Foreword

The first issue of the "Pesticides Monitoring Journal" is a direct result of cooperation among four Federal Departments, each of which is responsible for a distinctive mission in the field of pesticide usage. Because the ultimate objective of all of these programs is the enhancement of man's welfare, their collaboration is in the best public interest.

The initiative for this joint effort came from the Departments themselves. In 1961, the Secretaries of Defense, Interior, Agriculture, and Health, Education, and Welfare undertook the formation of the Federal Pest Control Review Board with the intent that it would review ". . . the various programs conducted by Federal agencies for control of forms of invertebrate and plant life which adversely affect man's interests, and 'shall' consider problems and developments in the field of chemical control, with particular reference to possible adverse effects and the adequacy of provisions for the proper use of pesticidal chemicals to insure the greatest public and national benefit." The Board was directed to turn its attention to all aspects of pest control, including the need; safety to man, domestic animals, wildlife, and the environment in general; and alternative methods. The Board was instructed to advise the Departments on modifications in plans that would be in the best public interest in view of these and related matters.

In 1964, in response to the report of the President's Science Advisory Committee on "Use of Pesticides" and with the advice and encouragement of the Executive Office of the President, especially the Office of Science and Technology and the Bureau of the Budget, these four Secretaries reorganized the Board as the Federal Committee on Pest Control. The reorganization was necessary to expand the collaboration in two directions: first, to permit the new Committee to cover all aspects of pest control — research, monitoring of the environment for pesticides, and public information programs — as well as to review operational programs; secondly, to extend their council to all Federal programs involving pests and their control.

The "Pesticides Monitoring Journal" is an outgrowth of one of the recommendations of the President's Science Advisory Committee that the concerned agencies "develop a continuing network to monitor residue levels in air, water, soil, man, wildlife, and fish."

To implement this recommendation the Federal Committee on Pest Control established a Subcommittee on Pesticide Monitoring which periodically evaluates the activities in this area throughout the Nation. Much of the work of monitoring levels of pesticides in the environment is being done by universities, State Agricultural Experiment Stations, conservation groups, and other non-Federal agencies. The results of many of these studies are not published, or appear in journals or individual reports that are scattered and difficult to locate. For this reason, the Subcommittee recommended that a Journal be established to assure accessibility of monitoring data to the scientists who need it. It is hoped that such data will not only provide information on the present levels of pesticide residues in various elements of the environment, including man, but will provide a base line from which we can determine whether these levels are increasing, decreasing, or remaining substantially unchanged.

There is an inherent risk in such an endeavor. Data of the type which will appear in this Journal are subject to misinterpretation. The significance of "parts per million" levels of a given chemical in the soil or in river water is not entirely clear. There is disagreement as to whether or not a particular level in a particular place at a particular time represents a signifi-

cant hazard to man or wildlife, or other environmental components. If then, such figures are published in a journal, is there not a possibility that special interest groups will quote them to "prove" their own preconceived biases? The Federal Committee on Pest Control has decided that such a risk must be taken. The alternative would be to encourage the scientists who gather such data to release only their own interpretations; however, no matter how well intentioned, such interpretations would not necessarily provide a sound basis for evaluation of changes in levels that may occur in the future. It will be the intent of this Journal to publish the data in a form that will permit each reader to interpret the results for himself. Information on sampling procedures and analytical methods used to gather each set of data will be included. Through this interdepartmental venture, the Federal Committee on Pest Control is demonstrating the practicability of collaboration between agencies with such diverse missions as food production, disease prevention, protection of human health and food supplies, and conservation of our natural resources.

The Committee has no direct appropriation to undertake such a venture. Therefore, the responsibility for staffing and financing this Journal has been delegated to one of the member agencies, but the editorial policy and guidance will continue to be the responsibility of an Editorial Board with members drawn from six different agencies of three of the cooperating Departments. The Editorial Board is appointed by and responsible to the Federal Committee on Pest Control. Thus the initiative of the four Federal Departments in establishing this Committee has been matched by the ingenuity of the Committee itself in finding a method of implementing programs without proliferating new authorities and agencies. It is for these reasons that the Office of Science and Technology has encouraged the Federal Committee on Pest Control to look beyond the four initiating Departments and to advise on all aspects of pests and their control in which any agency of the Federal Government is involved.

Ivan L. Bennett, Jr., M. D.
Deputy Director,
Office of Science and Technology,
Executive Office of the President

CONTENTS

Volume 1 June 1967 Number 1

The *Pesticides Monitoring Journal* is published quarterly under the auspices of the Federal Committee on Pest Control and its Subcommittee on Pesticide Monitoring as a source of information on pesticide levels relative to man and his environment.

The parent committee is composed of representatives of the U. S. Department of Agriculture, Defense, the Interior, and Health, Education and Welfare.

The Pesticide Monitoring Subcommittee consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Federal Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Federal Water Pollution Control Administration, Food and Drug Administration, Public Health Service, and the Tennessee Valley Authority.

Responsibility for publishing the *Pesticides Monitoring Journal* has been accepted by the Pesticides Program of the Public Health Service.

Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the Pesticide Monitoring Subcommittee which participate in operation of the national pesticides monitoring network, are expected to be principal sources of data and interpretive articles. However, pertinent data *in summarized form,* together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and non-government research institutions, both within and without the United States. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the Journal is not intended as a primary medium for the publication of basic research.

Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the Monitoring Subcommittee. Authors are given the benefit of review comments prior to publication.

Address correspondence to:

Editorial Manager

PESTICIDES MONITORING JOURNAL

Pesticides Program

National Communicable Disease Center

Atlanta, Georgia 30333

INTRODUCTION

PESTICIDES AND THE TOTAL ENVIRONMENT

John M. Geary[1]

The Federal Committee on Pest Control is concerned with assuring necessary control of pests without hazard to the environment and its inhabitants, including man. The Committee, while encouraging the use of other types of control, recognizes that chemical methods will continue to be needed for some time to come. It is therefore necessary to evaluate the long-term effects of such chemicals and their residues in the environment.

[1] Chairman, Federal Committee on Pest Control, Walter Reed Army Medical Center, Washington, D. C. 20012.

The effects of pesticides may be directly on organisms that are the target of control or on closely associated organisms. The effects may also be indirect or considerably delayed, with a certain amount of movement of pesticides in the environment after application. To evaluate the indirect effects it is necessary to know something about the distribution of pesticides in the various elements of the environment and the changes in these levels with time. The determination of this information is what the Federal Committee on Pest Control considers to be "pesticide monitoring."

The application of pesticides, depending on the target of control, may contaminate air, water, soil, plants, wildlife, and man. That portion which gets into the air may then settle on other parts of the environment or be carried for a considerable distance in air currents. It is likely that only a small amount will become uniformly distributed throughout a large mass of air. The selection of truly representative air samples is difficult even if the concentrations are large enough to make chemical detection easy.

The distribution of contaminating pesticides that may settle on water depends upon many factors — the solubility or suspendibility of the formulation in water, the movement of the water as well as its physical and chemical characteristics, and the presence of biological organisms.

Pesticides settling on soil may remain on the surface and later be moved by wind or washed off by rain — or they may penetrate to some depth. Penetration will depend on the characteristics of the material and the soil as well as on rainfall and other conditions. A portion of the pesticides in soil may be absorbed by plants or other organisms, and a small amount may be translocated.

Pesticides falling on plant surfaces may directly affect the plant, degrade with weathering, wash off into soil or water, or remain as residues. Residues may be carried away with falling leaves, consumed by animals, or harvested with crops. In the latter case they may be redistributed during processing or be ingested by the final consumer.

Pesticides making primary contact with man and animals may be absorbed and stored or excreted — or they may eventually reach other parts of the environment. Even that portion that reaches man directly, through handling of pesticides or by drift, may be washed off the skin and contaminate the soil or water.

An important consideration in the complex problem of the physical distribution of pesticides is the length of time required for breakdown of pesticides to other compounds. The time required for such degradation varies with each particular material. Some pesticides hydrolyze in the presence of moisture, and others may oxidize in air. Sunlight may act as a catalyst in decomposition, and this effect will certainly be modified by the concentration of the pesticide as well as by the amount and nature of the light. If pesticides occur in large aggregates, are absorbed in solid particles, or are deeply buried in the ground, the decomposition may be radically affected.

Finally, pesticides may be decomposed by biological processes. Such decomposition may vary greatly between organisms and may also be affected by chemical and physical conditions.

The interpretation of monitoring data must always be restricted to the exact portion of the environment of which it is representative. Within this limitation, the reliability of the interpretation will be affected by the adequacy of the sampling design, and the sensitivity and accuracy of the analytical procedures employed.

The many complexities of monitoring make it clear that there can be no simple and prompt answers to the questions of what pesticide residues are now present in our environment, where they are, and at what levels. The Federal Committee on Pest Control feels that such data must be permanently recorded in a form that will permit comparison between different studies and must be readily available to those who need such information. It is for these reasons that the "Pesticides Monitoring Journal" was proposed.

It is the intention of the Committee and the Editorial Board that the data published in the Journal be sufficiently detailed to show the precise portion of the environment sampled and to enable the user to judge the accuracy and dependability of the work. The reliability of the sampling, handling, cleanup, and chemical analyses is, of course, the responsibility of the author of each paper. The Editorial Board can only reject those papers in which the presentation leaves some doubt as to the adequacy of these procedures. The competence of the reader will be relied upon to avoid unwarranted generalizations from data that represent only a small segment of the total environment.

In an effort to provide a minimal base from which adequate information can be gained, the Subcommittee on Pesticide Monitoring recommended a national pesticide monitoring program which is described in this first issue of the "Pesticides Monitoring Journal." In addition to reporting the results of the national program, the Journal should serve as the publishing medium for essentially all pesticide monitoring efforts in this country. This should include individual work, as well as large programs, and foreign contributions also will be welcomed. The degree to which the goals of the Journal can be achieved will depend on the cooperation of interested workers.

The Federal Committee on Pest Control is the sponsor of the "Pesticides Monitoring Journal," but it was the members of the Subcommittee on Pesticide Monitoring who contributed long hours of devoted work to its establishment and to the initiation of the national program. The Subcommittee is made up of the people who most adequately represent the philosophy of the Federal government as to what a national program and journal should be.

The Federal Committee on Pest Control is not an independent agency of the Federal government with its own operating funds, but is an association of personnel designated by the Secretaries of the four departments which are most concerned with pesticides. Therefore, the actual publication of the Journal cannot be undertaken by the Committee. The Department of Health, Education, and Welfare, one of the member departments of the Committee, has agreed to be the publisher, through the Pesticides Program of the U. S. Public Health Service, National Communicable Disease Center, Atlanta, Georgia. The Federal Committee on Pest Control appreciates this cooperation and extends its thanks. The Federal Committee on Pest Control remains responsible for editorial policies, guidance, and general content.

NATIONAL PESTICIDE Monitoring PROGRAM

This initial issue of the PESTICIDES MONITORING JOURNAL is devoted in its entirety to a description of the National Pesticide Monitoring Program as recommended by the Subcommittee on Pesticide Monitoring of the Federal Committee on Pest Control and established by the responsible agencies. For the most part, the program as described represents the minimum effort necessary for adequate assessment of pesticide levels in man and his environment – it does not include all of the monitoring activities being conducted by the various Federal agencies.

Publication of original data derived from the National Pesticide Monitoring Program and from other Federal and non-Federal monitoring programs will commence with the second issue.

RESIDUES IN FOOD AND FEED

ASSESSMENTS INCLUDE RAW FOOD AND FEED COMMODITIES, MARKET BASKET ITEMS PREPARED FOR CONSUMPTION, MEAT SAMPLES TAKEN AT SLAUGHTER

R. E. Duggan[1] and F. J. McFarland[2]

The Federal program for monitoring pesticide residues in food and feed primarily is comprised of surveillance programs maintained by the Food and Drug Administration, U. S. Department of Health, Education, and Welfare. Data on residues in meat samples will be provided by the Livestock Slaughter Inspection Division, Consumer and Marketing Service, U. S. Department of Agriculture.

Monitoring Objective

The objective of this program is to determine the levels of pesticide residues in unprocessed and commercially processed consumer food commodities, animal feeds, and composites of food items prepared for human consumption. Studies being carried out to accomplish this objective include (1) a continuing Market Basket study to assay pesticide residues in the basic 2-week diet of a 19-year-old male, statistically the Nation's largest eater, and (2) nationwide surveillance of unprocessed food and feed. In addition, the Livestock Slaughter Inspection Division of the Consumer and Marketing Service, U. S. Department of Agriculture, will provide significant data on the analysis of meat samples taken from animals at slaughter.

Factors Influencing Program Design

Numerous interrelated factors necessarily have been considered and evaluated in defining a minimum monitoring effort for pesticide residues in food and feed.

Many individual commodities entering the Nation's food supply are produced in various geographical areas. Because the distribution system which brings these commodities to market is rapid, a constant shifting of commodity origins exists within a given consumption area. Since there are no crossroads in time or geography to permit concentrated or highly selective sampling which could be considered sufficiently representative of the food supply, monitoring of residues in food and feed must be programmed on a continuing and broadly geographical basis.

It should be recognized that the important impact of pesticide residues in human and animal food, insofar as environmental effects are concerned, lies in their consumption. Therefore, the examination of foods as they are prepared and ready for consumption is of special interest to this monitoring program. Residues in wastes from food processing, of course, may be of concern with regard to soil, water, or the atmosphere, depending upon their final disposition. Their effect on these elements of the environment, however, would be detected by other monitoring programs.

Because no uniformity may be expected within even a single food item due to extreme variations in local growing, harvesting, and processing procedures, sampling patterns taking geographical and seasonal variables into account must be used. Moreover, examination of the 82 individual food items in the Market Basket Survey was considered impractical because of the spectrum of unknown residues potentially present and the limitations in analytical methods to detect and measure more than one class of residues. The dilution factor, technical problems in methodology, and variations in dietary habits suggested that composites representing a "total diet" also would be unsatisfactory. To minimize these problems, a practical compromise was reached, that is, the compositing of foods by classes, e.g., meats, dairy products, green vegetables, etc.

Data yielded by this method, especially when correlated with that obtained on unprocessed foods, may be used to calculate the approximate residue intake associated with any diet pattern. Such correlations, however, would be a subject for special research projects and are not specifically contemplated as a function of the monitoring program.

[1] Office of Associate Commissioner for Compliance, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20204.
[2] Bureau of Science, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20204.

Geographical Distribution of Sampling Stations

Sampling in the Market Basket Survey (for analysis of composites of food items prepared for consumption) is carried out in five regions representing the northeastern, southeastern, north central, central, and western United States. Sampling sites within each region are chosen from different cities, one representing a standard metropolitan statistical area and one representing a smaller population center (less that 50,000 population).

Samples in the nationwide surveillance of unprocessed foods are collected at all major growing, processing, and marketing centers. Animal food ready for consumption is included in this part of the program. Collection headquarters are in each of the 18 Regional Districts of the Food and Drug Administration, with offices in Boston, New York City, Buffalo, Philadelphia, Baltimore, Atlanta, Cincinnati, Detroit, Chicago, St. Louis, New Orleans, Minneapolis, Kansas City, Dallas, Denver, Los Angeles, San Francisco, and Seattle.

Meat samples at slaughter will be taken at each of the Nation's major meat processing centers.

Sampling Frequency, Number of Samples

Market Basket samples are collected six times per year in each of the five geographic regions mentioned above, making a total of 30 Market Basket samples annually.

The surveillance program encompasses an estimated 2.5 million carloads of raw agricultural products annually shipped in interstate commerce. In addition, there are thousands of lots of other foods, e.g., milk, eggs, fish, and processed animal feeds, produced each year.

A minimum coverage sampling procedure has been designed whereby product samples will be collected throughout the year in the following broad categories:

Leaf and stem vegetables	*Fish and shellfish*
Root vegetables	*Eggs and egg products*
Fruits	*Fluid Milk*
Grains	*Manufactured Dairy Products*
Hay and forage	

Sampling locations are selected at random from wholesale markets and warehouses located in [illegible] cities. Approximately 12,000 random samples[3] are examined annually. This provides 95% confidence that the true percentage of samples exceeding guidelines will not be greater than 3.1% if the observed percentage is 2%. When the observed percentage of samples exceeding guides approaches 3%, the sampling rate is increased to provide more reliable estimates.

Commodities to be Sampled

In the Market Basket Survey, samples are collected according to a series of 82 items listed by commodity groups in Appendix A. Adjustments are made in this list to reflect local dietary patterns in each geographical area. The list also will be evaluated periodically and changed as necessary to reflect changes in dietary patterns, particularly in the area of "convenience" and frozen foods.

Commodities sampled under the nationwide surveillance of unprocessed foods are listed in Appendix C. A sampling schedule for these commodities is included as Appendix D.

Sample Preparation

Market Basket items which normally require further processing by cooking, such as fresh meats and certain raw vegetables, or preparation for eating raw, such as tomatoes, carrots, celery, lettuce, cucumber, cabbage, and fresh fruits are delivered to a diet kitchen for preparation under the direction of a dietician. Some food items, e.g., cabbage, are included in both the raw and cooked forms. Instructions to the diet kitchen for preparing these food items are contained in Appendix B.

Market Basket items normally consumed as purchased or which do not otherwise require further processing—e.g., dairy products, luncheon meats and frankfurters, canned meats, some fruits and vegetables, potato chips, canned fruit juices, concentrated fruit juices, and frozen fruits—are to be retained by the examining laboratory for compositing by commodity groups with the foods prepared by the diet kitchen.

Guidelines for compositing food items sampled

[3] The Food and Drug Administration also examines about the same number of samples selected because of suspected residues as an accompanying part of its enforcement activities. This is not considered a monitoring activity, and the program is not described.

in the surveillance of unprocessed foods are given in Appendix E.

Sample Analysis Procedures

All analytical procedures used in this program are described in FDA's Pesticide Analytical Manuals.

For the Market Basket Survey, procedures for examining each commodity group are outlined as follows:

1. *Chlorinated Organic Pesticides — Examine all commodity groups at sensitivity levels equivalent to 0.003 parts per million heptachlor epoxide using electron capture, gas-liquid chromatography. Residues above these limits are to be checked by thin-layer chromatography or Dohrmann glc, and results reported to the nearest 0.001 ppm. Multiple detection procedures are to be used to detect the 25 chlorinated organic compounds included in Appendix F.*
2. *Organic Phosphate Pesticides — Examine all commodity groups in conjunction with chlorinated organic residue analyses at a sensitivity level of 0.05 ppm of parathion. Confirm positive findings by thin-layer chromatography.*
3. *Herbicides — Examine all commodity groups by Dohrmann glc, confirm by paper chromatography. Examine all commodity groups in Appendix A, except groups 1, 2, and 10 for 3-AT (3-aminotriazole). Confirm residues by paper chromatography.*
4. *Carbamates — Examine all commodity groups for carbaryl, except groups 1, 2, and 10, Appendix A, and confirm positive findings. Use general methods for dithiocarbamates in examining above groups and report results as zineb.*
5. *Bromides — Examine all commodity groups.*
6. *Arsenic — Examine all commodity groups.*

For the nationwide surveillance of unprocessed food and feed, all samples are to be examined for chlorinated organic pesticides and organic phosphate pesticides (See Appendix F) using multiple detection procedures at sensitivity levels equivalent to 0.03 ppm heptachlor epoxide using electron capture, gas-liquid chromatography. Individual samples selected at random are to be examined for residues of chlorophenoxy compounds, carbaryl, and carbamates. Analytical procedures are described in the Food and Drug Administration's Pesticide Analytical Manuals.

Appendix A

MARKET BASKET COMPOSITION BY COMMODITY GROUPS

1. Dairy Products	*Milk, Fresh Fluid; Evaporated Milk; Nonfat Dry Milk; Ice Cream; Cottage Cheese; Processed Cheese (American); Natural Cheese; Butter or (alternate) Margarine*
2. Meat, Fish, and Poultry	*Lamb or Mutton; Roast Beef; Ground Beef; Pork Chops; Pork Sausage (cured); Bacon; Chicken (eviscerated—fresh or frozen); Fish Fillet, fresh or frozen; Tuna or Salmon, canned; Luncheon Meat; Frankfurters; Liver, beef; Eggs, large*
3. Grain and Cereal Products	*Flour, General Purpose; Flour, Self-Rising; Pancake Mix; Corn Flakes; Shredded Wheat or Wheat Cereal; Rice Flakes or Puffed Rice; Oatmeal; Rice; Corn Meal; Macaroni, elbow; Bread, White (enriched); Bread, White (unenriched); Bread, Italian style; Bread, Whole Wheat; Rolls (sweet, cinnamon, Bismarcks, doughnuts); Saltines; Cookies, Plain; Rolls (frankfurter); Graham Crackers; Pie Crust (fruit filling—item 9); Cake; Corn, raw; Corn, canned; Corn, frozen*
4. Potatoes	*Potatoes, white (bake ½); Potatoes, white (boil ¼); Potatoes, white (fry ¼); Potato Chips; Sweet Potatoes or Yams, fresh or canned*
5. Leafy Vegetables	*Beet Tops, raw; Beet Tops, canned; Collards, raw; Collards, canned; Mustard, raw; Mustard, canned; Spinach, raw; Spinach, canned; Spinach, frozen; Celery, raw; Lettuce, raw; Cabbage (raw ½); Cabbage (boil ½); Broccoli, fresh; Broccoli, frozen; Asparagus, canned; Asparagus, fresh; Asparagus, frozen; Mushrooms, raw; Mushrooms, canned; Cauliflower, raw; Cauliflower, frozen*
6. Legume Vegetables	*Peas, raw; Peas, canned; Peas, frozen; Green Beans, raw; Green Beans, frozen; Green Beans, canned; Beans, w/pork, canned; Lima Beans, raw; Lima Beans, canned; Lima Beans, frozen*
7. Root Vegetables	*Carrots, raw; Carrots, canned; Onions, dry, (raw ½); Onions, dry, (boil ½); Beets, w/o tops, raw; Beets, w/o tops, canned; Turnips, w/o tops, raw; Rutabagas, raw*
8. Garden Fruits	*Pepper, raw; Tomatoes, fresh; Tomatoes, canned; Catsup; Cucumbers, raw; Eggplant, raw; Summer Squash, raw; Summer Squash, frozen*
9. Fruits	*Fruit filling from pie; Oranges, fresh; Orange Juice frozen conc., canned; Bananas; Raisins; Peaches, fresh; Peaches, canned; Apples, fresh; Apples, canned; Strawberries, raw-fresh or frozen; Apricots, raw; Apricots, canned; Cherries, raw; Cherries, canned; Cherries, frozen; Grapes, raw; Pears, raw; Pears, canned; Pineapple, raw; Pineapple, canned; Pineapple, frozen; Plums, raw; Plums, canned; Rhubarb, w/o top, raw-fresh or frozen; Watermelon, raw*
10. Oils, Fats, and Shortening	*Salad Dressing - French; Mayonnaise; Salad Oil; Shortening; Peanut Butter*
11. Sugar and Adjuncts	*Sugar, White; Jam or Jelly; Pudding Mix; Syrup, blended; Molasses; Candy Bar; Baking Powder; Salt; Vinegar, cider*
12. Beverages	*Tea Leaves; Cocoa; Drinking Water; Coffee, ground; Soft Drinks*

Appendix B

INSTRUCTIONS FOR FOOD PREPARATION AND CHECK LIST OF ITEMS IN SAMPLE (Market Basket Survey)

The food items listed below are those requiring preparation. The preparation may consist of roasting, baking, broiling, frying, or boiling. Some vegetables are to be prepared to eat raw. After processing, wrap in aluminum foil and place in labeled containers.

FOOD ITEM	INSTRUCTIONS
Roast beef	*Roast, medium-well done. Remove bone and discard. Save drippings.*
Ground Beef	*Make into patties, broil, save drippings.*
Pork chops	*Broil. Remove bone and discard. Save drippings.*
Pork sausage	*Make into patties, broil.*
Bacon	*Broil.*
Chicken	*Discard neck and tail portion. Bake. Remove edible meat from bone after baking. Save drippings.*
Fish fillet	*Broil.*
Liver, beef	*Broil, save drippings.*
Potatoes, white	*Bake. Leave skin on. (5 lbs.)* *Fry. (2½ lbs.)* *Boil. Peel and discard skin before boiling. (2½ lbs.) Discard cooking water.*
Tomatoes, fresh	*Wash, remove core, do not peel.*
Oranges, raw	*Remove peel and seeds.*
Carrots, raw	*Wash, scrape, slice ready-to-eat raw.*
Greens (Beet tops, Collards, Mustard, Spinach)	*Fresh or frozen. Wash, trim, cook fresh item. Cook frozen item. Discard cooking water.*
Green Pepper or broccoli	*One item only. Pepper, fresh. Broccoli, fresh or frozen. Pepper, prepare to eat raw. Fresh broccoli—wash, trim, and cook. Frozen broccoli, cook. Discard cooking water.*
Sweet potatoes	*Wash, bake, and peel.*
Celery	*Wash, trim, cut.*
Lettuce	*Trim, quarter.*
Cucumber	*Wash with detergent to remove wax.*
Cabbage	*(1) Raw. Trim and chop for slaw.* *(2) Cook after trimming. Discard cooking water.*
Onions, dry	*(1) Raw. Clean and quarter.* *(2) Cooked. Clean and boil. Discard cooking water.*
Peas	*Fresh in season. Remove pods, cook. Frozen, cook. Discard cooking water from both.*
Green Beans	*Fresh if available. Wash and cook fresh or frozen. Discard cooking water.*
Corn, sweet	*Fresh if available. Remove husk, trim, cook in boiling water. Discard water. Remove cooked corn from ear. Cook frozen corn and discard water. Discard cobs.*
Peaches, raw	*Fresh when available. Wash, peel, remove pits, and halve.*
Apples	*Wash, remove core, do not peel.*
Strawberries	*Fresh in season. Wash, remove stems. Halve.*
Other Vegetables	**Fresh and frozen vegetables will be cooked.**
Asparagus	*Fresh in season. Wash and cook. Frozen, cook. Discard cooking water.*
Beets	*Fresh beets. Wash, trim, and cook. Discard cooking water.*
Mushrooms	*Wash, trim, and boil. Discard cooking water.*
Turnips	*Wash, trim, and cook. Discard cooking water.*
Lima Beans	*Fresh, shell and cook. Frozen, cook. Discard cooking water.*
Cauliflower	*Fresh, wash, trim, and cook. Frozen, cook. Discard cooking water.*
Eggplant	*Trim and cook. Discard cooking water.*
Rutabagas	*Trim and cook. Discard cooking water.*
Summer Squash	*Fresh, trim and cook. Frozen, cook. Discard cooking water.*
Other Fruits	**Fresh fruits only to be processed.**
Apricots	*Wash and pit.*
Cherries	*Wash and pit.*
Grapes	*Wash, remove seeds, and stems.*
Pears	*Wash and core.*
Pineapple	*Trim and core.*
Plums	*Wash and pit.*
Rhubarb	*Trim.*
Watermelon	*Trim and remove seeds.*
Cooking Oil	*(Unused cooking oil to be returned with processed foods and included in composite.)*

Appendix C

NATIONWIDE SURVEILLANCE COMMODITIES

Large fruit
Small fruit
Leaf and stem vegetables
Vine and ear vegetables
Beans
Root vegetables
Nuts
Hay and silage
Wheat
Corn
Oat
Rye
Sorghum
Barley
Flax
[illegible]
[illegible]
[illegible]
F... a...
Meat (be... a... b... an...
M... An.ma...
Vegetable Oil
F... l... Liver Oil
Other (e.g., coffee, ... beans, ... pepper, ginger, ...)

Appendix D

SAMPLING SCHEDULE FOR NATIONWIDE SURVEILLANCE COMMODITIES

Treat each identifiable grower's mark or lot number in the shipment as a separate sample. Sample, as a single lot, shipments containing commingled and unidentifiable lots from several growers. Be careful not to collect more than the proportional amount from facing layers. When sampling from loading cars, select subsamples at intervals to obtain a sample representative of the carload. For bulk lots select subs at random throughout the lot.

Collect a composite sample closely approximating 20 lbs. by taking a 2 lb. sub from each of 10 different shipping containers selected at random. DO NOT cut or divide individual produce items to adjust sub weights.

SPECIAL NOTE: Some produce items weighing 2 lbs. or more each, such as melons, pineapples, large heads of cabbage, large cauliflower, large celery stalks, large rutabagas, etc., do not lend themselves to the above sampling approaches. In such cases, collect a total composite sample of 10 subs taking one item from each shipping container.

For light bulky produce, e.g., collards, spinach, leaf lettuce, other leafy products, hay, etc., collect a 10-lb. composite sample taking 1 lb. from each of 10 different shipping containers selected at random.

Hold samples in cold storage until ready to be shipped or delivered to the laboratory only if normally held or shipped under refrigeration in commercial practice.

Appendix E

GUIDELINES FOR COMPOSITING UNPROCESSED FOOD SAMPLES

ANIMAL TISSUE		*Grind about half of each sub (meat grinder); composite 100 g from each sub and grind again.*
DAIRY PRODUCTS		*Equal weight from each sub. Grind, dice, or blend.*
EGGS		*Equal number of units from each sub, for total of 6-12. Blend.*
FEED, ANIMAL		*200 g from each sub (quarter subs down to 200 g where necesary); wet feeds (silage) 100 g from each sub.*
FORAGE		*Quarter each sub down to 200 g; composite 200 g from each sub. Chop fine. Where necessary, grind in Wiley Mill without screen; then with screen in.*
FRUITS	LARGE	*(apples, pears, tomatoes, etc.). Equal number of units from each sub. Chop or blend.*
	SMALL	*200 g from each sub. Chop or blend.*
GRAINS		*100 g from each sub. Grind in Wiley Mill or equivalent.*
HAY		*200 g from each sub. Chop or grind.*
MILK		*100 g (ml) from each sub after thorough shaking.*
NUTS		*Remove shells. Composite equal number of units (equal weight) from each sub. Chop or grind.*
OILS		*Equal weight or volume from each sub.*
SEEDS		*100 g from each sub. Grind.*
SPICES		*200 g from each sub. Grind or chop.*
VEGETABLES	HEAD	*Quarter each head in the sub. Take two opposite quarters from each head and chop into 1- to 2-inch pieces with a knife. Mix well. Composite 200 g of chopped product from each sub and chop entire composite in a food chopper.*
	LEAFY	*Leaf Cut — Mix sub well and select leaves at random until a 200-g portion is obtained. Composite in a food chopper.*
	POD	*(beans, peas, etc., also asparagus) 200 g from each sub. Chop or grind.*
	ROOT	*Equal number of units from each sub. Chop or grind.*
	STALK	*(celery, broccoli, etc.) Quarter each sub lengthwise and proceed as in "Head Vegetables."*

Appendix F

QUANTITATIVE AND QUALITATIVE

COMMON OR TRADE NAME	CHEMICAL NAME
1. Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
2. BHC (benzene hexachloride)	1,2,3,4,5,6-hexachlorocyclohexane
3. Bulan®	2-nitro-1,1-bis(*p*-chlorophenyl) butane
4. Butyl ether ester, 2,4-D	butyl ether ester of 2,4-dichlorophenoxyacetic acid
5. *n*-Butyl ester, 2,4-D	*n*-butyl ester of 2,4-dichlorophenoxyacetic acid
6. *n*-Butyl ester, 2,4,5-T	*n*-butyl ester of 2,4,5-trichlorophenoxyacetic acid
7. Chlorbenside	*p*-chlorobenzyl-*p*-chlorophenyl sulfide
8. Chlorobenzilate	ethyl 4,4′-dichlorobenzilate
9. Chlordane	1,2,3,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
10. Chlorothion	0,0-dimethyl 0(3-chloro-4-nitrophenyl)phosphorothioate
11. CIPC	isopropyl N-(3-chlorophenyl) carbamate
12. Dacthal®	dimethyl 2,3,5,6-tetrachloroterephthalate
13. DDE	dichlorodiphenyl dichloroethylene
14. DDT (o,p′+p,p′; o,p′; p,p′)	dichloro-diphenyltrichloroethane
15. Diazinon	0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate
16. Dichloran	2,6-dichloro-4-nitroaniline
17. Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
18. Dilan (See Bulan® and Prolan®)	
19. Dyrene®	2,4-dichloro-6-(*p*-chloroanilino)-*s*-triazine
20. Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
21. Ethion	0,0,0′,0′-tetraethyl-S-S′-methylene bis-phosphorodithioate
22. Ethyl hexyl ester, 2,4-D	ethyl hexyl ester of 2,4-dichlorophenoxyacetic acid
23. EPN	0-ethyl 0-*p*-nitrophenyl phenylphosphonothioate
24. Folpet	N-trichloromethylthiophthalimide
25. Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-*endo*-methanoindene
26. Heptachlor Epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
27. Hexachlorobenzene	Same
28. Isobutyl ester, 2,4-D	isobutyl ester of 2,4-dichlorophenoxyacetic acid
29. Iso-octyl ester 2,4,5-T	iso-ocytl ester of 2,4,5-trichlorophenoxyacetic acid
30. Iso-octyl ester, 2,4-D	iso-ocytl ester of 2,4-dichlorophenoxyacetic acid
31. Isopropyl ester, 2,4,5-T	isopropyl ester of 2,4,5-trichlorophenoxyacetic acid
32. Isopropyl ester, 2,4-D	isopropyl ester of 2,4-dichlorophenoxyacetic acid
33. Kelthane®	1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol
34. Lindane	γ isomer of benzene hexachloride
35. Malathion	S-[1,2-bis(ethoxycarbonyl) ethyl]0,0-dimethyl phosphorodithioate
36. Methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane
37. Methyl parathion	0,0-dimethyl 0-*p*-nitrophenyl phosphorothioate
38. Ovex	*p*-chlorophenyl *p*-chlorobenzenesulfonate
39. Parathion	0,0-diethyl-0-*p*-nitrophenyl phosphorothioate
40. PCNB	pentachloronitrobenzene
41. Perthane® & olefin	1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane
42. Prolan®	2-nitro-1,1-bis(*p*-chlorophenyl) propane
43. Ronnel	dimethyl 2,4,5-trichlorophenyl phosphorothioate
44. Strobane®	terpene polychlorinates
45. TCNB	1,2,4,5-tetrachloro-3-nitrobenzene
46. TDE	tetrachlorodiphenylethane
47. Tedion®	*p*-chlorophenyl-2,4,5-trichlorophenylsulfone
48. Telodrin®	1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan
49. Tetraiodoethylene	Same
50. Thimet®	0,0-diethyl S-(ethylthio) methyl phosphorodithioate
51. Thiodan I®	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide
52. Toxaphene	octachlorocamphene
53. Trithion®	S-[[(*p*-chlorophenyl) thio]methyl]0,0-diethyl phosphorodithioate
54. Vegadex®	2-chloroallyl diethyldithiocarbamate

PESTICIDES IN PEOPLE

CRITERIA FOR MONITORING PESTICIDES IN PEOPLE INCLUDE HIGH- AND LOW-EXPOSURE CONDITIONS, AGE, SEX DIFFERENCES

Anne R. Yobs[1]

As described here the program for assessing pesticide residue levels in the Nation's populace is being carried out by the Pesticides Program, National Communicable Disease Center, Bureau of Disease Prevention and Environmental Control, Public Health Service, U. S. Department of Health, Education, and Welfare.

Monitoring Objective

The purpose of the human monitoring program is to determine on a national scale the levels and trends of certain more commonly used pesticide chemicals, both in the general population and in population segments where the occurrence of more extensive exposure to pesticides is known or suspected.

In the past, studies were made by various investigators assessing the concentration of pesticides and or their metabolites in human beings. These studies have provided a useful body of information concerning the relationship of exposure to the human body's storage of pesticides. However, such assessments were limited in regard to the geographic coverage of the sampling, the variety of conditions of exposure, and the spectrum of pesticides investigated. They were also limited in the age range, the sex distribution, and the size of the sampled population. Probably the greatest weakness in the earlier studies was the limited variety of body tissues tested. In fact, this earlier work was essentially limited to body fat. The present monitoring program is to provide statistically and epidemiologically sound information for use in the evaluation of the significance of man's total exposure to pesticides.

[1] Pest[illegible] communicable Disease Center, Public He[illegible] Prevention and Environmental Con[illegible] Health, Education, and Welfare, [illegible]

Program Design, Samples, and Sampling Sites

Monitoring studies will be of two types, a limited national survey of the general population and an in-depth study of selected communities in high-use areas.

In the survey being activated in calendar year 1967, tissues will be collected regularly from the general population in 12 different areas of the country. The number of specimens will be relatively small at first to permit evaluation of the approach and correction of any problem areas. The program will be expanded later as data indicate. Samples will be collected at post-mortem examinations and from hospitalized patients. Only body fat samples will be analyzed at this time from post-mortem examinations. Sample tissues from living patients will include blood serum and adipose tissue removed incidentally at surgery.

In-depth community studies, including monitoring, are in progress at these locations:

Arizona — Pima and Maricopa Counties
California — State-wide
Colorado — Weld County
Florida — Dade County
Hawaii — Island of Oahu
Iowa — Johnson County
Louisiana — LaForche and Jefferson Parishes
Michigan — Berrien County
New Jersey — Monmouth County
Texas — Cameron and Hidalgo Counties
Washington — Wenatchee and Quincy Basins

Plans are under way for the initiation of additional studies in Idaho, South Carolina, Mississippi, and Utah.

These studies are sampling three population groups: (1) occupationally exposed workers, (2) individuals not occupationally exposed but known to be repeatedly exposed, and (3) the general urban population. The occupationally exposed group consists of one or more of the following: agricultural applicators, workers in pesticide formulating plants, pest control operators, greenhouse workers, and aerial spray pilots. Representatives in the repeatedly exposed population are people living in environments where they may be expected to have repeated nonoccupational exposure—these areas are usually heavily agricultural. The general urban population group represents individuals whose exposures are largely limited to pesticide traces in food, water, and air plus occasional household or garden use of pesticides. Since the occupationally exposed group consists predominantly of men, sampling of this group will be restricted to adult males. However, the two remaining groups will be equally divided between males and females with a reasonable age spread.

Study procedures for each participant include a detailed history of pesticide exposure and usage, a complete medical history and physical examination, and hematologic and biochemical testing. Pesticide residue analyses will be performed on urine and blood of all participants and, when available, on body fat and other tissues also. In addition, each study performs an area pesticide-usage profile and analyzes samples from the local environment. Tissues taken by the Community Studies for general population studies will be secured at postmortem examinations of accidental deaths.

Pesticides and Analytical Methods

Chlorinated hydrocarbon pesticides are known to concentrate in animal and human fat and to persist there for prolonged periods. Primary emphasis will be given to detecting residues from these chemical compounds and assessing their levels. A serious problem is the lack of suitable analytical procedures for detecting different classes of pesticide chemicals in the ranges expected in the general population and applicable to several tissues. It is recognized that human tissue samples may well contain several pesticides of the same or other classes, and the analysts will be expected to be alert to identify them. As research progress may indicate and require, and as technological developments permit, other tissues and other pesticides may be added to this monitoring program.

