SO_4 on Dry Peel, %: Treated and incubated	Incubated only	Nonincubated
S*[a]	77.1	0.0857	0.0787	0.0742
H_2S*	51.1	0.1500	0.1067	0.0987
S*O_2	5.6	0.2045	0.1027	0.0779
H_2S*O_4	..	0.1068	....	0.0666

[a] Incubated at 41° C.

In all treated fruit the percentage of sulfur determined as sulfate in peel increased over the controls (Table IV). Of the four different treatments, the sulfate having the highest percentage specific activity was where elemental sulfur* was applied. Specific activity was next highest in the hydrogen sulfide* and lowest for the sulfur* dioxide treatment.

The percentage of specific activities recovered as sulfates in the exudates in the various treatments was slightly higher due perhaps to absorption of the externally applied activity, but otherwise they roughly paralleled the activities recovered in the peel sulfates from the corresponding treatments (Table V).

Table V. SO_4 Exudation and Activity of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated ot 44° C.

Treatment	Sp. A. Exudate/ Sp. A. Treatment, % (Treated and Incubated)	SO_4, γ/Ml.		
		Treated and incubated	Incubated only	Nonincubated
S*[a]	82.7	167	309	0
H_2S*	60.0	468	..	..
S*O_2	7.5	240	21	0
H_2S*O_4	40.8	556	13	..

[a] Incubated at 41° C.

The pH values of lemon exudates and of extracts of the peels were lower in the various sulfur treatments than in the corresponding incubated or nonincubated controls (Table VI). The lower pH values of fruit treated with elemental sulfur and sulfur acids together

Table VI. pH of Exudates and Water Extracts (1 to 4) of Peel of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated at 44° C.

Treatment	Treated and Incubated		Incubated Only		Nonincubated
	Exudate, pH	Extract, pH	Exudate, pH	Extract, pH	Extract, pH
S*[a]	3.4	3.7	3.5	4.7	5.3
H_2S*	3.9	3.5	...	5.1	5.3
S*O_2	...	3.3	...	3.9	5.5
H_2S*O_4	3.3	3.8	4.5	4.6	5.0

[a] Incubated at 41° C.

with the increase in sulfate in the peel (Table IV) and that contained in the exudate (Table V) suggests that a fraction of the elemental sulfur or sulfur acids used in the treatments is converted by lemon peel to free sulfur acids, possibly sulfuric acid if it is not already in that state of oxidation. Incubation of the fruit alone, however, increases the free acid or hydrogen ion concentration. The pH is further lowered in the treatments with sulfur and sulfur acids, and ionization of the cell solution is increased as conditions favorable to sulfur injury are approached. Barron (*1*) has shown that sulfhydryl enzymes are inactivated in water solution irradiated with x-rays because of the ionization of the water. He postulated the formation of hydrogen peroxide by combination of the ionized water and the dissolved oxygen it contained. This mechanism may also function in sulfur injury since catalase was shown to be inactivated by elemental sulfur treatments (*18*) and reduced glutathione oxidized (*18*). Lemon juice protein has an isoelectric point at pH 5.6 to 5.7 (*10*) which is higher than the pH values of the cell solution of sulfur-treated fruit; lemon peel protein may also fall in this category. The buffer capacity of lemon peel is very small, however, because of its comparatively low content of organic acids and their salts (*12*) and may be expected to reflect small changes in pH.

While it is conceivable that an excess of bases in the cell solution might be protective against mild sulfur burn, this possibility has not yet been tested. On the other hand, a small increase in buffer capacity might reduce sulfur burn. An example of this effect may be seen in the buffer curves of the leaf sap in two of the United States Department of Agriculture's muskmelon varieties. No. 5, which is susceptible to sulfur burn, has a buffer curve which lies 0.2 to 0.3 pH unit closer to the acid side than the buffer curve of the sulfur burn–resistant variety, No. 11353 (Figure 1).

A peel section of a lemon treated with elemental radioactive sulfur was killed in formalin–acetic acid–alcohol mixture and then washed successively in water, in a graded series of ethanol-butanol-water mixtures from 10 to 95%, 100% ethanol, and xylene. The insoluble substances remaining in the section would be primarily the cellulose of the cell walls and alkali-soluble protein of the protoplasts, the acid-soluble protein having been washed out. Comparison of the photomicrograph and radioautograph of this peel section indicated that the alkali-soluble (acid-insoluble) proteins were weakly labeled with radioactive sulfur (*21*). Because of the indications of protein labeling in the radioautograph (Figure 3), the alkali-soluble and acid-soluble proteins were isolated from the peel in the hydrogen sulfide*, sulfur* dioxide, and sulfuric* acid treatments. Radioautographs of tissue of fruit subjected to the latter three treatments, but killed in formalin, are shown in Figures 5, 7, and 9. Figures 2 to 9 are photomicrographs (×9.5) of radial sections of peel of lemons, and radioautographs of the same sections from fruit treated with elemental S^{35} and S^{35} acids. The right-hand sides of radioautographs correspond to left-hand sides of sections.

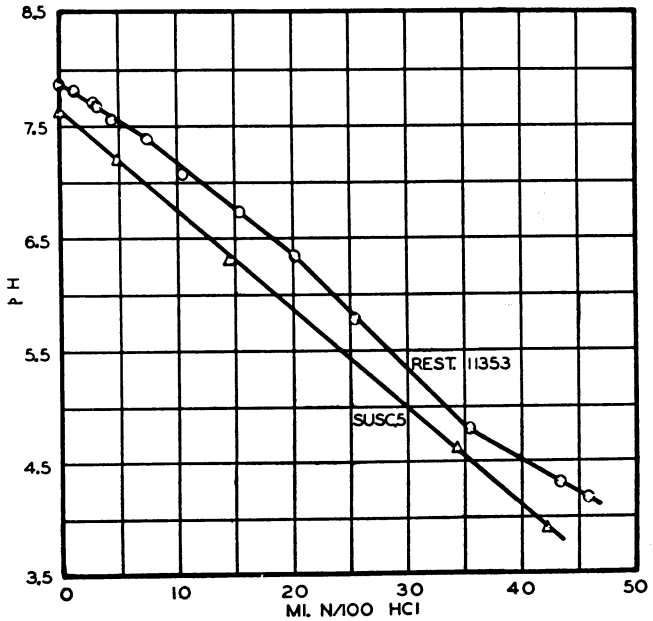
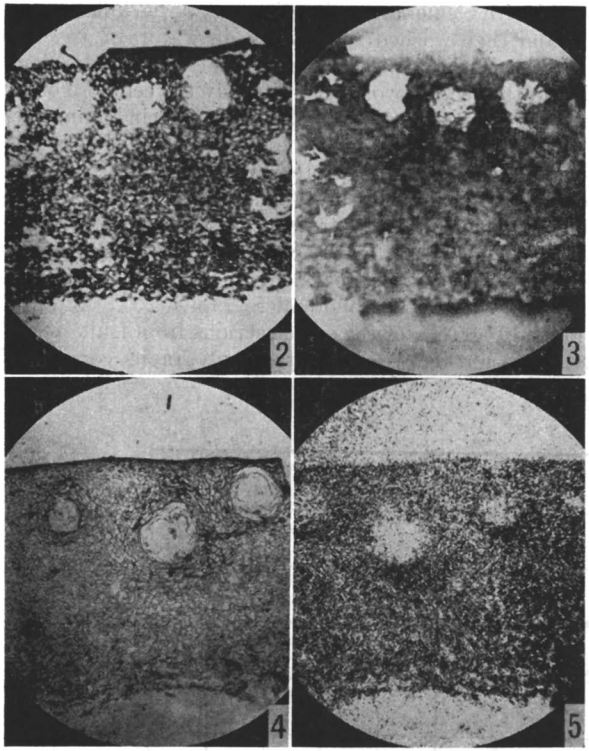


Figure 1. Titration Curves of Leaf Tissue Fluids of Sulfur Burn–Susceptible and Sulfur Burn–Resistant Muskmelon Leaves

The alkali-soluble protein of the peel of lemons treated with hydrogen sulfide*, sulfur* dioxide, and sulfuric* acid contained radioactive sulfur, but the fruit treated with hydrogen sulfide* had a significantly lower per cent specific activity in the alkali-soluble protein fraction than did the sulfur* dioxide or sulfuric* acid treated fruits (Table VII). These results suggest that sulfur dioxide and sulfuric acid react with protein more directly, while hydrogen sulfide perhaps must be oxidized first, as indicated in Table III. It also appears (from Table VII) that the alkali-soluble protein may have been dismuted as the amounts isolated were less in both the hydrogen sulfide* and sulfur* dioxide treated fruit than in the incubated or nonincubated controls. Other evidence of dismutation has been obtained in experiments where incubation at 60° C. was accompanied by the production of free ammonia (*18*), and the recovery of free ammonia and six amino acids in the exudates of incubated and sulfur-dusted fruits (*18*).



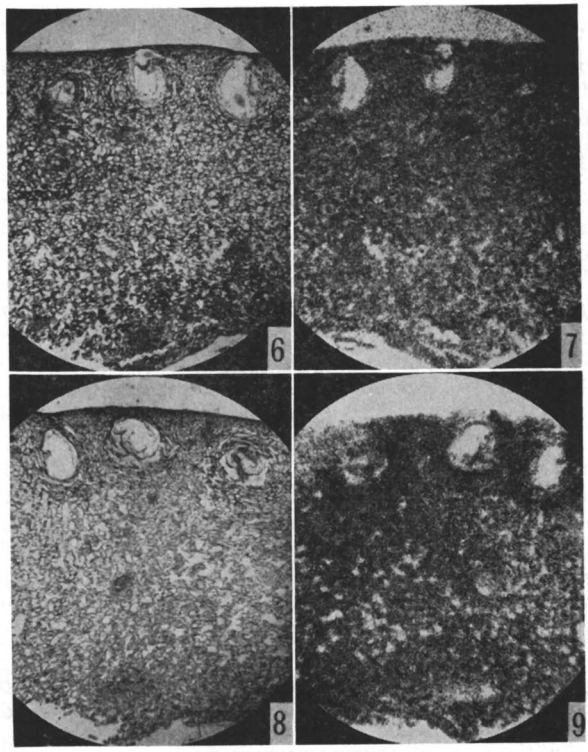
Figures 2 and 3. Peel Section and Radioautograph from Elemental S^{35} Treatment

Figures 4 and 5. Peel Section and Radioautograph from H_2S* Treatment

Acid-soluble protein, like the alkaline-soluble protein, was labeled with radioactive sulfur by the several sulfur* treatments. The specific activity of the protein increased from 29 in the hydrogen sulfide* to 52% in the sulfur* dioxide, to 68% in the sulfuric* acid treatment (Table VIII). Here again, as in the case of the alkali-soluble protein, there is an indication that hydrogen sulfide* may have required more processing by the fruit than sulfur* dioxide, which in turn required more processing than sulfuric* acid before it reacted with the protein. Comparison of the specific activities in Tables VII and VIII indicates that the labeling of the acid-soluble protein is much heavier than the alkali-soluble protein. The percentage of acid-soluble protein decreased in the peel in the hydrogen sulfide* treatment and increased in the sulfur* dioxide treatment. Sufficient data are not available to evaluate properly the latter shift, and further work must be done on this point, especially in view of Barron's (*1*) findings which show a diminution of plasma albumins and an increase of α-globulins in the blood sera of x-ray–treated dogs.

Discussion

It is unfortunate that the results of these experiments could not have been predicted or anticipated prior to their completion, so that all of them might have been carried out simultaneously with the same kind of material, genetically and physiologically, and with the same incubation temperatures, incubation times, gas pressures, osmotic pressures, activities, and complete chemical analyses for all treatments. Because of these variables an attempt at further interpretation of the results may be hazardous, but because of several possible factors that may offset the above-mentioned variables an attempt at further interpretations may be desirable. The differences in concentration of the treating materials offer the greatest hazard, but if results of fumigation of plants with sulfur dioxide (*15*) are used as a basis of judgment, the long incubation times tend to validate some dis-



Figures 6 and 7. Peel Section and Radioautograph from S^*O_2 Treatment

Figures 8 and 9. Peel Section and Radioautograph from $H_2S^*O_4$ Treatment

Table VII. Alkali-Soluble Protein Content and Activity of Peel of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated at 44° C.

Treatment	Sp. A. SO_4 (Protein)/ Sp. A. Treatment, % (Treated and Incubated)	Protein in Peel (Wet Wt.), %		
		Treated and incubated	Incubated only	Nonincubated
S*[a]	..	....	....	....
H_2S^*	2.7	0.0005	0.0079	0.0092
S^*O_2	31.8	0.0175	0.0311	0.0483
$H_2S^*O_4$	24.1	0.0253	....	....

[a] Incubated at 41° C.

Table VIII. Acid-Soluble Protein Content and Activity of Peel of Lemons Treated with Sulfur^{35} and Sulfur^{35} Acids and Incubated at 44° C.

Treatment	Sp. A. SO_4 (Protein)/ Sp. A. Treatment, % (Treated and Incubated)	Protein in Peel (Wet Wt.), %		
		Treated and incubated	Incubated only	Nonincubated
S*[a]	..	....	....	....
H_2S^*	29.0	0.0072	0.0202	0.0237
S^*O_2	51.6	0.1646	0.1468	0.0955
$H_2S^*O_4$	68.4	0.1756	...	...

[a] Incubated at 41° C.

cussion. With our present state of knowledge of sulfur metabolism in plants it is impossible to explain the cell reactions which result in the products formed when lemons are treated with sulfur or sulfur acids. The constitution of alkali- or acid-soluble proteins in citrus is not known. Likewise, there is little information concerning the sulfur enzymes present in citrus fruit. In fact, the essentiality of sulfur to plants has been known only since the time of Liebig (*3*, *8*).

However, Phinney (*6*) has suggested on the basis of experiments with mutants of *Neurospora* that the biosynthesis of cysteine involves the coupling of sulfate with an organic compound (presumably cysteine sulfinic acid) followed by reduction to sulfide. He noted also that sulfate may be reduced stepwise to sulfide and may then enter the protein as such. But he observed this process proceeds less readily than when sulfate combines first with protein.