Each Community Study has or is developing laboratory competence in pesticide analysis and the required hematological and biochemical testing. They perform all testing for their own locations, and some will perform the analytical testing for the general monitoring program using standardized procedures. All participating laboratories are required to maintain a satisfactory standard of technical performance as demonstrated in a quality control program conducted by the Pesticide Research Laboratory (Florida) of the Pesticides Program.

Standardization of Procedures

Guidelines and forms have been developed to standardize the collection and recording of information, the sampling and handling of tissue specimens, and laboratory test procedures. This will permit the comparison of data among the several participants in the monitoring program. Information from the several study areas will be combined to give an overall picture for the Nation as a whole.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

INDICATOR SPECIES NEAR TOP OF FOOD CHAIN CHOSEN FOR ASSESSMENT OF PESTICIDE BASE LEVELS IN FISH AND WILDLIFE—CLAMS, OYSTERS, AND SEDIMENT IN ESTUARINE ENVIRONMENT

R. E. Johnson[1], T. C. Carver[2], and E. H. Dustman[3]

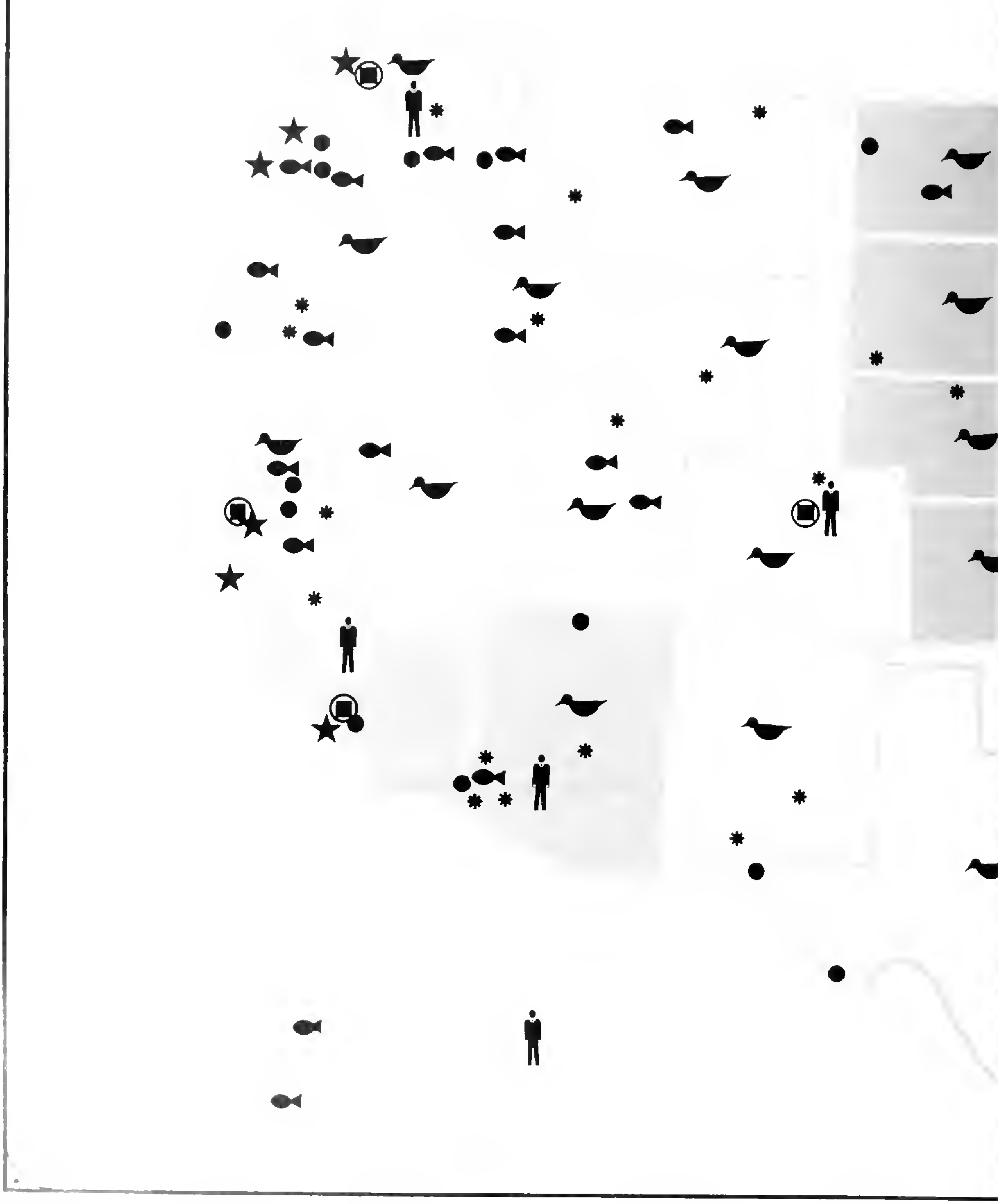
Federal efforts to determine pesticide levels in fish and wildlife are being carried out by the Bureau of Sport Fisheries and Wildlife, U. S. Department of the Interior. Monitoring estuarine pesticide levels in clams, oysters, and sediments is a joint endeavor of the Bureau of Commercial Fisheries, U. S. Department of the Interior, and the Water Supply and Sea Resources Program of the National Center for Urban and Industrial Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

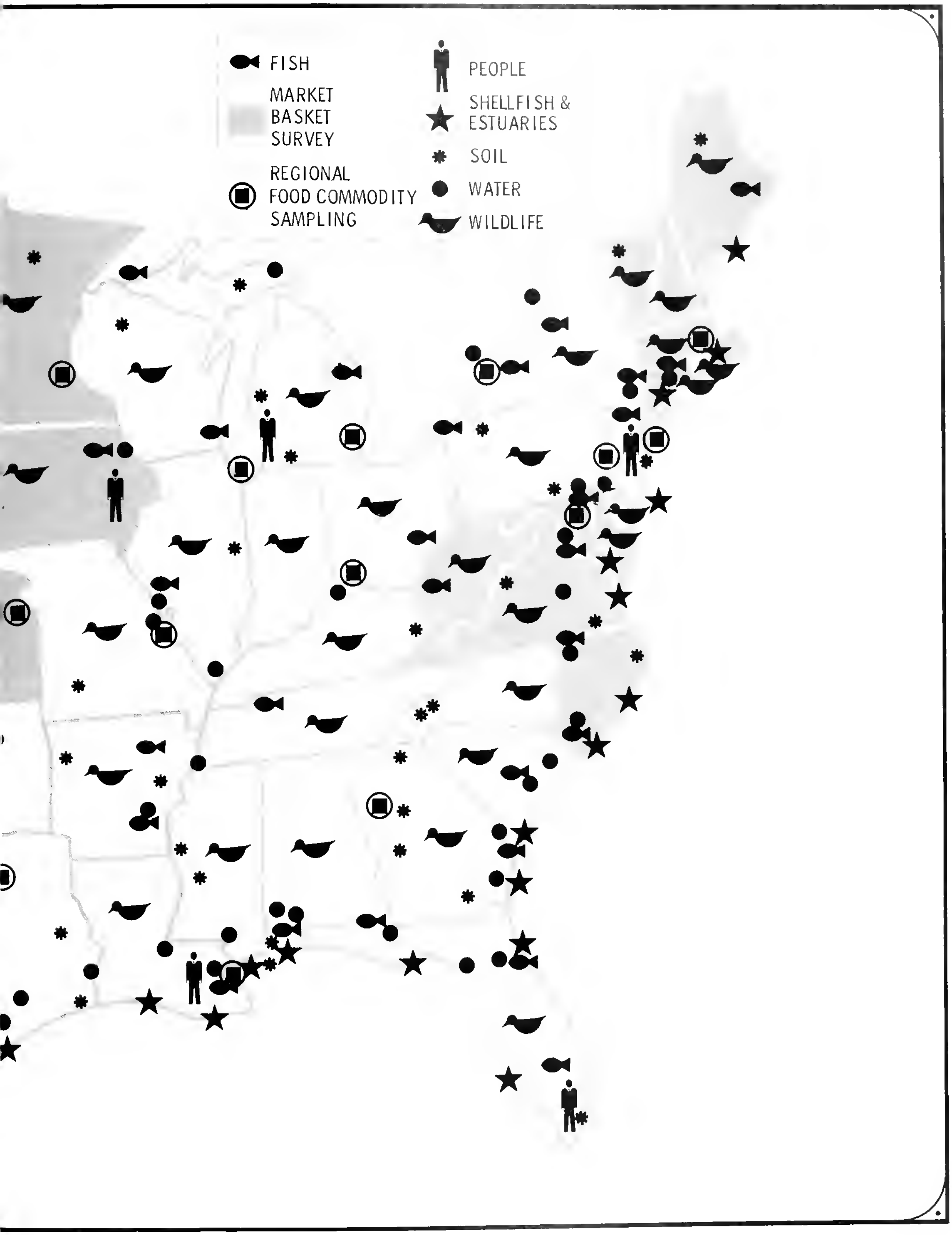
Monitoring Objective

These monitoring programs will ascertain on a national scale and independent of specific treatments the levels and trends of certain pesticidal chemicals in the bodies of selected forms of animals and in estuarine sediments.

[1]Bureau of Sport Fisheries and Wildlife, U. S. Department of the Interior, Washington, D. C. 20240.
[2]Patuxent Wildlife Research Center, Bureau of Commercial Fisheries, U. S. Department of the Interior, Laurel, Maryland 20810.
[3]Patuxent Wildlife Research Center, Bureau of Sport Fisheries and Wildlife, U. S. Department of the Interior, Laurel, Maryland 20810.

National Pesticide Monitoring Sites




FISH
MARKET BASKET SURVEY
REGIONAL FOOD COMMODITY SAMPLING
PEOPLE
SHELLFISH & ESTUARIES
SOIL
WATER
WILDLIFE

MONITORING FISH

Complete standardization of one fish species for nationwide analysis is not possible; therefore, a minimum of three species has been designated for collection at each sampling site. As with the wildlife forms, fish being sampled are at or near the top of the food chain. These include—listed in their order of preference, depending upon availability at individual collection sites—carp, buffalo, black bass, channel catfish, green sunfish, yellow perch, rainbow trout, and squawfish.

Collection Sites and Sampling Frequency

Fifty locations have been chosen as collection sites. These sites were selected to coincide wherever possible with sampling locations for monitoring pesticides in estuarine environments and in fresh water. In some instances selection of alternate locations was necessary to provide for collection sites at points where appropriate resident fish populations can be sampled, where nets can be placed in streams with some permanence and where commercial fishermen may be relied upon to take desired fishes if State or Federal crews are not available to do so. Some collection sites are in the immediate vicinity of other U. S. Fish and Wildlife Service facilities where manpower and equipment are readily available.

Collections are taken twice a year, as close to April and October as possible, at each of the 50 sampling locations; the amount of fish per collection is from 15 to 25 lbs. Measurement of pesticide levels at these times of the year indicates body burdens immediately prior to spawning of some fish species and at a time of maximum body fat content of nearly all species. Sampling at these times also reflects levels before and after major seasonal uses of pesticides.

Sampling locations are listed by regional drainage systems:

Atlantic Coastal Drainage

- Penobscot River, *vicinity of Orono, Maine*
- Connecticut River, *Windsor Locks, Connecticut*
- Hudson River, *Poughkeepsie, New York*
- Delaware River, *Camden, New Jersey*
- Susquehanna River, *Conowingo Dam, Maryland*
- Potomac River, *Little Falls, Maryland*
- Roanoke River, *Weldon, North Carolina*
- Cape Fear River, *Wilmington, North Carolina*
- Cooper River, *Lake Moultrie or Marion, South Carolina*
- Savannah River, *above Savannah, Georgia*
- St. Johns River, *Welaka, Florida*
- St. Lucie Canal, *Indiantown, Florida*

Gulf Coastal Drainage

- Apalachicola River, *Jim Woodruff Dam, Florida*
- Tombigbee River, *above Mobile, Alabama*
- Mississippi River, *commercial fisheries, New Orleans, Louisiana*
- Rio Grande, *above Brownsville, Texas*

Great Lakes Drainage

- Genessee River, *near Avon, New York*
- *Commercial fishery landings at:*
  - Port Ontario, New York
  - Erie, Pennsylvania
  - Bay Port, Michigan
  - Port Washington, Wisconsin
  - Bayfield, Wisconsin

Mississippi River System

- Kanawha River, *Winfield, West Virginia*
- Ohio River, *near Marietta, Ohio*
- Cumberland River, *Clarksville, Tennessee*
- Illinois River, *Beardstown, Illinois*
- Upper Mississippi River, *Guttenberg, Iowa*
- Arkansas River, *near Pine Bluff, Arkansas*
- Arkansas River, *Keystone, Oklahoma*
- White River, *near De Valls Bluff, Arkansas*
- Missouri River, *Nebraska City, Nebraska*
- Missouri River, *Garrison Dam, North Dakota*
- Missouri River, *Fort Benton, Montana*

Hudson Bay Drainage

- Red River, *near Noyes, Minnesota*

Colorado River System

- Green River, *near Vernal, Utah*
- Colorado River, *Imperial Dam, Arizona*

Interior Basins

- Lower Truckee River, *Derby Dam, Nevada*
- Utah Lake, *near Provo, Utah*

California Streams

- Sacramento River, *Sacramento, California*
- San Joaquin River, *near Los Banos, California*

Columbia System

- Snake River, *near Hagerman, Idaho*
- Snake River, *Lewiston Dam, Lewiston, Idaho*
- Salmon River, *near Riggins, Idaho*
- Yakima River, *near Prosser, Washington*
- Willamette River, *above Oregon City, Oregon*
- Columbia River, *Bonneville Dam, Oregon*

Pacific Coastal Streams

- Klamath River, *near Klamath River, California*
- Rogue River, *near Grants Pass, Oregon*

Alaskan Streams

- Yukon-Tanana system, *Fairbanks or Tanana, Alaska*
- Kenai River, *Soldatna, Alaska*

Methods of Collecting, Preserving, and Shipping Specimens

Fish are collected by seining, gill-netting, electric shocking, or by any other means which insures that no extraneous chemicals are introduced to complicate the analysis. Poisons such as rotenone are not being used for collecting samples, because they may interfere with the analysis. Approximately 1 lb. of fish is taken for each sample when the whole fish is to be ground for analysis. When individual tissues are to be analyzed, more than a pound of whole fish may be required to furnish large enough specimens of specific tissues.

Fish are wrapped in aluminum foil for preservation by freezing. Samples of fish are wrapped separately to avoid contamination between and among samples. Labels are made for each sample on paper of a durable quality and printed with soft black pencil rather than ink to avoid smearing when wet. Each label shows the name of the collector, specimen number, species, sex and age if known, and date and place of collection.

Frozen specimens are packed in a strong cardboard carton or drum with crumpled newspaper or styrofoam for insulation and kept refrigerated with dry ice at the rate of 10 lbs. for 10-15 lbs. of fish. Samples are transported by air freight or air express.

A telegram is sent to the receiving laboratory before or at the time of shipment to prevent delay in the pickup of specimens at destination points. Also at the time of shipment, or soon thereafter, a detailed list of the specimens is forwarded to the laboratory.

MONITORING WILDLIFE

Since it is impossible to sample representatives from each major group of animals occurring in the United States, it is necessary to select, at least for the time being, several species of wildlife which occur reasonably close to the top of a food chain. Later, as more is learned about the significance of pesticide residues in animal tissues, and as more is learned about effects in various groups of animals, adjustments can be made in the breadth and scope of monitoring coverage in keeping with new knowledge.

Several criteria are important in the selection of forms for monitoring. The species selected should not be extremely sensitive to chemicals to be monitored. They should be geographically well distributed, reasonably numerous, and easy to collect. Residues in species close to the top of a food chain also will reflect residues in organisms at lower levels.

Species chosen for monitoring pesticides in wildlife include the mallard duck, starling, and the bald and golden eagles. The closely related black duck is being substituted for the mallard in States where adequate samples of the mallard cannot be obtained.

Duck Sampling

In cooperation with State agencies, the U. S. Bureau of Sport Fisheries and Wildlife annually collects thousands of waterfowl wings of game species from all parts of the United States. These are sent to several collection sites throughout the country where they are housed in freezers until they are examined by waterfowl biologists to determine sex and age ratios.

The mallard is universally distributed in the United States. It is a migrant form, moving each spring into the northern United States and Canada to breed. In the fall it moves southward to winter. It is omnivorous and feeds in both aquatic and terrestrial environments. Approximately fifty thousand mallard wings, and where necessary black duck wings, are collected annually during the hunting season in each of the 48 conterminous States.

Through a process of systematic subsampling, a series of wings are drawn from each State. Approximately 12,500 wings annually are composited into samples of 25 wings each for analysis.

Starling Sampling

The starling is a ubiquitous bird which lends itself well to sampling. Being omnivorous, it feeds heavily on many kinds of insects and fruits in spring, summer, and fall, and on crop remnants and a miscellanea of other foods in winter months.

Beginning in calendar year 1967, starlings will be collected from various areas of the country at two periods of the year, late summer and winter. Three composite samples of 10 birds each will be collected per seasonal sampling period at each of 41 collection sites widely distributed geographically. One set of samples will be taken by trapping or shooting in August when pesticide body burdens will reflect applications made during the growing season. Another set will be taken in December or January to assess body burdens during a period of minimum pesticide usage throughout most of the country.

Eagle Sampling

The golden and bald eagles currently are being monitored by the U. S. Bureau of Sport Fisheries and Wildlife. Specimens found dead or incapacitated and beyond recovery are submitted to Bureau laboratories for analysis. No well-established sampling pattern is possible with these forms, largely because of their protected status and their relatively low population levels. They have been included in the pesticide monitoring program because they are carnivorous and at the top of food chains.

Methods of Handling Specimens

Wildlife specimens are handled and packaged in the same manner as fish samples.

MONITORING ESTUARIES

The Federal estuarine pesticide monitoring program is conducted jointly by the Bureau of Commerical Fisheries of the U. S. Department of the Interior and the Water Supply and Sea Resources Program of the National Center for Urban and Industrial Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

Shellfish of interest are oysters and clams. These filter-feeding pelecypod mollusks of commercial value occur in all large estuarine systems in the United States. They are particularly well suited for pesticide monitoring because, as sessile forms, they filter vast quantities of water. They also are abundant and easily obtained. Preliminary experimental work indicates that oysters and clams will tolerate chlorinated hydrocarbon pesticides and retain the residues of these chemicals for extended periods following exposure.

Sediment is of keen interest, because it is an important part of the total aquatic environment. Pesticides adsorbed to soil particles usually are chemically inactive although a slight decrease in pH values can result in release of the chemical from the soil particles. A similar pH change is usually encountered in the upper animal digestive tract. Many estuarine forms are susceptible to this type of exposure.

While the basic orientation of this program is toward commercial estuarine fisheries, other areas of interest related to sediment monitoring are recognized. For this program, sampling of sediment is at the interface, which is the uppermost portion of bottom sediments.

Sampling Sites

Samples for analyses are collected by agencies at both the Federal and State level from estuarine systems and major river drainages containing commercial quantities of shellfish. In the interest of continuity, uniform sampling procedures are observed by each cooperating organization. A total of 24 sampling locations have been selected which serve as collection sites for both shellfish and sediment. The sampling point within each estuary is selected on the basis of available hydrographic data, particularly current patterns, and on the availability of suitable shellfish populations.

Estuaries being studied, chosen on the basis of water mass, are:

Penobscot Bay	*Tampa Bay*
Narragansett Bay	*Apalachicola Bay*
Long Island Sound	*Mobile Bay*
Peconic Bay	*Mississippi Sound*
Delaware Bay	*Lake Calcasieu*
Raritan Bay	*Barataria Bay*
Mid Chesapeake Bay	*Galveston Bay*
Lower Chesapeake Bay	*Humboldt Bay*
Palmico Sound	*San Francisco Bay*
Cape Fear River	*Willipa Harbor*
Savannah River	*Lower Puget Sound*
Indian River	*Tillamook Bay*

Sampling Frequency, Number of Samples

In order to evaluate adequately the annual trend of pesticide residues in shellfish and estuarine sediment, a minimum of three samples per year are planned—in mid-March, mid-September, and mid-November. However, existing programs of cooperating agencies are being incorporated in this program resulting in monthly samplings at most sampling sites.

At each sampling station, 3 pools of 10 oysters constitute the mollusk sample. Samples taken 3 times yearly from all collection sites will total approximately 2,200 specimens per year.

Sampling Procedures

Oysters are the preferred mollusk at all monitoring sites. If commercial oysters are not available, any two species of local clams will be substituted. Only adult oysters are taken. If not endemic to the area, oysters are selected that have a 1-year history in the water mass from which they are taken. Persons collecting samples are requested to furnish a complete history of the oyster stock, a description of the sampling site, and of the collection gear used.

Samples are preserved immediately and forwarded without delay to appropriate regional residue testing centers. Data accompanying the samples include hydrographic observations as well as the station data previously described.

Sampling procedures for sediment are the same as those for mollusks with regard to frequency, area, sample treatment, and shipment. Sample origin is at the interface or uppermost layer of the bottom sediment.

CHEMICALS MONITORED IN FISH, WILDLIFE, AND ESTAURIES

Of the many pesticidal chemicals now in use and occurring in natural ecosystems, the following are considered most important to fish, wildlife, and estuaries: DDT (dichloro-diphenyltrichloroethane) and its metabolites, dieldrin (1, 2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8, 8a-octahydro-1,4-*endo-exo*-5,8-methanonaphthalene), endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene), heptachlor (1,4,5, 6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-*endo*-methanoindene), heptachlor epoxide (1,4, 5,6,7,8,8-heptachloro-2,3, epoxy-3a, 4,7,7a-tetrahydro-4,7-methanoindan), benzene hexachloride, lindane (gamma isomer of benzene hexachloride), chlordane (1,2,3,5,6,7,8-octachloro-2,3,3a,4,7,7a-hexahydro-4, 7-methanoindene), and toxaphene (octachlorocamphene). Each of these compounds is included in the guide to chemicals which has been established for the overall national pesticide monitoring program (p. 20). This list may be modified as new chemicals make their appearance or as new information on present chemicals dictates.

CHEMICAL METHODOLOGY

The primary method of analysis will be the latest methodology associated with gas chromatographic techniques, with a randomized system of cross checking with thin-layer chromatography.

The degree of sensitivity of residue determinations will be no less than 1 x 10^{-6} (parts per million) on a wet weight basis. Dry weights of samples also will be obtained.

PESTICIDES IN WATER

NETWORK TO MONITOR HYDROLOGIC ENVIRONMENT COVERS MAJOR DRAINAGE RIVERS

by R. S. Green[1] and S. K. Love[2]

This program for continuous surveillance of pesticides in surface waters has been proposed for joint operation by the Federal Water Pollution Control Administration and the Geological Survey of the U. S. Department of the Interior. The proposal has been partially implemented.

1 Division of Pollution Surveillance, Federal Water Pollution Control Administration, U. S. Department of the Interior, Washington, D. C. 20242.

2 Quality of Water Branch, Geological Survey, U. S. Department of the Interior, Washington, D. C. 20240.

Purpose and Objective

The purpose of this program is to provide continuing information on the overall extent of pesticide contamination of the Nation's water resources. The objective has been to develop the minimum program that will enable an adequate assessment of conditions. Within this objective, monitoring currently is confined to the examination of surface waters in the major drainage rivers of the United States through a nationwide network of sampling locations. Over a period of years, it is expected that data obtained from this network will reflect important changes in pesticide levels in these rivers.

Design of River Network

Selection of sampling locations for a minimum network to detect long-term or other significant changes in pesticide levels in the water environment has required that consideration be given primarily to area coverage, and only in a secondary sense to the factors of pesticide use or production.

Thus, the following criteria were used to select locations on rivers for pesticide monitoring: (1) locations to be near the mouths of rivers that represent major river drainages throughout the country; (2) river systems to be sampled at other points where there is reason to believe that a reasonable measure of pesticide contamination cannot be obtained merely by sampling at the mouth; (3) stations to be at or near stream-gauging sites; (4) consideration to be given to locating sites where the quality of river water is now being affected by use of pesticides; (5) wherever practicable, stations to be located at sites where other kinds of water-quality data have been or are being collected; (6) wherever practicable, stations to be coordinated with suitably located points from which historical data in the form of carbon filter extracts are available.

Location of Sampling Sites

Within the framework of the above criteria, 53 water sampling locations have been chosen to provide preliminary information on the discharge of pesticides in fresh water draining from the conterminus United States. Monitoring stations are located near the river mouths, except on those streams discharging to tidal waters. The latter stations are above areas of salt-water intrusion.

Sampling points near the mouths of the following streams were selected:

Connecticut River	*Sabine River*
Hudson River	*Trinity River*
Delaware River	*Brazos River*
Susquehanna River	*Colorado River (Texas)*
Potomac River	*Nueces River*
James River	*Rio Grande*
Roanoke River	*Colorado River (Arizona-California)*
Cape Fear River	*Los Angeles Aqueduct*
Pee Dee River	*San Joaquin River*
Santee River	*Sacramento River*
Savannah River	*Klamath River*
Altamaha River	*Columbia River*
St. Johns River	*Ohio River*
Suwanee River	*Illinois River*
Apalachiocola River	*Missouri River*
Alabama River	*Arkansas River*
Tombigbee River	*Yakima River*
Pearl River	*Willamette River*
Mississippi River	*Snake River*
Atchafalaya River	

Streams selected for sampling at other locations are as follows:

Middle Ohio River	*Upper Colorado River (Arizona)*
St. Mary's River (Michigan)	*Middle Rio Grande*
Lake Erie Outlet	*Upper Rio Grande*
St. Lawrence River	*Middle Missouri River*
Red River of the North (near Canadian Border)	*Upper Missouri River*
Middle Mississippi River (below Ohio and Missouri Rivers)	*Middle Arkansas River*
Upper Mississippi River (above Ohio and Missouri Rivers)	*Upper Arkansas River*

Sample Collection Procedures, Frequency, Preparation

Distinctive types of sampling programs are required when monitoring the effects of a point source of pollution as compared to monitoring a stream at a site subject to a diffused source of pollution. Because of its geographically broad but minimal scope, this program is concerned only with procedures for monitoring the latter kind of sites.

The number and frequency of sampling is one grab sample collected once a month at each sampling site.

The following sampling procedures have been prescribed:

All samples are to be collected in glass bottles. Prior to collection, scrupulous cleansing of sample containers is required. Chromic acid cleaning solution or other suitable cleansing agents are to be used, followed by several rinsings with organic-free distilled water. Containers are to be further treated as necessary to destroy remaining traces of organic matter; heat treating of containers at 300° C. has been found satisfactory. Bottles are to be stoppered immediately to prevent air-borne contamination. The sample must have no contact with rubber, cork, and most plastics; Teflon, however, will not contaminate the sample. Rubber or cork stoppers may be used if wrapped carefully with a double layer of organic-free tinfoil or aluminum foil, taking care to avoid rupture of the foil cover when stoppering the bottle.

The sample is to be collected in the prepared glass bottle by lowering it in a weighted bottle holder in a vertical section of the stream which is representative of the stream cross section. The bottle is to be lowered as nearly as possible to the bottom of the stream and returned to the surface so that all points in the vertical section are represented in the sample.

Local conditions may prevent, or make unnecessary, fulfillment of all of the above conditions. However, prior reconnaissance sampling at several vertical sections of the stream may be required to determine degree of uniformity in the cross section. If lack of complete mixing (including floating of pesticides at or near the surface) is suspected, notation of this effect should be made.

It is important that the sample not be transferred from one container to another. Separate containers must be used for determination of any parameters that may be desired in addition to pesticide levels. A sample tag or label providing appropriate identification is then completed and firmly affixed to each sample container. Recorded information is also to include river flow or stage, temperature, physical appearance of the water, or unusual physical stream features.

Sample Shipment

Samples are to be shipped in suitable packing cases to the laboratory for analysis as soon as possible after collection, using parcel post, railway express, air parcel post, or air express. It is highly desirable that samples arrive at the laboratory and extraction be commenced within 2 days after collection.

Sample Storage

When it is not possible for samples to be analyzed within 1 week, the samples or their extracts are to be stored in the dark and in a cool place to retard growth of algae.

Extraction and Analysis of Compounds

Water analysis techniques involving instrumentation with electron capture and coulometric titration are sensitive in the parts per trillion range, but interferences from organic contaminants in the laboratory air, reagent solutions, and sampling containers often pose problems that must be overcome to utilize this degree of sensitivity. Therefore, extreme care must first be taken to maintain the sample as pure as possible while handling and during the analytical procedure.

Principal chemicals for identification include lindane (gamma isomer of benzene hexachloride); heptachlor (1,4,5,6,7,8,8-heptachloro-3a, 4,7,7a-tetrahydro-4,7-*endo*-methanoindene); heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2, 3,epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan); aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5. 8,8a-hexahydro-1, 4-*endo-exo*-5, 8-dimethanonaphthalene); dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1, 4-*endo-exo*-5,8-dimethanonaphthalene); endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6, 7,8, 8a-octahydro-1, 4-*endo-endo*-5,8-dimethanonaphthalene); *o,p'*-DDT, *p,p'*-DDT (dichlorodiphenyltrichloroethane); and also the herbicides, 2, 4-D (2, 4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). When other primary pesticides are known to be used in the drainage areas, these are to be sought as will other compounds listed in the standard guide for the national pesticide monitoring program (p. 20).

Reporting Results

Results are to be reported in parts per trillion. Because of varied and rapidly evolving analytical methodology, detailed records should be maintained, giving the methods used for each analysis. In addition to normal information about dates of sampling and analysis and sample location, such additional details as the following are to be maintained: volume of sample extracted, volume of extract injected, methods used (i.e., electron capture, coulometric, infrared, etc.), and columns used (i.e., QF-1 fluorinated silicone coated 5% by weight, etc.).

Beyond the Minimum Program

In addition to the overall national pesticide monitoring program, more specific studies on many aspects of pesticide contamination of water resources are needed. These studies are required to enable official and private agencies to understand and predict the behavior of the total water system and to discharge their responsibilities in evaluating, regulating, and managing the Nation's water resources.

Detailed studies are especially necessary to understand pesticide contamination in relation to groundwater aquifers, sediment transport, irrigation return flows and other drainage from agricultural lands, near-ground precipitation, industrial waste discharges, and other factors.

Although not a part of this national program, the Great Lakes and key inland bodies of water should be sampled with proper attention being paid to major lake currents. Because individual sampling points in lakes are less valuable than sampling points in rivers, lake sampling is best correlated with points of water use. This provides useful information with respect to water use and contributes to the general understanding of pesticide contamination.

River sampling at low flows provides clues on the pesticide content of ground water entering the stream. Special studies of these complex hydrologic situations may be required.

Observations in estuarine waters and bays should be correlated with the sampling of major river systems, thus providing a base for relating contamination of the marine environment with fresh-water contamination. Because of the complexity of sampling within estuaries, the approach taken should be similar to that employed in lake sampling; that is, most sampling points should be associated with some beneficial use.

Public Water Supplies

Monitoring of pesticides in finished waters of public water supplies does not yield significantly more information about contamination of the environment than would already be known from monitoring of raw waters associated with the systems. Finished water sampling, therefore, is not considered an essential part of this program. It is recognized, however, that a sufficient number of finished waters should be examined to establish the general level of pesticides in finished supplies, to show the extent of removal of pesticides in the treatment process, and to forestall potential problem areas. Sampling of raw and finished water at a few river locations that coincide with sources of water supplies for large cities, for example, will help delineate areas of significant pesticide levels.

PESTICIDES IN SOIL

NATIONAL SOIL MONITORING PROGRAM STUDIES HIGH-, LOW-, AND NONUSE AREAS

F. F. Sand[1], J. W. Gentry[1], J. Bongberg[2], and M. S. Schechter[3]

Much of the described soil monitoring program is being carried out by the U. S. Department of Agriculture as an established program. Other phases of monitoring are to be conducted by the USDA in cooperation with State and other Federal agencies.

[1] Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture, Hyattsville, Maryland 20782.
[2] Division of Forest Pest Control, Forest Service, U. S. Department of Agriculture, Arlington, Virginia 22209.
[3] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705.

Monitoring Objective

The objective of this program is to determine existing levels of pesticide residues in soils of selected areas in the conterminous United States and to detect any significant changes in these levels. Soil monitoring sites were chosen whereever possible to coincide with sampling sites of other agencies in the Federal pesticide monitoring network so that soil data may be correlated with pesticide levels in other environmental media.

Samples and Sampling Locations

In monitoring the effects of pesticides on the agricultural environment, soils are being studied extensively in areas of high pesticide usage, as well as in areas of low use and nonuse.

Selection of high-use areas for monitoring purposes was based on pesticide-use records obtained from the literature and through local surveys. In each case, responsible State agencies were consulted concerning site selection before the program was undertaken in any particular area. Next, an intensive, direct survey was conducted among landowners or commercial pesticide applicators. Only those farms having accurate pesticide-use records over a period of years were chosen for the high-use studies. Wherever possible, these records were compiled by year for the past 10 years.

Intensive study areas are currently at single locations in Alabama, Arizona, and in the Red River Valley of North Dakota. Studies in these areas were set up to run for a 3-year period and will be phased out at the end of the 1967 season. Operations at Stuttgart, Arkansas; Greenville, Mississippi; and Utica, Mississippi, were phased out in the fall of 1966 after a 3-year sampling period was completed. Special soil monitoring activities have been extended to numerous other areas of the country where pesticides are extensively employed in agriculture. Five farms are included at each location.

These areas, and the principal crops they produce, include:

- *Texas Lower Rio Grande Valley* — cotton*
- *Dade County, Florida* — vegetables*
- *Western North Carolina — apples*
- *Eastern South Carolina — vegetables*
- *Central Georgia — peaches*
- *Eastern Virginia — peanuts*
- *Monmouth County, New Jersey* — vegetables*
- *Adams County, Pennsylvania — fruits*
- *Berrien County, Michigan* — fruits and vegetables*
- *Urbana, Illinois — corn*
- *Western Iowa — corn and soybeans*
- *Weld County, Colorado* — root crops*
- *Yuma County, Arizona — citrus fruits*
- *Wenatchee Basin area, Washington* — fruits and root crops (2 locations)*
- *Kern County, California — cotton, vegetables*
- *Tulelake area, California — small grains, root crops*

** Soil monitoring sites coincide with U. S. Public Health Service sites to monitor pesticides in human beings.*

Altogether, 23 study locations have been established and maintained in high-use areas.

For comparative purposes, areas that have received only one or two applications of pesticides also have meaning in the study of residues in soils. These conditions were found in forest areas where insect infestations have required only periodic control and on western rangelands where insecticides have been used periodically to control grasshoppers and Mormon crickets.

Areas in which there was no known previous use of pesticides were included to indicate possible distribution of pesticides in soil environments not directly exposed to pesticide application. Monitoring sites that were selected for low- and nonuse phases of this program meet the following specific criteria:

- *Sites are on noncultivated lands, and no site is included which has been cultivated within the past 10 years;*
- *Some, but not all of the sites, are located near areas chosen for high-use studies;*
- *Sites are at least 1 mile from any known treated areas and, where possible, include lands subject to contamination from treated areas*
- *Sites are in locations remote from current cultivation, e.g., ranges, forests, and wildlands.*

In selecting these areas, cooperation again was sought from Federal and State agencies responsible for management of public lands. Such areas as parks, forests, and western rangelands afforded preferable sites for these soil studies. Records of land use were available from appropriate agencies. If suitable sites could not be obtained through public land agencies, desirable sites were determined by direct survey on a local basis.

A total of 35 sites were selected for low- and nonuse area studies. Sites are distributed evenly within each category to include forest areas, arid rangeland, plains areas, and the eastern hardwood region.

Sites selected for low-use studies, listed according to their principal insect control efforts, are:

- *Grasshopper control (dieldrin[4], aldrin[5]) — Klamath County, Oregon; Lincoln County, Idaho; Phillips County, Montana; and Fremont County, Wyoming*
- *Japanese beetle control (dieldrin) — Pike County, Kentucky*
- *Mosquito control (DDT[6]) — Camp Drum, New York*
- *Forest insect control (DDT) — Davy Crockett National Forest, Texas; Manistee-Huron National Forest, Michigan; Thomas Jefferson National Forest, Virginia; Chippewa National Forest, Minnesota; Coconino National Forest, Arizona; Lincoln National Forest, New Mexico; Stanislaus National Forest, California; Chequamegon National Forest, Wisconsin; Allegheny State Forest, Pennsylvania; Eagle Lake State Forest, Maine; and Chattahoochee National Forest, Georgia*

Sites for nonuse studies include:

- *Forest Service Lands — Pisgah National Forest, North Carolina; Oconee National Forest, Georgia; Francis Marion National Forest, South Carolina; Cross Timbers Grasslands, Texas; Ozark National Forest, Arkansas; Mark Twain National Forest, Missouri; Buffalo Gap Grasslands, South Dakota; Cache National Forest, Utah*
- *National Wildlife refuges — Gulf Islands National Wildlife Refuge, Mississippi; Kofa National Wildlife Refuge, Arizona; Okefenokee National Wildlife Refuge, Georgia; Seney National Wildlife Refuge, Michigan; Ravalli National Wildlife Refuge, Montana; Ft. Niobrara National Wildlife Refuge, Nebraska; San Andres National Wildlife Refuge, New Mexico; Anahuac National Wildlife Refuge, Texas; Missisquoi National Wildlife Refuge, Vermont; Pea Island National Wildlife Refuge, North Carolina*

The Agricultural Research Service has developed a plan for expanding the national soil monitoring program. The proposed program has been designed on a statistical basis for the conterminous United States to provide information that will pinpoint major trouble areas which then will require additional monitoring. The objectives of the program are:

1. *To establish the level of pesticide residues in soils in reference to major land-use areas in the United States.*
2. *To continue sampling the same sites over a period of time to provide information on rates of change of pesticide residue levels in soils.*

It is planned to initiate this program in fiscal year 1968. Soil will be collected from approximately 15,000 sites over the conterminous United States during a 4-year period.

Sampling Frequency, Number of Samples

Under the present program: samples are collected once a year in high-use areas after seasonal control measures. Approximately 2,600 soil samples were collected annually when all 23 sites were being sampled.

One sampling per year also is made in each of the 35 low- and nonuse monitoring areas. Ten samples are collected for each site, totaling 350 samples per year.

About 2,950 soil samples have been collected annually for all phases of the soil monitoring program.

Sample Collection Procedures

Each of the large-scale study areas in Alabama, Arizona, and North Dakota contains approximately 1 square mile (640 acres) of agricultural land. Each area is divided for soil sampling purposes into 12 to 15 blocks of approximately 35-50 acres each. Collection procedures involve both block and plot sampling.