From Table IX it can be seen that the authors' experiments with radioactive sulfur seem to be in accord with those of Phinney (*6*), in so far as they are parallel. They suggest that elemental sulfur is first reduced to hydrogen sulfide. This reaction appears to be primarily one with reduced glutathione and results in glutathione in the disulfide form (*18*). However, the hydrogen sulfide formed in the other sulfur treatments must be largely a dismutation product. Hydrogen sulfide when produced by treatment of fruit with elemental sulfur possibly reacts with protein or cysteine and may then be oxidized to sulfate in a series of steps from cysteine sulfenic acid to cysteine sulfinic acid to sulfate. According to Medes and Floyd (*5*), these reactions occur in rats and appear to be enzymatically controlled except that cysteine sulfenic acid gives rise to cysteine sulfinic acid spontaneously. This process appears to occur in plants (*14*) though the reverse process (*6*) appears to be the major one by which sulfate is utilized in the plant. The experiments described here further indicate on the basis of the per cent specific activity of the products formed that hydrogen sulfide* is oxidized primarily to sulfate* which then combines with the protein. Likewise, they suggest that in fruit treated with sulfur* dioxide the sulfur* dioxide is oxidized to sulfate*, and then the sulfate* is coupled with the protein. Sulfate* as in sulfuric* acid, on the other hand, appears to react chiefly with the protein when applied directly to plant tissues.

Table IX. Relative Specific Activities of Products from Treatments of Lemons with Sulfur^{35} and Sulfur^{35} Acids

	Sp. A. Product/Sp. A. Treatment, %					Diff. pH Peel Extract (Incubated and Incubated and Treated)
Treatment	H_2S^*	S^*O_2	S^*O_4 (peel)	Acid-soluble protein	Alkali-soluble protein	
S^*	102	14	77	..	Weak	1.0
H_2S^*	..	21	51	29	3	1.6
S^*O_2	11	..	6	52	32	0.6
$H_2S^*O_4$	5	13	..	68	24	0.8

No evidence has been obtained as to the source of the sulfur* dioxide formed by the action of sulfur* and sulfur* acids on lemons but tentatively it may be assumed, since sulfur* dioxide appears in all the treatments and since it has such a low activity, that it may arise from sulfinic acid in the formation of sulfate or that it is a dismutation product of the protein which disappeared.

Acknowledgment

The authors wish to express their appreciation for the grants-in-aid made by the Texas Gulf Sulphur Company in the early stages of this work, without which it could not have been undertaken. The authors are also indebted to J. T. Middleton for the samples of muskmelon leaves. The radioactive sulfur 35 as $H_2S^*O_4$ was obtained from the Atomic Energy Commission, Oak Ridge, Tenn., and subsequently processed by Tracerlab, Boston, Mass.

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Colorimetric Determination of Small Quantities of 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)-ethane

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In a sensitive and specific colorimetric method 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane is extracted from plant or animal tissue, using benzene or petroleum ether as the solvent. The solvent is evaporated at room temperature by a current of air and the residue dehydrohalogenated with 2% alcoholic potassium hydroxide. By petroleum ether extraction the resulting 1,1-dichloro-2,2-bis(*p*-methoxyphenyl)-ethylene is removed from the reaction mixture. After the solvent is removed by air evaporation the dehydrohalogenated methoxychlor is isolated from the nonsaponifiable portion of the fats and waxes by dissolving the residue in hot acetone, chilling, and filtering. After the acetone is removed by air evaporation, the residue is treated with 85% sulfuric acid. This produces a red solution with an absorption maximum at 555 mμ, the intensity of which can be read on a colorimeter and is a function of the methoxychlor concentration. Beer's law is obeyed over the range of 1 to 50 micrograms.

A need has arisen for a sensitive and specific method of estimating microgram quantities of the insecticide methoxychlor in plant and animal products. Methoxychlor, 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane, is an analog of DDT and therefore procedures for the determination of DDT should be applicable to methoxychlor as well. Such has actually been found to be the case.

The pyridine-xanthydrol method of Stiff and Castillo (*4*) and Claborn (*1*) will indicate the presence of methoxychlor, but it has a low order of sensitivity and does not distinguish between methoxychlor and DDT.

Much more promising is the method of Schechter and Haller (*3*), which depends upon the coupling of the tetranitro derivative of DDT with methanolic sodium methylate. Under conditions of intensive nitration methoxychlor will yield by this procedure a red color with an absorption maximum at 535 mμ.

It may be generally stated that the Schechter-Haller procedure will distinguish methoxychlor from *p,p'*-DDT, because the latter compound gives a blue color. However, *o,p'*-DDT and various breakdown products of DDT yield red colors with absorption maxima close to that of methoxychlor, so that for practical purposes it is impossible to distinguish between them.

It appears that the greatest value of the Schechter-Haller method applied to the determination of methoxychlor is in the analysis of materials whose previous spray history is unknown. The production of a red color in such analysis indicates the possibility of the existence of methoxychlor, and warrants a further specific test.

Upon treatment with alcoholic potassium hydroxide, methoxychlor will undergo

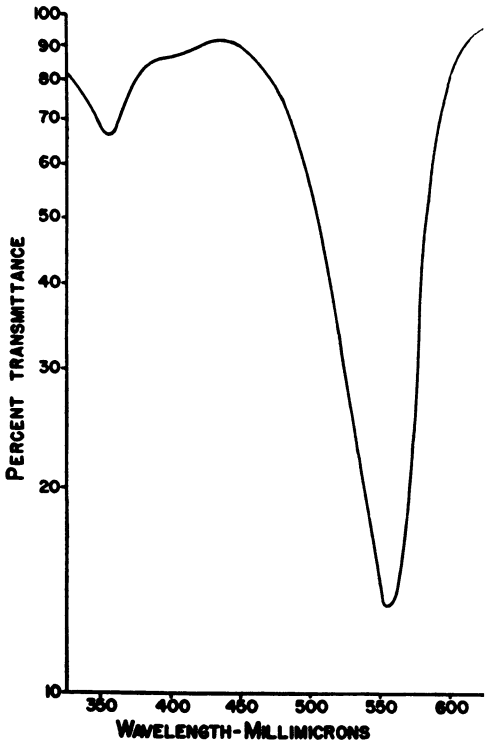

Figure 1. Absorption of Red Complex

quantitative dehydrohalogenation. The dehydrohalogenated product reacts with 85% sulfuric acid to produce a red complex of unknown composition with an absorption maximum at 555 mμ (Figure 1). No other organic insecticide now in use produces any color under similar conditions. Therefore, the method is specific for methoxychlor. Fats and waxes, however, yield strong brown colors which will completely mask the methoxychlor reaction. In the method described this interference has been reduced to a point where it introduces an error of less than 1% when the methoxychlor concentration is between 5 and 50 micrograms. Quantities of methoxychlor of less than 1 microgram may be determined by this method.

There are six steps involved in the procedure:

1. Extraction of the methoxychlor from the material under examination with a suitable solvent.
2. Dehydrohalogenation with alcoholic potassium hydroxide.
3. Separation of the dehydrohalogenated derivative from the reaction mixture with petroleum ether.
4. Further isolation of this material from the nonsaponifiable portion of fats and waxes with acetone.
5. Reaction of the dehydrohalogenated methoxychlor with 85% sulfuric acid.
6. Spectrophotometric measurement at 555 mμ of the optical density or per cent transmittance of the resulting color.

In order to prepare the methoxychlor for analysis, it must be removed from the material under examination by means of a solvent extraction. The procedures used for the stripping of DDT are applicable to methoxychlor (*2*, *5*, *6*). The solvent chosen should readily dissolve methoxychlor, be sufficiently volatile to be evaporated by an air current at room temperature, and leave no residue upon evaporation that will interfere with the analysis.

Benzene and petroleum ether satisfy these requirements rather well. It is, however, essential that the solvents be absolutely pure. Reagent grade or C.P. chemicals are usually adequate for the initial extraction, but each batch should be checked to see that it does not introduce an error into the determination. The extracted methoxychlor is isolated by evaporating the solvent with a current of air. No measurable loss of methoxychlor has been found when this evaporation takes place at room temperature. However, precautions should be taken to avoid prolonged exposure to the air stream after the solvent has evaporated.

Dehydrohalogenation is readily accomplished by heating with 2% potassium hydroxide in 95% ethyl alcohol. In order to separate the dehydrohalogenated methoxychlor from the reaction mixture, the alcohol is allowed to evaporate, and the residue is taken up in petroleum ether and washed with water. Most batches of even reagent grade petroleum ether contain substances which after contact with potassium hydroxide will yield

colors with sulfuric acid. It is therefore essential to purify the petroleum ether used for this extraction.

It is necessary to isolate the dehydrohalogenated methoxychlor from the nonsaponifiable portion of the fats and waxes. To do this, advantage is taken of the fact that both methoxychlor and its dehydrohalogenated product are readily soluble in acetone, whereas fats are relatively insoluble. The residue is first dissolved in hot acetone, and then the acetone is chilled to −15° C., which causes precipitation of the fats. After the fats are filtered off, the acetone is removed by evaporation.

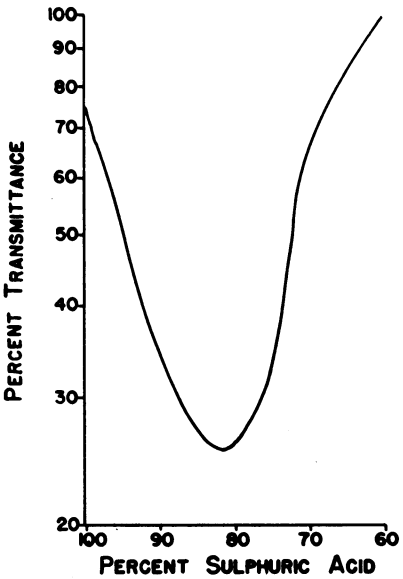

Figure 2. Effect of Acid Concentration on Color Intensity

The intensity of color produced by a given amount of dehydrohalogenated methoxychlor is dependent upon the acid concentration, as shown in Figure 2. The optimum concentration is about 82.5%, with the intensity falling off rapidly at lower concentrations. Acid at a nominal concentration of 85% is used, so that the sensitivity of the reaction is not decreased by the absorption of small amounts of moisture from the air. It is difficult to prepare sulfuric acid of an exact concentration and even more difficult to keep such acid at a constant concentration for any length of time. Therefore it is advisable to run a standard of known methoxychlor content along with each set of samples being analyzed in order to correct for changes in the acid strength. Because Beer's law is obeyed over the range of 1 to 50 micrograms of methoxychlor, it is not essential to plot more than two or three points on the standard curve during the daily recheck of the acid. The color develops slowly with acid of less than 82% strength and not at all with 60% acid. The addition of water destroys the color. In the absence of atmospheric moisture the color is stable for several days. The reaction rate varies with different batches of acid. With some lots the color is fully developed in 15 minutes, but occasionally 60 or more minutes are required. A trace of ferric chloride added to the acid assures rapid color development.

Analytical Procedure

Reagents. Petroleum ether, C.P., boiling range 35° to 60° C.; acetone, C.P., dry; and ethyl alcohol, C.P., 95%. Each reagent is purified before use by eluting through a column of chromatographic alumina or by shaking with activated charcoal and filtering.

Benzene, C.P., or petroleum ether, C.P., for stripping.

Potassium hydroxide (2%) in 95% ethyl alcohol, freshly prepared before use.

Sulfuric acid, 85%. The actual concentration may range between 82.5 and 88%, but under no conditions should it be less than 82.5%. To 1 liter of this are added 10 mg. or more of ferric chloride.

Sodium chloride, saturated solution.

Sodium sulfate, C.P., anhydrous.

Standard methoxychlor solution, 10 mg. of recrystallized methoxychlor made up to 1 liter with 95% ethyl alcohol.

Standard dehydrohalogenated methoxychlor solution is prepared from 44.71 mg. of recrystallized 1,1-dichloro-2,2-bis(*p*-methoxyphenyl)-ethylene made up to 1 liter with 95% ethyl alcohol. One milliliter of this solution is equivalent to 50 micrograms of methoxychlor.

The 1,1-dichloro-2,2-bis(*p*-methoxyphenyl)-ethylene is prepared by refluxing recrystallized methoxychlor in 95% ethyl alcohol for 2 hours with an excess of potassium hydroxide. It is recrystallized from methanol.

Procedure

Extract the methoxychlor from the material under consideration with C.P. grade solvents (benzene, petroleum ether, etc.) that have been checked and found free of interfering substances.

Shake stripping with about $^1/_{20}$th of its weight of anhydrous sodium sulfate.

A small quantity of some filtering aid may be used if necessary to obtain a clear filtrate, but its use should be limited and it should be checked to see that no loss of methoxychlor occurs with the particular product under analysis.

Filter the solution through rapid fluted filter paper.

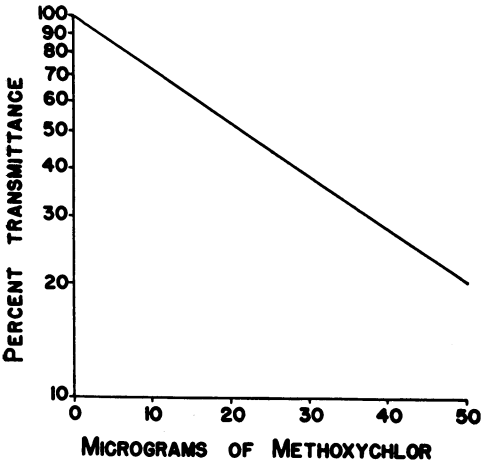

Figure 3. Standard Curve

Transfer an aliquot of the filtrate containing between 5 and 50 micrograms of methoxychlor to a 125-ml. standard-taper Erlenmeyer flask.

Remove the solvent with a gentle current of dry, oil-free air, preferably at room temperature but never over 35° C.

Add 50 ml. of 2% potassium hydroxide in 95% ethyl alcohol.

Connect a short length (12 cm.) of glass tubing equipped with a male standard-taper joint to the flask and immerse flask in a boiling water bath until the volume of the liquid is reduced to about 5 ml. Avoid prolonged heating after the alcohol has evaporated and run the next step as soon as possible. It is undesirable to hold this alcoholic solution overnight.