- *Block Sampling — Three samples are taken per block at each sampling. Each sample consists of a composite of one core per acre per block. Cores are spaced as equally as possible throughout the block and are taken both from the row and between rows in cultivated crops.*
- *Plot Sampling — Intensive sampling of 20-acre plots within certain designed blocks is made to obtain more precise data on accumulation or depletion of pesticide residues, and to develop data on rate of movement of pesticides with water.*

The 20-acre plot is divided into 1-acre sections, each to be sampled and analyzed separately. One representative sample consisting of 50

[4] 1,[illegible]10-Hexachloro[illegible]1,4,4a,5,6,7,8,8a-octahydro-1,[illegible]-5,8-dime[illegible]
[5] 1,[illegible],10,10-Hexachloro[illegible]-1,4-endo-exo-5,[illegible]naphthalene.
[6] [illegible]

cores is taken from each acre. The location of the intensive study plot within a selected block is determined by watershed surveys. Where possible, samples of each crop grown within a study area are analyzed for pesticide residues as a part of this study.

In the special soil studies in the high-use areas, one representative field of 20 acres or more on each of the five study farms is chosen for sampling. Five 1-acre plots are laid out in this field and samples collected on a stratified random basis throughout the plot. Establishment of these plots is made in reference to the field's topography.

Ten 1-acre plots are laid out in each of the low- and non-use areas. Fifty cores are collected on a stratified random basis as in the high-use area plots.

A uniform procedure for taking the cores, compositing the sample, and general handling of the sample has been developed. Soil is sampled to a depth of 3 inches with a corer 2 inches in diameter. All cores contain the surface cover of the soil, e.g., debris, sod, leaves, or any other material which penetrates through normal sampling. In forest areas of heavy duff, samples are taken in spots where cover is lightest. Cores are collected in a large container, such as a 5-gallon pail, and the combined cores are passed twice through a ¼-inch screen to facilitate mixing. Stones, roots, twigs, grass, etc., that do not pass through the screen are discarded; however, lumps of soil are forced through the screen. A ½-gallon container is filled with the mixed, screened soil and sealed with an air-tight lid. The container then is labeled with sample number and date. A sample data sheet, in an envelope, is fastened securely with tape to the outside of the container. Equipment is thoroughly cleaned after each sample collection, and other measures are taken to guard against contamination in all phases of the operation.

Pesticides and Analytical Methods

Analyses are performed to detect and identify as many pesticides and important degradation or metabolic products as possible. A general guide for pesticides to be identified is that developed for the national pesticide monitoring program (p. 20). This is augmented by such knowledge and records as can be obtained of pesticides used for agriculture, control of forest pests, and for any other uses.

In the laboratory, subsamples of soil are used for analysis; the remainder of each sample is then stored until it is determined to be of no further use. The latest and most sensitive methods of analysis are employed, including various procedures based on gas chromatography and paper and thin-layer chromatography. In doubtful cases, infrared spectrophotometry or colorimetric analyses also are employed if a sufficient amount of pesticide is present to permit using these techniques.

In addition to other sources, the *Guide to the Analysis of Pesticide Residues* (H. P. Burchfield, 1965, Supt. of Docs., U. S. Government Printing Office, Washington, D. C.), is used to select appropriate analytical procedures. Sample sizes and sensitivity of the methods employed are sufficient to permit reasonable detection without making the analytical method unduly cumbersome or complicated.

Factors which affect the sensitivity of the analytical methods include:

1) sample size; 2) efficiency of extraction; 3) efficiency of cleanup procedure (solvent partitions, column chromatography, etc.); 4) background due to naturally occurring interferences; 5) interference from instrument noise or fluctuations; 6) interferences from solvents and reagents; 7) cross interference of one pesticide (or its degradation and metabolic products) with another; 8) sensitivity of the final detection step, such as gas chromatography with its various attached detectors as well as spectrophotometry in the visible, ultraviolet, or infrared regions, etc.

Importantly, sensitivities of the analytical method may vary from one insecticide to another, and even for the same insecticide from one soil type to another. Sandy soils with low organic content usually are less troublesome to analyze than muck soil with high organic content. Due consideration also is given to significant pesticide degradation and metabolic products to the extent that suitable analytical procedures are available.

Reporting Results

Results of analyses are expressed in parts per million on a dry-weight basis, and where possible in pounds per 3-inch acre.

CHEMICALS MONITORING GUIDE FOR NATIONAL PESTICIDE MONITORING PROGRAM[1]

Milton S. Schechter[2]

The purpose of this guide is to focus attention on certain pesticides of special concern to the national pesticide monitoring program. Two lists of chemicals are presented as an aid to participating Federal agencies in designating pesticides to be identified in their initial monitoring studies. The primary listing contains chemicals believed at present to be of most interest because of their (1) extent and/or volume of usage; (2) degree of hazard to man, fish, and wildlife; and (3) degree of persistence. Pesticides on the secondary list are considered to be of lesser importance or interest at present. Both are minimal listings in keeping with the minimum scope of the national pesticide monitoring program; however, these lists are not to be considered exclusive. All identifiable pesticides found in significant quantities should be reported. This includes metabolic and/or breakdown products which are pesticidal or toxic.

Not all of the pesticides listed, of course, can or should be determined in all samples. Available information on the use patterns of pesticides in the areas where samples are taken should be used as a guide and consideration given to possible movement of pesticides in the air (drift), water (run-off), or soil (percolation).

These lists may be revised periodically to allow for addition or deletion of pesticides according to changes in their use, introduction of new pesticide chemicals, and advances in analytical methodology.

Because of difficulties involved in screening samples for a multiplicity of pesticides and their important metabolites and degradation products, care should be used not only in the sampling and quantitative aspects of monitoring studies but especially in the identification aspects in order to assure reliability of reported results.

[1] This guide was drawn up by a group of representatives (with Milton S. Schechter as chairman) from the U. S. Departments of Agriculture, Defense, the Interior, and Health, Education, and Welfare, under the sponsorship of the Subcommittee on Pesticide Monitoring of the Federal Committee on Pest Control.

[2] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705.

Primary List of Chemicals for Monitoring[3]

Common or Trade Name	Chemical Name
Aldrin	not less than 95% of 1,2,3,4,10,10-hexa=chloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene
Amitrole	3-amino-*s*-triazole
Arsenic-containing pesticides (inorganic and organic)	
Azinphosmethyl (Guthion®)	*0,0*-dimethyl phosphorodithioate *S*-ester with 3-(mercaptomethyl)-1,2,3-benzotriazin-4(3*H*)-one
Benzene hexachloride (BHC)	1,2,3,4,5,6-hexachlorocyclohexane, consisting of several isomers and containing a specified percentage of *gamma* isomer
Chlordane	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan; at least 60% of 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene and not over 40% of related compounds
2,4-D (including sales, esters, and other derivatives)	2,4-dichlorophenoxyacetic acid
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)=ethane; technical DDT consists of a mixture of the *p,p'*-isomer and the *o,p'*-isomer (in a ratio of about 3 or 4 to 1)
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexa=chloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa=hydro-1,4-*endo*-*exo*-5,8-dimethanano=naphthalene
Dithiocarbamate pesticides:	
Maneb;	[ethylenebis(dithiocarbamato)]manganese;
Ferbam;	tris(dimethyldithiocarbamato)iron;
Zineb;	[ethylenebis(dithiocarbamato)]zinc;
etc.	
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-*endo*-5,8-dimethanonaphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Lindane	1,2,3,4,5,6-hexachlorocyclohexane, *gamma* isomer of not less than 99% purity
Malathion	diethyl mercaptosuccinate *S*-ester with *0,0*-dimethyl phosphorodithioate
Mercury-containing pesticides (inorganic and organic)	
Methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)=ethane; technical methoxychlor contains some *o,p'*-isomer also
Methyl parathion	*0,0*-dimethyl *0*-*p*-nitrophenyl phosphorothioate
Mirex	dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene
Parathion	*0,0*-diethyl *0*-*p*-nitrophenyl phosphorothioate
Silvex (including salts, esters, and other derivatives)	2-(2,4,5-trichlorophenoxy)propionic acid
Strobane®	terpene polychlorinates containing 65% chlorine
2,4,5-T (including salts, esters, and other derivatives)	2,4,5-trichlorophenoxyacetic acid
TDE (DDD) (including its isomers and dehydrochlorination products)	1,1-dichloro-2,2-bis(*p*-chlorophenyl)=ethane; technical TDE contains some *o,p'*-isomer also
Toxaphene	chlorinated camphene containing 67-69% chlorine

Secondary List of Chemicals for Monitoring

Demeton (Systox®)	mixture of *0,0*-diethyl *S*(and *0*)-[2-(ethylthio)ethyl] phosphorothioates
Disulfoton (Di-Syston®)	*0,0*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate
Endosulfan (Thiodan®)	1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite
Inorganic Bromide from bromine-containing pesticides	
Triazine-type herbicides:	
Atrazine;	2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine;
Simazine;	2-chloro-4,6-bis(ethylamino)-*s*-triazine;
etc.	

[3] The first chemical name given after the common or trade mark name is according to *Chemical Abstracts*. The second chemical name, if one is given, is taken from "A List of Insect Control Chemicals to be Used in Entomology Research Division Manuscripts" compiled by E. M. Osborne and Ruth L. Busbey, Pesticide Chemicals Research Branch, Entomology Research Division, ARS, USDA, June 1966.

Note: *A chemical name occupying two lines separated by an equal(=) sign is joined together as one word, without the equal sign, if written on one line.*

Information For Contributors

The *Pesticides Monitoring Journal* welcomes from all sources qualified data and interpretive information which contributes to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

The publication is distributed principally to scientists and technicians associated with pesticide monitoring, research, and other programs concerned with the fate of pesticides following their application. Additional circulation is maintained for persons with related interests, notably those in the agricultural, chemical manufacturing, and food processing industries; medical and public health workers; and conservationists.

Authors are responsible for the accuracy and validity of their data and interpretations, including tables, charts, and references. Accuracy, reliability, and limitations of the sampling and analytical methods employed must be clearly demonstrated through the use of appropriate procedures, such as recovery experiments at appropriate levels, confirmatory tests, internal standards, and interlaboratory checks. The procedure employed should be referenced or outlined in brief form, and crucial points or modifications should be noted. Check or control samples should be employed where possible, and the sensitivity of the method should be given, particularly when very low levels of pesticides are being reported. Specific note should be made regarding correction of data for percent recoveries.

Preparation of manuscripts should be in conformance to the *Style Manual for Biological Journals*, American Institute of Biological Sciences, Washington, D. C., and/or the *Style Manual* of the United States Government Printing Office, and an abstract (not to exceed two hundred words) should accompany each manuscript submitted.

Pesticides ordinarily should be identified by common or generic names approved by national scientific societies. The first reference to a particular pesticide should be followed by the chemical or scientific name in parentheses—assigned in accordance with *Chemical Abstracts* nomenclature. Structural chemical formulas should be used when appropriate.

Published data and information require prior approval by the Editorial Advisory Board; however, endorsement of published information by any specific Federal agency is not intended or to be implied. Authors of accepted manuscripts will receive edited typescripts for approval before type is set. After publication senior authors will be provided with 100 reprints.

Manuscripts are received and reviewed with the understanding that they previously have not been accepted for technical publication elsewhere. If a paper has been given or is intended for presentation at a meeting, or if a significant portion of its contents has been published or submitted for publication elsewhere, notation of such should be provided.

Correspondence on editorial and circulation matters should be addressed to: Editorial Manager, *Pesticides Monitoring Journal*, Pesticides Program, National Communicable Disease Center, Atlanta, Georgia 30333.

The *Pesticides Monitoring Journal* is published quarterly under the auspices of the Federal Committee on Pest Control and its Subcommittee on Pesticide Monitoring as a source of information on pesticide levels relative to man and his environment.

The parent committee is composed of representatives of the U. S. Departments of Agriculture, Defense, the Interior, and Health, Education, and Welfare.

The Pesticide Montoring Subcommittee consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Federal Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Federal Water Pollution Control Administration, Food and Drug Administration, Public Health Service, and the Tennessee Valley Authority.

Responsibility for publishing the *Pesticides Monitoring Journal* has been accepted by the Pesticides Program of the Public Health Service.

Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the Pesticide Monitoring Subcommittee which participate in operation of the national pesticides monitoring network, are expected to be principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernment research institutions, both within and without the United States. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the Monitoring Subcommittee. Authors are given the benefit of review comments prior to publication.

Trade names appearing in the *Pesticides Monitoring Journal* are for identification only and do not represent endorsement by any Federal agency.

Address correspondence to:
Mrs. Sylvia P. O'Rear
Editorial Manager
PESTICIDES MONITORING JOURNAL
Pesticides Program
National Communicable Disease Center
Atlanta, Georgia 30333

CONTENTS

Volume 1 September 1967 Number 2

EDITORIAL

PUBLICATION of original papers and data on pesticide monitoring begins with this issue. We have been pleased by the submission of many excellent articles and by the gracious response of the authors to the comments and suggestions of the review board.

Since the original announcement of plans for publication, there have been numerous questions concerning the type and scope of reports acceptable to the Journal. We are hesitant to define limits of subject matter acceptability except in the rather broad terms of significant information and data on the levels of pesticides in all portions of the environment.

Monitoring, as used in the title of this Journal, may be defined as the systematic recording of information relating to the distribution and movement of pesticides and their residues in the environment. Reports concerned with this broad field are within the purview of the Journal. There is no publication specializing in baseline data and the everchanging levels of pesticides in influential and significant environmental factors. The policy of this Journal is to publish reliable information on this broad subject in such a form as to be readily used, compared, interpreted, and correlated by scientists and others concerned with pesticides and their residues.

The National Pesticide Monitoring Program can be only a frame to support and maintain a continuity with other monitoring activities. Data derived from many sources other than the National Pesticide Monitoring Program described in the first issue are needed for a more complete understanding of the effects of pesticides on the environment. Informative articles may be prepared from certain methodology and research projects where the monitoring part of the investigation is secondary, and the monitoring data cannot be properly reported elsewhere in sufficient detail for use by others. Also, surveillance data from water, forest, or wildlife conservation programs and that obtained during the enforcement of tolerances for residues should be valuable sources of information. The Editorial Advisory Board wishes to renew the invitation for manuscripts concerned with monitoring pesticides from all sources.

There have been a few expressions of opinion that the requirements for information on confirmatory analyses, recovery experiments, sensitivity, and other items mentioned in *Information to Contributors* are too stringent. We do not have requirements beyond sound and reasonable criteria for scientific investigations. The uses for monitoring data are extensive, and information in sufficient depth is needed to permit the reader to make an independent judgment concerning the data and their range of usefulness. The editorial policy of the Journal is to fulfill this need insofar as practical in order that the readers will find the Journal a dependable, accurate, and useful source of information. For example, we have suggested that lengthy tabular data be presented in a summarized form. The participants in the National Pesticides Monitoring Program have agreed to respond to requests from readers for more detailed data. We hope other contributors will be equally responsive to such requests. In future issues, we plan to devote this page to discussions by members of the Editorial Advisory Board on special requirements and important aspects of definitive monitoring programs.

R. E. Duggan
Chairman, Editorial Advisory Board

RESIDUES IN FOOD AND FEED

Pesticide Residues in Total Diet Samples (II)

R. E. Duggan[1], H. C. Barry[2], and L. Y. Johnson[3]

ABSTRACT

Pesticide residue levels detected in ready-to-eat foods remained at low levels during the second year of the total diet study. Food samples were taken in 36 different markets which were located in 25 different cities representing five geographical regions. Averages and ranges of pesticides found are reported for each year by region and food class.

THE study of pesticide residues in ready-to-eat foods which was conducted by the Food and Drug Administration from June 1964 through April 1965 was described in an earlier report *(1)*. The sampling, compositing, and analytical schemes were given in detail in the Food and Feed section of the initial issue of the Pesticides Monitoring Journal which described the National Pesticide Monitoring Program *(2)*. This paper presents data collected from June 1965 through April 1966. More complete data for both periods are included in tabular form.

The study was expanded beginning in August 1965 to include samples from Minneapolis, Minn., and Baltimore, Md. Sampling was not confined to the five metropolitan areas; some samples were collected from smaller cities.

Two significant procedural changes were made in August 1965. One was the use of an improved analytical procedure in which gas-liquid chromatography *(3)*, the thermionic detector, and the isolation procedure for chlorinated organic compounds *(4)* were used to detect organic phosphorus compounds. This procedure determines ethion, ronnel, carbophenothion, malathion, di[illegible]zinon, methyl parathion, and parathion at a sensitivity [illegible] ppm. Thin layer chromatography *(5)* was [illegible] confirmatory analyses. The second procedural [illegible] the analysis of carbaryl by a thin layer [illegible]

[1] [illegible] Commissioner for Compliance, Food and Drug [illegible] U. S. Department of Health, Education, and Welfare [illegible] D. C. [illegible]

[2] [illegible] Food and Drug Administration, New Orleans, [illegible]

[3] [illegible] Food and Drug Administration, Cincinnati, Ohio [illegible]

All methods used in these studies are described in the FDA Pesticide Analytical Manual (1965), and may include refinements not described in the basic references listed above. Recoveries of specific pesticide chemicals vary within product classes, usually within a range of 85% to 115% at these levels. No correction was made for recovery.

Quantitative values reported for chlorinated organic compounds were obtained by electron capture gas-liquid chromatography and confirmed by thin layer chromatography, microcoulometric gas-liquid chromatography, or both.

Results

Thirty-three different residues were found in the samples in 1966. The frequency of the residues and the ranges of their amounts are shown in Table 1. The most common residues, maximum levels of these residues, and residues reported less frequently are discussed for each food class.

DAIRY PRODUCTS: Thirteen chlorinated organic pesticides in varying combinations were detected in 21 of 22 composites. The most common and their maximum values on a fat basis were DDE (0.58 ppm), DDT (0.19 ppm), dieldrin (0.06 ppm), heptachlor epoxide (0.077 ppm), and TDE (0.065 ppm). Aldrin, BHC, lindane, methoxychlor, 2,4,5-TP, 2-4-DB, and PCP and MCP were also present. Bromides were found (0.5 ppm to 21.4 ppm) in 25 of 28 composites.

MEAT, FISH, AND POULTRY: Nine chlorinated organic pesticides were present in varying quantities in 22 of 26 composites. DDT, DDE, TDE, heptachlor epoxide, dieldrin, and BHC were the most common, with maximum values of 1.39 ppm, 1.0 ppm, 0.78 ppm, 0.29 ppm, 0.20 ppm, and 0.20 ppm, respectively, on a fat basis. Lindane, tetradifon, and PCP were also present. Bromides were detected (0.5 ppm to 44 ppm) in 23 of 28 composites, and arsenic (0.1 ppm to 0.5 ppm As_2O_3) in 5 of 28 composites. Diazinon (0.051 ppm) and ronnel (0.011) were found in 1 composite each.

TABLE 1.—*Number of composites where pesticide residues were found and ranges in the amounts (June 1965 - April 1966)*

Pesticide	No. composites with residue	No. of positive composites with residues below sensitivity level [1]	Range at and above sensitivity level (ppm)
BROMIDES	244	0	0.5-117.0
DDT 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	119	7	0.004-1.39
DDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene	119	32	0.003-1.00
TDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	83	13	0.003-0.78
DIELDRIN not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	75	22	0.003-0.20
LINDANE 1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer	49	21	0.003-0.080
HEPTACHLOR EPOXIDE 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan	42	9	0.004-0.29
BHC 1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers	21	3	0.008-0.20
MALATHION diethyl mercaptosuccinate, *S*-ester with *0,0*-dimethyl phosphorodithioate	16	13	0.053-0.15
ALDRIN not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	13	3	0.005-0.070
KELTHANE® 4, 4′-dichloro-*a*-(trichloromethyl) benzhydrol	12	0	0.013-0.21
PCP pentachlorophenol	10	5	0.036-0.31
ARSENIC (As_2O_3)	10	0	0.1-0.5
2,4-D 2,4-dichlorophenoxyacetic acid	9	3	0.051-0.10
DIAZINON *0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	9	8	0.051
CARBARYL 1-naphthyl methylcarbamate	8	5	0.2-0.4
ENDRIN 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene	6	0	0.004-0.052
ENDOSULFAN 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	5	2	0.006-0.016
METHOXYCHLOR 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane	5	2	0.004-0.073
MCP 4-chloro-2-methyl-phenoxyacetic acid	4	1	0.039-0.58
PERTHANE® 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane	4	0	0.007-0.057
PARATHION *0,0*-diethyl *0-p*-nitrophenyl phosphorothioate	4	3	0.089
TOXAPHENE chlorinated camphene containing 67% to 69% chlorine	3	0	0.048-0.38
RONNEL *0,0*-dimethyl *0*-2,4,5-trichlorophenyl phosphorothioate	3	3	
CIPC isopropl *N*-(3-chlorophenyl) carbamate	2	0	0.20-0.36
TETRADIFON *p*-chlorophenyl 2,4,5-trichlorophenyl sulfone	2	0	0.011-0.076
2,4,5-TP 2-(2,4,5-trichlorophenoxy) propionic acid	2	0	0.018-0.029
TCNB 1,2,4,5-tetrachloro-3-nitrobenzene	2	1	0.37
2,4-DB 4-(2,4-dichlorophenoxy) butyric acid	2	2	
DACTHAL® dimethyl ester of tetrachloroterephthalic acid	2	2	
CHLORDANE 1,2,4,5,6,7,8,8-octachloro-3a, 4,7,7a-tetrahydro-4,7-methanoindane	1	0	0.37
PCNB pentachloronitrobenzene	1	0	0.005
ETHION *0,0,0′,0′*-tetraethyl *S,S′* methylene bisphosphorodithioate	1	1	

[1] Pesticide chemicals capable of being detected by the specified analytical methodology may be confirmed qualitatively but are not quantifiable when they are present at concentrations below the sensitivity level.

GRAIN AND CEREAL PRODUCTS: Thirteen chlorinated organic pesticides were found in 23 of 26 composites, with the most common being lindane, DDT, and DDE at maximum values of 0.028 ppm, 0.024 ppm, and 0.004 ppm, respectively. Other chlorinated organic pesticides present were aldrin, BHC, dieldrin, heptachlor epoxide, methoxychlor, TDE, 2,4-DB, PCNB, Perthane, and PCP. Bromides were present (1.1 ppm to 66.7 ppm) in 27 of 28 composites, and arsenic at 0.1 (As_2O_3) ppm in 1 composite. Eleven composites contained detectable malathion with the maximum level 0.15 ppm. Diazinon and ronnel were also present.

POTATOES: Dieldrin and DDT at maximum values of 0.003 and 0.010 ppm, respectively, were the most common of 10 chlorinated organic pesticides found in 14 of 26 composites. Also, BHC, DDE, endrin, heptachlor epoxide, lindane, TCNB, CIPC, and TDE were present. Bromides were found (0.7 ppm to 68.5 ppm) in 20 of 28 composites, and parathion at 0.003 ppm in 1 composite.

LEAFY VEGETABLES: While DDT, DDE, and TDE with maximum values of 0.048 ppm, 0.033 ppm, and 0.024 ppm, respectively, were the most common of 10 chlorinated organic pesticides found in 20 of 26 composites, others were also detected. They were Dacthal, dieldrin, lindane, endosulfan, toxaphene, 2,4-D, and MCP. Bromides were found (0.6 ppm to 14.8 ppm) in 22 of 28 composites. Diazinon and parathion were detected in 3 composites with maximum levels of 0.031 ppm and 0.089 ppm, respectively; malathion was found in 1 composite.

LEGUME VEGETABLES: Ten of twenty-six composites were found to contain seven chlorinated organic pesticides. The most common were DDE and TDE, with maximum values of 0.003 ppm and 0.064 ppm, respectively; but DDT, aldrin, heptachlor epoxide, and trace amounts of dieldrin and lindane were also detected. Bromides were found (0.5 ppm to 14.5 ppm) in 22 of 28 composites.

ROOT VEGETABLES: The 6 chlorinated organic pesticides found in 9 of 26 composites were DDE, dieldrin (most common: maximum values of 0.011 ppm and 0.028 ppm, respectively), DDT, endrin, TCNB, and TDE. Bromides were found (0.6 ppm to 17.0 ppm) in 21 of 28 composites, and arsenic (0.1 ppm As_2O_3) was found in 1 composite. Malathion (0.22 ppm) and carbaryl (0.1 ppm) were also found in 1 composite.

GARDEN FRUITS: A total of 11 chlorinated organic pesticide residues were found in 22 of 26 composites. DDT, TDE, lindane, and DDE were most common, with maximum levels of 0.17 ppm, 0.34 ppm, 0.012 ppm, and 0.064 ppm, respectively. The remainder were dieldrin, aldrin, endrin, heptachlor epoxide, endosulfan, toxaphene, and chlordane. Bromides were found (0.5 ppm to 7.5 ppm) in 24 of 28 composites; arsenic (0.1 ppm As_2O_3) in 1 composite; and diazinon (0.005 ppm) in 1 composite.

FRUITS: The most common of 11 chlorinated organic pesticide residues found in 19 of 26 composites were DDE, Kelthane, and DDT; their maximum levels were 0.043 ppm, 0.21 ppm, and 0.045 ppm, respectively. Less frequently present were aldrin, Dacthal, dieldrin, Perthane, lindane, TDE, tetradifon, and endosulfan. Bromides were present (0.7 ppm to 25.2 ppm) in 19 of 28 composites, carbaryl (maximum level 0.2 ppm) in 4 composites, and ethion (maximum level 0.019 ppm) in 1 composite.

OILS, FATS AND SHORTENING: A total of 9 chlorinated organic pesticide residues were found in 17 of 26 composites. DDE, DDT, and TDE were the most common, with maximum levels on a fat basis of 0.029 ppm, 0.038 ppm, and 0.12 ppm, respectively. Aldrin, dieldrin, endrin, heptachlor epoxide, Perthane, and PCP made up the other 6. Bromides were present (0.9 ppm to 90.8 ppm) in 21 of 28 composites. The organophosphate, malathion (maximum level 0.18 ppm), was found in 4 composites.

SUGARS AND ADJUNCTS: Of 7 chlorinated organic pesticide residues found in 9 of 26 composites, 2,4-D, at a maximum level of 0.1 ppm, was the most common. DDE, DDT, dieldrin, MCP, and trace amounts of lindane and heptachlor epoxide were also found. Bromides were present (0.7 ppm to 117 ppm) in 25 of 28 composites, but arsenic was found (0.1 ppm As_2O_3) in only 1. Carbaryl was found in 2 composites at a maximum level of 0.2 ppm, and ronnel was found in 1 composite in trace amounts.

BEVERAGES: A total of 3 chlorinated organic pesticides were found in 2 of 26 composites. PCP (0.02 ppm) was found in 1 composite, and trace amounts of lindane and heptachlor epoxide were also reported. Bromides in concentrations ranging from 0.5 ppm to 13.7 ppm were found in 9 of 28 composites. Carbaryl was found in 1 composite at 0.4 ppm.

Bromide residues were in excess of the quantitative sensitivity limits established for the investigation in 258 of 336 composite samples. This incidence is 76.8% and does not differ significantly from the 1964-1965 results; however, there was a lower incidence of residues exceeding 25 ppm—3.8% compared to 11.6%. The presence of chlorinated organic pesticides was confirmed in 168 of 312 composites examined (53.8%) for this class of chemicals; this percentage of incidence also is not significantly different from the 1964-1965 data. In contrast, the finding of residues of chlorophenoxy compounds in 24 composites was an increase over the previous period. Carbaryl was found in 8 composites—again a lower incidence than in the preceding period. Organic phosphorus compounds were found in 27 composites; we attribute the finding of this class of compound primarily to the improved analytical procedures used. Amitrole and dithiocarbamate residues were not found at or above the prescribed sensitivity limits.

Discussion

Levels of residues for the interval of this study remain on the same order of magnitude as those reported in the earlier studies. In addition, the frequency of residues encountered has not changed significantly as a whole or within food classes. Residues of toxaphene, Dacthal, endosulfan, 2,4,5-TP, diazinon, ronnel, malathion, parathion, 2,4-DB, CIPC, and ethion have not been reported previously in studies of foods ready for consumption.

Data obtained during both periods of study are reported in more detail in Table 2a where the findings are arranged by food class, region, and sampling period. Period average, number of positive composite samples and range of positive findings are given for those pesticide residues commonly found. Similar information on pesticide residues found infrequently is given in Table 2b. Trace amounts, <0.001 ppm, were not included in the averages. Where no average value is given, results on individual composites are shown.

On the basis of these data, average daily pesticide intake from the diet was calculated and is reported elsewhere *(7)*. There was no statistically significant difference in the dietary intake between the two reporting periods, and the calculated levels were not seen to be approaching the acceptable daily intakes established for certain pesticides chemicals by the World Health Organization.

TABLE 2a.—*Levels of Pesticide Residues Commonly Found—by Food Class, Region, and Sampling Period*

[T = Trace<0.001 ppm]

PESTICIDE	BOSTON 1965	BOSTON 1966	KANSAS CITY 1965	KANSAS CITY 1966	LOS ANGELES 1965	LOS ANGELES 1966	BALTIMORE 1966	MINNEAPOLIS 1966
1. DAIRY PRODUCTS (8-13% fat)[1] Residues in Parts Per Million—Fat Basis								
DDT								
Average	0.010	0.029	0.065	0.039	0.031	0.073	0.035	
Positive Composites								
Number	2	4	6	6	2	4	3	None examined.
Range	0.011-0.048	T(a)-0.173	0.019-0.153	0.014-0.112	0.060-0.080	0.046-0.190	0.021-0.076	
DDE								
Average	0.009	0.025	0.020	0.044	0.150	0.251	0.030	
Positive Composites								
Number	4	4	4	6	6	6	4	do.
Range	T-0.037	0.028-0.050	0.005-0.063	0.007-0.091	0.072-0.222	0.073-0.579	0.013-0.077	
TDE								
Average	0.006	0.020	0.014	0.012		0.018	0.018	
Positive Composites								
Number	2	3	3	5	1	2	2	do.
Range	0.005-0.031	0.031-0.050	0.010-0.050	0.004-0.029	0.053	0.051-0.058	0.008-0.065	
DIELDRIN								
Average	0.019	0.013	0.019	0.031	0.015	0.023	0.002	
Positive Composites								
Number	3	3	3	6	4	5	2	do.
Range	0.012-0.056	0.013-0.038	0.027-0.045	0.011-0.059	0.013-0.028	0.014-0.039	0.003-0.005	
HEPTACHLOR EPOXIDE								
Average	0.004	0.008	0.010	0.019	0.003		0.031	
Positive Composites								
Number	3	3	3	6	2	1	3	do.
Range	0.004-0.014	0.010-0.019	0.009-0.028	0.012-0.029	0.008-0.009	0.022	0.014-0.077	
BHC								
Average			0.019	0.026				
Positive Composites								
Number	0	1	4	5	0	1	0	do.
Range		0.032	0.008-0.072	0.016-0.057		0.026		
TOTAL BROMIDES								
Average	15.8	2.8	9.4	4.8	3.7	3.3	4.8	1.9
Positive Composites								
Number	5	5	6	6	6	6	4	4
Range	3.5-31.7	0.5-8.6	4.9-13.8	1.5-8.2	1.1-9.3	1.0-9.8	1.0-21.4	0.5-5.4

TABLE 2a.—Levels of Pesticide Residues Commonly Found—by Food Class, Region, and Sampling Period—Continued

Pesticide	Boston 1965	Boston 1966	Kansas City 1965	Kansas City 1966	Los Angeles 1965	Los Angeles 1966	Baltimore 1966	Minneapolis 1966
II. MEAT, FISH AND POULTRY (17-23% fat)[2] — Residues in Parts Per Million—Fat Basis								
DDT								
Average	0.247	0.679	0.096	0.313	0.228	0.437	0.096	
Positive Composites								
Number	6	6	6	6	4	6	3	1
Range	0.010-0.862	0.503-1.180	0.068-0.182	0.056-0.874	0.306-0.406	0.128-1.[illegible]	[illegible]68-0.165	0.476
DDE								
Average	0.113	0.317	0.095	0.252	0.437	0.513	0.048	
Positive Composites								
Number	6	5	6	6	6	6	4	1
Range	0.003-0.328	0.230-0.726	0.041-0.156	0.028-0.868	0.020-0.915	0.231-0.997	0.017-0.066	0.580
TDE								
Average	0.098	0.446	0.057	0.092	0.109	0.158	0.016	
Positive Composites								
Number	4	5	5	6	4	5	2	1
Range	0.055-0.251	0.361-0.781	0.031-0.115	0.018-0.246	0.118-0.204	0.047-0.697	0.030-0.034	0.533
DIELDRIN								
Average	0.046	0.116	0.013	0.039	0.068	0.030		
Positive Composites								
Number	6	5	3	5	4	5	1	0
Range	0.003-0.142	0.097-0.203	0.012-0.036	0.024-0.070	0.006-0.141	0.028-0.053	0.008	
HEPTACHLOR EPOXIDE								
Average	0.015	0.097	0.012	0.021	0.031	0.017		
Positive Composites								
Number	5	5	3	6	4	4	1	0
Range	0.002-0.059	0.053-0.290	0.011-0.049	0.010-0.030	0.018-0.082	0.006-0.051	0.006	
BHC								
Average		0.140	0.047	0.022				
Positive Composites								
Number	0	6	5	5	1	1	0	0
Range		0.070-0.203	0.027-0.141	T-0.063	0.065	0.043		
LINDANE								
Average	0.001	0.024						
Positive Composites								
Number	3	2	0	0	1	1	0	0
Range	[illegible]-0.002	0.080-0.061			0.111	0.027		
ORGANIC BROMIDES								
Average	9.0	4.0	14.8	8.7	4.7	2.9	[3] 8.7	[3] 8.1
Positive Composites								
Number	4	6	6	3	6	5	4	5
Range	1-23.4	2.2-6.2	3.5-35.5	2.4-44.0	2.2-7.1	0.9-6.6	0.5-34.3	1.0-30.1
III. GRAIN AND CEREAL[2] — Residues in Parts Per Million								
DDT								
Average	0.003	0.011	0.010	0.007	0.012	0.004		
Positive Composites								
Number	4	5	5	5	5	3	1	0
Range	T-0.010	0.006-0.024	0.005-0.021	T-0.015	0.008-0.026	0.004-0.012	0.007	
DDE								
Average		0.001	0.002	0.002	0.001	0.001	0.002	
Positive Composites								
Number	0	2	3	4	2	2	2	0
Range		T-0.002	T-0.007	T-0.004	T-0.002	0.001-0.002	0.002-0.004	
[illegible]								
Average			0.004	0.001				
Positive Composites								
Number	0	1	3	3	1	1	1	0
Range		T	T-0.013	T-0.005	0.003	0.002	0.002	
[illegible]								
Average				0.005		0.001		
Positive Composites								
Number		1	1	5	1	2	1	0
Range		[illegible]	T	T-0.018	0.004	0.002-0.004	0.006	

TABLE 2a.—*Levels of Pesticide Residues Commonly Found—by Food Class, Region, and Sampling Period*—Continued

Pesticide	Boston 1965	Boston 1966	Kansas City 1965	Kansas City 1966	Los Angeles 1965	Los Angeles 1966	Baltimore 1966	Minneapolis 1966
III. GRAIN AND CEREAL [2]—(Continued) Residues in Parts Per Million								
LINDANE								
Average	0.004	0.007	0.009	0.018	0.007	0.001	0.004	0.006
Positive Composites								
Number	4	5	6	6	4	3	3	3
Range	T-0.012	0.004-0.014	T-0.016	0.005-0.028	0.001-0.032	0.002-0.003	0.002-0.009	0.006-0.010
MALATHION								
Average				0.018		0.041		0.011
Positive Composites								
Number	0	1	0	4	0	4	0	2
Range		0.035		0.013-0.035		0.025-0.153		0.024-0.018
TOTAL BROMIDES								
Average	20.7	15.7	52.0	12.2	6.5	6.8	[8] 17.4	[8] 19.5
Positive Composites								
Number	6	6	6	6	6	6	4	5
Range	10.0-31.2	11.2-20.4	10.4-111.0	1.1-18.6	4.4-9.6	2.4-11.7	4.0-51.4	2.3-66.7
IV. POTATOES [2] Residues in Parts Per Million								
DDT								
Average		0.003						
Positive Composites								
Number	0	4	0	0	1	1	1	0
Range		T-0.010			0.006	0.007	0.004	
DDE								
Average					0.001			
Positive Composites								
Number	0	1	0	1	4	1	1	0
Range		T		0.010	T-0.002	0.003	0.005	
DIELDRIN								
Average				<0.001		0.001		
Positive Composites								
Number	0	1	1	3	1	3	0	0
Range		0.003	0.006	T-0.002	0.003	T-0.002		
TOTAL BROMIDES								
Average	9.0	4.1	18.4	7.7	5.6	1.8	[3] 9.7	[3] 15.2
Positive Composites								
Number	5	5	6	4	4	3	3	5
Range	7.4-28.0	1.1-13.1	1.5-38.0	1.1-41.0	4.0-17.6	0.7-5.7	4.0-38.3	1.3-68.5
V. LEAFY VEGETABLES [2] Residues in Parts Per Million								
DDT								
Average		0.017	0.004	0.004	0.017	0.022	0.007	0.016
Positive Composites								
Number	1	2	2	2	4	5	2	2
Range	0.008	0.009-0.099	0.004-0.017	0.006-0.016	0.010-0.047	0.009-0.048	0.006-0.022	0.028-0.035
DDE								
Average	0.007	0.004	0.007	0.007	0.002	0.005	0.002	
Positive Composites								
Number	2	2	3	2	3	5	2	1
Range	0.019-0.025	0.002-0.023	0.003-0.032	0.006-0.033	0.003-0.006	0.004-0.008	0.002-0.006	0.015
TDE								
Average			0.061	0.005		0.004	0.001	0.011
Positive Composites								
Number	1	0	3	3	1	2	2	3
Range	T		0.006-0.291	0.001-0.024	T	0.008-0.014	0.002-0.003	0.012-0.017
TOTAL BROMIDES								
Average	4.6	3.3	4.4	2.0	1.9	1.3	[3]	[8] 1.7
Positive Composites								
Number	5	6	6	6	5	5	1	4
Range	1.3-16.3	1.3-7.2	1.7-10.5	0.7-3.4	0.7-6.8	0.6-2.9	14.8	0.7-5.0