Transfer the contents of the flask to a separatory funnel with the aid of 200 ml. of water and 75 ml. of purified petroleum ether. Add 10 ml. of saturated sodium chloride solution.

Shake well, and separate and discard the aqueous layer.

Wash two or more times with 200 ml. of water and 10 ml. of saturated sodium chloride solution, discarding the aqueous layers.

Filter the petroleum ether extract through 1 or 2 cm. of anhydrous sodium sulfate contained in a glass Büchner funnel equipped with a 30-mm. fritted-glass plate of coarse porosity. This filtration may be carried out rapidly without the aid of suction. (A small amount of filtering aid may be mixed with the sodium sulfate to remove plant pigments.)

Wash the separatory funnel with 20 ml. of petroleum ether. Pass this washing through the filter and combine it with the filtrate.

Transfer the filtrate in portions to a test tube and remove the solvent at room temperature, either with a current of air or by means of reduced pressure.

When dry, add 5 ml. of dry acetone and heat from 2 to 4 seconds in a boiling water bath to effect a complete solution of the entire residue.

Chill the tube of acetone thoroughly, to −15° C. if possible. At the same time chill some dry acetone for washing the filter.

Filter the cold acetone rapidly into a glass-stoppered test tube by means of suction through a 2- or 3-ml. micro Büchner funnel, fitted with a fritted-glass plate of medium porosity. Wash the tube and funnel with 3 ml. of cold acetone and combine it with the filtrate.

Evaporate the filtrate with a current of air. Avoid excessive drying, as it makes the subsequent dissolving of the residue difficult; 0.01 to 0.05 ml. of moisture is frequently desirable.

Add 10 ml. of 85% sulfuric acid (preferably by means of an automatic pipet).

Stopper the tube with a glass stopper and shake well at intervals for 10 minutes. Let the tube stand for 15 minutes to 3 hours.

If undissolved particles are in suspension, filter through a plug of glass wool. This is generally unnecessary.

Read the per cent transmittance at 555 $m\mu$ in a colorimeter or spectrophotometer against 85% sulfuric acid.

Preparation of Standard Curve

By means of a microburet add 0, 0.1, 0.2, 0.5, 1, 2, and 5 ml. of the standard methoxychlor solution to a volume of the stripping solvent corresponding to the average size of aliquot taken. Evaporate this mixture to dryness and carry it through the above procedure. Plot the resulting per cent transmittance semilogarithmically against concentration (Figure 3). From this standard curve calculate the original concentration of methoxychlor in the unknown in parts per million from the formula:

$$\frac{W_x V_o}{W_o V_x} = \text{p.p.m.}$$

where W_x = weight of residue in micrograms determined from the standard curves, W_o = weight of sample in grams, V_x = aliquot of solvent in ml., and V_o = original volume in ml. of solvent used for stripping.

If a series of determinations is to be made on the same type of material, it is advisable to make a standard curve by adding known amounts of methoxychlor to a stripping of the material under consideration which is known to be free of methoxychlor. Any slight variation introduced into the technique by the presence of other extracted substances will be compensated for in this way.

Discussion

The recovery of methoxychlor has been found to be quantitative from strippings of apples, green beans, peaches, carrots, celery, pears, peas, lamb fat, beef fat, pork fat, and milk. Table I shows the recovery from apples.

Table I. Recovery of Methoxychlor from Benzene Solutions of Apple Wax

Methoxychlor Added, γ	Methoxychlor Recovered, γ	Recovery, %
0	0	...
2.5	2.3	92
	2.5	100
	2.9	116
5.0	4.8	96
	4.8	96
	5.0	100
10.0	9.8	98
	10.3	103
	10.4	104
15.0	15.3	102
	15.8	105
20.0	20.2	101
	20.8	104
25.0	24.1	96
	24.2	97
50.0	50.0	100
		Av. 100.6

The dehydrohalogenation proceeds rapidly with a yield of better than 98%. The over-all yield of the entire procedure is better than 95%. Because high yields are obtained, it is possible to prepare a standard solution of dehydrohalogenated methoxychlor to be used in the daily recheck of the calibration curve. The use of such a standard greatly reduces the time required and makes possible the frequent checking of the curve at several points with but little additional effort. One gram of methoxychlor is equivalent to 0.8942 gram of the dehydrohalogenated product.

In the authors' experience it has not been possible to obtain a completely colorless blank, for even the most carefully purified reagents will yield a light yellow color. However, this color has no effect upon the determination of methoxychlor, for if the solvents have been properly purified this reagent blank will not lower the transmittance at 555 mμ by more than 0.25%.

Even with the best technique, however, some interference will still be encountered from fats and waxes. With the acetone separation method described this interference is reduced to a point where it seldom lowers the transmittance by as much as 1% and never by more than 3%. It has been found that the off-colors introduced by any one type of biological material are remarkably constant and it is therefore thoroughly practical to apply a correction for this interference.

The over-all errors caused by solvents and fats reach significant proportions only when determinations are being made at the very limit of sensitivity of this test. The authors have been able to measure less than 1 microgram of methoxychlor with errors not in excess of 15%.

For some time the acetone separation step was performed before dehydrohalogenation. However, great difficulty was experienced in preventing rather sizable losses, because some methoxychlor would dissolve in the precipitated fat. With milk in particular these losses became prohibitive. After dehydrohalogenation most of the fat is saponified and removed, so that only a small nonsaponifiable portion remains to be separated. If any methoxychlor does dissolve in this remaining fraction it is too small to be measured.

Further separation of the methoxychlor from fatty contaminants may be accomplished by diluting the acetone, after filtering, with an equal volume of water and again filtering. This step is seldom necessary, but when employed it does provide a means for the removal of the last traces of fatty impurities. Because water will be present, it is necessary to extract the dehydrohalogenated methoxychlor from the solution with petroleum ether. This adds another step to the procedure. Such additional work is hardly warranted.

Considerable variation is possible in the technique of this procedure for the determination of methoxychlor. Several steps may be eliminated or shortened for rapid routine work, and with suitable biological materials this may be done without appreciably sacrificing the accuracy of the test.

Acknowledgment

The authors wish to thank E. I. du Pont de Nemours & Company for supplying the samples of methoxychlor used in this work, and Mary L. Wilson for carrying out much of the routine experimental work.

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Partition Chromatography in Analysis of Insecticide Formulations

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A procedure for the determination of γ-benzene hexachloride and DDT in benzene hexachloride–DDT-sulfur formulations, employing partition chromatography, is described. The procedure has also been applied to the assay of 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)-ethane in technical methoxychlor. Results of separation of other insecticidal ingredients are discussed.

Partition chromatography, developed by Martin and Synge (*3*) for the separation of amino acid derivatives, was employed by Ramsey and Patterson (*4*) for the separation of isomers of benzene hexachloride (1,2,3,4,5,6-hexachlorocyclohexane) in the technical product. The work of Ramsey and Patterson was extended by Aepli, Munter, and Gall (*1*) in the development of a quantitative method for the determination of γ-benzene hexachloride. A modification of the method of Aepli and co-workers was recently published (*2*). The essential feature of this modified method is the addition of a small quantity of dye, D and C Violet No. 2 (1-hydroxy-4-*p*-toluinoanthraquinone), to the solution to be chromatographed. The dye forms a violet band on the column and serves as a visible marker for the position of the gamma isomer band on the column.