Pesticide	Boston 1965	Boston 1966	Kansas City 1965	Kansas City 1966	Los Angeles 1965	Los Angeles 1966	Baltimore 1966	Minneapolis 1966
VI. LEGUME VEGETABLES [2] Residues in Parts Per Million								
DDT								
Average					0.024			
Positive Composites								
Number	1	1	0	0	4	1	1	0
Range	0.039	0.004			T-0.126	0.010	0.149	
TDE								
Average						0.001		
Positive Composites								
Number	1	1	0	1	1	3	1	0
Range	0.051	0.010		T	0.006	0.001-0.004	0.064	
TOTAL BROMIDES								
Average	4.4	3.4	6.1	1.4	3.3	0.7	[3] 2.3	[3] 4.6
Positive Composites								
Number	4	5	6	6	4	4	3	4
Range	2.1-14.1	1.6-12.0	1.0-17.9	0.5-2.4	0.9-15.2	0.7-1.7	0.5-9.9	1.1-14.5
VII. ROOT VEGETABLES [2] Residues in Parts Per Million								
DDT								
Average			0.018	0.003	0.002	0.005		
Positive Composites								
Number	1	0	2	2	2	2	0	0
Range	0.009		0.039-0.071	0.007-0.011	0.003-0.009	0.006-0.021		
DDE								
Average			0.009	0.003	0.010	0.003		
Positive Composites								
Number	1	0	2	4	4	4	0	0
Range	0.010		0.020-0.051	T-0.011	0.004-0.045	T-0.009		
DIELDRIN								
Average				0.008		0.002		
Positive Composites								
Number	1	0	1	4	1	3	0	0
Range	0.008		0.018	0.003-0.028	0.004	0.001-0.005		
TOTAL BROMIDES								
Average	5.6	4.1	10.9	3.1	1.5	2.8	[3] 3.0	[3] 4.1
Positive Composites								
Number	5	4	6	5	3	5	3	4
Range	4.3-9.3	5.5-7.2	2.8-22.1	2.1-5.4	2.6-3.5	1.5-7.5	0.7-8.9	0.6-17.0
VIII. GARDEN FRUITS [2] Residues in Parts Per Million								
DDT								
Average	0.011	0.014	0.034	0.059	0.032	0.048	0.013	0.007
Positive Composites								
Number	3	4	4	4	5	6	2	2
Range	0.008-0.033	T-0.034	0.018-0.149	0.011-0.168	0.018-0.086	0.022-0.115	0.012-0.038	0.014-0.030
DDE								
Average		0.001		0.011	0.003	0.004	0.002	
Positive Composites								
Number	1	2	1	4	6	3	2	0
Range	T	T-0.002	0.002	T-0.064	T-0.009	T-0.023	0.003-0.005	
TDE								
Average	0.017	0.006	0.010	0.072	0.001	0.006		0.005
Positive Composites								
Number	3	3	2	6	3	2	1	2
Range	0.012-0.048	0.005-0.020	0.009-0.049	T-0.338	T-0.003	0.011-0.023	0.015	0.013-0.017
[illegible]								
[illegible]	0.002		0.004	0.003	0.002	<0.001		0.006
[illegible]								
[illegible]	2	1	2	3	4	2	1	2
[illegible]	[illegible]	0.004	0.010-0.011	0.002-0.013	T-0.005	0.001-0.003	0.004	0.006-0.017
[illegible]								
Average	[illegible]	0.001	0.007	0.003	0.003	<0.001		0.002
Positive Composites								
Number	[illegible]	[illegible]	3	4	2	2	1	2
Range	[illegible]	T-0.005	0.008-0.025	T-0.012	0.002-0.017	T-0.001	0.002	0.004-0.005

TABLE 2a.—*Levels of Pesticide Residues Commonly Found—by Food Class, Region, and Sampling Period*—Continued

Pesticide	Boston 1965	Boston 1966	Kansas City 1965	Kansas City 1966	Los Angeles 1965	Los Angeles 1966	Baltimore 1966	Minneapolis 1966
VIII. GARDEN FRUITS [2]—(Continued) Residues in Parts Per Million								
TOTAL BROMIDES								
Average	9.1	3.0	6.4	3.5	1.5	2.5	[3] 2.6	[3] 2.3
Positive Composites								
Number	5	5	6	6	4	5	3	5
Range	5.7-18.9	1.1-7.5	4.0-8.3	1.2-7.5	1.7-2.8	1.7-7.1	1.5-9.2	0.5-7.2
IX. FRUITS [2] Residues in Parts Per Million								
DDT								
Average	0.008	0.012			0.008	0.010	0.006	
Positive Composites								
Number	3	4	1	1	5	2	3	0
Range	0.008-0.027	0.007-0.035	0.006	T	0.007-0.019	0.012-0.045	0.004-0.014	
DDE								
Average	<0.001	0.001		0.001	0.001	0.008	0.003	
Positive Composites								
Number	2	2	1	4	5	3	3	0
Range	T-0.003	0.002-0.004	0.005	T-0.005	T-0.003	0.003-0.043	0.001-0.006	
TDE								
Average							0.003	
Positive Composites								
Number	0	0	0	1	1	1	3	0
Range				0.007	0.004	0.007	0.002-0.006	
ALDRIN								
Average	0.008	0.015			0.001		0.004	
Positive Composites								
Number	4	3	1	0	3	1	2	0
Range	0.003-0.020	0.007-0.070	0.007		0.002	0.002	0.005-0.012	
KELTHANE								
Average		0.032		0.068		0.041		
Positive Composites								
Number	0	3	1	6	0	2	1	0
Range		0.013-0.107	0.166	0.016-0.161		0.032-0.212	0.015	
TOTAL BROMIDES								
Average	3.1	3.2	7.9	3.2	1.0	0.6	[3]	[3] 6.0
Positive Composites								
Number	2	5	6	6	4	3	1	4
Range	3.2-15.3	0.8-10.2	1.2-31.4	0.7-6.4	0.7-2.4	0.7-1.9	20.4	1.1-25.2
X. OILS, FATS AND SHORTENING [2] Residues in Parts Per Million								
DDT								
Average		0.010	0.008	0.008		0.011	0.010	
Positive Composites								
Number	1	3	2	5	1	2	2	0
Range	0.028	T-0.032	0.008-0.038	T-0.018	0.049	0.027-0.038	0.009-0.031	
DDE								
Average		0.003	0.003	0.004		0.011	0.006	
Positive Composites								
Number	1	2	2	6	1	3	3	0
Range	0.014	0.009-0.010	0.004-0.012	T-0.011	0.006	0.013-0.029	0.007-0.009	
TDE								
Average		0.025	0.010	0.007		0.021		
Positive Composites								
Number	1	2	2	5	1	3	1	0
Range	0.018	0.034-0.117	0.025-0.037	T-0.027	0.032	0.025-0.068	0.010	
HEPTACHLOR EPOXIDE								
Average			0.001	0.001				
Positive Composites								
Number	1	0	3	3	1	0	0	0
Range	0.004		0.001-0.004	T-0.004	0.002			
TOTAL BROMIDES								
Average	12.6	5.3	53.0	7.5	4.4	6.2	[3] 12.8	[3] 23.0
Positive Composites								
Number	6	5	5	6	5	2	3	5
Range	1.1-29.0	1.9-11.8	7.2-261.0	0.9-15.2	1.7-9.8	5.7-7.3	1.3-48.8	1.1-90.8

TABLE 2a.—*Levels of Pesticide Residues Commonly Found—by Food Class, Region, and Sampling Period*—Continued

PESTICIDE	BOSTON 1965	BOSTON 1966	KANSAS CITY 1965	KANSAS CITY 1966	LOS ANGELES 1965	LOS ANGELES 1966	BALTIMORE 1966	MINNEAPOLIS 1966
XI. SUGARS AND ADJUNCTS [2] Residues in Parts Per Million								
2, 4-D								
Average			0.020	0.030	0.07	0.038		
Positive Composites								
Number	0	1	4	4	4	3	0	0
Range		0.01	0.020-0.040	0.020-0.058	0.04-0.16	0.057-0.100		
TOTAL BROMIDES								
Average	12.8	11.2	30.8	10.5	4.3	3.8	[3] 12.8	[3] 29.7
Positive Composites								
Number	6	6	6	6	6	5	3	5
Range	4.0-26.4	7.0-17.7	12.0-55.1	6.0-16.5	0.7-9.2	2.3-7.7	2.1-55.4	0.7-117.0
XII. BEVERAGES [2] Residues in Parts Per Million								
TOTAL BROMIDES								
Average	5.7	4.1	7.9		1.5		[3]	[3] 0.5
Positive Composites								
Number	3	4	5	1	2	0	1	3
Range	1.2-16.2	1.3-13.7	2.8-15.0	3.2	0.9-8.2		8.7	0.5-1.2

[1] Six composite samples examined each year at Boston, Kansas City, and Los Angeles; four composite samples examined October 1965-April 1966 at Baltimore; for bromides, five composite samples examined beginning August 1965-April 1966 at Baltimore and Minneapolis.

[2] Six composite samples examined each year at Boston, Kansas City, and Los Angeles; four composite samples examined October 1965-April 1966 at Baltimore and Minneapolis.

[3] Five composites examined beginning August 1965.

NOTE: 1965 = June 1964–April 1965
1966 = June 1965–April 1966

TABLE 2b.—*Pesticides Found Infrequently—by Food Class, Region, and Sampling Period*

PESTICIDE	DISTRICT	NO. COM-POSITES	YEAR	AMOUNT (PPM)
I (a). DAIRY PRODUCTS (8-13% fat) [1] Residues in Parts Per Million—Fat Basis				
ALDRIN	Kansas City	1	1966	T
LINDANE	Kansas City	2	1965-1966	T, 0.210
	Boston	1	1965	0.006
MCP	Boston	1	1966	0.583
	Kansas City	1	1966	0.039
METHOXY= CHLOR	Kansas City	3	1966	T, T, 0.073
PCP	Boston	1	1966	0.310
	Kansas City	1	1966	0.009
2,4-DB	Los Angeles	1	1966	0.025
2,4, 5-TP	Boston	2	1966	0.018, 0.029
II (a). MEAT, FISH AND POULTRY (17-23% fat) [1] Residues in Parts Per Million—Fat Basis				
ALDRIN	Kansas City	1	1965	0.008
DIAZINON	Kansas City	1	1966	0.051
ENDRIN	Kansas City	1	1965	T
HEPTACHLOR	Kansas City	1	1965	0.008
P[illegible]	Kansas City	2	1965	0.01, 0.03
	Boston	2	1966	0.005, 0.051
	Los Angeles	1	1966	0.051
RONNEL	Kansas City	1	1966	0.011
TETRADIFON	Los Angeles	1	1966	0.076
ARSENIC (As_2O_3)	[illegible]eles	6	1965-1966	0.12, 0.2, 0.1, 0.1, 0.1, 0.2
	[illegible]	1	1966	0.5

PESTICIDE	DISTRICT	NO. COM-POSITES	YEAR	AMOUNT (PPM)
III (a). GRAIN AND CEREAL [1] Residues in Parts Per Million				
ALDRIN	Boston	1	1965	0.001
	Baltimore	1	1966	0.016
	Los Angeles	1	1966	0.014
BHC	Kansas City	1	1966	T
CARBARYL	Kansas City	3	1965	0.42, 0.20, 0.30
DIAZINON	Kansas City	2	1966	0.024, 0.024
	Minneapolis	2	1966	0.004, 0.030
DITHIOCAR= BAMATES	Kansas City	1	1965	0.5
ENDRIN	Boston	1	1966	0.001
	Los Angeles	1	1966	0.004
HEPTACHLOR	Kansas City	1	1965	0.006
	Los Angeles	1	1965	T
HEPTACHLOR EPOXIDE	Boston	1	1966	0.005
	Kansas City	2	1966	T, 0.005
MCP	Boston	1	1965	0.10
METHOXY= CHLOR	Boston	1	1966	0.004
	Kansas City	1	1966	0.007
PCNB	Boston	1	1966	0.005
PCP	Kansas City	2	1965-1966	0.02, 0.036
	Boston	1	1966	0.004
PERTHANE	Kansas City	2	1966	0.057, 0.049
2,4-DB	Kansas City	1	1966	0.013
RONNEL	Kansas City	1	1966	T
ARSENIC (As_2O_3)	Boston	1	1965	0.10
	Los Angeles	1	1966	0.10

IV (a). POTATOES[1]
Residues in Parts Per Million

Pesticide	District	No. Composites	Year	Amount (PPM)
BHC	Kansas City	1	1966	0.008
CARBARYL	Kansas City	1	1965	0.28
CIPC	Kansas City	2	1966	0.360, 0.199
ENDRIN	Kansas City	1	1965	T
	Los Angeles	3	1965-1966	0.005, 0.002, 0.006
	Boston	1	1966	0.004
HEPTACHLOR EPOXIDE	Kansas City	4	1965-1966	0.015, 0.020, 0.002, T
LINDANE	Boston	1	1965	0.008
	Baltimore	1	1966	0.011
	Kansas City	2	1966	T, 0.002
	Los Angeles	1	1966	T
PARATHION	Los Angeles	1	1966	0.003
TCNB	Boston	1	1965	0.216
	Baltimore	1	1966	0.370
TDE	Los Angeles	2	1965-1966	T, 0.001
ARSENIC (As_2O_3)	Los Angeles	1	1966	4 7

V (a). LEAFY VEGETABLES[1]
Residues in Parts Per Million

Pesticide	District	No. Composites	Year	Amount (PPM)
BHC	Kansas City	1	1965	0.015
CARBARYL	Kansas City	2	1965	0.3, 0.2
CHLOR=BENSIDE	Kansas City	2	1965	0.023, 0.038
	Los Angeles	1	1965	0.002
DACTHAL	Los Angeles	1	1966	0.006
DIAZINON	Los Angeles	2	1966	0.015, 0.012
	Minneapolis	1	1966	0.031
DIELDRIN	Baltimore	1	1966	0.002
	Kansas City	1	1966	T
DITHIOCAR=BAMATES	Kansas City	3	1965	0.4, 0.7, 0.8
2, 4-D	Kansas City	1	1965	T
	Boston	1	1966	0.017
ENDRIN	Kansas City	1	1965	T
HEPTACHLOR EPOXIDE	Kansas City	1	1965	0.004
LINDANE	Los Angeles	1	1965	0.004
	Boston	2	1966	T, 0.005
	Minneapolis	1	1966	0.012
MALATHION	Kansas City	1	1966	0.017
MCP	Boston	1	1966	0.114
PARATHION	Boston	1	1966	0.012
	Los Angeles	1	1966	0.016
	Minneapolis	1	1966	0.089
ENDOSULFAN	Los Angeles	1	1966	0.016
TOXAPHENE	Baltimore	1	1966	0.386

VI (a). LEGUME VEGETABLES[1]
Residues in Parts Per Million

Pesticide	District	No. Composites	Year	Amount (PPM)
ALDRIN	Boston	1	1966	0.006
DDE	Los Angeles	4	1965-1966	0.003, T, 0.002, 0.002
	Baltimore	1	1966	0.003
	Boston	2	1966	T, 0.003
	Kansas City	1	1966	T
DIELDRIN	Los Angeles	1	1965	0.002
	Kansas City	1	1966	T
HEPTACHLOR EPOXIDE	Los Angeles	1	1966	0.001
LINDANE	Boston	1	1966	T
ARSENIC (As_2O_3)	Boston	1	1965	0.11

VII (a). ROOT VEGETABLES[1]
Residues in Parts Per Million

Pesticide	District	No. Composites	Year	Amount (PPM)
CARBARYL	Kansas City	2	1965-1966	0.20, 0.10
CHLOR=BENSIDE	Kansas City	1	1966	0.010
ENDRIN	Kansas City	2	1965-1966	T, 0.052
MALATHION	Kansas City	1	1966	0.022
TCNB	Kansas City	1	1965	0.011
	Los Angeles	1	1966	T
TDE	Kansas City	2	1965-1966	0.021,0.009
	Los Angeles	1	1965	0.004
ARSENIC (As_2O_3)	Boston	1	1965	0.10
	Minneapolis	1	1966	0.10

VIII (a). GARDEN FRUITS[1]
Residues in Parts Per Million

Pesticide	District	No. Composites	Year	Amount (PPM)
ALDRIN	Boston	1	1966	0.005
ARSENIC (As_2O_3)	Minneapolis	1	1966	0.10
BHC	Kansas City	1	1965	0.004
CARBARYL	Kansas City	1	1965	0.19
CHLORDANE	Boston	2	1965-1966	0.033, 0.006
	Los Angeles	1	1966	0.002
DIAZINON	Minneapolis	1	1966	0.005
ENDRIN	Kansas City	2	1965-1966	0.007, 0.005
HEPTACHLOR EPOXIDE	Los Angeles	1	1965	T
	Boston	1	1966	T
ENDOSULFAN	Boston	2	1966	0.006, T
	Los Angeles	1	1966	0.002
TOXAPHENE	Boston	1	1966	0.048
	Los Angeles	1	1966	0.050

TABLE 2b. *Pesticides Found Infrequently—by Food Class, Region, and Sampling Period*—Continued

Pesticide	District	No. Composites	Year	Amount (ppm)
IX (a). FRUITS[1] Residues in Parts Per Million				
CARBARYL	Kansas City	4	1965-1966	0.19, 0.20, 0.10, 0.20
	Boston	1	1966	0.17
	Los Angeles	1	1966	0.05
DACTHAL	Boston	1	1966	0.004
DIELDRIN	Boston	3	1965-1966	0.004, T, 0.002
	Kansas City	3	1966	T, T, T
ETHION	Boston	1	1966	0.019
LINDANE	Kansas City	1	1965	0.009
	Los Angeles	2	1966	0.002, 0.005
	Boston	2	1966	T, 0.002
PCNB	Kansas City	1	1965	0.003
PERTHANE	Boston	2	1965-1966	0.016, 0.007
TETRADIFON	Boston	1	1965	0.044
	Los Angeles	2	1965-1966	0.006, 0.011
ENDOSULFAN	Kansas City	1	1966	0.014
ARSENIC (As_2O_3)	Boston	1	1965	0.18

Pesticide	District	No. Composites	Year	Amount (ppm)
X (a). OILS, FATS AND SHORTENING[1] Residues in Parts Per Million				
ALDRIN	Kansas City	2	1965-1966	T, T
BHC	Kansas City	1	1965	0.007
DIELDRIN	Kansas City	3	1965-1966	T, 0.005, T
	Los Angeles	4	1965-1966	0.007, 0.070, 0.005, 0.009
ENDRIN	Kansas City	2	1965-1966	0.017, 0.006
	Los Angeles	1	1966	0.012
HEPTACHLOR	Kansas City	1	1965	0.002
LINDANE	Kansas City	1	1965	0.003
	Los Angeles	1	1965	0.004
MALATHION	Boston	1	1966	0.053
	Kansas City	2	1966	0.053, 0.013
	Minneapolis	1	1966	0.18
PCP	Boston	1	1966	0.012
	Los Angeles	1	1966	0.193
X (a). OILS, FATS AND SHORTENING[1]—(Continued) Residues in Parts Per Million				
PERTHANE	Kansas City	1	1966	0.032
2,4-D	Boston	1	1965	0.030
TBA	Kansas City	1	1965	0.02

Pesticide	District	No. Composites	Year	Amount (ppm)
XI (a). SUGARS AND ADJUNCTS[1] Residues in Parts Per Million				
ALDRIN	Kansas City	1	1965	0.003
BHC	Kansas City	1	1965	0.015
CARBARYL	Kansas City	4	1965-1966	0.7, 0.2, 0.1, 0.2
DDT	Kansas City	2	1965	0.021, 0.085
	Los Angeles	3	1965-1966	T, 0.005, 0.008
DDE	Los Angeles	3	1965-1966	0.003, T, 0.004
DIELDRIN	Los Angeles	2	1965-1966	T, 0.002
HEPTACHLOR EPOXIDE	Los Angeles	2	1965	0.002, T
	Kansas City	1	1966	T
LINDANE	Kansas City	2	1965-1966	T, T
	Los Angeles	1	1965	0.001
	Boston	1	1966	T
MCP	Boston	1	1966	0.022
RONNEL	Los Angeles	1	1966	T
TDE	Los Angeles	2	1965	0.012, T
ARSENIC (As_2O_3)	Boston	1	1966	0.1
XII (a). BEVERAGES[1] Residues in Parts Per Million				
CARBARYL	Kansas City	4	1965-1966	0.37, 0.50, 0.50, 0.40
DDE	Los Angeles	1	1965	T
HEPTACHLOR EPOXIDE	Kansas City	1	1966	T
LINDANE	Kansas City	1	1966	T
PCP	Boston	1	1966	0.02

[1] Six composite samples examined each year at Boston, Kansas City, and Los Angeles; four composite samples examined October 1965–April 1966 at Baltimore and Minneapolis.

NOTE: 1965 = June 1964–April 1965
1966 = June 1965–April 1966

Acknowledgment

The authors gratefully acknowledge the analytical work from the FDA Laboratories in Baltimore, Md.; Boston, Mass.; Kansas City, Mo.; Los Angeles, Calif.; and Minneapolis, Minn.

LITERATURE CITED

[illegible] *H. C. Barry, and L. Y. Johnson. 1966.* [illegible] total diet samples. Science 151:101.

[illegible] *McFarland. 1967.* Assessments [illegible] food and feed commodities, market basket [illegible] for consumption, meat samples taken [illegible]

[illegible] *1962.* A flame ionization detector [illegible] to phosphorus—a sodium thermionic detector. J. Ass. Offic. Agr. Chem. 47:293; *ibid. 1964.* Investigation of two gas chromatographic techniques for the determination of organophosphate pesticide residues. 47:1112.

(4) *Mills, P. A., J. H. Onley, and R. A. Gaither. 1963.* Rapid method for chlorinated pesticide residues in non-fatty foods. J. Ass. Offic. Agr. Chem. 46:186-191.

(5) *Kovacs, Martin F., Jr. 1964.* Thin layer chromatography for organo thiophosphate pesticide residue determination. J. Ass. Offic. Agr. Chem. 47:1097.

(6) *Finocchiaro, J. M., and W. R. Benson. 1965.* Thin layer chromatographic determination of carbaryl (Sevin) in some foods. J. Ass. Offic. Agr. Chem. 48:736.

(7) *Duggan, R. E., and J. R. Weatherwax. 1967.* Dietary intake of pesticide chemicals. Science (Accepted for publication).

Chlorinated Hydrocarbon Pesticide Residues in or on Alfalfa Grown in Soil With a Previous History of Aldrin and Heptachlor Application

R. J. Moubry[1], G. R. Myrdal[1], and H. P. Jensen[2]

ABSTRACT

Samples of soil, alfalfa, and alfalfa roots were collected from acreage with a past history of aldrin and heptachlor application. The samples were analyzed with the final determination by gas liquid chromatography (GLC). Data obtained are presented on both the wet, or as is, and the dry weight basis.

LOW level dieldrin residues in milk from herds located in corn producing areas of the State prompted an investigation into the possible contamination of alfalfa grown in soil with a past history of aldrin use. The common use of aldrin involved the placement of insecticidal granules in the corn row as a narrow band behind the seed shoe. In an effort to determine the effect of this practice, personnel of the Wisconsin Department of Agriculture, in cooperation with J. W. Apple, Professor of Entomology, College of Agriculture, University of Wisconsin, collected samples from an alfalfa field which had a known soil insecticide application and cropping history.

A 40-acre field in Columbia County was selected for this study. Corn had been grown on this field in 1962, 1963, and 1964. Pesticide application was by band treatment, with 1 lb/acre heptachlor in 1962, 1 lb/acre aldrin in 1963, and 1 lb/acre aldrin in 1964. In 1965 the field was seeded with alfalfa, with oats planted as a nurse crop.

The east one-half of the field was sampled in a diagonal pattern on August 30, 1965. Samples of soil (Carrington silt loam), alfalfa and alfalfa roots were randomly collected at approximately 20-foot intervals. Composites of the samples of alfalfa and alfalfa roots were extracted and cleaned up by the acetonitrile extraction procedure *(1)*. Composites of the samples of soil were extracted by the hexane-acetone procedure *(2)*. The soil extracts were cleaned up with Florisil *(1)*. Prior to analysis the alfalfa and root samples were ground and mixed in a Hobart food chopper. A portion of each homogeneous sample was selected for a moisture determination. Analysis was made on the wet weight basis. The dry weight residue results were obtained by calculation, using the percent moisture obtained for each sample.

The determination of the amount of pesticide residue present in the sample was by GLC.

Conditions of Gas Liquid Chromatography Determination

Instrument — Jarrell-Ash, Model 28-710
Column — 4 ft. x 0.156 in. bore glass
Packing — 10% DC200 on 80-90 mesh Anakrom ABS
Detector — Electron affinity, source 100 μc H^3
Amplifier — Sensitivity 1 x 10^{-9} A, voltage, 18 v
Flow Rate — 196 ml N_2/min.; pressure, 30 lbs/sq. in.
Temperatures — Injector, 240 C; oven, 203 C; detector, 209 C

The presence of the pesticide residue in the samples was confirmed by GLC, using different column systems. These were (a) mixed bed consisting of one part 10% DC200 on 80-90 mesh Anakrom ABS and two parts 5% QFl on 60-80 mesh Chromosorb W, AW; and (b) 5% QFl on 60-80 mesh Chromosorb W, AW.

The sample size used for GLC injection was selected to provide detection and confirmation of residues at or above 0.001 ppm on the wet weight or as is basis.

[1] Wisconsin Department of Agriculture, General Laboratory Division, 4702 University Avenue, Madison, Wisc. 53705.
[2] Present address: Ciba Corporation, Agricultural Chemicals Test Laboratories, Vero Beach, Fla. 32960.

TABLE 1. *Detection of cyclodiene insecticide residues in alfalfa and soil following use on corn*

Sample	Residues in ppm: Wet Weight Basis: Heptachlor	Heptachlor Epoxide	Aldrin	Dieldrin	Dry Weight Basis: Heptachlor	Heptachlor Epoxide	Aldrin	Dieldrin
Soil, Top ¼″ Layer					0.139	0.226	0.007	0.019
Soil, 6″ Cores					0.211	0.221	0.032	0.036
Alfalfa Roots [1]—Group 1	0.098	0.460	0.014	0.142	0.293	1.370	0.041	0.424
Alfalfa Roots with Tops [1]—Group 2	0.049	0.360	0.010	0.120	0.146	0.970	0.030	0.358
Alfalfa [2]		0.020		0.003		0.111		0.015
Alfalfa, Lower Half [3]		0.031		0.004		0.165		0.020
Alfalfa, Upper Half [3]		0.010		0.001		0.059		0.008
Alfalfa [4]		0.010		0.002		0.061		0.012

[1] These roots were washed and separated into two groups. The aerial portions of the plants were removed from the first group; ½ to 1 inch of the aerial portions were left remaining on the second group.

[2] This alfalfa was cut ½ inch above ground. It was thoroughly washed before analysis.

[3] A portion of the alfalfa plants cut ½ inch above ground was randomly selected from the original sample (b). These plants ranged between 4 and 10 inches in height. Each plant was water washed and cut in half. The lower and upper halves were grouped, ground, and analyzed.

[4] This sample of alfalfa was collected at the same time and in the same manner as that defined in (b) except that it was cut 1½ inches above ground. This alfalfa was not washed prior to analysis.

Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-dimethanonaphthalene
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene

Inasmuch as this was an exploratory survey, recovery studies were not conducted in conjunction with these samples. The data presented are the results obtained using the methodology specified. The results are detailed in Table 1.

In the analysis of forages in this laboratory we customarily report results in the range of 0.005 ppm to 0.001 ppm as a "trace." In this study results below this level are defined numerically, because they present information useful in comparison to the amount of residue found in the different portions of the plants. Data obtained in this initial investigation showed aldrin residues in the root and aerial portions of alfalfa grown in soil where aldrin had been applied as a band treatment in corn rows 1 to 2 years previously; similar treatment with [illegible] years previously also resulted in residues [illegible]

[illegible] of [illegible] forage to dairy animals will provide [illegible] of dieldrin to the diet which could be a contributing [illegible] of low level residue in the milk production of the [illegible] animals (3). These data also indicated that the residue levels were higher in the roots than in the aerial portion of the plant. Analysis of the aerial portion of the plants divided into upper and lower halves showed higher residues in the lower halves of the plants. Washing of the aerial portions did not shown any effect on the residue present. The data also showed that residues in the soil were appreciably lower in the top ¼-inch layer than in the upper 6 inches of the soil.

LITERATURE CITED

(1) *Barry, Helen C., Joyce G. Hundley, and Loren Y. Johnson.* Pesticide Analytical Manual Vol. I, 2.21(B), U. S. Department of Health, Education and Welfare, Food and Drug Administration, Washington, D.C. 20204.

(2) *Lichtenstein, E. P., G. R. Myrdal, and K. R. Schultz. 1964.* Effect of formulation and mode of application of aldrin on the loss of aldrin and its epoxide from soils and their translocation into carrots. J. Econ. Entomol. 57:133-136.

(3) *Williams, S., P. A. Mills, and R. E. McDowell. 1964.* Residues in milk of cows fed rations containing low concentrations of five chlorinated hydrocarbon pesticides. J. Ass. Offic. Agr. Chem. 47(6): 1124-1128.

PESTICIDES IN PEOPLE

Storage of DDT in The People of Israel[1]

M. Wassermann, Dora Wassermann, L. Zellermayer, and M. Gon

MEASUREMENT of the storage of chlorinated hydrocarbon insecticides in the body fat constitutes a valuable tool for the appraisal of exposure of the general population to these compounds.

Their storage is encountered in populations of different continents all over the world. The main source of insecticide absorption is the dietary intake, but air pollution produced by the household use of insecticides may also contribute to storage. Use of new analytical techniques, especially gas chromatography, has revealed that, besides DDT and its metabolite DDE, other organochlorine insecticides are stored in the body fat of people without known occupational exposure. The compounds include: DDD and β-isomer of BHC in the general population of the USA (New Orleans) *(14)*; BHC in body fat in the USA *(1,15,16)* in France *(13)*, and India *(2)*; γ-isomer of BHC in the general population of England *(23)*; dieldrin in body fat in the USA *(1,15,16,24)*, in Southern England *(17,23)* and in India *(2)*; heptachlor epoxide in persons in the USA *(14,29)*, and in India *(2)*; and DDD and dieldrin—in some cases also γ-isomer of BHC—in the general population and in farm workers of USA (Dade County, Florida) *(8)*. This paper reports on a further study that has been carried out on the general population of Israel in order to follow up the evaluation of organochlorine insecticide storage in this country. A previous study was performed by us on 254 specimens of fat tissue, obtained in 1963-64, from persons without occupational exposure *(26)*. It revealed that, at that time in the body fat of the general population of Israel, the mean concentration of DDT was 8.5 ppm, that of DDE was 10.7 ppm and that of DDT-equivalent (expressed as the numerical sum of DDT and DDE) was 19.2 ppm; DDE averaged 55.6% of the total DT-derived material.

Material and Methods

The survey was carried out on 204 samples (144 autopsy and 60 biopsy specimens) from five hospitals and the Forensic Medicine Institute. They were obtained in 1965 and 1966 from persons without known occupational exposure to pesticides. A survey sheet containing information regarding name, sex, country of origin, occupation, dietary habits, and operative diagnosis or cause of death was completed for each sample. Each specimen was preserved in 4% formaldehyde (Hayes *et al. (12)* have shown that this method of preservation is suitable for such survey purposes). The analyses for DDT were performed by the Schechter-Haller spectrophotometric method with the modification described by the Technical Development Laboratories, Communicable Disease Center *(25)*. A Shimadzu XV type automatic Recording Spectrophotometer model SV.50A served to record the visible spectrum. Since the method fails to distinguish between DDE and DDD, the values given for DDE must be considered to represent the sum of the two compounds.

The distribution of samples according to age and geographic origin is summarized in Table 1. From the

TABLE 1.—*Distribution of samples according to age and geographic origin*

Age Group (in years)	No. of Samples	Area of Origin			
		Israel	Europe	Asia	Africa
0 - 9	71	69	—	1	1
10 - 19	2	1	—	—	1
20 - 29	5	1	1	1	2
30 - 39	11	—	3	5	3
40 - 49	24	1	13	6	4
50 - 59	31	4	23	2	2
60 - 69	37	4	15	9	9
70 - 79	18	2	8	5	3
80 - 89	5	—	5	—	—
TOTAL	204	82	68	29	25

The systematic names of compounds mentioned in this paper are:

DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexa=chloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa=hydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Heptachlor epoxide	1,4,5,6,7,8,8-hepta=chloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan

[1] From the Department of Occupational Health, the Hebrew University, Hadassah Medical School, Jerusalem, Israel.

epidemiological point of view, it was important to determine that these samples were from persons who themselves, or whose parents, immigrated at least 8 years previously, and it can be assumed they have had unchanged living conditions since immigration. There are great differences in the dietary and cooking habits among the different ethnic groups of this country. However, the basic foods for the entire population of the country have a similar origin, and it appears therefore that these people have similar exposure to the dietary intake of DDT.

In the previous study we found no significant differences between the mean values of DDT-derived material in different ethnic groups. In this study, likewise, there were no differences by ethnic group. For this reason the data in this paper are presented by sex and age only. For purposes of comparison with the previous study in which there were only three cases in the age group 0 - 9 years, mean values are given separately for the groups 0 - 9 years and 10 - 89 years.

The group 0 - 9 years, consisting of 71 cases, or 34.4% of the total number of cases, provided a valuable opportunity for studying storage at these ages (Table 2).

Results

A total of 204 samples of fat tissue originating from persons without known occupational exposure have been analyzed for DDT-derived material. The results are summarized in Table 2.

There are no significant differences between the mean values found in different ethnic groups.

In the general population of Israel, age 10 - 89 years, the mean of total DDT-derived material in 133 samples is 18 ± 12.6 ppm. DDT is present in an average concentration of 8.2 ± 6.1 ppm, and DDE in 9.9 ± 7.1 ppm. DDE averages 53.9 ± 6.8% of the total DDT-derived material.

There is no significant difference between the present results and those obtained in our previous study on Israelis ($p > 0.1$). In the period 1965-66, the storage of DDT-derived material continued to be maintained at a high level in comparison with those levels reported during the last decade in other countries with the exception of India (Table 3).

[illegible] aged 10 - 89 years, there is no significant [illegible] the 5% level in the DDT-derived [illegible] age groups.

[illegible] amounts of DDT-derived material [illegible] for DDT, p. 0.02; DDE [illegible] DDT [illegible] DDE [illegible] 0.01. For percent of DDE the difference was [illegible] significant (p [illegible] 0.05).

When the age group considered [illegible] restricted to 60 - 69 years, there is a significant difference ($p < 0.05$) for DDE and for DDT + DDE. There is no significant difference for DDT and for the DDE percent ($p > 0.05$).

In the age group 0 - 9 years, the average concentration of DDT + DDE is 10.2 ± 9.2 ppm. The mean for DDT is 4.6 ± 4.2 ppm and for DDE is 5.6 ± 5.9 ppm. The DDE percent is 53.3 ± 7.9.

As far as concentrations of DDT, DDE, and DDT + DDE are concerned, a significant difference ($p < 0.001$) does exist in the storage of DDT-derived material between the group aged 0 - 9 years and that of 10 - 89 years even though certain unusually high values found in the 0 - 9 age group are used in the calculations. From the total of 71 cases belonging to this age group, 69 were aged from 0 - 2 years (Table 2). No significant difference was found in the storage of DDT-derived material among stillborns, neonates, and infants.

Mathematically there is a significant difference ($p < 0.05$) for the DDE percent in the female sex between the stillborn, neonate, and infant categories. In stillborns, there is a significant difference ($p < 0.05$) between sexes for DDE percent.

However, the number of cases in every category is too small to justify a biological conclusion.

Comments

The data presented in Table 3 suggest that DDT is a current constituent of human fat in the general population of the world at this time.

Twelve samples of this study were from stillborns and 24 from neonates (15 in the first week of life). DDT-material was found in all these samples and, in fact, in all the samples analyzed in this study. Dénes *(3)* found DDT in a day-old neonate and Halacka *et al. (9)* in three neonates. In our previous study in 1963-64, we found 16.1 ppm DDT-derived material in the fat tissue of a day-old neonate.

A note on the results of 50 samples as part of the present study has been presented at the First World Congress on Air Pollution *(27)*. At that time we found a mean of 10.2 ppm DDT-derived material in the fat tissue of stillborns, neonates, and infants of Israel.

Fiserova-Bergerova *et al. (8)* found in the fat tissue of four stillborns and two fetuses 5.65 ppm total DDT. Zavon *et al. (30)* in 64 samples originating from children dying between the 36th week of gestation and 2 weeks postpartum, have found DDT-derived material, heptachlor epoxide, and dieldrin. All these studies show that the stored DDT-derived material in fetuses, newborns, and infants in their first weeks are in a lower concentration than in the adult population. In animal studies Finnegan *et al. (7)* found DDT in the offspring of dogs and Pillmore *et al. (20)* in rabbits.

TABLE 2.—*Concentration of DDT-derived material in the body fat of people in Israel*

Age Group		Total DDT (PPM)	Total DDE (PPM)	Total Total as DDT (PPM)	Total DDE as DDT (%)	Males DDT (PPM)	Males DDE (PPM)	Males Total as DDT (PPM)	Males DDE as DDT (%)	Females DDT (PPM)	Females DDE (PPM)	Females Total as DDT (PPM)	Females DDE as DDT (%)
Age group 0 - 9 years													
Stillborns	Range	0.5—19.3	0.5—19.8	1.0—39.1	37.3—65.0	0.5— 7.3	0.5—13.0	1.0—20.0	47.3—65.0	2.5—19.3	2.0—19.8	4.5—39.1	37.3—55.1
	Mean-SD	5.4± 4.8	6.1± 5.6	11.5±10.2	50.9± 8.0	3.6± 2.6	5.1± 4.4	8.7± 7.0	55.5± 6.9	7.3± 5.7	7.1± 6.3	14.4±11.9	46.3± 6.2
	N	12	12	12		6	6	6		6	6	6	
Neonates I (1 - days)	Range	0.5—12.2	0.3—20.6	0.8—32.8	37.5—65.0	5.0— 7.0	5.5—13.0	10.5—20.0	44.0—65.0	0.5—12.2	0.3—20.6	0.8—32.8	37.5—65.0
	Mean-SD	4.9± 3.4	6.2± 5.4	11.1± 8.6	51.7± 8.4	6.3± 0.9	8.0± 3.5	14.3± 4.1	53.8± 8.6	4.5± 3.7	5.7± 5.6	9.2±10.2	51.2± 8.2
	N	15	15	15		3	3	3		12	12	12	
Neonates II (8 - 30 days)	Range	0.6— 8.2	0.4— 6.0	1.0—14.0	32.8—54.6	1.0— 8.2	1.1— 4.0	2.1—12.2	32.8—53.1	0.6— 8.0	0.4— 6.0	1.0—14.0	40.0—54.6
	Mean-SD	4.1± 2.9	3.2± 1.8	7.3± 4.6	46.1± 6.9	3.3± 2.6	2.5± 1.0	5.7± 3.5	47.3± 7.5	5.1± 2.9	4.2± 2.2	9.2± 5.0	44.6± 5.9
	N	9	9	9	9	5	5	5		4	4	4	
Infants (30 days - 2 years)	Range	0.5—27.1	0.5—32.9	1.0—60.0	42.9—74.0	1.1—27.1	0.9—32.9	2.0—60.0	45.0—74.0	0.5—10.1	0.5—11.0	1.0—21.1	42.9—65.8
	Mean-SD	4.4± 4.7	5.8± 7.1	10.2±11.4	54.2± 7.2	5.0± 5.6	6.9± 8.6	11.2±13.8	54.1± 7.7	3.5± 2.5	4.1± 2.7	7.6± 5.1	54.4± 6.2
	N	33	33	33		20	20	20		13	13	13	
Children (3 - 9 years)	Range	2.2— 6.1	2.6— 7.2	4.8—13.3	54.0—54.2	—	—	—	—	—	—	—	—
	Mean	4.2	4.9	9.1	54.1	6.1	7.2	2.2	54.6	2.2	2.6	4.8	54.2
	N	2	2	2		1	1	1		1	1	1	
Total	Range	0.5—27.1	0.3—32.9	0.8—60.0	32.8—74.0	0.5—27.1	0.5—32.9	1.0—60.0	32.8—74.0	0.5—19.3	0.3—20.6	0.8—39.1	37.3—65.8
	Mean-SD	4.6± 4.2	5.6± 5.9	10.2± 9.2	53.3± 7.9	4.6± 4.5	6.0± 7.2	19.6±11.4	53.3± 8.0	4.7± 3.9	5.2± 4.7	9.9± 8.5	50.8± 7.8
	N	71	71	71		35	35	35		36	36	36	
Age group 10 - 89 years													
10 - 19 years	Range	3.1— 8.9	2.8— 9.4	5.9—18.3	47.5—51.4	3.1— 8.9	2.8— 9.4	5.9—18.3	47.5—51.4	—	—	—	—
	Mean-SD	6.0± 2.9	6.1± 3.3	12.1± 6.2	49.5± 2.0	6.0± 2.9	6.1± 3.3	12.1± 6.2	49.5± 2.0	—	—	—	—
	N	2	2	2		2	2	2		—	—	—	—
20 - 29 years	Range	3.2—11.5	3.3—21.5	6.5—33.0	49.0—65.7	3.2—11.5	3.3—21.5	6.5—33.0	50.8—65.7	5.9— 7.6	6.4— 7.3	12.3—14.9	49.0—52.0
	Mean-SD	7.1± 2.7	9.3± 6.3	16.4± 8.9	54.2± 5.9	7.3± 3.4	11.0± 7.7	18.3±11.0	56.7± 6.5	6.8± 0.8	6.9± 0.5	13.6± 1.3	50.5± 1.5
	N	5	5	5		3	3	3		2	2	2	
30 - 39 years	Range	3.1— 9.2	3.2—15.5	6.4—23.3	47.8—71.1	3.2— 9.0	3.2—15.5	6.4—23.3	47.8—66.5	3.1— 9.2	5.4—10.6	8.5—19.0	50.0—71.1
	Mean-SD	5.8± 2.2	7.9± 3.9	13.6± 5.7	56.3± 7.5	6.1± 2.2	8.2± 4.9	14.3± 6.9	54.5± 6.8	5.3± 6.1	7.5± 2.3	12.8± 3.6	58.6± 7.8
	N	11	11	11		6	6	6		5	5	5	
40 - 49 years	Range	1.9—16.0	1.8—20.3	3.7—35.5	44.1—66.8	1.9—16.0	1.8—19.5	3.7—35.5	47.4—62.4	1.9—16.0	2.0—20.3	4.9—34.0	44.1—66.8
	Mean-SD	7.3± 4.2	8.9±55.8	16.2± 9.9	53.1± 5.1	8.1± 4.3	10.0± 5.8	18.1±10.0	53.9± 4.4	6.5± 3.9	7.8± 5.7	14.3± 9.3	52.2± 5.8
	N	24	24	24		12	12	12		12	12	12	
50 - 59 years	Range	1.9—50.5	2.0—40.6	3.9—82.6	38.9—68.6	1.9—50.5	2.0—40.6	3.9—82.6	38.9—68.6	3.5—14.2	3.5—26.7	7.0—40.9	40.6—65.3
	Mean-SD	10.3± 9.3	13.1±10.1	23.4±18.4	55.1± 8.3	11.5±11.1	13.8±11.3	25.3±21.2	54.9± 8.9	8.3± 3.5	11.7± 7.4	20.0±10.6	55.4± 8.3
	N	31	31	31		20	20	20		11	11	11	
60 - 69 years	Range	1.0—25.3	1.0—27.0	2.0—52.3	31.0—74.8	1.0—25.3	1.0—27.0	2.0—52.3	31.0—67.5	2.0—15.7	2.8—16.5	4.9—32.2	50.0—74.8
	Mean-SD	8.5± 5.2	9.9± 5.6	18.4±10.3	54.2± 7.0	9.8± 5.4	11.4± 5.9	21.2±10.7	53.9± 7.3	6.4± 4.0	7.5± 3.9	13.9± 7.7	54.6± 6.4
	N	37	37	37		23	23	23		14	14	14	
70 - 79 years	Range	0.9—15.0	1.0—16.2	1.9—31.9	43.6—58.5	0.9—10.0	1.0—11.5	1.9—21.5	49.7—58.5	1.2—15.0	1.0—16.2	2.2—31.2	43.6—52.7
	Mean-SD	6.4± 3.6	6.8± 4.0	13.2± 7.6	51.2± 3.1	6.5± 2.6	7.3± 3.2	13.7± 5.8	52.7± 2.4	6.2± 4.3	6.4± 4.6	12.6± 9.0	49.6± 3.1
	N	18	18	18		9	9	9		9	9	9	
80 - 89 years	Range	4.5—18.0	4.7—23.5	9.2—41.5	50.5—66.7	4.5—18.0	4.7—23.5	9.2—41.5	50.5—66.7	7.2— 9.0	9.2—14.6	18.2—21.9	50.5—66.7
	Mean-SD	10.7± 4.9	13.7± 6.4	24.3±11.1	55.5± 6.0	12.4± 5.7	14.8± 7.8	27.2±13.7	53.5± 2.3	8.1± 0.9	11.9± 2.7	20.0± 2.3	58.6± 8.1
	N	5	5	5		3	3	3		2	2	2	
Total years	Range	0.9—50.5	1.0—40.6	1.9—82.6	31.0—74.8	0.9—50.5	1.0—40.6	1.9—82.6	31.0—68.6	1.2—16.0	1.0—26.7	2.2—40.9	40.6—74.8
	Mean-SD	8.2± 6.1	9.9± 7.1	18.1±12.6	53.9± 6.8	9.3± 7.1	11.2± 7.8	20.5±14.2	54.1± 6.6	6.7± 3.8	8.2± 5.5	14.9± 9.0	53.8± 6.9
	N	133	133	133		77	77	77		56	56	56	

TABLE 3.—*Concentration of DDT and its metabolite DDE in the body fat of the general population of various countries in the period 1955-67*

Country	Year	No. Samples	DDT (ppm)	DDE as DDT (ppm)	Total as DDT (ppm)	DDE as DDT (%)	Reference
USA	1955	49	7.4	12.5	19.9	62.8	(11)
USA	1954-56	61	4.9	6.8	11.7	58.1	(12)
USA	1961-62	130	4.0	8.6	12.6	68.3	(21)
USA	1963	28	2.4	4.2	6.7	62.0	(1)
USA	1963	282	2.9	8.2	11.1	73.9	(15, 16)
USA [1]	1955-56	16	2.3	3.6	5.9	61.0	(12)
USA [2]	1960	20	0.8	2.2	3.0	73.3	(5)
USA	1964	25	2.4	7.9	10.3	77	(14)
USA [3]		6	3.9	8.3			Fiserova-Bergerova *et al.* (1967)
USA [4]		10	7.2	12.7			Fiserova-Bergerova *et al.* (1967)
Canada	1959-60	62	1.6	3.3	4.9	67.0	(22)
Germany	1958-59	60	1.0	1.3	2.3	56.5	(18)
France	1961	10	1.7	3.5	5.2	67.3	(13)
Hungary	1960	50	5.7	6.7	12.4	51.3	(3)
England	1961-62	131			2.2		(17)
England	1964	100			4.0		(23)
India, Group I	1964	67	16.9	10.1	26.0	39.0	(2)
India, Group II	1964	19	20.3	10.7	31.0	34.0	(2)
India, Group III	1964	16	8.1	4.7	12.8	37.0	(2)
USA	1964-65	13			3.1-8.6		(24)
Czechoslovakia	1963-64	229	5.5	3.7	9.2	40.2	(9)
Israel	1963-64	254	8.5	10.7	19.2	55.6	(26)
Israel, Group I [5]	1965-66	71	4.6	5.6	10.2	52.1	This paper
Israel, Group II [6]	1965-66	133	8.2	9.9	18.1	53.9	This paper

[1] Persons eating no meat.
[2] Eskimos (Alaska).
[3] Age group 31–83, M.
[4] Farm workers, age group 18–57, M.
[5] Age group 0–9 years.
[6] Age group 10–89 years.

The further observations in this study support the concept of placental transmission of DDT, which can be considered a characteristic feature of pregnancy in our epoch.

Neonates continue to receive DDT through their dietary intake and probably in other ways too, since the storage level of this insecticide does not diminish but, on the contrary, increases with age.

Storage of high amounts of DDT-derived material can occur in very early childhood. Thus, in this study, the following amounts of total DDT have been found: 39.1 ppm in a female stillborn; 32.8 ppm in a day-old neonate; 42.0 ppm in a male infant of 7 months and 60.0 ppm in a male infant of 11 months. Our epidemiological investigations carried out in order to clarify the occurrence of these relatively high storage levels, did not provide any meaningful explanation. In the fat of two Indian children Dale *et al.* (2) found 291 ppm in a 3-year-old boy and 180 ppm in a 7-year-old girl. These authors attribute the high storage level of these two children to the possibility of unusual exposure.

The population sampled for this study is exposed to presumably similar environmental and living conditions, but analysis of the storage of DDT-derived material reveals age and sex differences.

If one considers the results of the whole group 10 - 89 years, higher amounts of DDT, DDE, and DDT + DDE are stored in comparison with the 0 - 9 year age group (and particularly 0 - 2 years).

The results of this study also reveal a significant difference between the sexes in the storage of DDT-derived material in the 10 - 89 year group, the men storing more DDT and more DDE than the females. These results accord with those of Hunter *et al.*, *(17)* who in a sample of 131 necropsy fats in England found that the total DDT concentration in specimens from males was significantly higher than that in specimens from females. In a further study on a sample of 100 (50 biopsy and 50 necropsy) specimens, Robinson *et al.* *(23)* found that the mean DDT-derived material was 4.9 ppm in males and 3.4 ppm in females. The mean concentrations of *p,p*-DDE in the male samples, both biopsy and necropsy, were significantly greater than those in the female samples.

In this study sex differences are observed in all age groups and even in the aged people in whom it is thought that hormonal differences tend to disappear. Higher storage of DDT-derived material in males could be attributed to the fact that males generally eat larger amounts of food than females.

In rats submitted to substantial dosage levels, Ortega *et al.* *(19)* and Durham *et al.* *(4)* have found that the female rat stores more DDT and much more DDE than the male rat. Other species have shown small or no differences between sexes in the storage of DDT: Woodard *et al.* *(28)* in dogs, Harris *et al.* *(10)* in hogs, and Durham *et al.* *(6)* in monkeys.

Regarding the intensity of the population exposure to DDT, the results of this study do not differ significantly from those of our previous study (1963-64). As can be seen from Table 3, the storage level of DDT-derived material in the fat of people in Israel is persistently high.

Summary and Conclusions

A total of 204 samples of human body fat were collected in 1965-1966 from Israelis with no known occupational exposure. The samples were analyzed by the Schechter-Haller spectrophotometric method. Since the method fails to distinguish between DDE and DDD, the values given for DDE must be considered to represent the sum of the two compounds. The mean total DDT-derived material in 133 samples from persons aged 10 - 89 years is 18.1 $\pm$ 12.6 ppm; the mean for DDT is 8.2 $\pm$ 6.1 ppm; and that for DDE is 9.1 $\pm$ 7.1 ppm. DDE averages 53.9 $\pm$ 6.8% of the total DDT-derived material. No significant difference was found between the results obtained in this study and those of the previous study (1963-64) on Israelis. There is no significant difference for the DDT-derived material stored in different age groups within the group aged 10 - 89 years.

In this group (10 - 89 years) males store higher amounts of derived material than females, namely, DDT 9.3 ppm versus 6.7 ppm; DDE 11.2 ppm versus 8.2 ppm; DDT + DDE 20.5 ppm versus 14.8 ppm.

In the age group 0 - 9 years, the mean total DDT is 10.2 $\pm$ 9.2 ppm; DDT averages 4.6 $\pm$ 4.2 ppm; and DDE 5.6 $\pm$ 5.9 ppm. DDE constitutes 53.3 $\pm$ 7.9% of the total DDT-derived material. In this group of 71 cases, there were 12 stillborns, 15 neonates aged 0 - 7 days, 9 neonates aged 7 - 30 days, 33 infants aged 30 days - 2 years, and 2 older children. No significant difference was found in the storage of DDT-derived material among stillborns, neonates, and infants.

There is a significant difference ($p<0.001$) in the storage process of DDT-derived material between the 0 - 9 year group and that aged 10 - 89 years, as far as the concentrations of DDT, DDE, and DDT + DDE are concerned.

In the light of the data published during the past 10 years on the storage of DDT-derived material in the body fat of people from various countries, this study supports the contention that DDT represents a current constituent of human body fat in the general population, and that it is transmitted through the placenta to the fetus. Its storage may present variations according to sex. Further research is needed to clarify the mechanism of this last feature.

This research study was supported by the U. S. Department of Health, Education, and Welfare, Public Health Service, National Communicable Disease Center, Atlanta, Georgia, research grant No. BSS-CDC-IS-9.

LITERATURE CITED

(1) *Dale, W. E. and G. E. Quinby. 1963.* Chlorinated insecticides in the body fat of people in the United States. Science 142(3592):593-5.

(2) *Dale, W. E., M. F. Copeland, and W. J. Hayes, Jr. 1965.* Chlorinated insecticides in the body fat of people in India. Bull. World Health Organ. 33:471-7.

(3) *Dénes, A. 1962.* Problems of food chemistry concerning residues of chlorinated hydrocarbons. Die Nahr. 6:48-56.

(4) *Durham, W. F., C. Cueto, Jr., and W. J. Hayes, Jr. 1956.* Hormonal influences on DDT metabolism in the white rat. Amer. J. Physiol. 187(2):373-7.

(5) *Durham, W. F., F. F. Armstrong, W. M. Upholt, and C. Heller. 1961.* Insecticide content of diet and body fat of Alaskan natives. Science 134(3493):1880-1.

(6) *Durham, W. F., P. Ortega, and W. J. Hayes, Jr. 1963.* The effect of various dietary levels of DDT on liver function, cell morphology, and DDT storage in the rhesus monkey. Arch. Intern. Pharmacodyn 141(1-2): 111-29.

(7) *Finnegan, J. K., H. B. Haag, and P. S. Larson. 1949.* Tissue distribution and elimination of DDD and DDT following oral administration to dogs and rats. Proc. Soc. Exp. Biol. Med., 72:357-60.

(8) *Fiserova-Bergerova, V., J. L. Radomski, J. E. Davies, and J. H. Davis. 1967.* Levels of chlorinated hydro-

carbon pesticides in human tissues. Ind. Med. Surg. 36(1):65-70.

(9) *Halacka, H., J. Hakl, and I. Vymetal. 1965.* Effect of massive doses of DDT on human adipose tissue. Cesk. Hyg. 10(3-4):188-92.

(10) *Harris, T. T., J. R. Harris, L. L. Mangelson, D. A. Greenwood, C. Biddulph, W. Binns, and M. L. Minner. 1953.* Effect of feeding DDT-treated alfalfa hay to swine and of feeding the swine tissues to rats. J. Nutr. 51(4):491-505.

(11) *Hayes, W. J., Jr., W. F. Durham, and C. Cueto, Jr. 1956.* The effect of known repeated oral doses of chlorophenothane (DDT) in man. J. Amer. Med. Ass. 162(9):890-7.

(12) *Hayes, W. J., Jr., G. E. Quinby, K. C. Walker, J. W. Elliott, and W. M. Upholt. 1958.* Storage of DDT in people with different degrees of exposure to DDT. Amer. Med. Ass. Arch. Ind. Health 18(5):398-406.

(13) *Hayes, W. J., Jr., W. E. Dale, and R. LeBreton. 1963.* Storage of insecticides in French people. Nature 199(4899):1189-91.

(14) *Hayes, W. J., Jr., W. E. Dale, and V. W. Burse. 1965.* Chlorinated hydrocarbon pesticides in the fat of people in New Orleans. Life Sci. 4(16):1611-15.

(15) *Hoffman, W. S., W. I. Fishbein, and M. B. Andelman. 1964.* The pesticide content of human fat tissue. Arch. Environ. Health 9:387-94.

(16) *Hoffman, W. S., W. I. Fishbein, and M. B. Andelman. 1964.* Pesticide storage in human fat tissue. J. Amer. Med. Ass. 188(9):819.

(17) *Hunter, C. G., J. Robinson, and A. Richardson. 1963.* Chlorinated insecticide content of human body fat in Southern England. Brit. Med. J. 1:221-4.

(18) *Maier-Bode, H. 1960.* DDT in human body fat. Med. Exp. 1:146-52.

(19) *Ortega, P., W. J. Hayes, Jr., W. F. Durham, and A. Mattson. 1956.* DDT in the diet of the rat. Public Health Monograph No. 43, PHS. Publ. No. 484,1-27.

(20) *Pillmore, R. E., J. O. Keith, L. C. McEwen, M. H. Mohn, R. A. Wilson, and G. H. Ise. 1963.* Cottontail rabbit: feeding test. US Fish Wildlife Serv. Circ. No. 167,47-50.

(21) *Quinby, G. E., W. J. Hayes, Jr., J. F. Armstrong, and W. F. Durham. 1965.* DDT storage in the United States population. J. Amer. Med. Ass. 191(3):175-9.

(22) *Read, S. T. and W. P. McKinley. 1961.* DDT and DDE content of human fat. Arch. Environ. Health 3:209-11.

(23) *Robinson, J., A. Richardson, C. G. Hunter, A. N. Crabtree, and H. J. Rees. 1965.* Organo-chlorine insecticide content of human adipose tissue. Southeast Engl. Brit. J. Ind. Med. 22:220-9.

(24) *Schafer, M. L. and J. E. Campbell.* Distribution of residues in human body tissues from Montgomery County, Ohio. In: *Organic Pesticides in the Environment.* Edited by R. F. Gould. 1966. Advances in Chem. Ser. No. 60, Amer. Chem. Soc., Wash., D. C., p. 89-98.

[illegible] *Development Laboratories. 1953.* Method [illegible] analysis of DDT and DDE in samples [illegible] Mem. No. 1, Commun. Dis. Center, USPHS [illegible] Ga. (mimeogr.)

[illegible] *W. M. Gon, D. Wassermann, and L. Z[illegible]* DDT and DDE in the body fat of the peo[illegible] Arch. Environ. Health 11(3):375-9.

(2[illegible]) *Wasserman[illegible] Hal[illegible] 1965.* The role of air pollution [illegible] body burden for the general populat[illegible] World Congr. on Air Pollution, Buenos [illegible]

(28) *Woodard, G., R. R. Ofner, and C. M. Montgomery. 1945. Accumulation* of DDT in the body fat and its appearance in the milk of dogs. Science, 102:177-8.

(29) *Zavon, M. R., C. H. Hine, and D. K. Parker. 1965.* Chlorinated hydrocarbon insecticides in human body fat in the United States. J. Amer. Med. Ass. 193(10):837-9.

(30) *Zavon, M. R., R. Tyre, and L. Latorre. 1967.* Chlorinated hydrocarbon pesticide levels in the newborn. Proc. Con. Biol. Effects Pesticides Mammalian Syst., N. Y. Acad. Sci. (To be published).

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Insecticide Concentrations In Wildlife At Presidio, Texas

Dudley D. Culley[1] and Howard G. Applegate[2]

ABSTRACT

Data are given on insecticide concentrations in representative species of reptiles, birds, and mammals from Presidio, Texas. Various tissues and organs were examined by the means of gas chromatography. As specimens were taken farther from the cultivated area, the insecticide concentrations decreased. Within and adjacent to the cultivated area, insecticide concentrations showed a complex pattern. Possible reasons for the complexity are discussed.

Introduction

PRELIMINARY studies on insecticides in an ecosystem at Presidio, Texas during June, July, and August 1965, have been reported in a series of papers *(1-3,5,8)*. This paper is an expansion of the paper by Culley and Applegate *(5)* giving more data and reporting on an extended period of study.

The study was undertaken to provide training to students in Departments of Wildlife, Plant Sciences, and Meteorology in the detection of insecticides and to assess the value of the Presidio Valley as an area in which to conduct long-term studies on the movement of insecticides through an ecosystem and the effects and fate of insecticides within the ecosystem. The data gathered during the year must be regarded as preliminary and, perhaps, indicative. They cannot be considered as conclusive.

Methods and Materials

The Presidio Valley has approximately 384,000 acres, of which 2,900 acres are in cultivation. The area is geographically part of the Chihuahuaian Desert. The valley supports excellent populations of a variety of reptiles, birds, and mammals. The valley itself is at an elevation of 2,600 feet, while the surrounding mountains in the United States are above 7,000 feet, and in Mexico above 8,000 feet. Thus, we have essentially a point source of insecticide application within a large enclosed area.

A map of the area is presented in Fig. 1. Sites 1 and 4 were the westernmost and easternmost farms, respectively, in the Presidio growing region. Site 2, located between sites 1 and 4, was near the town. Each of these sites (1,2, and 4) had two sampling stations: one in the cotton fields and one in the desert peripheral to the fields. Sites 7 through 9 were in the desert and located

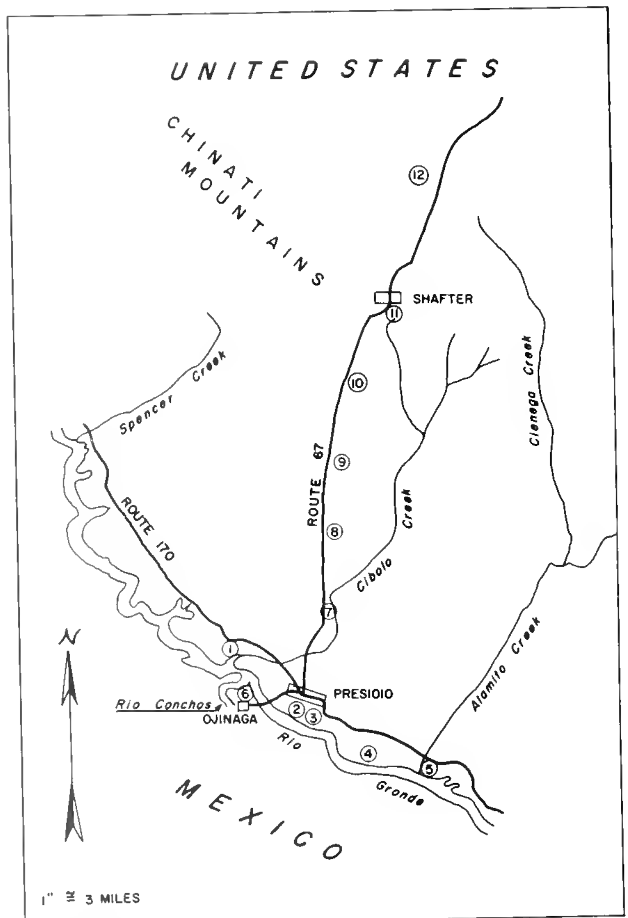


FIGURE 1. *Diagram of sampling area at Presidio, Texas. Sites 1, 2, 3, and 4 are in cotton fields; sites 5 and 6 are water sampling areas; sites 7, 8, 9, and 10 are in the desert and aerodynamically related to the Presidio region; site 11 is in the Chinati Mountains and site 12 north of the Chinati Mountains.*

[1] Mississippi State University, State College, Mississippi.
[2] Texas A & M University, College Station, Texas.

3, 6, and 9 miles from the center of the growing region. Site 12 (30 miles from the growing area) was located on the north site of the Chinati Mountains and not normally related aerodynamically or hydrographically to the Presidio Valley; this was used as the control area.

Representative specimens of reptiles, birds, and mammals were obtained by shooting or trapping. Birds and lizards were shot and placed on ice within an hour of death. Mammals were trapped and killed by freezing. All specimens were kept frozen until tissues were taken for analysis. A minimum of four specimens per site of each kind were collected on each date. Most of the values presented represent mean insecticide concentrations of five to six specimens.

Samples were analyzed by the method of Langlois, Stemp, and Liska *(9)*. Briefly, 1 g of frozen tissue was ground with 20 g of Florisil. The sample-Florisil mixture was placed on top of 25 g of partially deactivated Florisil in a chromatographic column. Partial deactivation of the Florisil was done by heating it to 90 C for 48 hours, adding 5% water (v/w) and then stoppering tightly for 48 hours. Insecticides were eluted from the column with 1 liter of a mixture of 20% methylene chloride in petroleum ether (v/v). The eluate was evaporated to dryness, the residues taken up in 5 ml of hexane and 5 μl injected into a gas chromatograph. The gas chromatograph was an Aerograph 680 with an electron capture detector. The column was 5 ft. x 1/8 in. Pyrex and packed with 5% Dow-11 on Chromasorb W. The column temperature was 180 C, nitrogen pressure was 20 psi, and the gas flow rate was 50 ml/min. Thin layer chromatography was used to confirm the identification of the insecticides.

All solvents used for extraction and chromatography were purified by the methods of Burke and Giuffrida *(4)*.

Periodically, samples were spiked with all the insecticides and their breakdown products for which data are reported in this paper. The spiked samples were then carried through the proper analytical procedure and percent recovery of the compounds calculated. Recoveries ran from 83% to 96%.

In 1965, the first application of insecticides in the Presidio Valley was to onions in March. This application consisted of a fungicide (usually sulfur) and DDT. Cantaloupes were sprayed with a fungicide and DDT starting in May. Only small acreages were devoted to these crops. The vast bulk of insecticides was applied on cotton from late June to the middle of September. During 1965, the following amounts of insecticides (calculated as pounds of the pure chemical) were applied by the commercial growers: DDT - 20,750 lb.; methyl parathion - 15,900 lb.; Sevin® - 2,600 lb.; BHC - 2,585 lb.; ethyl parathion - 2,000 lb.; endrin - 200 lb. In addition, seven sprays (three in late September and four in October) of low volume, high concentration malathion were applied under a Federal program. A total of 17,640 lb. of malathion was applied in the seven sprays to the Presidio Valley.

Results

No differences could be detected in insecticide concentrations among three species of lizards — *Cnemidophorus Tessellatus* Say, *C. tigris* Baird and Girard, and *C. inornatus* Baird. The data presented here are a composite for all species. The data on insecticide concentrations in lizard tail muscle (Table 1), brain tissue (Table 2), and liver tissue (Table 3), in general, show that an increase in insecticide concentrations occurred during June, July, and August. There appeared to be little difference in concentrations between samples from the various sites in the cotton fields or from the desert peripheral to the cotton fields. Concentrations decreased up to 9 miles from the cotton fields. Thereafter they remained essentially static.

Insecticide concentrations in post-coelomic fat bodies of lizards are given in Table 4. Since many of the samples contained no fat bodies, caution must be used in drawing conclusions. There appeared to be a sharp rise in concentrations from June through July followed by a drop during August. In general, the concentrations decreased up to 9 miles from the cotton fields. Thereafter they remained essentially static.

The analysis of the stomach contents of the lizards (Table 5) also showed that as specimens were gathered at greater distances from the cotton fields, the insecticide concentrations decreased. With the exception of DDE, there were only slight increases in concentrations from June through August. DDE showed pronounced increases at all sites from July to August.

In the latter part of July many of the female lizards that were collected contained eggs. Separate determinations were made of the gravid female muscle tissue and of the egg. In every case, the egg contained higher insecticide concentrations than did the gravid female muscle tissue (Table 6). Then, concentrations in the muscles of gravid females were compared with concentrations in the muscles of non-gravid females collected on the same dates and at the same sites. No significant differences could be detected.

The chemical names of compounds mentioned in this paper are:

BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
Methyl Parathion	*O,O*-dimethyl *O-p*-nitrophenyl phosphorothioate
Parathion	*O,O*-diethyl *O-p*-nitrophenyl phosphorothioate
Malathion	diethyl mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate
Sevin®	1-naphthyl methylcarbamate

TABLE 1.—*Mean insecticide concentrations (ppm) in lizard tail muscle; each sample is five to six tails*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	2.0	0.5	0.8	2.9	0.3	0.6	0.9	1.2	1.8	3.9	0.9	0.8	0.9	0.3	0.8	3.1	0.0	0.4
July	0.1	0.6	1.0	1.5	1.7	0.5	0.0	1.1	0.7	2.0	1.9	2.0	0.0	0.7	1.8	1.9	1.9	2.2
August	0.0	1.1	1.7	2.5	2.5	0.5	0.0	1.5	2.8	2.8	4.7	0.0	0.0	1.1	1.5	2.7	1.2	0.8

	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.4	0.9	0.5	4.1	1.8	0.4	0.6	1.7	0.2	3.7	1.3	0.7	0.7	1.7	1.2	2.2	0.9	0.1
July	1.2	3.7	2.9	2.6	1.5	2.1	1.6	3.7	4.1	3.4	2.2	2.5	1.2	1.8	2.5	3.0	1.4	1.4
August	0.6	4.9	4.6	7.2	6.0	3.4	1.3	1.6	3.8	3.0	3.1	2.5	0.2	4.4	4.4	6.9	5.6	4.7

	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.2	0.6	0.3	0.9	0.4	0.6	0.3	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.2	0.2
July	0.6	1.2	1.0	1.9	1.1	1.4	0.2	0.6	0.4	0.3	0.2	0.4	0.0	0.1	0.1	0.3	0.1	0.1
August	0.5	1.5	2.4	2.5	1.4	1.7	0.4	0.6	1.1	1.1	1.0	0.4	0.2	0.7	0.8	0.6	0.4	0.3

TABLE 2.—*Mean insecticide concentrations (ppm) in lizard brain tissue; each sample is five to six brains*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.1	0.7	1.4	1.9	0.6	0.4	0.0	0.4	0.4	1.1	0.9	0.0	0.3	0.6	0.0	1.0	0.0	1.6
July	0.1	0.6	1.0	1.5	1.7	0.5	0 0	1.1	0.7	2.0	1.9	2.0	0.0	0.7	1.8	1.9	1.9	2.2
August	0.0	1.1	1.7	2.5	2.5	0.5	0.0	1.5	2.8	2.8	4.7	0.0	0.0	1.1	1.5	2.7	1.2	0.8

	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	0.5	0.3	1.6	0.4	0.2	0.4	0.2	0.5	0.7	0.6	0.1	0.3	0.7	0.0	1.8	0.4	0.2
July	0.0	0.6	1.3	1.8	0.7	0.9	0.0	0.8	1.8	1.5	1.4	0.9	0.2	0.6	0.3	3.1	1.2	1.3
August	0.0	1.5	1.9	2.4	0.0	2.7	0.0	1.1	1.8	2.5	1.0	1.1	0.0	1.1	1.5	3.8	1.4	0.0

	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	0.2	0.4	0.3	0.6	0.1	0.0	0.0	0.0	1.5	0.5	1.1	0.0	0.1	0.3	0.3	0.1	0.2
July	0.0	0.7	0.6	0.8	1.2	1.2	0.0	1.2	1.1	0.6	1.2	1.6	0.0	0.1	0.9	0.6	0.4	0.1
August	0.0	1.0	0.7	1.6	1.4	0.8	0.0	0.4	1.8	0.7	1.5	0.6	0.0	0.1	0.9	0.8	0.0	0.0

TABLE 3.—*Mean insecticide concentrations (ppm) in lizard liver; each sample is five to six livers*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	1.1	0.6	1.0	1.2	0.3	0.0	1.0	1.3	2.1	1.3	1.0	0.0	0.2	0.1	2.5	0.1	0.2
July	1.0	1.0	0.8	2.1	1.6	0.6	1.1	0.4	0.8	3.3	1.6	0.5	0.3	0.4	0.4	1.5	0.6	0.6
August	0.0	1.5	1.2	3.7	2.0	1.3	0.1	1.0	1.1	3.4	0.9	0.6	0 0	0.9	0.6	3.8	2.1	0.7

TABLE 3.—*Mean insecticide concentrations (ppm) in lizard liver; each sample is five to six livers*—Continued

	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	1.1	1.3	0.6	3.1	0.4	0.0	0.1	0.5	0.2	0.5	0.2	0.0	0.1	0.4	0.7	1.8	0.4	0.3
July	0.3	0.9	0.9	2.4	0.9	0.8	0.1	0.1	0.3	1.7	0.3	0.2	0.0	0.1	0.2	0.5	0.1	0.1
August	0.0	0.8	1.7	2.7	0.7	1.1	0.0	0.5	0.3	1.6	1.0	0.0	0.0	1.5	0.6	1.7	0.0	1.0
	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	0.4	0.3	0.1	0.4	0.0	0.3	0.3	0.1	0.4	0.0	0.0	0.1	0.1	0.1	0.2	0.6	0.5
July	0.5	0.4	0.5	1.0	0.6	0.8	0.0	0.4	0.6	0.2	0.4	0.6	0.3	0.6	0.5	0.3	0.4	0.7
August	0.0	0.8	0.7	1.4	0.2	0.5	0.8	0.6	1.5	1.3	1.3	0.0	0.0	0.5	0.1	0.5	0.1	0.1

TABLE 4.—*Mean insecticide concentrations (ppm) in coelom fat of lizards; each sample is five to six coelom fat bodies*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June			No Data				1.5	0.0	0.3	31.5	19.2	14.5			No Data			
July	11.5	4.2	2.8	17.4	34.3	43.0	8.9	0.0	1.4	45.9	32.0	44.3			No Data			
August	8.5	2.1	1.0	21.2	20.8	25.1	1.2	0.0	0.0	18.8	7.6	7.6	0.4	0.3	0.6	5.8	3.4	4.0
	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June			No Data				0.7	0.0	0.0	25.2	0.0	2.8	0.2	0.0	0.3	19.4	2.1	1.5
July	6.8	3.7	4.1	31.4	7.9	26.1	2.1	1.6	3.6	35.4	10.3	8.4			No Data			
August	7.0	0.0	0.0	22.4	10.4	16.2	2.3	0.0	0.0	28.2	7.3	4.0	0.1	0.1	0.1	17.7	0.7	1.9
	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	1.9	1.1	8.4	3.1	4.8	0.0	1.0	0.0	7.9	3.7	3.6			No Data			
July	1.0	1.8	2.3	10.4	4.0	4.3	1.1	2.0	1.3	10.1	6.9	3.6	1.2	0.6	0.0	5.4	2.2	1.7
August	1.4	2.4	1.8	15.7	5.1	4.0			No Data				0.0	0.0	0.0	9.4	1.5	0.0

TABLE 5.—*Mean insecticide concentrations (ppm) of the stomach contents of lizards; each sample is five to six stomach contents*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	1.1	0.7	0.9	0.6	0.0	0.1	0.9	0.8	1.2	0.7	0.2	0.1	0.4	0.2	1.3	0.2	0.2
July	0.6	0.8	1.5	2.0	2.7	4.2	0.6	0.6	1.1	2.7	1.8	1.4	0.0	0.5	0.9	1.7	0.6	0.3
August	1.5	1.4	1.3	7.7	1.4	1.3	1.1	1.7	1.3	4.7	1.5	1.6	0.2	1.9	1.7	3.4	0.9	0.7
	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
[illegible]	0.0	0.6	0.6	0.4	0.4	0.3	0.0	0.6	0.4	0.6	0.4	0.0	0.1	0.9	0.2	0.4	0.1	0.1
[illegible]	0.0	0.3	0.4	0.2	0.4	0.4	0.0	0.2	0.1	0.3	0.3	0.3	0.0	0.3	0.4	0.5	0.3	0.2
[illegible]	[illegible]	[illegible]	1.3	4.5	0.2	0.0	0.3	1.0	0.3	3.2	0.2	0.5	0.3	1.7	0.5	1.9	0.8	0.4
	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	0.4	[illegible]	0.4	0.4	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.0	0.0
July	[illegible]	[illegible]	0.3	0.9	0.3	0.3	0.0	0.5	0.1	0.4	0.3	0.2	0.0	0.0	0.2	0.2	0.1	0.0
August	[illegible]	[illegible]	0.4	1.5	0.3	0.4	0.0	0.5	0.4	1.0	0.2	0.2	0.0	0.1	0.2	0.5	0.1	0.1

TABLE 6.—*Mean Insecticide concentrations (ppm) in muscle of four gravid and four non-gravid female lizard species and eggs; all lizards were collected in the same field*

	BHC	MP	P	DDE	TDE	DDT
Females, gravid	1.1	2.5	1.4	3.4	2.8	2.1
Eggs	5.6	11.6	8.5	16.4	7.3	10.7
Females, non-gravid	0.8	2.3	2.1	3.4	2.8	2.0

Insecticide concentrations found in the breast muscle (Table 7), brains (Table 8), livers (Table 9), and gizzard contents (Table 10) of English sparrows (*Passer domesticus* L.) are given for specimens collected in the cotton fields. Not enough birds were collected from the desert to furnish reliable data. In general, insecticide concentrations increased from June through August at all three sites. Birds collected from the west end of the valley, however, had lower MP, P, DDT, DDE, and TDE concentrations in their breast muscles in August. Most samples contained less insecticides in November than they did in August. There appeared to be a slight rise in concentrations from November to January for DDT, DDE, and TDE. Two species of pocket mice (*Perognathus penicillatus* Woodhouse and *P. intermedius* Merriam) and one species of kangaroo rat (*Dipodomys merriami* Mearns) were used for analysis. Approximately 2% of all mammals collected were *D. merriami*, 8% were *P. intermedius*, and 90% were *P. penicillatus*.

None of the above mammals could be found in the cotton fields. They were collected in the desert less than 30 meters from the fields, however. In general, at all sites on the periphery of the cotton fields insecticide concentrations increased from June to July, remained essentially static in August, and dropped in November. In January an increase is apparent at all sites except site 4 (Table 11) and the 9- and 30-mile stations (Tables 11 and 12). Mammals collected from the desert at the western periphery of the growing area had greater insecticide concentrations than did those collected from the desert peripheral to the center of the growing area. Animals from the desert adjacent to the easternmost cotton fields had the least concentrations of insecticides. The farther the specimens were collected from the cotton fields the less were the insecticide concentrations.