Methoxychlor [1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane], benzene hexachloride (BHC), chlordan, and toxaphene are chlorinated compounds of recognized importance in the insecticide field. Other chlorinated compounds now undergoing field testing experiments will, no doubt, soon be added to the list. The need for specific analytical methods is familiar to those concerned with the analysis of formulations containing one or more of the above insecticides.

In applying the modified partition chromatographic method to the determination of γ-benzene hexachloride in formulations of widely varying composition, this analytical technique appeared promising for the separation and determination of other insecticidal ingredients. It was noted, for example, that 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane (90 to 93% of the technical product), γ-benzene hexachloride, and technical DDT could each be separated and determined in the mixture after chromatography on a single partition chromatographic column. A red dye, D and C Red No. 18 (1-xylylazoxylylazo-2-naphthol), added to the solution, forms a red band on the column and serves to mark the exact position of the DDT band in the same way that D and C Violet No. 2 locates the γ-benzene hexachloride.

The present paper deals with certain separations that have been accomplished thus far and gives a detailed procedure for the determination of DDT and γ-benzene hexachloride in DDT–benzene hexachloride–sulfur formulations.

Apparatus

Partition Chromatographic Column. The column was constructed of standard-wall borosilicate glass pipe by the Scientific Glass Apparatus Company. It is 83 cm.

long, 2.5 cm. in inside diameter, and tooled at the top to fit a No. 7 rubber stopper. A fritted-glass disk, 4 mm. thick and of medium porosity, is sealed in place at the bottom of the column and about 5 cm. below the fritted disk is sealed the male part of a No. 18/7 ball joint. The female part of the joint is pulled out in a flame to form a short adapter. The joint is held together by a pinch clamp.

Air-Pressure Reduction Valve. The column is operated by air pressure. The valve used in this work was purchased from the Southern Oxygen Company and was provided with a reduced pressure indicating gage reading in pound units. The valve is connected to the column by means of a length of pressure rubber tubing and a rubber stopper.

Solvent Evaporator. The fractions are evaporated under reduced pressure obtained with an efficient and high-capacity water pump and with heating in a water bath at 60° C. A glass manifold provides multiple vacuum connections and is constructed by sealing four glass tubes at equidistant points around a 50-ml. round-bottomed borosilicate glass flask. This permits the simultaneous evaporation of four fractions. The solvent is recovered by trapping in a 800-ml. Kjeldahl flask immersed in a Dewar flask containing a dry ice–acetone mixture. Photographs of both the column assembly and solvent evaporator have been published (*2*).

Other apparatus includes a Waring Blendor, 1-quart mixing cup; 24 or more 125-ml. Erlenmeyer flasks; two 10-ml. graduated cylinders; a 50-ml. glass-stoppered volumetric flask; and 10-ml. volumetric and 5-ml. serological-type pipets.

Reagents

n-Hexane, Phillips Petroleum Company, commercial grade.

Nitromethane, Commercial Solvents Company, redistilled before use.

Silicic acid, Mallinckrodt's reagent grade.

Mixed dye solution made by dissolving 25 mg. each of D and C Violet No. 2 and D and C Red No. 18 in 50 ml. of mobile solvent, stored in a glass-stoppered bottle. Dyes are available from Pylam Products Company, 799 Greenwich St., New York 14, N. Y.

Mobile solvent is a saturated solution of nitromethane in *n*-hexane. Two liters of *n*-hexane are shaken vigorously with an excess of nitromethane in a glass-stoppered bottle. The mobile solvent is decanted as needed.

Determination of γ-Benzene Hexachloride and DDT in Benzene Hexachloride–DDT–Sulfur Formulations

A number of official samples have been analyzed in the insecticide laboratory of the Production and Marketing Administration, which contained among the active ingredients γ-benzene hexachloride, DDT, and sulfur in the proportions of 3, 5, and 57%, respectively. The following detailed procedure was adopted after testing with authentic formulations of this type.

Preparation of Sample. Weigh a quantity of sample sufficient to provide 0.750 gram of γ-benzene hexachloride and 1.25 grams of DDT, and extract the sample vigorously overnight in a Soxhlet extractor with ethyl ether. This will remove total benzene hexachloride, DDT, and part of the sulfur. Evaporate most of the ether on the steam bath and the remaining few milliliters under vacuum. Extract dry residue remaining in the flask (250-ml. standard taper) with mobile solvent, as follows: Add 25 ml. of mobile solvent, heat just to boiling, and allow to cool to room temperature with occasional shaking. Then decant the extract through a small, fast, conical filter paper into a 50-ml. volumetric flask containing 1 ml. of the mixed dye solution. Make a second hot extraction, using 10 ml. of solvent, and follow with two to three successive extractions with 5-ml. portions of solvent. Finally dilute to volume and mix.

The extraction procedure described by Aepli, Munter, and Gall (*1*) may be used, if preferred, as an alternate extraction procedure for the residue after ether extraction.

Preparation of Column. Weigh 100 ± 0.5 grams of silicic acid and transfer to a Waring Blendor. Add 300 ml. of mobile solvent and with vigorous mixing add 55 ml. of nitromethane. After mixing for 15 seconds in the blender, pour the mixture quickly into the column through a large glass funnel. Apply 8 pounds (3.6 kg.) of pressure to the column until the silicic acid–solvent boundary ceases to drop. Release the pressure, cautiously remove the rubber stopper, and, by means of a 25-ml. pipet, remove most of the upper solvent layer by gentle suction. The latter operation reduces the time required

in preparing the column. Again apply 8 pounds of pressure to the column and just force the solvent into the silicic acid. Release the pressure again cautiously and continue as directed under operation of column.

Operation of Column. Pipet 10 ml. of the solution to be chromatographed and allow it to flow slowly down the inside of the column without disturbing the surface of the silicic acid. Force the solution just into the column of silicic acid. Wash down the inside of the column with 1 to 2 ml. of mobile solvent and force into the silicic acid by applying pressure. Now fill the column up to within 1 inch (2.5 cm.) of the top with mobile solvent, but add the first 10 to 15 ml. very slowly, so that the surface of the silicic acid is not disturbed. This is done by using a pipet for the first portion. Insert the rubber stopper tightly in the top of the column and apply 8 pounds of pressure. The separation of the two dyes will be noted soon after starting the column. Adjust the rate of flow through the column to 3 to 4 ml. per minute with the pressure regulator.

Collection of Fractions. Begin to collect 10-ml. fractions by using alternately two 10-ml. graduated cylinders when the red dye band is about 2 inches above the fritted-glass disk. Pour the fractions cautiously into numbered 125-ml. Erlenmeyer flasks and wash the cylinder with a small quantity of *n*-hexane contained in a dropping bottle. (This washing operation is necessary only at points where a cut is expected. Contamination of alternate fractions is thus minimized.) The small amount of sulfur which dissolves in the mobile solvent will be found concentrated in one of the first fractions collected. The residue of sulfur will decrease to a negligible quantity in the succeeding fractions and the first portion of the DDT will be recognized after evaporation of the fractions by the appearance of a small amount of oily residue. This first fraction of DDT frequently precedes the red dye. Continue to collect 10-ml. fractions until only a trace of red dye remains on the column, then begin to collect 5-ml. fractions. Collect five 5-ml. fractions that contain no visible red dye and stop the column operation. Open the column, refill with mobile solvent, and continue the operation of the column to collect the γ-benzene hexachloride fraction. While the column is operating, work up the DDT fractions as directed below.

A small portion of the DDT frequently comes off the column ahead of the red dye, but 94 to 98% of the technical product is located within the red band. The cut between the DDT and benzene hexachloride will be indicated either by the absence of any residue after evaporation of one of the 5-ml. fractions or the appearance of a small amount of crystalline material which sublimes up around the neck of the flask, leaving no residue, and which is apparently the first portion of the heptachloro- and octachlorocyclohexane fraction. On cooling and stirring with a stirring rod the DDT residues will crystallize readily. This offers another means of determining where to cut, for the quantity of crystalline residue will rapidly decrease to a negligible amount in the succeeding flasks.

When the cut is thus determined, combine the DDT fractions by dissolving the residues with a minimum of *n*-hexane and pour into a weighed 125-ml. Erlenmeyer flask. Evaporate the solvent by using the solvent evaporator. Then immerse the flask up to the neck in a water bath at 60° C. and evacuate with a high vacuum pump for 15 minutes. Remove the flask, wipe with a clean moist towel, and allow it to attain equilibrium near the balance for 10 minutes. Weigh and calculate the percentage of DDT in the original sample. A second pumping is frequently necessary to attain constant weight. At this point the residue is usually almost entirely crystalline.

Table I. Determination of γ-Benzene Hexachloride and DDT in Benzene Hexachloride–DDT–Sulfur Formulations

Formulation	Source of γ-BHC	Sulfur	Added, %		Found, %	
			γ-BHC[a]	DDT	γ-BHC	DDT
1	Lindane[b]	0	50.0	50.0	50.1	50.6
2	Lindane[b]	50	25.0	25.0	25.1	25.4
3	Lindane[b]	50	18.8	31.2	18.6	30.9
4	Lindane[b]	60	3.0	37.0	3.1	37.2
5	Technical BHC	0	12.9	20.8	13.0	20.3
6	Technical BHC	0	10.9	18.2	10.9	18.4 (18.5)[c]
7	Technical BHC	0	11.6	11.1	11.8	11.2 (11.1)[c]
8	Technical BHC	60	11.0	18.8	11.4	18.5
9	Technical BHC	60	11.3	18.8	11.6	18.4
10	Technical BHC	60	10.9	15.7	10.7	15.6

[a] γ-BHC in each technical BHC sample determined by partition chromatography.
[b] The common name for the γ-isomer of benzene hexachloride of a purity of not less than 99%.
[c] Calculated from total chlorine determination on DDT fraction.

When all the violet dye is off the column, begin to collect and evaporate 10-ml. fractions. Two or three fractions, which on evaporation yield little or no residue, will precede the appearance of the γ-benzene hexachloride. Continue to collect 10-ml. fractions and evaporate until no more γ-benzene hexachloride is obtained. Stop the column operation, dissolve the several γ-benzene hexachloride residues with a minimum quantity of *n*-hexane, and pour into a tared 125-ml. Erlenmeyer flask. Evaporate under vacuum, using the solvent evaporator, at 60° and finally at room temperature for 5 minutes with a high vacuum pump. Release the vacuum, wipe the flask with a clean, moist towel, and weigh after allowing the flask to come to equilibrium near the balance. Calculate the percentage of γ-benzene hexachloride in the original sample.

Authentic formulations of benzene hexachloride–DDT–sulfur were analyzed, following the above procedure, and the results are shown in Table I.

In preparation for the next experiment, invert the column and extrude the silicic acid by applying gentle pressure. Clean the column with a long-handled brush, wash with water and acetone, and dry by attaching to a vacuum line.

Assay of 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)-ethane in Technical Methoxychlor

With minor modification—that is, shorter column and one half the reagents—the procedure previously described was followed in chromatographing solutions of technical methoxychlor. The 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane was found to move down the column more slowly than γ-benzene hexachloride, and in one experiment the two compounds were separated and recovered quantitatively. The oily residue of 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane remaining after evaporation of the solvent crystallized readily and completely when the bottom of the flasks was scratched, and the purity of the combined residues was indicated by the melting point. The material melted at 87° to 88° C., which is the melting point reported in the literature for pure 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane. Table II gives the percentages of 1,1,1-trichloro-2,2-bis (*p*-methoxyphenyl)-ethane in the technical product found after chromatographing different quantities of the technical material.

Table II. Assay of 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)-ethane in Technical Methoxychlor

Detn.	Wt. of Material Chromatographed, Mg.	1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)-ethane Found, %
1	100	91.0
2	200	92.5
3	400	93.0
4	500	92.8

Notes on Procedure. In partition chromatography as well as adsorption experiments, successful results require a certain amount of skill which is acquired after some practice.

If the surface of the silicic acid is not flat, stir gently with a long stirring rod. The particles of silicic acid will settle evenly and a more horizontal band will be formed when the sample is introduced. The column should be clean and dry; otherwise, the silicic acid will tend to adhere to the inner wall.

In evaporating the fractions, do not allow the mobile solvent to boil. A two-way stopcock of 4-mm. bore inserted between the trap and pump will make it convenient to stop the evacuation of the flasks if boiling occurs. If the system is tight, the fractions will continue to evaporate evenly and there will be no danger of loss of material through spattering.

Other Chromatographic Separations

The method has been applied to the separation of other insecticidal ingredients.

Chromatography of Pyrethrins Concentrate. Five hundred milligrams of concentrate consisting mainly of the two pyrethrins and the two cinerins were dissolved

in mobile solvent and chromatographed. Forty-three 10-ml. fractions were collected. The fractions which made up three bands on the column were combined and evaporated, and the residues were tested with Denigés' reagent. A positive test for chrysanthemum monocarboxylic acid was obtained on two residues, which separated widely on the column. Because pyrethrin I and cinerin I are both esters of this monocarboxylic acid, it may be assumed that these two ingredients had been separated.

Chromatography of Chlordan. Attempts to separate technical chlordan and DDT by the above procedure were unsuccessful. Most of the chlordan came off the column with the DDT fraction. A solution of technical chlordan alone was separated into several fractions, some of which gave crystalline residues after evaporation of the solvent; however, the separations were not sharp.

Discussion

The formulations shown in Table I were made to contain approximately 3 parts of γ-benzene hexachloride to 5 parts of technical DDT, because this is the ratio in which they have been present in many of the formulations encountered by the author. For these ratios the separation and recovery of γ-benzene hexachloride and DDT are satisfactory. However, if the ratio of γ-benzene hexachloride to DDT is increased much beyond 3 to 5, a small amount of impurity from the technical benzene hexachloride contaminates the DDT fraction and leads to high DDT results. This is not the case with lindane-DDT mixtures, as will be seen by the results in Table I. Because pure γ-benzene hexachloride and DDT separate so widely on the column, it should be possible to separate and determine these two ingredients in almost all proportions.