Discussion

No malathion was detected in any sample obtained during the period of study reported in this paper. Since some of the samples (those reported for November) were obtained within 6 weeks after malathion applications, this indicates that the compound rapidly disappeared from the Presidio ecosystem.

Only a small amount of endrin was applied during the 1965 spraying season. It was detected in human urine and cotton leaves, but not in leatherstem leaves, Rio Grande water, or silt in the Rio Grande water (1,5,8). Endrin was not detected in any of the reptiles, birds, or mammals.

An attempt was made to obtain samples from each class of vertebrates, except fish. Amphibians were not found in sufficient numbers to sample during the 3-month period. From birds, the English sparrow was selected due to both its numbers and year-round residence in the valley *(13)*. The nearest areas in which sparrows established residence were at Shafter and Redford—19 miles north and east of the valley, respectively. It is unlikely there is interchange between these three areas. *P. penicillatus*, *P. intermedius*, and *D. merriami* were selected to represent the mammals due to their small home range—0.12 acres to 0.46 acres *(12,14)*. Reptiles were originally supposed to be represented by *C. tigris*, which has an estimated home range of 0.26 acres *(12)*. However, there were areas where this species was not abundant. Therefore, *C. tesselatus* and *C. inornatus*, which were abundant in such areas, were also sampled. Milstead *(11)* has reported that all three species have similar food habits and apparently occupy similar ecological niches.

TABLE 7.—*Mean insecticide concentrations (ppm) in breast muscle of sparrows; each sample is five to six breasts*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.0	2.0	1.7	5.2	4.1	3.4	0.0	3.2	2.0	4.4	2.7	1.9	0.3	2.7	3.9	1.6	2.7	3.1
July	1.7	4.4	6.5	7.0	8.1	5.0	0.0	3.5	2.7	8.0	6.0	5.0	0.6	3.6	3.2	4.5	2.6	4.6
August	1.9	2.1	1.5	6.8	4.6	3.2	1.9	4.9	5.3	16.2	10.2	7.1	0.9	5.5	3.4	11.8	7.8	6.6
Nov.	0.0	2.3	0.5	1.8	0.5	0.7	0.0	1.5	1.6	2.7	0.4	0.6	0.0	1.6	1.1	6.4	0.2	0.5
Jan.	0.5	4.3	2.5	3.9	1.5	0.9	0.3	2.9	2.0	5.7	0.4	1.2	0.0	1.9	1.0	7.2	1.2	1.0

TABLE 8.—*Mean insecticide concentrations (ppm) in brains of sparrows; each sample is five to six brains*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.5	0.0	0.2	5.4	0.2	0.3	0.5	1.0	0.8	3.9	0.3	0.0	0.0	0.1	0.0	1.6	0.1	0.0
July	0.0	0.8	0.8	1.7	0.2	0.0	0.0	1.5	1.1	1.2	1.2	1.0	0.6	0.3	0.8	1.2	0.2	0.5
August	2.0	0.9	0.7	6.3	0.0	0.5	1.7	1.3	0.9	5.0	0.5	0.5	0.2	0.7	0.9	1.8	0.8	0.5
Nov.	0.1	0.8	0.6	2.5	0.3	0.3	0.1	0.6	0.8	1.2	0.5	0.4	0.0	0.4	0.6	0.8	0.9	1.0
Jan.	0.2	1.5	1.1	4.9	1.2	1.1	0.0	2.4	1.2	5.3	0.9	0.2	0.3	2.6	1.8	5.9	0.4	0.4

TABLE 9.—*Mean insecticide concentrations (ppm) in livers of sparrows; each sample is five to six livers*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.3	0.2	0.9	5.8	1.9	0.1	0.0	0.4	0.3	3.5	0.2	0.3	0.3	0.9	0.7	2.7	0.3	0.4
July	1.9	1.2	0.8	6.7	3.3	0.9	0.0	2.8	1.1	5.5	4.6	2.1	0.4	0.4	0.3	3.5	0.6	0.4
August	1.2	1.4	1.7	9.6	1.6	0.4	0.9	1.6	1.3	6.5	0.7	0.0	0.5	0.8	1.2	2.9	0.0	0.0
Nov.	0.0	1.0	0.5	4.6	1.1	0.8	0.4	1.0	1.7	3.5	0.9	0.4	0.0	0.5	0.3	1.4	0.3	0.3
Jan.	0.0	1.6	1.9	7.9	1.5	0.9	0.0	2.8	1.9	3.8	0.1	0.1	0.0	1.6	0.8	3.0	0.1	0.2

TABLE 10.—*Mean insecticide concentrations (ppm) in gizzards of sparrows; each sample is five to six gizzards*

	Site 1 West Cotton Field						Site 2 Center Cotton Field						Site 4 East Cotton Field					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.5	0.9	0.6	2.1	0.0	0.4	0.0	0.3	0.3	2.3	0.6	0.5	0.0	0.4	0.2	2.6	0.2	0.2
July	0.1	1.2	1.1	3.2	0.0	0.3	0.2	3.2	0.8	5.4	1.5	0.8	0.1	0.7	1.3	3.6	0.3	0.3
August	1.1	1.7	1.8	8.6	1.0	0.0	1.9	0.8	2.5	6.5	0.0	0.3	0.0	1.6	1.3	4.1	0.0	0.3
Nov.	0.7	0.5	0.8	5.1	0.8	0.3	0.0	0.9	0.9	2.3	0.4	0.4	0.0	0.6	0.8	3.1	1.1	0.8
Jan.	0.4	0.7	1.2	5.8	0.5	0.9	0.0	0.9	0.5	2.7	0.3	0.3	0.2	0.9	0.2	1.3	0.5	0.8

TABLE 11.—*Mean insecticide concentrations (ppm) in leg muscles of pocket mice and kangaroo rats; each sample is five to six leg muscles*

	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.3	2.9	2.8	1.2	2.2	1.8	1.6	1.4	0.7	0.7	1.6	1.2	0.6	0.5	0.2	2.6	0.6	0.9
July	0.2	4.0	5.6	3.2	4.3	2.7	0.1	5.4	5.1	3.2	3.6	3.1	0.5	1.6	1.8	3.3	1.4	1.2
August	0.5	4.6	5.1	5.2	5.8	5.7	1.9	3.8	3.1	2.2	0.8	0.1			Lost			
Nov.	0.0	0.7	0.9	0.8	1.1	1.2	0.1	1.3	0.9	2.6	0.3	0.2	0.4	1.1	1.2	3.3	0.7	0.7
Jan.	0.0	1.3	1.1	5.1	0.6	0.9	0.1	2.5	1.9	4.3	0.7	0.3	0.0	1.2	0.5	2.0	0.9	1.0
	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	[illegible]	[illegible]	0.1	0.7	0.4	0.5	0.1	0.1	0.1	0.3	0.2	0.4	0.1	0.0	0.0	0.1	0.1	0.0
July	[illegible]	[illegible]	[illegible]	1.0	1.0	1.0	0.1	0.3	0.2	0.5	0.6	0.5	0.2	0.1	0.1	0.2	0.2	0.2
August	[illegible]	[illegible]	[illegible]	1.3	1.2	1.2	0.3	0.5	0.5	0.6	0.6	0.8	0.0	0.3	0.2	0.3	0.3	0.3
Nov.	[illegible]	[illegible]	[illegible]	0.3	0.2	0.3	0.2	0.6	0.8	0.4	0.1	0.5	0.0	0.9	0.4	0.2	0.3	0.4
Jan.	[illegible]	[illegible]	[illegible]	[illegible]	1.0	0.4	0.0	0.8	0.7	1.0	0.5	0.4	0.0	0.3	0.2	0.4	0.2	0.1

TABLE 12.—*Mean insecticide concentrations (ppm) in livers of pocket mice and kangaroo rats; each sample is five to six livers*

	Site 1 West Desert Periphery						Site 2 Center Desert Periphery						Site 4 East Desert Periphery					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.1	0.9	0.8	2.6	0.9	1.7	0.2	0.6	0.7	2.1	0.7	0.7	0.0	0.4	0.6	1.9	0.4	0.8
July	0.1	1.4	1.2	0.8	1.0	0.8	0.1	0.7	0.5	2.6	0.4	0.8	0.0	1.1	1.2	2.9	0.8	0.7
August	0.3	1.2	1.5	4.2	0.3	0.9	0.0	1.1	1.2	3.7	0.6	0.9	Lost					
Nov.	0.0	0.4	0.7	1.2	0.4	0.4	0.0	0.8	0.9	1.2	0.3	0.3	0.0	1.1	0.8	2.4	0.7	0.5
Jan.	0.0	1.1	0.6	2.9	0.2	0.9	0.3	1.7	1.9	2.8	2.0	1.2	0.8	1.9	1.8	2.9	3.4	1.5
	Site 7 3 Mile Desert						Site 9 9 Mile Desert						Site 12 30 Mile Prairie					
	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT	BHC	MP	P	DDE	TDE	DDT
June	0.2	0.6	0.7	0.8	0.5	0.1	0.0	0.2	0.4	0.3	0.7	0.3	0.0	0.2	0.2	0.3	0.2	0.0
July	0.3	0.8	0.6	1.3	0.5	0.4	0.0	0.7	0.4	0.6	0.1	0.5	0.0	0.3	0.4	0.2	0.4	0.2
August	0.3	0.9	0.7	1.8	0.3	0.7	0.0	1.0	0.5	1.3	0.4	0.5	0.0	0.7	0.2	0.3	0.5	0.1
Nov.	0.1	0.7	0.4	0.9	0.5	0.3	0.5	0.9	0.4	0.8	0.5	0.4	0.0	0.1	0.3	0.3	0.2	0.2
Jan.	0.0	0.8	0.6	1.3	0.6	0.5	0.1	0.1	0.4	0.6	0.9	0.0	0.0	0.1	0.3	0.6	0.4	0.2

It should be noted that 15,900 lb. of methyl parathion and 2,000 lb. of ethyl parathion were sprayed in the Presidio Valley in 1965. This is an 8:1 ratio. However, an inspection of our data shows that this ratio of methyl parathion to ethyl parathion was not found in either the biological material or in the soil and water. The reason for the unbalance between the ratios applied and the ratios detected is not apparent. In tail muscle, brain, and liver tissues of the lizards, BHC concentrations decreased from June through August. During this same period, MP, P, DDT, DDE, and TDE increased in concentration in these tissues. With the exception of DDE (insecticide concentrations in the stomach contents increased only slightly in the same period. DDE residues in the stomach contents showed pronounced increases during this period. The stomach contents of lizards from the cotton fields consistently had higher insecticide concentrations than did the stomach contents of lizards collected adjacent to the fields. An examination of the stomach contents showed that lizards in the cotton fields ate mainly grasshoppers, while those in the desert ate mainly termites. The grasshoppers were more directly exposed to insecticide applications than the termites. In addition, the grasshoppers' diet of fresh foliage as contrasted to the termites' diet of organic debris could also account for the differences in stomach concentrations between the two lizard populations.

Insecticides in the coelomic fat increased sharply from June to about the first of August in lizards from the cotton fields. Thereafter, they dropped greatly in concentration until, at the end of August, the concentrations were, in many cases, less than at the end of June. However, the coelomic fat even in August contained greater concentrations of chlorinated insecticides than did the muscle, liver, brain, and stomach contents. The storage of chlorinated insecticides in the fat of mammals has been well documented. It was not surprising to find that reptiles also store these compounds in fat.

Whiptails in the Presidio area hibernate from September to May *(Milstead, personal communication and our observations)*. The adults of any given year were born the previous year. Breeding appears to take place during the entire summer *(11)*. In contrast to Milstead, who found mature eggs complete with shell in early June, we did not find any eggs until the latter part of July. These eggs were small and immature. Milstead *(personal communication)* believes that a post-coelomic fat body is used in egg development. Hahn and Tinkle *(6)* reported that post-coelomic fat may serve a reproductive function with *Uta stansburiana*, a species living in a habitat similar to *C. tesselatus and tigris*. Hoddenbach *(7)* confirmed this in female race runners *(C. sexlineatus)* in western Texas. It appears likely that the post-coelomic fat body in *Cnemidophorus* spp. has a similar reproduction function. This being the case, insecticide concentrations in coelomic fat would be transported directly to the developing egg. The peak concentrations in the coelomic fat occurred in late July—concomitant with the appearance of eggs.

Tinkle *(15)* and Maslin *(10)* have reported on the prevalence of females in *C. tesselatus*. We found two immature males in 3 months' collecting in 1965 and one mature male in 2 weeks' collecting in 1966. All three specimens were collected adjacent to the cotton fields. Only one male, *C. tesselatus*, has been reported previously *(10)*.

There is a much closer correlation between insecticide concentrations found in the food taken from the gizzard of sparrows and their tissue concentrations than between the insecticide concentrations in the stomach contents of lizards and their tissue concentrations. As the sparrow tissue varied in insecticide concentration from date to date and from site to site, the gizzard content concentrations varied in a similar manner. The variations in insecticide concentrations in all tissues and food from site to site are difficult to assess due to lack of information concerning movement, behavior, feeding habits, and food quality of the sparrows and lizards.

Concentrations in samples fell in November (after all spraying had stopped) as would be expected. The slight rise noted in January may or may not be significant, but appeared in mammals as well as birds. The use of fat reserves might cause such a rise. Further work is needed to clarify this point.

Due to the use of bait, no concentrations in stomach contents could be obtained for mammals. In general, the variations for the mammals followed the variations observed in the birds rather than those of lizards. Variations of insecticide concentrations from site to site were more closely correlated with changes in soil and vegetations *(2,8)* than were changes in the birds and lizards.

There were cases where methyl and ethyl parathion residues in June exceeded those in July in reptiles, birds, and mammals. Applegate *(2)* reported that leaves of leatherstem (a perennial) had higher methyl and ethyl parathion concentrations in June than did leaves of cotton (an annual). This was interpreted as an indication that these insecticides could accumulate in leatherstem. It would appear, in the Presidio area, that reptiles, birds, and mammals can also accumulate methyl and ethyl parathion from one spraying season to the next spraying season.

It is apparent that very complex interactions exist in the movement of insecticides through the air to soil, plants, water, insects and, ultimately, into reptiles, birds, and mammals. More information is needed about the concentrations of insecticides present at various levels [illegible] chains. The entrance of insecticides into organisms [illegible] dicted by the organisms' shelters, movements, and behavior is not presently available.

Technical Article No. 5762, Texas Agricultural Experiment Station, College Station, Texas. This research was supported in part by Grant AP 28-02 from the Division of Air Pollution, Bureau of State Services, Public Health Service.

Acknowledgments

We wish to thank Dr. W. B. Davis, Department of Wildlife Science, Texas A & M University, for aid in identification of the mammals and Dr. T. Paul Maslin, Curator of Herpetology, University of Colorado Museum, Boulder, for aid in identification of the lizards. We appreciate the critical reading of this paper by Dr. Denzel E. Ferguson, Department of Zoology, Mississippi State University and Dr. Jack Inglis, Department of Wildlife Sciences, Texas A & M University.

LITERATURE CITED

(1) Applegate, H. G. 1966. Pesticides at Presidio I. General survey. Tex. J. Sci. 18:171-178.

(2) Applegate, H. G. 1966. Pesticides at Presidio II. Vegetation. Tex. J. Sci. (Accepted for publication).

(3) Applegate, H. G. 1967. Pesticides at Presidio V. Summary. Tex. J. Sci. (Accepted for publication).

(4) Burke, J., and L. Giuffrida. 1964. Investigations of electron capture gas chromatography for the analysis of multiple chlorinated pesticide residues in vegetables. J. Ass. Agr. Chem. 47:326-342.

(5) Culley, D. D., and H. G. Applegate. 1967. Pesticides at Presidio IV. Reptiles, birds, and mammals. Tex. J. Sci. (Accepted for publication).

(6) Hahn, W. E., and D. W. Tinkle. 1965. Fat body cycling and experimental evidence for its adaptive significance to ovarium follicle development in the lizard *Uta stansburiana*. J. Exp. Zool. 158:79-86.

(7) Hoddenbach, G. A. 1966. Reproduction in Western Texas *Cnemidophorus sexlineatus* (Sauria: Teiidae). Copeia 1:110-113.

(8) Lahser, C., and H. G. Applegate. 1966. Pesticides at Presidio III. Soil and water. Tex. J. Sci. (Accepted for publication).

(9) Langlois, B. E., A. R. Stemp, and B. J. Liska. 1964. Analysis of animal food products for chlorinated insecticide residues I. Column clean-up of samples for electron capture gas chromatographic analysis. J. Milk and Food Tech. 27:202-204.

(10) Maslin, T. P. 1962. All-female species of the lizard genus Cnemidophorus, Teiidae. Science 135:212-213.

(11) Milstead, W. W. 1957. Some aspects of composition in natural populations of whiptail lizards (genus Cnemidophorus). Tex. J. Sci. 9:410-447.

(12) Milstead, W. W. 1961. Observations of the activities of small animals (Reptillia and Mammalia) on a quadrat in southwest Texas. Amer. Midland Natur. 65:127-138.

(13) Peterson, R. T. 1963. A field guide to the birds of Texas. Houghton Mifflin Co. Boston. 304 p.

(14) Reynolds, H. G. 1960. Life history notes on Merriam's Kangaroo rat in southern Arizona. J. Mammalogy. 41:48-58.

(15) Tinkle, D. W. 1959. Observations on the lizards *Cnemidophorus tigris*, *Cnemidophorus tesselatus* and *Crotaphytus wislizeni*. Southwestern Natur. 4:195-200.

An Evaluation of the Effects of the Aedes aegypti Eradication Program on Wildlife in South Florida[1]

Philip N. Lehner[2], Thomas O. Boswell[3], and Frank Copeland[4]

Introduction

THE objective of this study was to evaluate the effects of the Aedes aegypti Eradication Program in South Florida on Wildlife other than *Aedes aegypti*, the target organism. The field investigations and collection of specimens for this evaluation were carried out during the period May 10 to August 27, 1965.

The authors were furnished a selected group of written complaints from among those received by the Aedes aegypti Eradication Program in Atlanta, Georgia. These complaints were then categorized by time and type. Newspaper and magazine articles were reviewed to determine the extent and type of problems inciting public antagonism. Naturalists, civic leaders, and personnel conducting the *Aedes aegypti* eradication operations were interviewed personally or by telephone.

Sick and dead birds of various species were collected from the treated areas, and healthy house sparrows and mockingbirds from both treated and untreated zones. Bird eggs also were collected. Specimens were shipped at intervals to the National Communicable Disease Center, Toxicology Laboratory, Atlanta, Georgia, to be analyzed for chlorinated hydrocarbon insecticides. Pathological analyses were conducted on some specimens by the Animal Diseases Diagnostic Laboratory, Miami Section, Florida Department of Agriculture. In addition, bioassay tests were made by the Diagnostic Laboratories Section, Division of Animal Industry, Florida Department of Agriculture, Kissimmee, Florida.

Field Investigation

OBSERVATIONS OF SPRAY OPERATIONS

The Dade County spray operations of the Aedes aegypti Eradication Program are conducted on a zone basis. The zones generally are those delineated for city census purposes. There is a great variance in the size of zones; they usually contain 50 to 100 blocks and hundreds of premises. The premises likewise vary widely in size, ranging from individual home sites to large parcels of vacant land, some as large as 20 acres.

Before spraying is begun, the zones are checked by inspectors to determine the number of premises in the zone that contain *Aedes aegypti* larvae. From these data are derived two indices: (1) block index—the percent of positive blocks in the zone—and (2) premises index—the percent of positive premises in the zone.

Using these indices, a decision is made as to how the zone will be sprayed. Following are definitions of the three degrees of application employed.

---

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, Bureau of Disease Prevention and Environmental Control, National Communicable Disease Center, Aedes aegypti Eradication Program, Atlanta, Georgia 30333.

[2] Presently graduate research assistant with the Utah Cooperative Wildlife Research Unit, Utah State University, Logan, Utah 84321.

[3] Presently graduate research assistant with the Department of Biology, New Mexico State University, University Park, New Mexico 88070.

[4] Formerly Analytical Chemist, Toxicology Section, Technology Branch, Communicable Disease Center, Atlanta, Georgia; present address, NCDC Pesticide Research Laboratory, P. O. Box 490, Perrine, Florida 33157.

1. *Comprehensive Spraying*. The treatment of all breeding and potential breeding containers and the area immediately around these containers on all premises of all blocks in a given area. In areas where excessive vegetational growth precludes positive detection of hidden containers, spray applications will be applied.

2. *Encompassment Spraying*.—The treatment of all breeding and potential breeding containers and the area immediately around these containers on all premises in infested blocks and in the blocks immediately adjacent to infested blocks.

3. *Spot Treatments*.—This type of treatment refers to a special situation, for example, application of insecticides to bromeliads or to areas around fish ponds where routine spraying might be impractical.

Besides the various degrees of application employed, there are three basic methods of application: spraying by truck-mounted power sprayers, spraying by hand compression sprayers, and dispensing of dust by hand equipment. The decision as to method of application in specific situations must often be made by spraymen, using general guidelines provided by the area supervisors.

The spray formula used during this study was a xylene-water emulsion containing 1.25% DDT by weight. Spray used during 1964 and early 1965 was 2.50% DDT. The 1.25% spray contains approximately 0.1 lb. of DDT/gallon of spray.

A total of 64 hours was spent observing spray operations. The following is a list of the spray applications that were inconsistent with operational standards and represent a hazard to wildlife:

No. of Observations	*Misuse of Spray*
9	Spraying areas obviously clean of containers
5	Blanket spraying
3	Spraying pond or waterway
3	Carelessness with equipment resulting in excess spray deposits

[illegible] there was not only great inconsistency in [illegible] tion between and within spray crews, but [illegible] complete lack of knowledge as to prescribed [illegible] procedure. This can be partly accounted for by the numerous individual circumstances that arise in the field; however, there was also a great difference observed in the treatment of similar objects, such as birdbaths, animal dishes, shrubbery, and ornamental plants.

COLLECTION OF SPECIMENS

Seventy-four specimens—55 birds and 19 bird eggs—were collected by the authors in the Miami area. Of the 55 bird specimens, 41 were taken in healthy condition, 7 were found sick and later died, and 7 were dead when found. Four additional bird specimens were received from a member of the Florida Audubon Society in West Palm Beach. Of these one was found dead following spraying, and the other three were found sick and ultimately died. Unfortunately, all of the eggs collected were in such poor condition upon arrival at the Atlanta laboratory that analysis for insecticide content was not practical.

Laboratory Analysis of Specimens

All of the bird specimens were analyzed for brain levels of chlorinated hydrocarbons. Also, pathological analyses were made on 11 of the 14 specimens found sick or dead. Tables 1, 2, and 3 present data for the 59 specimens analyzed. For the purposes of this study, only the levels of DDT and its metabolites have been listed. Previous work at the Patuxent Wildlife Research Center suggests that the brain level of DDE is not a good indication of lethality *(1)*.

In the laboratory, the bird brain samples were ground with sodium sulfate and then extracted with 25 ml of nano-grade *n*-hexane for 1 hour with the aid of a wrist action shaking machine. After extraction, the samples were filtered through a small plug of glass wool into 50-ml test tubes. The solvent was then evaporated down to 4 ml in a 40 C water bath with the aid of a gentle stream of clean dry air. As a clean-up procedure the 4-ml hexane extract was partitioned three times with 4 ml of nano-grade acetonitrile which had been equilibrated with hexane (1:1). The acetonitrile phases were then combined and evaporated down to 1 ml as described above. Two ml of distilled water were added, and the acetonitrile-water phase was extracted three times with 2-ml portions of nano-grade hexane. The hexane extracts were dried with sodium sulfate and then combined in a 15-ml centrifuge and evaporated down to 0.2 ml and appropriate aliquots subjected to gas chromatography.

A Microtek 2503R gas chromatograph equipped with a microcoulometric detector and a strip chart recorder was used. In addition to the microcoulometric detector which is specified for chlorine, two columns were also used to confirm the identity of the materials in the effluent gas. Both columns consisted of an aluminum tube 6 ft. x 1/4 in. O.D. Column No. 1 was packed with 2.5% diethylene glycol succinate (D.E.G.S.) on 60/80 mesh, acid-washed chromosorb G. Column No. 2 was packed with 3% QF-1 on 60/80 mesh acid-washed chromosorb G. Both columns were operated under the following conditions: inlet and outlet blocks 230 C; column oven 170 C; transfer line 230 C; combustion furnace 800 C; carrier gas N_2 60 cc/min; oxygen 100 cc/min; bias 250 mv. The retention times in minutes of columns 1 and 2, respectively, were as follows: *p,p'*-DDT, 24.0 and 33.8; *o,p'*-DDT, 12.8 and 22.4; *p,p'*-DDE, 9.2 and 17.6; *o,p'*-DDE, 7.2 and 13.4; *p,p'*-DDD, 30.5 and 31.2; alpha-BHC, 3.8 and 5.6; beta-BHC, 18.9 and 8.3; gamma-BHC, 5.9 and 7.0; delta-BHC, 17.1 and 9.2; heptachlor epoxide, 8.0 and 16.3; dieldrin, 11.9 and 25.4.

Results and Discussion

Tables 1 through 3 report "less than" values in order to give the reader an idea of the sensitivity of our method. The "less than" values show variation because the sample sizes and therefore the weight represented by any given aliquot varied. The "less than" values give more information to the reader than a simple "not detectable" notation. It was also felt that to show a "zero" would have been false. The authors believe that with larger samples or with more sensitive detectors, the number of positive readings could have been increased.

Since gas chromatography is a more sensitive technique than paper, thin layer, or infrared, and since we were not able to detect anything by gas chromatography in many samples, pesticides would not have been detected by these less sensitive methods. Therefore we resorted to the use of two columns of different polarity and the microcoulometric detector which is specific for halogens as reasonable proof of identity of the compounds in the effluent gas.

Tables 1 and 2 show the results of analyses of 33 nestling house sparrows, 18 from zones treated twice with DDT and 15 from untreated zones. House sparrows obtained from treated zones were collected at least a block inside the periphery of the zone. It is immediately apparent that there is no observable difference between the insecticide levels in brain tissue found in these two sets of samples. The insecticide levels for Specimen No. 29 were much higher than for the remainder of the population from the treated area, indicating that although this bird was probably not killed by DDT, it had received amounts far above what would be expected in that zone.

Treatment of Zone 9C for the fifth time was begun just previous to termination of the study and did not allow sufficient time for thorough investigation. Because Zone 9C was receiving treatments greatly in excess of other zones observed, five specimens were collected from it (four alive and one dead).

Specimen No. 68 was a young domestic turkey allowed to run loose in a yard in Zone 9C. It was taken inside the house while the premises were sprayed in the morning, but was released into the yard again that same afternoon and died by mid-afternoon. The owner's description of the turkey's death suggested loss of motor control and periodic muscle spasms indicative of neurotoxic poisoning. The brain level of DDT + DDD was 21.82 ppm. Although a few birds have been known to die of DDT poisoning with brain levels this low, the level does not approach the tentatively accepted minimal lethal brain level of 30 ppm *(1)*. Circumstantial evidence indicates DDT poisoning but is not fully supported by results of brain analysis in light of the available knowledge today.

Specimen No. 75 was an adult loggerhead shrike collected in Zone 9C to serve as an indicator of contamination of the food chain, since shrikes are almost exclusively carnivorous and insectivorous. The shrike gets most of its food from one trophic level higher than songbirds. The high level of DDE and very low level of DDT + DDD found in this specimen suggest a long-term buildup of DDE from the environment but little recent exposure to DDT. Present available knowledge does not permit an interpretation of the significance of this level of DDE; however, this shrike was apparently healthy when collected.

Three dead birds (a duck, a mockingbird, and a coot) were received by the Diagnostic Laboratories Section, Florida Department of Agriculture, from cities sprayed by the Program. Results of bioassay tests made on these birds were all negative. There was no evidence, however, that these birds were from actual sprayed areas within the cities.

Four specimens, Nos. 69-72, received from a resident of West Palm Beach (Resident No. 1) on August 12, 1965, were alleged to have been killed by heavy spray applications made by the Aedes aegypti Eradication Program in late 1964 and early 1965. Specimen No. 69 (myrtle warbler) was found dead by this individual at her residence 2 days after the surrounding premises had been sprayed. Brain analysis showed that death cannot be attributed to DDT poisoning.

Specimen Nos. 70-72 were collected by another resident of West Palm Beach (Resident No. 2), who froze each of them after death and sent them with an accompanying letter describing the deaths to a third resident of the city (Resident No. 3). The available information indicates that Resident No. 3 then sent the specimens to Resident No. 1, who kept them frozen until she turned them over to the authors on August 12.

Brain analysis of specimen No. 70 (crow) showed a sizeable quantity of DDE but only small amounts of DDT and DDD. This indicated either a heavy exposure to DDE through the food chain or a past heavy exposure to DDT and/or DDD and their metabolism to DDE and storage in the bird.

The brain level of DDT + DDE in specimen No. 71 (cardinal) was 27.31 ppm. Thus, it is probable that the

TABLE 1.—*Pesticide analyses of brain tissue (ppm) from nestling sparrow collections from zones treated twice in 1965 with 1.25% DDT*

Specimen No.	Condition When Collected	Date Collected	Date of Last Treatment [1]	Aggregate Avg. Gal Premises [2]	Pesticide levels in brain tissue (ppm)			
					DDT	DDD	*p,p'*-DDE	*o,p'*-DDE
2	Healthy	5/11	4/22	4.92	< .58	< .30	.40	< .30
6	Healthy	5/13	4/22	4.92	< 1.16	< .58	< .58	< .58
7	Healthy	5/14	4/22	4.92	.63	.25	1.07	< .24
8	Healthy	5/14	4/22	4.92	< .78	.39	.25	< .39
9 [3]	Healthy	5/14	4/22	4.92	< 1.08	.54	1.56	< .54
10 [4]	Healthy	5/14	4/22	4.92	< .88	< .44	2.67	< .44
26	Healthy	6/24	6/18	11.78	.44	< .22	.33	< .22
27	Healthy	6/24	6/18	11.78	< .50	< .25	.86	< .25
28	Dead	6/24	6/18	11.78	< 1.36	< .68	1.50	< .68
29	Dead	6/24	6/18	11.78	2.81	< .54	5.68	< .54
33 [5]	Healthy	7/6	6/6	6.06	.55	< .86	1.19	< .86
37	Healthy	7/14	6/6	6.06	< .88	< .44	.56	< .44
38	Healthy	7/14	6/6	6.06	.60	< .30	.12	< .30
39	Healthy	7/14	6/6	6.06	.31	< .53	.26	< .53
40	Healthy	7/14	7/28	1.46	< .53	< .26	.65	< .26
41	Healthy	7/14	7/28	1.46	.18	< .30	.57	< .30
46	Healthy	7/20	6/6	6.06	< .88	< .44	.21	< .44
47	Healthy	7/22	6/6	6.06	< .85	< .43	.66	.19

[1] Type of treatment: Comprehensive.
[2] The sum of the two averages, one for each treatment.
[3] Sample contains three nestlings.
[4] Sample contains five nestlings.
[5] Sample contains four nestlings.

TABLE 2.—*Pesticide analyses of brain tissue (ppm) from nestling house sparrow collections from untreated zones, 1965 (healthy)*

Specimen No.	Date Collected	Approx. distance (miles) to nearest treated zone	Pesticide levels in brain tissue (ppm)			
			DDT	DDD	*p,p'*-DDE	*o,p'*-DDE
19	6/15	13	.34	< .62	.39	< .62
20	6/15	13	< .82	< .41	.22	< .41
21	6/15	13	< 1.07	< .53	.66	< .53
22	6/16	7	< .36	< .18	.25	.10
23	6/16	7	< .38	< .19	.28	< .19
24	6/16	7	< .37	< .19	.16	< .19
[illegible]	7/22	4	< .50	< .25	.72	< .25
[illegible]	7/22	4	< .70	< .35	.81	< .35
[illegible]	7/22	4	< .62	< .31	.43	< .31
[illegible]	7/22	4	< .91	< .45	.70	< .45
[illegible]	7/23	10	< 2.04	< 1.02	.32	< 1.02
[illegible]	7/23	10	< .74	< .37	.18	< .37
[illegible]	[illegible]	10	.80	< .47	.33	< .47
[illegible]	[illegible]	10	.79	< .40	.25	< .40
[illegible]	[illegible]	10	< .57	< .28	.18	< .28

TABLE 3.—*Pesticide analyses of brain tissue (ppm) from miscellaneous specimens collected from treated zones*

Specimen No.[1,2]	Species	Age	Condition when collected	Date collected	No. of spray applications	Date of last treatment	Type of treatment and percent DDT: First	Second	Third	Fourth	Aggregate avg. gal./premises	Pesticide levels in brain tissue (ppm): DDT	DDD	p,p'-DDE	o,p'-DDE
69	Myrtle Warbler (*Dendroica coronata coronata*)	Ad.	dead	1964 Mid. Nov.	1	1964 11/10	comp. 2.50				6.80	< .42	.55	2.29	< .21
71	Cardinal (*Richmondena cardinalis*)	Ad.	sick	12/16	1	12/3	comp. 2.50				2.90	11.35	15.96	10.86	.42
72	Ground Dove (*Columbigallina passerina passerina*)	Ad.	sick	12/20	1	12/11	comp. 2.50				4.00	17.39	34.58	12.68	< .26
70	Crow (*Corvus brachyrhynchos*)	Juv.	sick	1965 4/19	1	1965	not known					< .21	1.11	15.97	.40
1	Red-bellied Woodpecker (*Centurus carolinus*)	Ad.	sick	5/6	3	5/17	comp. 2.50	spot 1.25	comp. 1.25		2.74	< .50	< .25	.17	< .25
4	Rock Dove (*Columba livia*)	Ad.	dead	5/11	1	5/21	comp. 1.25				1.00	< .33	< .16	< .16	< .16
30	Rock Dove	Nestl.	healthy	7/2	2	4/22	comp. 1.25	comp. 1.25			4.92	< .33	< .16	.22	< .16
5	Mockingbird (*Mimus polyglottos polyglottos*)	Juv.	dead	5/13	2	5/28	comp. 1.25	comp. 1.25			2.89	.56	1.33	14.40	1.85
15	Mockingbird	Juv.	sick	6/2	2	2/15	comp. 1.25	comp. 1.25			4.40	< .40	< .20	1.32	< .20
18	Mockingbird	Juv.	dead	6/11	1	2/22	comp. 1.25				11.78	< .43	< .22	1.30	.21
32	Mockingbird	Juv	sick	7/2	4	7/7	comp. 2.50	comp. 1.25	comp. 1.25	comp. 1.25	12.28	8.73	2.72	4.04	.48
65	Mockingbird	Ad.	healthy	8/10		untreated						< .34	< .17	.49	< .17
66	Mockingbird	Ad.	healthy	8/10		untreated						< .28	< .14	.62	< .14
73	Mockingbird	Ad.	healthy	8/17		untreated						< .30	< .15	1.99	< .15
74	Mockingbird	Ad.	healthy	8/17		untreated						< .32	< .16	1.36	< .16
76	Mockingbird	Ad	healthy	8/19	4	8/12	comp. 2.50	comp. 2.50	comp. 1.25	comp. 1.25	60.96	< .32	< .16	1.37	< .16
77	Mockingbird	Ad.	healthy	8/25	4	8/12	comp. 2.50	comp. 2.50	comp. 1.25	comp. 1.25	60.96	< .36	< .18	< .18	< .18
78	Mockingbird	Ad	healthy	8/25	4	8/19	comp. 2.50	comp. 2.50	comp. 1.25	comp. 1.25	60.96	< .25	< .12	2.56	.12
16	Blue Jay (*Cyanocitta cristata*)	Juv.	dead	6/2	2	2/15	comp. 1.25	comp. 1.25			4.40	< 1.50	< .75	.09	< .75
35	Muscovy Duck (*Cairina moschata*)	Ad.	sick	7/12	1	7/8	encom. 1.25				73	.15	.02	.28	< .06
42	Muscovy Duck	Ad.	sick	7/15	1	7/8	encom. 1.25				.73	.28	.07	.55	< .11
43	Muscovy Duck	Juv	sick	7/20	1	7/8	encom. 1.25				.73	.13	< .07	.33	< .07
44	Muscovy Duck	Juv.	sick	7/20	1	7/8	encom. 1.25				.73	.40	.14	.84	.10
67	Red-winged blackbird (*Agelaius phoeniceus*)	Juv.	healthy	8/11		untreated						< .25	.12	1.31	< .12
68	Turkey (*Meleagris pallopavo*)	Juv.	dead	8/13	3	8/13	comp. 2.50	comp. 2.50	comp. 1.25		60.96	12.14	9.68	24.24	< .08
75	Loggerhead Shrike (*Lanius ludovicianus*)	Ad.	healthy	8/19	4	8/12	comp. 2.50	comp. 2.50	comp. 1.25	comp. 1.25	60.96	< .23	< .11	27.68	.80

[1] Specimen No. 1—male; specimen No. 71—female; all other specimens—sex unknown.

[2] Pathological examination was performed on 11 of the birds found sick or dead and was negative in all cases except two (No. 15 and 16), which showed some degree of liver necrosis. Two (No. 1 and 15) showed staphylococcal infection (staphylococcosis), and *Escherichia coli* was recovered from two others (No. 16 and 35).

bird died of DDT poisoning; if it did not, it certainly was very close to reaching a lethal brain level. Cardinals were most often mentioned in reports of past wildlife damage. Their disappearance particularly was related to spraying, although some people mentioned finding dead cardinals. Specimen No. 71 was the only cardinal that was saved during the period when wildlife damage was supposed to be greatest. No cardinals were collected during the present study period. A letter dated December 16, 1964, which accompanied the dead bird when it was submitted as a specimen, stated that when the bird was collected, it could not balance itself but repeatedly fell on its back until its death. This description of the bird's death would fit several types of poisoning wherein the organism loses motor control. It does not include a description of the tremors that accompany DDT poisoning and are usually obvious to the observer. This bird was collected less than 13 days after the area was sprayed, which is a reasonable time lapse in which to expect detrimental effects to wildlife to appear.

Brain analysis of the ground dove (specimen No. 72) collected by the same resident showed that this bird was carrying 52 ppm of DDT + DDD, an amount well in excess of what is tentatively considered to be the lower lethal level. This bird was collected 8 days after the zone was sprayed. In her accompanying letter of December 21, 1964, the collector described the bird's death as being accompanied by uncontrolled twitching and convulsive movements of the feet. This description is consistent with the symptoms of DDT poisoning, which are similar, of course, in any neurotoxic poisoning. With the combination of high brain level of DDT + DDD and the 8-day time lapse after spraying, it can be stated with some certainty that this bird died of DDT poisoning—circumstantial evidence indicated that the Aedes aegypti Eradication Program could have been the source of the DDT.

Conclusions

No evidence was found of mass kills of vertebrates that could be attributed to the Aedes aegypti Eradication Program. Because of the operational methods employed by the spray program during this investigation (with the exception of Zone 9C), there was little reason to suspect immediate and widespread damage to wildlife. By far the greatest number of personal complaints was directed at the use of DDT and not first-hand accounts of wildlife damage.

[illegible] of specimens from West Palm Beach tended [illegible] some wildlife damage reported [illegible] the investigation. Brain analysis of [illegible] indicated a high probability that this bird was killed by DDT poisoning. In one cardinal, the brain level of DDT + DDD was high enough to seriously endanger the bird, if not to cause its death. Circumstantial evidence in these cases indicated that the Aedes aegypti Eradication Program could have been the source of the DDT. One crow showed heavy exposure to DDE, probably through the food chain, and probably not mainly from the Aedes aegypti spray program.

In the Miami area, a domestic turkey showed a brain level of DDT + DDD high enough to seriously endanger the bird, possibly to be the cause of its death; and one healthy loggerhead shrike showed heavy exposure to DDE, probably through the food chain.

Seven other sick birds and five other dead birds collected in the treated areas had brain levels of pesticides that were so low as to rule out DDT as being the cause of their illness or death.

Brain levels of DDT and its metabolites were not significantly different between house sparrow nestlings from treated zones and those from untreated zones in the Miami area.

The great decrease in complaints and reports of wildlife damage was probably correlated with the change in operational spray methods employed: i.e., to more selective applications and reduced rate of dosage with insecticide.

The data herein presented are limited in sample size. Specimens were collected over a span of only 4 months and within only a small portion of the total area covered by the spray program. The data are presented solely as an indication of the effects of the Aedes aegypti Eradication Program on wildlife, with the recommendation that studies of this type continue for the duration of the program.

The chemical names of compounds mentioned in this paper are:

DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,*endo-exo*-5,8-dimethanonaphthalene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan.

Acknowledgment

The authors are indebted to the Patuxent Wildlife Research Center of the U. S. Fish and Wildlife for assistance in directing the study, especially to Mr. William H. Stickel and Dr. Lucille F. Stickel, who provided timely direction and assistance in the interpretation of findings.

LITERATURE CITED

(1) *Stickel, Lucille F., William H. Stickel, and R. Christensen. 1966.* Residues of DDT in brains and bodies of birds that died on dosage and in survivors. Science 151(3717):1549-1551.

Pesticides in Hatchery Trout—Differences Between Species and Residue Levels Occurring in Commercial Fish Food

H. Cole[1], A. Bradford[2], D. Barry[1], P. Baumgarner[1], and D. E. H. Frear[1]

ABSTRACT

Samples of commercial fish food from four manufacturers and trout of three species, brook, brown, and rainbow, were analyzed for persistent chlorinated pesticides. The trout were in the 8- to 9-inch size range at the time of analysis. Small quantities of heptachlor, heptachlor epoxide, dieldrin, DDE, DDD (TDE), o,p'-DDT, and p,p'-DDT were found in the fish food. One source contained all of the pesticides except DDD. The trout were analyzed on the basis of chloroform-methanol extractable lipids from the whole fish. The rainbow trout with one exception contained all seven pesticides. The rainbow trout contained greater quantities of all pesticides than the brook or brown trout. The brown trout contained significantly more heptachlor, heptachlor epoxide, dieldrin, DDE, and DDD than the brook trout.

Introduction

VARIOUS investigations have demonstrated the presence of trace quantities of DDT and other persistent chlorinated pesticides in feed stuffs including grains, meat scraps, alfalfa meals, and fish oils. Many of these ingredients are normally included in the manufacture of commercial fish foods used in the production of trout. In the investigation reported here an attempt was made to determine the levels of certain persistent chlorinated pesticides in commercial fish food and the levels of pesticides occurring in trout being fed this food at the Pennsylvania Fish Commission's Benner Spring Research Station.

Sampling Methods

The fish meal samples were collected from commercial packages of pellets. A 2-lb composite sample of pellets was collected from lots of each of four different manufacturers. Three species, rainbow *(Salmo gairdneri)*, brown *(Salmo trutta)*, and brook trout *(Salvelinus fontinalis)* were collected from hatchery pools. Seven fish of each species ranging in size from 8 to 9 inches were used for analysis. All three species had been fed the same brand of food (listed in Table 1 as source No. 1) in the same manner throughout their growth from the fingerling stage until collected for analysis.

Analytical Methods

FISH FOOD

The samples of food consisting of 2 lb of pellets were ground in a Wiley Mill. A 100-g subsample of the resulting meal was extracted for 16 hours in a large Soxhlet apparatus with chloroform-methanol (2:1, v/v). The extract was filtered with suction through a Büchner funnel and placed in a flask equipped with a Synder column. This was heated on a steam bath to evaporate the chloroform. Two hundred ml of n-hexane were added to the methanol and after thorough shaking the mixture was washed three times with water to remove the methanol. The n-hexane extract was then passed through an anhydrous sodium sulfate column to remove the water. The extract was then passed through a column of alumina, Celite, and Nuchar activated carbon (2:1:1). This removed any pigments and other interfering substances. The purified extract was concentrated in a Kuderna-Danish evaporator to a volume of 2 ml and an aliquot injected into the gas chromatograph.

FISH

Each fish was weighed and then macerated in a Waring Blendor with 300 ml of chloroform-methanol (2:1); approximately 100 g of anhydrous sodium sulfate were added during the blending process. The liquid was decanted, and the blending repeated with another 300-ml portion of chloroform-methanol. The extracts and slurry were combined and filtered with suction through filter paper in a Büchner funnel. The filtered extract was then placed in a flask equipped with a Snyder column. This was placed on the steam bath and the chloroform removed. One hundred ml of n-hexane were added, the liquid transferred to a separatory funnel and washed three times with 200-ml portions of water to remove the methanol. The washed extract was filtered through anhydrous sodium sulfate, then evaporated to a small volume in a flask on the steam bath and then to dryness with a stream of air at room temperature.

---

[1] Pesticide Research Laboratories, Departments of Plant Pathology and Entomology, The Pennsylvania State University, University Park, Pennsylvania 16802. (Authorized for publication as paper No. 3265 in the Journal Series of the Agricultural Experiment Station on May 24, 1967.)

[2] Chief Fishery Pathologist, Pennsylvania Fish Commission, Benner Spring Research Station, Bellefonte, Pennsylvania 16823.

At this point the residue consisted of lipid material in a semisolid state. Two g of this were weighed into a small separatory funnel and dissolved in 25 ml of petroleum ether. This solution was extracted by shaking for 1 minute with 25 ml of acetonitrile saturated with petroleum ether: the acetonitrile layer was drawn off and the lipid solution re-extracted with three additional 25-ml portions of acetonitrile saturated with petroleum ether. The combined acetonitrile extracts were evaporated to a small volume and taken up in n-hexane. This was then evaporated in a Kuderna-Danish evaporator to exactly 2 ml and an aliquot injected into the gas chromatograph.

Instrumental Procedure

All analyses were made on a Research Specialties Gas Chromatograph Model 600, equipped with a 6-foot glass column packed with Gas Chrom Q impregnated with 5% DC-200. An electron capture detector was used in all studies reported in this paper. The column temperature was maintained at 210 C, the detector at 270 C, with a nitrogen flow of 60 ml per minute. Samples of standard solutions were run periodically to check on recovery. All results were calculated on the basis of ppm of pesticide in the 2-g aliquot of lipid material. Thus the results are on a "fat" basis derived from the chloroform-menthanol extractable lipids. Considering the size of sample and analytical method, the minimum level of detectability was considered to be 0.002 ppm. Residue traces less than 0.002 ppm were reported as NR (no residue).

The pesticides included for analysis were heptachlor, heptachlor epoxide, dieldrin, DDE, DDD (TDE), *o,p'*-DDT, and *p,p'*-DDT. The identities of questionable compounds were confirmed with a QF-1 column and by thin layer chromatography.

Results

Tables 1 and 2 summarize the findings from analysis of the fish meal and trout samples. The No. 1 food sample was of the same brand that composed the diet of the trout selected for analysis. It contained all the pesticides included for analysis except DDD.

All three species of trout contained pesticides. All rainbow trout samples contained with a few exceptions every pesticide included in the analysis. The rainbow trout contained greater quantities of all pesticides than the brook and brown trout. The brook and brown trout did not contain any of the *p,p'*-DDT isomer. Statistical treatment of the results by analysis of variance and studentized range test indicated that the rainbow trout contained significantly more (0.05 confidence level) heptachlor, heptachlor epoxide, dieldrin, DDE, DDD, *o,p'*-DDT, and *p,p'*-DDT than either the brook or brown trout, and that the brown trout contained significantly more heptachlor, heptachlor epoxide, dieldrin, DDE, and DDD than the brook trout. The lipids extractable in chloroform-methanol represented about 2.5% of the fresh weight of the fish. Thus, an approximate fresh weight pesticide content may be obtained by dividing the results in Table 2 by a factor of 40.

Discussion

Previous research has shown that fish vary in their tolerance to pesticides as evidenced by widely different LC_{50} values from species to species *(4)*. It has also been shown that pesticide resistance is present in certain strains of mosquitofish *(Gambusia affinis)* golden shiners *(Notemigonus crysoleucas)*, green sunfish *(Lepomis cyanellus)*, bluegills *(Lepomis macrochirus)*, and yellow bullhead *(Ictalurus natalis) (1-3)*.

It also has been shown that individual lots of fish from different sources vary markedly in their LC_{50} values. For example Marking *(4)* found that with *p,p'*-DDT, rainbow trout lots varied from LC_{50} ppb values of 2.4 to 17 and brook trout from 1.8 to 20. In the present study it has been shown that when three species of trout are fed the same diet throughout a prolonged period, the whole body accumulation of certain pesticides varies considerably with the species. All trout in the pools from which the samples were selected appeared to be in normal health and all trout in the hatchery including breeding stock were being fed the No. 1 brand of fish food. The hatchery at the Research Station has indicated no reproductive problems to date. Analyses of water from sources entering the hatchery have failed to show the presence of pesticides in waters entering the hatchery.

It is also of interest that while pesticide residue tolerances for fish have not been established, the levels found in rainbow trout were above the levels accepted by the FDA for beef fat.

It is not known whether the differences between species represent differences in uptake, excretion, or degradation of pesticides. It is also not known how much the diet of the fish may influence uptake and accumulation. However, it appears in the case of DDT at least, degradation abilities between species may vary since the brook and brown trout under the conditions in the study did not contain any *p,p'*-DDT isomer.

At present studies are underway to determine if various genetic lines within species with uniformity for other characters will exhibit uniform differences in pesticide accumulation when fed similar diets.

[illegible] names of compounds mentioned in this paper are:

[illegible]	[illegible] trichloro-2,2-bis(*p*-chlorophenyl)ethane
[illegible]	[illegible] chloro-2,2-bis(*p*-chlorophenyl)ethane
[illegible]	[illegible] chloro 2,2-bis(*p*-chlorophenyl)ethylene
BHC	[illegible] hexachlorocyclohexane, mixed isomers
[illegible]	[illegible] of 1,2,3,4,10,10-hexachloro-[illegible] 5,6,7,8,8a-octahydro-1,4-*endo*-[illegible] 5,8-dimethanonaphthalene
Hept[illegible]	[illegible] tetrahydro-4,7-methanoindene
Heptachlor epoxide	[illegible] heptachloro 2,3-epoxy 3a,4,7,7a-[illegible] 4,7-methanoindan

LITERATURE CITED

(1) *Boyd, C. E. and E. E. Ferguson. 1964.* Susceptibility and resistance of mosquitofish to several insecticides. J. Econ. Entomol. 57:430-431.

(2) *Ferguson, D. E. and C. R. Bingham. 1964.* Endrin resistance in the yellow bullhead, *Ictalurus natalis*. Trans. Amer. Fish Soc. 95:325-326.

(3) *Ferguson, D. E., D. D. Culley, W. D. Cotton, and R. P. Dodds, 1964.* Resistance to chlorinated hydrocarbon insecticides in three species of fresh water fish. Bioscience 14(11):43-44.

(4) *Marking, L. L. 1966.* Evaluation of *p,p'*-DDT as a reference toxicant in bioassays. USDI Resource Publication No. 14:1-10.

TABLE 1.—*Pesticides in commercial fish food*

Food source	PPM						
	Heptachlor	Heptachlor epoxide	Dieldrin	DDE	DDD	*o,p'*-DDT	*p,p'*-DDT
1	0.073	0.014	0.060	0.096	NR	0.305	0.087
2	0.198	NR	0.085	0.315	0.189	NR	NR
3	0.101	0.211	NR	NR	NR	NR	NR
4	0.031	NR	NR	0.016	NR	NR	NR

Notes: Each source represents the product of a different manufacturer. Samples were collected on 3/31/66.
NR = Less than 0.002 ppm.

TABLE 2.—*Pesticides in hatchery trout of three species from Benner Spring Hatchery*[1]

Sample No.	Species	PPM[2]						
		Heptachlor	Heptachlor epoxide	Dieldrin	DDE	DDT	*o,p'*-DDT	*p,p'*-DDT
1	Rainbow	0.5	1.3	0.8	1.3	3.2	1.1	4.0
2	Rainbow	0.5	1.0	0.7	1.3	1.8	1.0	2.7
3	Rainbow	0.4	0.9	0.6	1.0	2.1	0.9	2.7
4	Rainbow	0.5	1.3	0.8	1.3	3.2	1.2	4.0
5	Rainbow	0.7	1.4	0.8	1.4	2.2	0.8	2.8
6	Rainbow	0.4	1.2	0.9	1.3	3.3	0.9	3.5
7	Rainbow	2.8	7.5	0.5	NR	4.4	NR	5.8
Species mean		0.8	2.1	0.7	1.1	2.9	0.8	3.6
8	Brown	0.03	NR	0.05	0.81	1.3	0.02	NR
9	Brown	NR	NR	0.006	0.34	0.28	NR	NR
10	Brown	0.06	NR	NR	0.82	1.3	NR	NR
11	Brown	0.02	NR	NR	0.68	0.81	NR	NR
12	Brown	0.08	0.09	NR	0.12	0.19	NR	NR
13	Brown	0.003	0.13	0.008	0.20	0.40	NR	NR
14	Brown	0.006	0.14	0.03	0.19	0.41	NR	NR
Species mean		0.03	0.05	0.01	0.45	0.67	0.003	
15	Brook	0.48	NR	0.02	0.07	0.68	NR	NR
16	Brook	NR	NR	NR	0.45	NR	NR	NR
17	Brook	NR	NR	NR	0.15	0.24	NR	NR
18	Brook	NR	NR	NR	NR	NR	0.002	NR
19	Brook	NR	NR	0.10	0.15	0.70	NR	NR
20	Brook	0.009	NR	NR	NR	0.003	NR	NR
21	Brook	NR	NR	0.003	0.05	0.34	NR	NR
Species mean		0.007		0.002	0.12	0.28		

[1] Fish selected at random from hatchery pool of each species at Benner Spring. All fish in 8- to 9-inch size category. Each sample number represents a single fish.
[2] Based on amount of pesticide in the lipids extractable by chloroform-methanol
Note: NR = Less than 0.002 ppm.

PESTICIDES IN WATER

Pesticides in Selected Western Streams—A Contribution to the National Program[1]

E. Brown and Y. A. Nishioka

ABSTRACT

Since October 1965 samples of a water-suspended sediment mixture from 11 streams in the western United States have been analyzed monthly for 12 pesticides. The compounds determined include the insecticides aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane; and the herbicides 2,4-D; 2,4,5-T; and silvex. No herbicide was found at any station during the first year of the sampling program. All insecticides were found at one time or another, but not at all stations. The amounts observed were quite small, ranging from less than 5 parts per trillion of lindane to 110 parts per trillion of DDT.

Introduction

IN the fall of 1965, the U. S. Geological Survey initiated a limited program of pesticide monitoring on 11 streams in the western United States, selected from the Survey's program of water-quality studies of irrigation-network sites. Purpose of the program was to determine the extent and magnitude of pesticide contamination. To accomplish this, the streams were analyzed initially for nine of the more commonly used insecticides; analysis for herbicides was begun later in the program. Insecticides chosen for analysis included aldrin, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane. The herbicides consisted of 2,4-D; 2,4,5-T; and silvex.

Pesticides selected for analysis were chosen mainly from the primary list of pesticide compounds established in March 1964 by the Subcommittee on Monitoring, Federal Committee on Pest Control.

Data Collection Sites

[illegible] the actual sampling sites, consideration was [illegible] following criteria: (1) each station should [illegible] operating U. S. Geological Survey [illegible] station; (2) where practical, each [illegible] selected as one of the sites for the [illegible] monitoring program recommended by the Federal Committee on Pest Control; (3) each site should be one at which other types of data are being obtained; (4) no station should overlap the activities of other agencies. It was felt that irrigation-network stations were highly desirable because: (1) several years of record of inorganic water quality and stream discharge are available; and (2) these stations represent mainly agricultural areas where the probability of observing pesticide residues would be greater.

Stations selected for sampling are listed in Table 1 and their location shown in Fig. 1. Complete hydrologic

[1] [illegible] U. S. Survey, Sacramento, [illegible] Director, U. S. [illegible]

TABLE 1.—*Pesticide monitoring stations in western United States*

Irrigation Network No.[1]	Geological Survey Station Ident. No.	Stream and Location	Inorganic Analysis Started
4	6-8070	Missouri River at Nebraska City, Neb.	1-4-51
24	7-1305	Arkansas River below John Martin Reservoir, Colo.	1-10-51
27	-2505	Arkansas River at Van Buren, Ark.	10-1-45
37	8-1140	Brazos River at Richmond, Tex.	9-1-45
40	-1620	Colorado River at Wharton, Tex.	4-11-44
52	-4625	Rio Grande below Anzalduas Dam, Texas	1944
63	9-5255	Colorado River (Yuma Main Canal) below Colorado River Siphon, at Yuma, Ariz.	1042
86a	11-4255	Sacramento River at Verona, Calif.	
94	12-5105	Yakima River at Kiona, Wash.	12-30-52
97	13-1545	Snake River at King Hill, Idaho	3-27-51
102	14-1057	Columbia River near The Dalles, Ore.	12-1-50

[1] Number refers to list of irrigation-quality network stations (U. S. Geological Survey, 1954, p. 31).

data for these stations are published in annual reports of the U. S. Geological Survey entitled "Quality of surface waters for irrigation, western United States." These reports include inorganic water-quality data, drainage area and stream discharge figures, as well as an indication of the period of record available. The first report was issued in 1954 as U. S. Geological Survey Water-Supply Paper 1264 *(4)* and covers the period October 1, 1950 through September 30, 1951. The latest report in this series was released in 1966 as Water-Supply Paper 1946 *(5)* and contains data for the period October 1, 1961 through September 30, 1962.

Sampling Procedures

At the beginning of the program, samples were collected monthly in 1-gallon Pyrex bottles by personnel of the U. S. Geological Survey, with the exception of the station below Anzalduas Dam, Texas. In this case, samples were provided by personnel of the International Boundary and Water Commission, United States and Mexico, United States Section. The bottles were tightly stoppered with rubber stoppers, wrapped in aluminum foil, and promptly shipped in wooden boxes to the laboratory for analysis. The size and weight of the bottle and container required shipment by rail, so that in most cases 2 to 3 weeks elapsed between collection and analysis. In addition, containers were broken and samples lost in transit.

As soon as analytical methods improved to the point that a smaller sample could be used without sacrifice of accuracy, the duo-pak container was put into use. This container *(1)* is lightweight, small, and sufficiently sturdy

FIGURE 1.—*Pesticide stations in western United States*

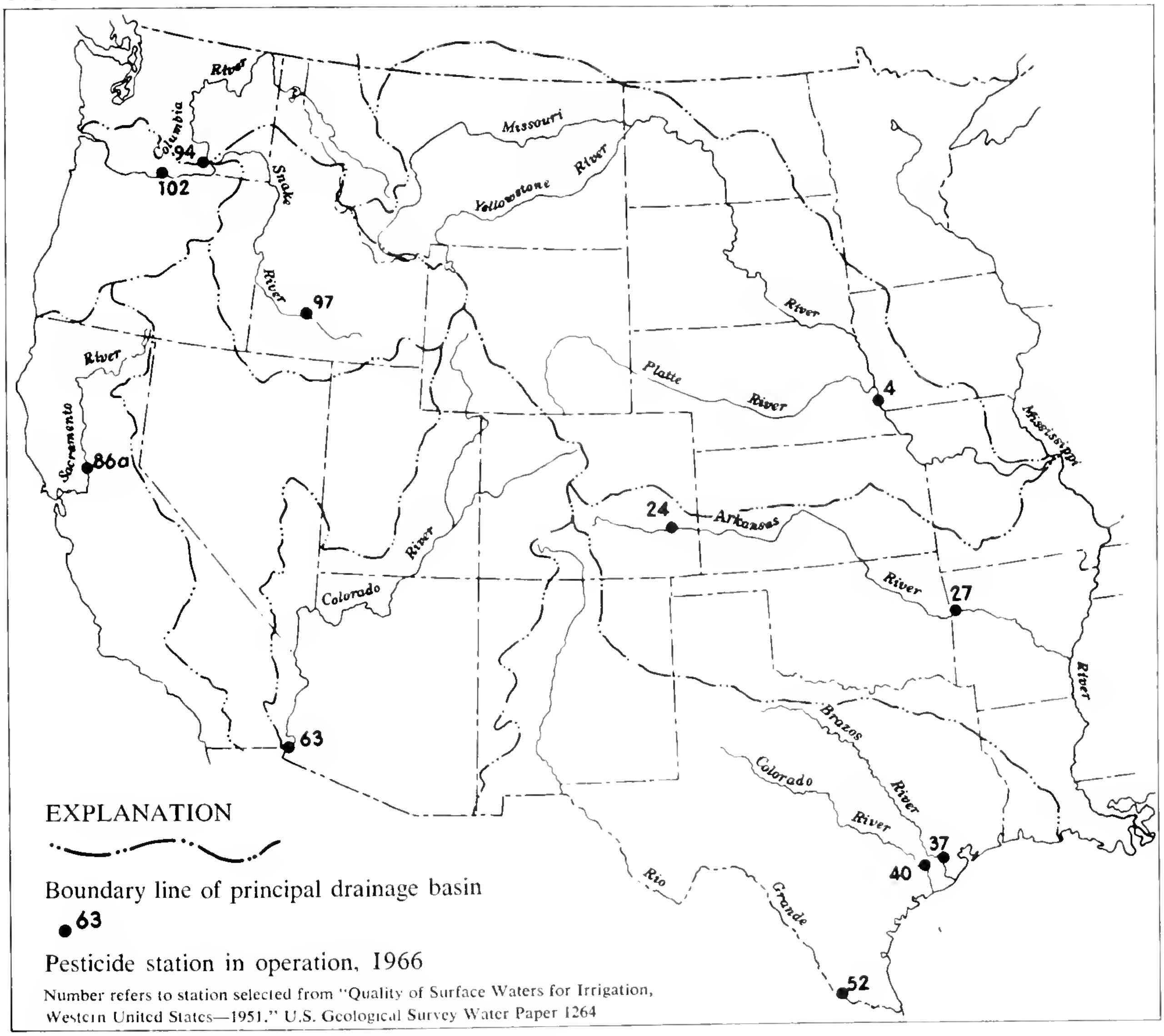
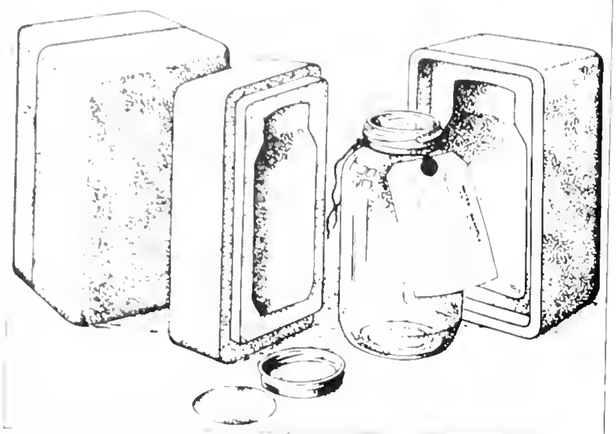


FIGURE 2.—*Glass sample container, screw-cap (Teflon liner), and expanded polystyrene protective case*



to permit shipment by air express. No breakage in transit has been observed during 6 months of use. The collection unit—consisting of plastic foam case, bottle, cap, and teflon capliner—is shown in Fig. 2.

Two bottles were collected at each station, one being used for insecticide analysis and the other for herbicides.

It was originally intended that a depth-integrated sample be collected to most nearly represent the average water-quality condition at the time of sampling. Small-mouthed gallon jugs provide a reasonable approximation of this type of sample; wide-mouth quart jars however, fill almost instantly when lowered into the water. The type of sample obtained is not representative of the vertical section, but only of the upper-most water layer.

At the present time, a study is underway to modify existing sediment-sampling equipment to provide both depth and point-integrated samples.

Analytical Procedures

Because the total insecticide or herbicide concentration [illegible] each sample was analyzed as received, [illegible] attempt to separate the water and sediment for [illegible] analysis. Chlorinated pesticides were analyzed [illegible] described by Lamar *et al. (3)* which is [illegible] one liter sample of water was extracted [illegible] times with equal volumes of hexane to a [illegible] hexane. Hexane was dried over anhydrous Na_2SO_4 [illegible] concentrated to 5 ml in a Kuderna-Danish [illegible]. Of the concentrated hexane, 5 μl were injected into the gas chromatograph. In all cases, injections were made into two different chromatographic columns to effect separation.

Recovery data reported by Lamar *et al. (3)* range from about 80% to 115%. No adjustments were made to the data reported in this paper, because many of the values are so near the lower limits of sensitivity that they are rounded off to the nearest 5 ppt.

Herbicide analysis was conducted according to a procedure developed by Goerlitz and Lamar *(2)*, using boron trifluoride methanol reagent for esterification. As in the analysis of insecticides, a liter sample was used, with the final volume of extractant being reduced to 5 ml prior to injection into the gas chromatograph. Recovery data reported by Goerlitz and Lamar *(2)* ranged from about 75% to 120%. No adjustments were made in the data reported for herbicides.

Operating conditions for the chromatographic procedure were as follows:

Instrument:	Aerograph Hy-Fl Model 600-D, with a Wilkens Model 328 Isothermal temperature controller
Columns:	1. 1/8″ x 5′ pyrex glass, packed with 60/80 mesh Gas-Chrom Q-coated with 5% DC 200. 2. 1/8″ x 5′ pyrex glass, packed with 60/80 mesh Gas-Chrom Q-coated with 5% QF-1.
Oven temperature:	187 C
Detector:	Electron-capture, concentric tube design, D.C. mode
Carrier gas:	Nitrogen at 40/min
Injection volume:	5 μl

Using these procedures, accurate analysis of most water is routinely practical if it contains the minimum concentration of pesticides indicated in Table 2. Amounts less than that can be detected, but are not considered accurately measurable unless a larger sample volume is taken for analysis, or the extractant volume is reduced to less than 5 ml. For example, water containing as little as 10 nanograms per liter of 2,4-D may be analyzed if the final extraction volume is reduced to 0.5 ml instead of 5.0 ml. Not all extracts, however, can be reduced to such a low volume without an accompanying buildup of excessive interferences. The extensive cleanup necessary to remove such interference is not always practical in routine analysis.

TABLE 2.—*Minimum measurable concentrations of pesticides in water*

Pesticides	Parts per trillion (nanograms per liter)	Pesticides	Parts per trillion (nanograms per liter)
ALDRIN not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	5	HEPTACHLOR 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	5
DDD 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	5	HEPTACHLOR EPOXIDE 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan	5
DDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene	5	LINDANE 1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer	5
DDT 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	10	2,4-D 2,4-dichlorophenoxyacetic acid	100
DIELDRIN not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	5	2,4,5-T 2,4,5-trichlorophenoxyacetic acid	5
ENDRIN 1,2,3,4,10,10-hexachloro-6,1-epoxy-1,4,4a,5,6,7,8,8a-Octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene	5	SILVEX 2-(2,4,5-trichlorophenoxy)propionic acid	5

Discussion of Results

Pesticide analyses of samples of a water-suspended sediment mixture obtained during the first year of this study are arranged in downstream order and presented in Table 3. Data in Table 3 have been summarized and presented in Table 4 to indicate frequently occurring pesticides, and the stations at which they occurred. Based on data obtained, the following observations can be made:

1. No herbicide was found at any time at any station. The absence of herbicides from any of the samples analyzed may be due at least in part to their susceptibility to degradation. Recent studies by Goerlitz and Lamar *(2)* on the stability of herbicides added to water samples, indicate that 2,4-D may be degraded rapidly, especially in samples obtained from areas repeatedly treated with 2,4-D.

2. All insecticides were found at one time or another, but not at all stations.

3. The insecticide concentrations found were very small, amounting to 5 ppt in slightly more than 50% of all positive results.

4. Although no definite pattern could be noted in pesticide occurrence, positive results were most frequently found in February, March, April, and May.

5. The most frequently found insecticide was lindane, which occurred 46 times out of a total of 165 positive results. The most infrequently occurring insecticide was aldrin, which was observed only four times at all stations during the year.

6. On a geographical basis, most frequent occurrence of pesticides was at the Rio Grande Station below Anzalduas Dam, Texas. Thirty-two positive results were obtained at this station, or about 20% of the total. The least number of positive results were noted at the Snake and Columbia River stations, each of which recorded only seven pesticide occurrences.

7. No relationship can be noted between pesticide occurrence and the various factors in pesticide application since the latter parameters cannot be ascertained in most areas.

8. No evaluation of other forms of pesticide movement, such as might be involved in sediment transport, can be made on the basis of the data obtained. Data presented will provide a basis for efforts into other areas of pesticide relationships, such as mode transport, time of travel, and improved sampling techniques.

LITERATURE CITED

(1) Breidenbach, A. W., J. J. Lichtenberg, C. F. Henke, D. J. Smith, J. W. Eichelberger, Jr., and H. Stierli. 1964. The identification and measurement of chlorinated hydrocarbon pesticides in surface waters. U. S. Dep. of Health, Educ., and Welfare, Public Health Serv., Pub. No. 1241, p. 11.

(2) Goerlitz, D. F., and W. L. Lamar. Determination of phenoxy acid herbicides in water by electron-capture and microcoulometric gas chromatography. U. S. Geol. Surv. Water-Supply Paper 1817-C. (in press).

(3) Lamar, W. L., D. F. Goerlitz, and L. M. Law. 1965. Identification and measurement of chlorinated organic pesticides in water by electron-capture gas chromatography. U. S. Geol. Surv. Water-Supply Paper 1817-B.

(4) U. S. Geological Survey. 1954. Quality of surface waters for irrigation, western United States, 1951. U. S. Geol. Surv. Water-Supply Paper 1264, 153 p.

(5) U. S. Geological Survey. 1966. Quality of surface waters for irrigation, western United States, 1962. U. S. Geol. Surv. Water-Supply Paper 1946, 143 p.

TABLE 3.—*Pesticide content of selected streams in western United States*

nd = not determined, — = not present

Date	Time	Instantaneous Discharge (CFS)	Aldrin	DDD	DDE	DDT	Dieldrin	Endrin	Heptachlor	Heptachlor Epoxide	Lindane	2,4-D	2,4,5-T	Silvex
			Parts per trillion (nanograms per liter, rounded to nearest 5 ppt)											
IRRIG. NETWORK NO. 4—USGS NO. 6-8070 MISSOURI RIVER AT NEBRASKA														
10/18/65	10:30A	38,700	—	—	—	—	10	—	—	—	—	nd	nd	nd
11/23/65	10:00A	39,700		—	—	—	—	—	—	—	5	nd	nd	nd
12/08/65	11:30A	25,900	—	—	—	—	—	—	—	—	—	nd	nd	nd
01/20/66	2:30P	20,400	—	—	—	—	—	—	—	—	—	nd	nd	nd
02/09/66	2:30P	47,900	—	—	—	—	5	—	—	—	—	nd	nd	nd
03/03/66	10:45A	32,600	—	—	—	—	15	—	—	—	5	nd	nd	nd
04/04/66	10:45A	40,000		—	—	—	5	—	—	—	5	—	—	—
05/17/66	1:30P	35,600	5	—	—	50	15	35	—	5	5	—	—	—
06/15/66	10:00A	37,200	—	—	—	—	—	—	—	—	—	—	—	—
07/07/66	10:45A	35,900	—	—	—	—	—	—	—	5	—	—	—	—
08/04/66	10:00A	37,200	—	—	—	—	—	—	5	—	—	—	—	—
09/16/66	10:30A	36,000	—	—	—	45	—	—	—	—	—	—	—	—
IRRIG. NET. NO. 24—USGS NO. 7-1305 ARKANSAS RIVER BELOW JOHN MARTIN RESERVOIR, COLO.														
10/16/65	7:45A	272	—	—	5	—	5	—	5	5	5	nd	nd	nd
11/26/65	3:00P	297	—	—	—	—	—	—	—	—	10	nd	nd	nd
12/21/65	4:00P	331	—	—	—	—	—	—	—	—	5	nd	nd	nd
01/20/66	4:00P	297	—	—	—	—	—	—	—	—	—	nd	nd	nd
02/19/66	8:30A	448	—	—	—	—	5	—	—	5	10	nd	nd	nd
03/22/66	8:40A	87	—	—	—	—	—	—	—	—	5	nd	nd	nd
04/18/66	2:40P	774	—	—	—	—	5	—	—	—	10	—	—	—
05/16/66	3:20P	1,030	—	10	5	75	—	—	10	—	10	—	—	—
06/13/66	3:15P	870		—	—	—	—	—	—	—	—	—	—	—
07/25/66	4:20P	978		—	15	—	—	—	5	5	—	—	—	—
08/23/66	9:45A	283	—	—	—	—	—	—	—	—	—	—	—	—
09/19/66	3:50P	506	—	—	—	—	—	—	—	—	5	—	—	—
IRRIG. NET. NO. 27—USGS NO. 7-2505 ARKANSAS RIVER AT VAN BUREN, ARK.														
10/21/65	3:40P	12,800	—	—	20	—	—	—	—	—	—	nd	nd	nd
11/22/65	11:00A	3,960		—	—	—	—	—	—	—	—	nd	nd	nd
[illegible]	9:05A	7,200			—	—	5	—	5	—	5	nd	nd	nd
[illegible]	12:20P	6,250			—	—	—	—	—	—	—	nd	nd	nd
[illegible]	[illegible]	5,070			—	—	5	—	—	—	5	nd	nd	nd
[illegible]	9:00A	11,000		5	5	—	—	—	—	5	10	nd	nd	nd
04/06/66	8:50A	5,520			—	—	5	—	—	—	5	—	—	—
05/10/66	1:05P	16,700				—	—	—	—	—	5	—	—	—
[illegible]	12:45P	23,100	5		—		—	—	5	—	—	—	—	—

TABLE 3.—*Pesticide content of selected streams in western United States*—Continued

nd = not determined; — = not present

Date	Time	Instantaneous Discharge (cfs)	Parts per trillion (nanograms per liter, rounded to nearest 5 ppt)											
			Aldrin	DDD	DDE	DDT	Dieldrin	Endrin	Heptachlor	Heptachlor Epoxide	Lindane	2,4-D	2,4,5-T	Silvex
IRRIG. NET. NO. 27—USGS NO. 7-2505 ARKANSAS RIVER AT VAN BUREN, ARK.—Continued														
07/12/66	9:20A	3,280	—	—	—	70	10	—	—	—	—	—	—	—
08/12/66	8:30A	24,400	—	10	—	110	10	—	—	—	—	—	—	—
09/07/66	8:30A	13,400	—	—	—	—	—	—	—	—	—	—	—	—
IRRIG. NET NO. 37—USGS NO 8-1140 BRAZOS RIVER AT RICHMOND, TEX.														
Oct. 1965	No sample													
Nov. 1965	No sample													
Dec. 1965	No sample													
02/01/66	12.00P	5,620	—	5	—	—	10	—	—	5	5	nd	nd	nd
Mar. 1966	No sample													
04/12/66	12:50P	1,930	—	—	5	—	—	—	—	5	5	—	—	—
05/19/66	2:00P	22,200	—	10	—	55	15	—	—	—	5	—	—	—
06/22/66	11:30A	2,900	—	—	—	45	10	—	15	—	—	—	—	—
07/26/66	3:15P	1,570	—	—	—	—	—	—	—	—	—	—	—	—
08/30/66	11:00A	3,460	—	—	10	105	—	—	—	—	—	—	—	—
IRRIG. NET. NO. 40—USGS NO. 8-1620 COLORADO RIVER AT WHARTON, TEX.														
10/22/65	11:30A	1,600	—	—	—	25	5	5	5	5	—	nd	nd	nd
Nov. 1965	No sample													
Dec. 1965	No sample													
01/07/66	9:00A	1,400	—	—	—	—	10	—	—	5	10	nd	nd	nd
02/02/66	3:15P	897	—	—	—	—	10	—	—	—	5	nd	nd	nd
Mar. 1966	No sample													
04/13/66	8:30A	1,630	—	—	15	70	—	—	—	—	20	—	—	—
05/24/66	5:30P	5,490	—	—	5	—	—	—	—	—	—	—	—	—
06/27/66	3:00P	1,320	—	—	—	—	—	—	—	—	—	—	—	—
07/28/66	5:30P	1,380	—	—	—	—	—	—	—	—	—	—	—	—
Aug. 1966	No sample													
09/01/66	9:30A	483	—	—	—	—	—	—	—	—	—	—	—	—
IRRIG. NET. NO 52—USGS NO. 8-4625 RIO GRANDE BELOW ANZALDUAS DAM, TEX.														
10/15/65	9:10A	500	—	5	5	—	10	—	10	5	10	nd	nd	nd
11/26/65	8:30A	300	—	10	—	—	15	—	—	—	5	nd	nd	nd
12/15/65	1:45P	260	—	—	—	—	15	—	15	—	10	nd	nd	nd
01/14/66	8:30A	300	—	—	—	—	10	—	5	—	5	nd	nd	nd
02/16/66	9:30A	850	—	—	5	—	—	10	—	—	5	nd	nd	nd
03/14/66	9:10A	920	—	—	—	—	—	—	—	10	10	nd	nd	nd