The results shown in Table II were obtained on a recently manufactured sample of technical methoxychlor.

The sharp separation of the 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)-ethane on the column and the purity of the material as evidenced by the melting point appear to recommend it as a method of assay for the technical product.

On the basis of results reported in this paper, it would appear that partition chromatography will find other applications in analysis of insecticide formulations. The method possesses a certain degree of specificity in that the partition behavior of the compounds being studied is a characteristic property.

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Organic-Chlorine Determinations as a Measure of Insecticide Residues in Agricultural Products

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Determination of organically bound chlorine is shown to be in general agreement with fly mortality tests of insecticide residues in agricultural products. Alfalfa hay that had received applications of toxaphene and beef fat from animals fed alfalfa hay containing toxaphene residues or sprayed with benzene hexachloride or DDT were used for tests.

The use of chlorinated hydrocarbon insecticides for the control of insects affecting field crops and farm animals has created interest in the amounts of these materials that may be found in products intended for human and animal consumption.

Colorimetric methods (*3*, *6–10*), some of which are specific, have been developed for the determination of DDT in small amounts. For benzene hexachloride (hexachlorocyclohexane), chlordan, and toxaphene, however, specific analytical methods have not been developed, and their residues have been evaluated by the determination of organically bound chlorine. The procedure comprises extraction of the insecticide residue from the sample with benzene or other suitable organic solvent, evaporation of the solvent, treatment of the residue with isopropyl alcohol and metallic sodium, and finally determination by standard methods of the amount of chloride ion formed.

Because the results obtained reflect the presence, not only of the insecticide in question, but also of any of its decomposition products or other organic compounds containing halogen, confirmatory evidence of the identity and amount of the residues was desired. Therefore, studies to correlate the results of organic-chlorine determinations with insecticidal activity were undertaken.

Evaluation of Toxaphene Residues on Alfalfa Hay by Biological Tests

A sample of alfalfa hay from Bozeman, Mont., that had received two spray applications, each of 4 pounds of technical toxaphene per acre, was found by the method recommended by Carter and Hubanks (*2*) to contain 225 p.p.m. of organic chlorine, equivalent to 331 p.p.m. of toxaphene. This sample was obtained from a bale approximately 5 months after the last spray application. A similar sample of untreated hay was found to contain 1.2 p.p.m. of organic chlorine.

Portions of the treated hay were extracted with benzene, and aliquots of the benzene solution were evaporated to dryness on the steam bath, with a gentle air current to remove the last traces of solvent. The residues were then taken up in deodorized kerosene and tested against houseflies by the turntable method. For comparison, extracts were made up to contain the same amounts of technical toxaphene as were indicated by the chlorine determinations to be present in the treated hay.

The results are reported in Table I. The mortalities are the averages of seven replicates, approximately 100 flies being used in each test.

Table I. Toxicity to Houseflies of Toxaphene Residues on Alfalfa Hay

Source of Extract	Concentration of Residue or Toxaphene, Mg./Ml.	% Knockdown	% Kill in One Day
Sprayed alfalfa	2	10	81
	1	10	56
Technical toxaphene (standard)	2	15	89
	1	15	65

The kills obtained from the residues from the sprayed alfalfa and from the known technical toxaphene are in close enough agreement to support the assumption that the chemically determined material was toxaphene and that the concentrations determined were essentially correct.

Toxaphene Stored in Animal Fat

Samples of abdominal fat from a steer that had been fed hay containing toxaphene residues and from a steer that had been fed untreated hay were analyzed for their organic-chlorine content by the method recommended by Carter (*1*). The organic-chlorine content of the fat from the treated steer in excess of the amount found in the untreated steer was equivalent to approximately 700 p.p.m. of toxaphene.

Extracts of these fat samples were treated with sodium sulfate–concentrated sulfuric acid mixture and fuming acid by the method described by Schechter *et al.* (*5*) in order to separate the organic-chlorine compound from the fatty materials. An infrared spectrum from 7 to 15 microns on carbon disulfide solutions of the residues from the fat qualitatively identified the organic-chlorine compound as toxaphene. All the bands of toxaphene in this spectral region were plainly seen in the treated steer extract, whereas none of the absorption bands were visible in the untreated steer extract.

Different samples of chlorinated camphene containing from 62 to 72% of chlorine all give the same infrared spectra. However, the toxicity to flies reaches a maximum at a chlorine content of 67 to 69% and drops off rapidly below 60% and above 72%. From the results of both the infrared spectroscopic examination and the fly-toxicity tests given below, it is concluded that the organic-chlorine compound in the fat was essentially unchanged toxaphene.

Toxaphene, Benzene Hexachloride, and DDT in Animal Fat

Samples of abdominal fat from a steer that had been fed on alfalfa hay containing toxaphene residues and from two steers that had been heavily sprayed, one with DDT and

Table II. Toxicity to Houseflies of Residues of Beef-Fat Extracts Containing Chlorinated Hydrocarbon Insecticides

(One test per sample)

Insecticide	Extract, Mg. per Flask	Sample No.	% Knockdown 1 hour	2 hours	3 hours	4 hours	% Kill in 24 Hours
Fat only (checks)	0	1	0	0	0	0	25
		2	0	0	0	0	25
Toxaphene	6.7	1	0	0	20	28	80
		2	0	0	20	65	95
		Known	0	0	25	60	94
Benzene hexachloride (technical)	2.7	1	80	95	100	100	100
		2	80	95	95	100	100
		Known	100	100	100	100	100
DDT	3.7	1	0	60	60	85	85
		2	0	60	75	90	92
		Known	85	100	100	100	100

the other with benzene hexachloride, were found to contain several hundred parts per million of organic chlorine. Portions of the fat were treated by the Schechter-Haller (*5*) method to separate the toxicant residue. The residues were then subjected to biological assay with houseflies, in comparison with the corresponding insecticides containing equivalent amounts of organic chlorine. A modification of the Laug (*4*) technique was used. The residues were placed in 500-ml. flasks, which were then turned on their sides. Approximately 100 flies, immobilized by chilling, were introduced into each flask. As soon as the flies revived, the flasks were set upright and observations made on knockdown and kill. Food (skim milk) was provided on strips of cotton hung from the mouths of the flasks, which were covered with cheesecloth. The results are reported in Table II.

The flasks used had narrow mouths, whereas the Laug technique specifies wide-mouthed beaker flasks. The small opening reduced air exchange and moisture condensed on the insides of the flasks. This condition is not good for flies and probably accounts for the mortality observed in the checks. However, this effect had not shown up 5 hours after the tests were started, and by that time most of the flies in the flasks containing insecticides were down. These tests are qualitative rather than quantitative, but the data indicate that the chemically determined quantities of the various insecticides in the fat extract were nearly equal in toxicity to known samples at the same level.

The tests reported in this paper were intended only to show general agreement between the insecticide level calculated from organic-chlorine determinations and fly mortality.

It is also shown that organic-chlorine residues on alfalfa hay resulting from insecticide applications of toxaphene and the organic-chlorine content of beef fat from animals fed alfalfa hay containing toxaphene residues or sprayed with benzene hexachloride or DDT approximate a true measure of the amounts of these compounds present.

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