TABLE 3.—*Pesticide content of selected streams in western United States*—Continued

nd = not determined; — = not present

Date	Time	Instantaneous Discharge (CFS)	Aldrin	DDD	DDE	DDT	Dieldrin	Endrin	Heptachlor	Heptachlor Epoxide	Lindane	2,4-D	2,4,5-T	Silvex
			Parts per trillion (nanograms per liter, rounded to nearest 5 ppt)											
IRRIG. NET NO. 52—USGS NO. 8-4625 RIO GRANDE BELOW ANZALDUAS DAM, TEX.—Continued														
04/15/66	9:30A	300		—	—	—	—	—	—	—	—	—	—	—
05/17/66	9:30A	280		10	10	—	—	40	15	—	10	—	—	—
06/16/66	8:30A	2,500	—	—	—	—	—	25	—	—	—	—	—	—
07/12/66	8:00A	7,950	—	15	—	50	10	—	—	—	—	—	—	—
08/15/66	9:00A	900	—	—	10	—	—	—	—	—	5	—	—	—
09/16/66	1:00P	12,360	—	10	—	—	—	—	—	—	—	—	—	—
IRRIG. NET NO. 63—USGS NO. 9-5255 COLORADO RIVER (YUMA MAIN CANAL) AT YUMA, ARIZ.														
10/12/65	10:30A	502	—	—	—	—	—	—	—	—	—	nd	nd	nd
12/01/65	2:00P	239	—	—	—	—	—	—	—	—	—	nd	nd	nd
01/05/66	10:00A	161	—	—	—	—	—	—	—	5	—	nd	nd	nd
02/01/66	1:00P	239	—	—	—	—	—	—	—	—	—	nd	nd	nd
03/02/66	9:30A	616	—	—	—	70	5	—	—	—	5	nd	nd	nd
04/06/66	11:00A	216	—	—	—	—	—	—	—	—	5	—	—	—
05/03/66	1:30P	687	—	—	5	—	—	—	—	—	5	—	—	—
06/02/66	3:30P	661	—	—	10	—	—	15	—	90	—	—	—	—
07/05/66	9:30A	557	—	—	—	—	—	—	—	—	—	—	—	—
08/08/66	9:00A	560	—	—	—	—	—	—	—	—	—	—	—	—
09/01/66	9:00A	671	—	—	—	—	—	—	—	—	—	—	—	—
IRRIG. NET. NO. 86a—USGS NO. 11-4255 SACRAMENTO RIVER AT VERONA, CALIFORNIA														
Oct. 1965	No sample													
11/29/65	10:00A	23,400	—	—	—	—	—	—	—	—	—	nd	nd	nd
12/17/65	10:05A	20,000	—	—	—	—	—	—	—	—	—	nd	nd	nd
01/06/66	10:00A	39,600	—	—	—	—	—	—	—	5	—	nd	nd	nd
02/04/66	3:00P	29,900	—	—	—	—	—	—	—	—	—	nd	nd	nd
03/03/66	10:20A	18,900	—	—	—	—	10	—	—	5	5	nd	nd	nd
04/01/66	2:00P	24,600	—	—	—	—	—	—	—	—	5	—	—	—
05/02/66	9:30A	11,700	—	—	—	—	5	—	—	—	5	—	—	—
06/13/66	11:00A	8,390		—	—	—	—	—	—	—	—	—	—	—
[illegible]	10:00A	8,940		—	—	—	—	—	—	—	—	—	—	—
[illegible]	9:00A	10,700		10	—	—	—	—	—	—	—	—	—	—
[illegible]	No sample													
IRRIG. NET NO. 94—USGS NO. 12-5105 YAKIMA RIVER AT KIONA, WASH.														
10/11/65	[illegible]:15P	2,100		—	—	—	—	—	[illegible]	—		nd	nd	nd
Nov. 1965	No sample													
12/[illegible]/65	[illegible]P	[illegible]10				—	—	—	—	—	—	nd	nd	nd

TABLE 3.—*Pesticide content of selected streams in western United States*—Continued

nd = not determined; — = not present

Date	Time	Instantaneous Discharge (cfs)	Parts per trillion (nanograms per liter, rounded to nearest 5 ppt)											
			Aldrin	DDD	DDE	DDT	Dieldrin	Endrin	Heptachlor	Heptachlor Epoxide	Lindane	2,4-D	2,4,5-T	Silvex
IRRIG. NET. NO. 94—USGS NO. 12-5105 YAKIMA RIVER AT KIONA, WASH.—Continued														
Jan. 1966	No sample													
02/02/66	4:45P	1,820	—	—	—	—	—	—	—	—	—	nd	nd	nd
03/04/66	3:45P	2,050	—	—	—	—	5	—	—	5	10	—	—	—
04/01/66	11:00A	6,390	—	—	10	—	—	—	—	—	10	—	—	—
05/09/66	11:55A	5,510	—	—	10	—	—	—	—	—	5	—	—	—
06/17/66	4:00P	1,680	—	5	—	—	—	—	—	—	—	—	—	—
07/25/66	11:15A	1,380	—	10	15	—	—	—	5	5	5	—	—	—
08/30/66	11:00A	1,820	—	—	—	65	—	—	—	—	—	—	—	—
Sep. 1966	No sample													
IRRIG. NET. NO. 97—USGS NO. 13-1545 SNAKE RIVER AT KING HILL, IDAHO														
10/17/65	2:00P	14,700	—	—	—	—	—	—	—	—	—	nd	nd	nd
11/29/65	2:55P	14,700	—	—	—	—	—	—	—	—	—	nd	nd	nd
Dec. 1965	No sample													
01/03/66	2:00P	15,900	—	—	—	—	—	—	—	5	—	nd	nd	nd
02/07/66	11:45A	14,400	—	—	—	—	—	25	5	—	—	nd	nd	nd
03/21/66	4:15P	15,000	—	—	—	—	—	—	—	—	—	nd	nd	nd
04/24/66	3:30P	11,400	—	—	—	—	5	—	—	—	5	—	—	—
May 1966	No sample													
06/09/66	1:20	10,200	5	—	—	—	—	—	—	—	—	—	—	—
07/08/66	12:30P	9,750	—	—	—	60	—	—	—	—	—	—	—	—
08/23/66	10:30A	4,100	—	—	—	—	—	—	—	—	—	—	—	—
09/27/66	11:00A	6,420	—	—	—	—	—	—	—	—	—	—	—	—
IRRIG. NET. NO. 102—USGS NO. 14-1057 COLUMBIA RIVER AT DALLES, ORE.														
10/27/65	5:30P	104,000	—	—	—	—	—	—	—	—	—	nd	nd	nd
11/22/65	9:30A	105,000	—	—	—	—	—	—	—	—	—	nd	nd	nd
12/27/65	11:15A	101,000	—	—	—	—	10	—	—	—	—	nd	nd	nd
01/27/66	4:45P	125,000	—	—	—	—	—	—	—	5	—	nd	nd	nd
02/21/66	8:45A	102,000	—	—	—	—	—	—	—	—	5	nd	nd	nd
03/25/66	1:30P	120,000	—	—	—	—	—	—	—	—	5	nd	nd	nd
04/29/66	9:00A	171,000	5	—	—	—	—	—	—	—	5	—	—	—
05/23/66	4:00P	280,000	—	—	—	—	—	—	—	—	—	—	—	—
06/22/66	7:45A	319,000	—	—	—	—	—	—	—	—	—	—	—	—
07/14/66	1:30P	286,000	—	—	—	—	—	—	—	—	—	—	—	—
08/15/66	8:00A	145,000	—	—	—	—	—	—	—	—	20	—	—	—
09/19/66	8:15A	96,000	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 4.—*Occurrence of insecticides*

	Missouri River at Nebraska City, Nebr.	Arkansas River below John Martin Reservoir, Colo.	Arkansas River at Van Buren, Ark.	Brazos River at Richmond, Tex.	Colorado River at Wharton, Tex.	Rio Grande below Anzalduas Dam, Tex.	Colorado River at Yuma, Ariz.	Sacramento River at Verona, Calif.	Yakima River at Kiona, Wash.	Snake River at King Hill, Idaho	Columbia River near The Dalles, Ore.	Totals
Aldrin	1	–	1	–	–	–	–	–	–	1	1	4
DDD	–	1	2	2	–	5	–	1	2	–	–	13
DDE		3	2	2	2	4	2	–	3	–	–	18
DDT	2	1	2	3	2	1	1	–	1	1	–	14
Dieldrin	5	3	5	3	3	5	1	2	–	1	1	29
Endrin	1	–	–	–	1	3	1	–	–	1	–	7
Heptachlor	1	3	2	1	1	4	–	–	1	1	–	14
Heptachlor epoxide	2	3	1	2	2	2	2	2	2	1	1	20
Lindane	4	8	5	3	3	8	3	3	4	1	4	46
Totals	16	22	20	16	14	32	10	8	13	7	7	165

Persistence and Movement of Parathion in Irrigation Waters[1]

C. W. Miller, W. E. Tomlinson, and R. L. Norgren

ABSTRACT

The occurrence, persistence, and movement of parathion (0,0-diethyl 0-p-nitrophenyl phosphorothioate) in cranberry bog irrigation waters was investigated. The chemical was found to persist for 96 hours at concentrations known to be toxic to certain aquatic organisms. Movement of the chemical from the irrigation waters to an associated water system was also demonstrated to occur; however, the degree of concentration and persistence was not as great as within the bog area.

Introduction

APPLICATION of parathion to cranberry bogs, either by helicopter or through overhead sprinklers.is often made with water impounded in the irrigation ditches. In such a situation it is impossible to avoid deposition of the chemical into these waters. As a result, a possible pollution problem exists since seepage of these waters through leaky floodgates often occurs, and, in the advent of heavy precipitation shortly after application, the water level must be lowered by draining to prevent prolonged soil saturation or flooding which is injurious to the cranberry vines. For these reasons, the following investigation was undertaken.

Materials and Methods

A section of cranberry bog measuring 2900 ft^2 was treated with parathion at a rate equal to 1 lb/acre. The treated area was completely surrounded by an irrigation ditch 3 feet wide by 3 feet deep. Water for this ditch was pumped from an adjacent pond up through a drainage canal a distance of 200 yards. The bog and the irrigation ditch surrounding it are separated from the drainage canal by a roadway 15 feet wide, the waters passing beneath the roadway in a culvert. A floodgate on the bog impounded the water in the irrigation ditch when the water level was approximately 1 to 2 inches below the bog surface. At this time, the pumping of water to the bog ceased and the water in the drainage canal allowed to recede to its normal level. When this happened, the level of the impounded irrigation water was approximately 1½ feet higher than that of the drainage canal.

The chemical was applied to the test area through overhead sprinklers when the irrigation ditch was full and the waters impounded. The sprinkler's pattern was such that no chemical-containing waters fell in the drainage canal, but deposition did occur in the irrigation water. Slight runoff of this application water from the bog surface into the irrigation water was observed. During the sampling period, seepage of the irrigation water through the floodgate occurred, lowering the level approximately 8 inches.

Two 1-liter water samples were collected, from each of two locations, from the irrigation waters prior to application (controls), immediately following application, and every 24 hours thereafter for a period of 96 hours. In addition, similar samples were collected from the drainage canal at the point where the seeping irrigation water mixed with the canal waters (bog-canal junction), and at 50 and 150 yards down from this point using the same sampling sequence as above.

[1] University of Massachusetts, Cranberry Experiment Station, E. Wareham, Massachusetts 02538.

[2] Present address: U. S Dept. of the Interior, Bureau of Commercial Fisheries, Biological Laboratory, Sabine Island, Gulf Breeze, Fla. 32561.

The experiment was repeated with 14 days elapsed time between the first and second experiment. Data reported herein represent the mean of the two experiments.

Extraction of the water samples was by the method of Teasley and Cox (3). Recovery from fortified samples average 83%, and all data have been corrected for this recovery value. Indentification and quantitation was made by gas-liquid chromatography using a Varian Aerograph model 204 equipped with an electron capture detector. Level of sensitivity was 0.1 ppb. Confirmation was made by thin layer chromatography. The samples were spotted on silica gel-coated plates and developed in chloroform containing 0.7% ethyl alcohol. Parathion was resolved by spraying with palladium ammonium chloride (0.5 g palladium ammonium chloride and 2 ml conc. HCl in 98 ml distilled water).

Results and Discussion

Samples from the irrigation ditch collected immediately after application contained considerable quantities of parathion (Table 1). The concentration of chemical decreased sharply (92%) after 24 hours, with a subsequent reduction of approximately 50% for each succeeding sample until, after 96 hours, the level was 5 ppb.

TABLE 1.—*Parathion concentrations, in ppb, in impounded irrigation waters and associated drainage waters following treatment of a cranberry bog*[1]

Location	Time (hours)				
	0[2]	24	48	72	96
Irrigation ditch	750.0 (650-850)	60.0 (50-75)	25.0 (10-35)	10.0 (5-20)	5.0 (1-9)
Drainage canal-bog junction	30.0 (15-45)	3.0 (2-8)	1.0 (0.6-1.5)	0.0	0.0
50 yards down from junction	0.0	0.3 (0.1-0.5)	0.0	0.0	0.0
150 yards down from junction	0.0	0.1 (0.08-0.14)	0.0	0.0	0.0

[1] Values reported are the mean of two separate tests with two samples per test. Figures in parentheses represented the range of the four analyses.

[2] Immediately following application.

It is possible that the high residue value obtained in the samples taken immediately after application is a result of the chemical being somewhat stratified and not uniformly distributed throughout the irrigation waters—the lower concentration detected after 24 hours being a [illegible] mixing and dilution rather than actual loss.

[illegible] (2) have reported that only trace [illegible] could be found in irrigation [illegible] application to a rice paddy. The [illegible] closely correlate with their [illegible]

In the [illegible] the presence of parathion at a [illegible] of [illegible] ppb was also demonstrated. [illegible] water seeping through the floodgate introduced the chemical into the untreated area, the difference in concentration between the two areas being a result of dilution. After 24 hours the concentration in the canal-bog junction had decreased to 3.0 ppb, the rate of disappearance being approximately the same as that in the irrigation waters. At this time the chemical was also detected at the locations 50 and 150 yards down from the junction and, although in lesser amounts, demonstrates movement away from the point of application. By the end of 48 hours, parathion could be detected only at the canal-bog junction and by 72 hours the chemical, if present, was below the level of sensitivity.

The presence of parathion in aquatic environments has been shown to cause undesirable effects in the associated biota. Mulla *et al.* (1) reported a high degree of mortality for mosquito fish (*Gambusia affinis* Baird and Girard) in shallow ponds treated with parathion at rates of 1 and 0.4 lb/acre. A 24-hour exposure to a concentration of 65 ppb parathion caused 50% mortality in a population of sheepshead minnows (*Cyprinodon variegatus* Lacepede), and a similar mortality value was recorded for the brown shrimp (*Penaeus aztecus* Ives) following a 24-hour exposure to only 5.5 ppb parathion (4).

It is evident, therefore, that introduction of parathion into non-target areas can occur under the conditions described and at concentrations which could be harmful to certain aquatic organisms. One solution to the problem would be to remove all waters from the bog area prior to chemical application. Where this is not feasible, lowering the water level in the bog to a point where a sudden heavy rain would not necessitate draining to prevent vine injury, and insuring absolute watertight floodgates will greatly lessen the chances of unintentional pollution.

Acknowledgment

This work was supported, in part, by funds provided by the U. S. Department of the Interior as authorized under the Water Resources Act of 1964, Public Law 88-379.

LITERATURE CITED

(1) *Mulla, M. S., J. O. Keith, and F. A. Gunther. 1966.* Pesistence and biological effects of parathion residues in waterfowl habitats. J. Econ. Entomol. 59:1085-1090.

(2) *Santo, R. and H. Kubo. 1965.* The water pollution caused by organo-phosphorus insecticides in Japan. Proc. Second Int. Water Pollut. Res. Conf. Tokyo, 1964. Pergamon Press, New York, N. Y. p. 95-99.

(3) *Teasley, J. I. and W. S. Cox. 1963.* Determination of pesticides in water by micro-coulometric gas chromatography after liquid-liquid extraction. J. Amer. Water Works Ass. 55:1093-1096.

(4) *Pesticide Wildlife Studies. 1963.* U. S. Department of the Interior Circ. 199.

GENERAL

Systemic Activity of Zectran, Matacil, and Bidrin Injected Into Conifer Trunks

John E. Larson[1], G. R. Pieper[1], and H. C. Ratsch[2]

ABSTRACT

Three insecticides—Zectran® [4-(dimethylamino) 3,5-xylyl methyl carbamate], Matacil® [4-(dimethylamino)-m-tolyl methylcarbamate], and Bidrin® (3-hydroxy-N,N-dimethyl-cis-crotonamide dimethyl phosphate)—were tested for systemic activity using spruce budworm as a bioassay organism. The materials were injected into the trunks of Douglas fir and grand fir trees of varying sizes.

In small Douglas fir trees (3 feet or less), movement was sufficient, and high mortality resulted from the injection of these three compounds at rates of 40 and 200 mg per tree. In larger trees (5 to 8 feet) treated with 0.2 to 1.0 g of these chemicals per tree, only Matacil and Bidrin yielded high mortality. Bidrin gave a higher percentage kill than Matacil 10 and 17 days after treatment. But after 38 days, Matacil treatments resulted in higher percentage kill than did Bidrin treatments.

Foliage and wood were analyzed for residues of Zectran and Matacil. Foliage residue levels of 50 ppm and more were consistently found for Matacil. Foliage residue levels of Zectran did not exceed 21 ppm in large trees but reached 308 ppm in the small-tree test. Analysis of trunk sections at points of injection revealed concentrations as high as 8,460 ppm of Zectran.

Poor results with Zectran were probably the result of its partitioning into the oleoresin of tree trunks.

THE U. S. Forest Service has underway an extensive research program aimed at finding safer, more specific chemical treatments for controlling destructive forest insects *(1)*. As part of this program, aerial spray tests with the carbamate Zectran were held on the Bitterroot National Forest in Montana in 1965 and 1966. The target insect was the spruce budworm [*Choristoneura fumiferana* (Clemens)], an important defoliator.

Along with the aerial test in 1966, a study was also made of three systemic insecticides injected into tree trunks. Zectran has previously shown systemic activity when applied to the soil *(2)*. Besides Zectran, Matacil and Bidrin were also tested. Matacil is a close analog of Zectran. Bidrin has been reported to have systemic action in controlling the European elm bark beetle [*Scolytus multistriatus* (Marsham)], vector of the Dutch elm disease *(3)* and of sawfly larvae [*Diprion similis* (Hartig)] in eastern white pine (*Pinus strobus* L.) *(4)*.

Methods and Materials

The test site on the Bitterroot National Forest consisted primarily of young Douglas fir [*Pseudotsuga menziesii* (Mirb) Franco] and grand fir [*Abies grandis* (Dougl.) Lindl.] at 5,000 to 6,000 feet elevation. Trunk injections were made in two ways: (a) in trees less than 5 feet, a small hole was drilled and a tight-fitting glass tube was inserted; (b) in larger trees, a Mauget injector was used *(5)*. Silicone rubber, diluted in heptane, was used to form a leak-proof seal where either device was inserted into the tree. Normally, Mauget capsules are compressed. But in our earlier tests in Montana, there were too many leaks when the capsules were compressed—even with the silicone rubber seals. Therefore, the Mauget injectors were used as gravity feeds by drilling a smaller hole in the lid. In later tests, in which smaller volumes of insecticide solution were uesd, the Mauget capsules were compressed successfully.

Nearly all spruce budworm larvae used in the tests were reared at the Pacific Southwest Forest and Range Experiment Station, Berkeley, Calif., *(Lyon, R. L., C. Richmond, and K. Pennington, unpublished data)* where they were fed on artificial media. However, spruce budworms obtained in the test area were used during one period in June.

The budworms were caged for 5 days before final observations were made. Two cages per tree were first used, but later as many as eight were used. As soon as evidence of budworm mortality was noted in any of these cages, additional cages were placed on the tree to increase sampling accuracy.

Zectran was applied as a 20% or 30% solution in acetone; Matacil, as a 20% solution in acetone; and Bidrin, as the technical form (7.9 lbs/gal or 79% w/w).

[1] Pacific Southwest Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Berkeley, California 94701.

[2] Commissioned Corps, U. S. Public Health Service, Cincinnati, Ohio 45226.

The foliage and wood samples for residue analysis of Zectran and Matacil were collected August 10, 1966, and stored in a coldroom (35 F) at Berkeley until they were prepared for analysis. Foliage samples were analyzed as described by Pieper and Miskus *(6)*. The wood samples were frozen in liquid nitrogen, then pulverized to pass a 30-mesh screen. Each 3-g sample was extracted four times with 30-ml portions of benzene for 10 minutes. After each extraction, the benzene was decanted and filtered through glass wool with a small amount of anhydrous Na_2SO_4.

To determine the water solubilities of Zectran and Matacil, each material in excess (about 20 mg Zectran and 100 mg Matacil) was added to 100 ml water and adjusted to pH 7.0 by adding three drops of Beckman 3581 concentrated buffer solution. The mixture was shaken for 21 hours, and excess insecticide was removed by filtration through a Whatman #1 filter and a #245 Nalgene filter unit (0.45 micron pores). To the Zectran solution was added 10 ml saturated $NaHCO_3$, thereby raising the pH to 9.7. The solution was then extracted three times with 30-ml portions of benzene.

The Matacil solution was diluted a thousandfold with acetone and analyzed directly by microcoulometric analysis for combusted nitrogen.

A partition coefficient for Zectran between oleoresin and water was determined, using ponderosa pine (*Pinus ponderosa* Laws.) oleoresin. Five g of oleoresin and 15 ml of double-distilled water were introduced into a 30-ml Squibb-type separatory funnel. To this amount 0.1 mc of Zectran (carbonyl-C^{14}) (specific activity of 6.3 mc/mmole) in 5 μl of methyl cellosolve was added. After 3 minutes of shaking, the funnel was spun in an International centrifuge (size 2/240 Head) to partially separate the tight emulsion formed between the water and the oleoresin. The water phase was next spun at 97,550 × g for 30 min in a Spinco Ultracentrifuge (Model L, #40 Head). An insignificant number of oleoresin droplets remained in the water phase. One ml aliquots of the water phase and 0.1 ml aliquots of the oleoresin dissolved in toluene were added to a PPO-POPOP-naphthalene-dioxane cocktail and counted in a Packard Tri-Carb liquid scintillation counter.

Results and Discussion

Before the field test in Montana, Zectran and other [illegible] had been injected into the trunks of small [illegible] 12 inches tall) white fir [*A. concolor* (Gord. [illegible]] in a greenhouse study at Berkeley. [illegible] Z[illegible] resulted in high percentage kill [illegible] of kill with Bidrin was less than [illegible] Z[illegible] Applied topically to spruce budworm, Z[illegible] M[illegible] were about equally toxic, and both [illegible] as Bidrin. *(Lyon, R. L. personal* [illegible] *1966)* Zectran was next tried in Montana in a large-scale field test on Douglas fir on May 27, 1966. The trees were divided into four sizes with three different concentrations of Zectran and three replications per treatment. The first spruce budworm bioassay, 2 weeks later, showed poor kill. In mid-June, a light, natural infestation was found in all but two of the treated trees. Although some scattered bioassays of these trees were made later, this test generally resulted in a low degree of mortality. There was incomplete uptake of the Zectran solution in about half the treatments. Bioassay data from these trees had no validity. In the other treatments where uptake was complete, spruce budworm mortality was still unsatisfactory. The most likely reason for this failure was the cool wet weather during June that did not favor rapid transpiration rates.

Warm dry weather conducive to good transpiration prevailed from June 27 to July 18, 1966. Several trunk injections of Bidrin, Matacil, and Zectran were made during this period on Douglas fir and on grand fir that ranged from 5 to 8 feet tall. These tests showed clearly that Bidrin and Matacil gave superior results while results with Zectran were mediocre (Table 1). No phytotoxicity resulted from these treatments. In two of the tests, both the compressed Mauget capsules and the gravity-type feed were used. There was low mortality in Zectran-treated trees whether the capsules were compressed or not. Results with Bidrin- and Matacil-treated trees were sometimes mediocre when the capsules were compressed—a finding different from what had been expected.

Residue data from Zectran-treated trees indicated very poor movement of the chemical from the point of injection, resulting in generally low budworm mortality

TABLE 1.—*Mortality of spruce budworm on 5- to 8-feet tall Douglas fir and grand fir resulting from three trunk-injected insecticides*

Chemical	Application (g/tree)	Replications (No.)	Percent mortality [1] (days after treatment) 3	10	17	24	38
ZECTRAN	1.0	4	—	33	31	30	14
ZECTRAN	0.6	9	0	25	39	44	—
ZECTRAN	0.2	2	—	—	—	20	—
MATACIL	1.0	2	—	—	57	89	92
MATACIL	0.2	2	—	—	—	62	—
BIDRIN	1.0	5	—	100	82	82	73
BIDRIN	0.2	4	—	—	—	82	—
CONTROL	—	4	0	0	0	0	0

[1] Based upon a 5-day exposure of an average of 12 spruce budworms (range 6-24).

TABLE 2.—*Chemical residues from 10 injected trees and results of spruce budworm bioassay on foliage*

Tree No.	Chemical	Application (g/tree)	Height of tree (feet)	Date of treatment	Final bioassay [1]	Residues (ppm)			Original amount remaining at POI [3] (Percent)
						Foliage	Trunk		
							4 feet above POI [2]	At POI [2]	
1	ZECTRAN	7.15	13.8	5/27	6/8	21.1	4.3	8460	51
2	ZECTRAN	7.15	13.0	5/27	2/6	—	2.7	4170	23
3	ZECTRAN	1.0	5.0	6/27	1/6	7.1	0.0	2820	20
4	ZECTRAN	1.0	5.9	6/27	1/8	1.9	1.1	1900	12
5	MATACIL	1.0	6.0	6/27	6/6	753.0	323.0	456	4.2
6	MATACIL	1.0	6.6	6/27	6/7	50.5	10.5	2320	15
7	ZECTRAN	1.5	10.2	7/6	3/7	1.8	0.0	2770	41
8	ZECTRAN	0.6	8.2	7/18	6/6	[4] [3.5 / 7.6]	6.0	2470	25
9	ZECTRAN	0.6	7.5	7/18	0/8	[1.4 / 10.1]	2.0	2670	34
10	ZECTRAN	0.6	7.0	7/18	0/7	[3.5 / 10.4]	67.5	1550	24

[1] Made within 1 week of August 10, 1966, when foliage and wood samples were collected.

Fraction represents: $\frac{\text{No. of dead budworm found after a 5-day exposure}}{\text{Total No. of budworm found after the 5-day exposure}}$

[2] POI = Point of injection. Wood samples collected were 4-inch sections.

[3] Chemical remaining in the 4-inch section.

[4] First figure is residue in top one-third of crown; second figure is residue in bottom one-third.

(Table 2). From the residues of the eight Zectran-treated trees, a large amount of Zectran was found in the 4-inch section at the point of injection. In one instance, 75 days after application, 51% of the original amount placed there still remained (Tree 1, Table 2). At the 4-inch section 4 feet above this point, only 6 ppm or less (with the exception of tree #10) was found. There was a slight accumulation of Zectran found in the comparable foliage samples from these trees. This condition indicates high retention at the point of injection and implies very little movement in the transpiration stream. These residues were 99% free, unmetabolized Zectran.

The bioassay data (Table 2) for trees #1 and #8 suggest rather high toxicity of Zectran to budworm. These bioassays were completed only 3 days before the residue samples were collected. This anomaly may be attributed to the too few caging sites used for the insect. In trees 8, 9, and 10 it is evident that the lower one-third of the crown received more Zectran than did the upper one-third. These results suggest the importance of the location of the cages during the bioassay, and they suggest the likely pattern occurring in the crown from trunk-injected materials. Homogeneity was and probably will always be difficult to attain.

The distribution pattern of Matacil in tree #5 would seem to answer the question: "What distribution of the chemical in trees is sought in trunk-injected systemic insecticides?" The relatively small amount remaining at the point of injection (4.2% of the original amount injected) showed much less retention than was true of Zectran. The superior movement of Matacil in the transpiration stream is strongly indicated by the presence of 323 ppm 4 feet up from the point of injection and by the high accumulation in the foliage (753 ppm). With this type pattern, much less chemical would give satisfactory results. That variations occurred even with Matacil was apparent in the pattern in tree #6, which showed an intermediate pattern between Matacil-treated tree #5 and the Zectran-treated trees. But even here, 50 ppm in the foliage gave a high kill.

To explain these differences in the behavior of these two carbamates, their chemical and physical differences may be considered. Matacil had superior mobility in the transpiration stream. In these tests, penetration of the bark, of course, was not necessary because both chemicals were injected directly into the sapwood of the trees. The formulations were the same (20% w/v in acetone), although in certain instances Zectran was 30% w/v in acetone. When the wood sections were cut for analysis, a large ring of discoloration appeared in the wood around the point of injection. This ring of discoloration was also in control trees receiving only acetone, and so it can be assumed that the discoloration was due to the solvent, acetone.

If it is assumed that Zectran and Matacil were carried with acetone throughout these rings of discoloration, then both insecticides were exposed to considerable resin of the ray parenchyma of Douglas fir and grand fir. In Douglas fir, this region of discoloration would also include the oleoresin of the resin canals. True firs of the genus *Abies* do not normally have resin canals but may form them in response to wounding. Whether this happened to the grand fir trees in this study is not known.

The above consideration would suggest that the more lipophilic compound tended to partition into the lipid phase, i.e., the resin and oleoresin. That this may have happened for Zectran is further substantiated by its low water solubility, 100 ppm, and by the fact that its partition coefficient between oleoresin and water was 80:1. The fact that this partitioning into the lipid phase was not so likely with Matacil is substantiated by its greater water solubility (1200 ppm). No determination of its partition coefficient between oleoresin and water has been made as yet. Since Bidrin is miscible in water, it is assumed that it would not be tightly bound in the lipid phase. The low water solubility of Zectran cannot necessarily be held responsible for its poor ascent in the larger trees. Herbicides with very low water solubilities, such as diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] (42 ppm), simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] (3.5 ppm), and others exert their toxic action only after ascending in the transpiration stream.

Chemically, the only difference between Zectran and Matacil is the presence of $-CH_3$ groups in both meta positions of the benzene ring in Zectran, with only one meta position being occupied by a $-CH_3$ group in the case of Matacil. The remainders of both compounds are identical. The single difference chemically does not affect their toxicity to spruce budworm (i.e., by topical application) *(Lyon, R. L., personal communication, April 1966)*. The differences noted in this study when both chemicals were trunk-injected, seem to be related to their different physical characteristics reflected in their partition coefficients between oleoresin and water.

The foregoing results and discussion of these tests conducted on trees 5 to 8 feet tall gave fairly uniform results and plausible conclusions. However, a final test was made in which nine small Douglas fir (less than 3 feet tall) were injected with Zectran, Matacil, and Bidrin. An attempt was made to duplicate the results from the earlier, previously mentioned greenhouse test conducted in Berkeley. Of the nine trees, six each received 200 mg of each of the three compounds—Zectran, Matacil, and Bidrin. One tree treated by each chemical at this rate was potted and watered regularly to determine if low soil moisture impeded transpiration. Three of the nine trees were injected at the rate of 40 mg of each chemical per tree. All chemicals performed well at either concentration, and the watering had no effect (Table 3).

TABLE 3.—*Mortality of spruce budworm*[1] *after trunk injections of insecticides into small Douglas fir trees*

Chemical	Applications (mg/tree)	Replications (No.)	Percent Mortality (days after treatment) 13	20	27
ZECTRAN	200	2	100	57	100
ZECTRAN	40	1	—	75	100
BIDRIN	200	2	100	100	100
BIDRIN	40	1	—	75	75
MATACIL	200	2	75	100	88
MATACIL	40	1	—	65	100
CONTROL	—	2	0	0	0

[1] Based upon a 5-day exposure to an average of six spruce budworms.

TABLE 4.—*Residue analysis of two small Douglas fir injected with Zectran and Matacil*

Chemical	Dosage/Tree (mg)	Height of Tree (inches)	Final Bioassay[1]	Residue (ppm): Distance of trunk sections above soil level (inches) 0-1	POI[2]	12-15	Top 10 Inches	Foliage[3]
[illegible]	200	34.5	4/4	23	3720	403	2	131 (308)
[illegible]	200	26.0	4/4	92	2480	113	595	437 (631)

[1] [illegible] 1966, when foliage and wood samples were collected. The fraction represents: [illegible] and after a 5 day exposure [illegible] after the 5-day exposure.
[2] POI = P[illegible]. In Zectran trial, POI was 5- to 6-inch section; in Matacil, it was 2.5- to 3.5-inch section.
[3] [illegible] after November 21, 1966, when wood analysis was made. Figure in parentheses is the earlier determination [illegible] 1966.

A residue analysis (Table 4) was made of two entire trees, one treated with Zectran and the other with Matacil at 200 mg each. The striking difference here was that the terminal 10 inches of the Zectran-treated tree contained only 2 ppm, and the same section of the Matacil-treated tree contained 595 ppm. The foliage analysis was high in both cases, but the Zectran-treated tree showed considerable increase in residue from that shown in the foliage analysis of larger trees. In an attempt to explain the behavior of Zectran in this test, it may be pointed out that the distance involved was much shorter and that young Douglas fir trees contain less oleoresin. However, the results of this test did confirm the results of the earlier greenhouse test in Berkeley on trees of similar size. It should be noted that the results from small trees in greenhouse tests may be misleading if the research is later to be applied to a field test.

A root analysis was made only of the Matacil-treated tree from this small-tree test. The roots were all deeper than 4 inches below the soil surface. The residue found was only 1.7 ppm. This low value occurring 35 days after application indicated essentially no recirculating by Matacil in the phloem tissue. The ability to translocate in the phloem is considered a very desirable property in a systemic chemical. One such compound with this proprty is the herbicide, amitrole (3-amino-1,2,4-triazole). *(Crisp, C. E., D. E. Bayer, H. C. Ratsch, and R. K. Glenn. Comparative tests on the uptake and distribution of labelled insecticides by* Pinus ponderosa, Pseudotsuga menziesii, *and* Abies concolor. *Unpublished data on file at Pacific Southwest Forest and Range Experiment Station, Berkeley, California.)* Finding a systemic insecticide having both apoplastic and symplastic mobilities, such as amitrole, could be a significant breakthrough in systemic insecticide research.

Acknowledgment

The authors are indebted to Mr. Richard Tierney and Mr. R. A. Youngberg for their technical help and to Dr. C. E. Crisp for his critical reading of the manuscript.

The Mauget injectors used were donated by J. J. Mauget of the Zero Mfg. Co., Burbank, California.

LITERATURE CITED

(1) *U. S. Forest Service. 1965.* Evaluation of chemicals for control of forest insects. Pacific Southwest Forest and Range Exp. Sta., Berkeley, Calif. 8 p., illus.

(2) *Kenaga, E. E., A. E. Doty, and J. L. Hardy. 1962.* Laboratory insecticidal tests with 4-dimethylamino-3,5-xylyl methylcarbamate. J. Econ. Entomol. 55:466-9.

(3) *Norris, Dale M., Jr. 1960.* Systemic insecticidal action in the cortical tissue of elm twigs. J. Econ. Entomol. 53:1034-6.

(4) *Coppel, Harry C., and Dale M. Norris, Jr. 1961.* Systemic insecticidal action of certain phosphates in Pinus strobus against Diprion similis. J. Econ. Entomol. 54:1061-2.

(5) *Coppel, Harry C., and D. M. Norris, Jr. 1966.* Bark penetration and uptake of systemic insecticides from several treatment formulations in white pines. J. Econ. Entomol. 59:928-31.

(6) *Pieper, G. R., and R. P. Miskus. 1967.* Determination of Zectran® residues in aerial forest spraying. J. Agr. Food Chem. (Accepted for publication).

Problems in Monitoring DDT and Its Metabolites in the Environment

Donald A. Spencer[1]

ABSTRACT

DDT is degraded to less harmful compounds by a number of biological and chemical factors in the environment, which conversion can take place in as little as a few hours. Involved are: (1) bacteria, soil fungi, and other microorganisms, (2) enzymatic action, and (3) conversion by reduced porphyrins. Biological samples should be acquired as quickly after the death of the organism as possible. If too long a period at seasonal temperatures has elapsed, there is little value in reporting the ratio of metabolites. Biological decomposition should be arrested by cold storage or dry processing within a few hours after collection is made. Avoid anaerobic conditions of storage, even at −20 C. Shorten the period between collection of sample and chemical analysis. In every case, report the interval and condition of storage.

RESIDUE problems from persistent pesticides such as DDT have generated a renewed interest in the means by which these organic compounds can be degraded and removed from the environment. Research emphasis in the past 4 years has focused on the role of microorganisms in converting DDT to progressively less toxic metabolites, and the mechanisms by which man, domestic animals, fish, and wildlife store, metabolize, and excrete the DDT-complex. Paradoxically, in the nationwide monitoring programs for pesticide residues in the environment there is a need to arrest these very same degradation processes in the interval between the collection of the sample and its eventual chemical analysis.

Bacteria and certain other microorganisms are highly effective in converting DDT to TDE, then more slowly continuing the degradation to simpler compounds. In an excellent paper recently presented to the Water Pollution Control Federation Meetings in Kansas City, Hill and McCarty [illegible] of Stanford University studied the degradation of DDT, TDE, lindane, aldrin, dieldrin, [illegible] endrin by sewage sludge. The active [illegible] methane producing and sulphate-[illegible] produce conditions where fatty acids are [illegible] DDT was converted to TDE almost immediately (DDT was detected only in samples taken 20 minutes after injection into anaerobic sludge held at 35 C). TDE then underwent further degradation, showing a half life of about 4 days. When DDT was injected into the same culture daily at 1.0 ppm for 57 consecutive days, instead of accumulating, the rate of conversion of DDT to lower metabolites improved. Following larger doses of DDT (100 ppm) there was slower degradation of TDE, ". . . possibly because a complexing capacity of the sludge for TDE became saturated, or because degrading organisms became poisoned."

Cope and Sanders *(2)* became interested in the possibilities of altering the structure of pesticides in water so as to reduce the hazard to fish. They experimented with five species of bacteria and found that four of them (*Micromonospora chalcea, Pseudomonas aeruginosa, P. fluorescens,* and *Corynebacterium pyogenes*) "appreciably reduced" concentrations of DDT in water within the period of 7 to 16 days. In studies with isotope-labeled DDT these investigators found that a high portion of the DDT present was taken up by the bacterial cells or by contaminating protozoans which were present, and that one or both microorganisms "apparently metabolized DDT to TDE and possibly to DDE."

At McGill University in Quebec, Barker and Morrison *(3)* isolated several microorganisms from the gut of a DDT-resistant mouse and plated them out on agar-brain-heart infusion media. When DDT was added and the cultures incubated for 5 days at 30 C, one isolate, *Proteus vulgaris*, dechlorinated DDT to TDE. Since this bacterium is one of the primary invaders of animal tis-

[1] [illegible] Chemical Association, 1155 Fif-[illegible] D.C. 20005

The chemical names of compounds mentioned in this paper are:

DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
Lindane	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-exo-5,8-dimethano=naphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene

sues after death, it is of particular import in the handling of samples for residue analysis. Mendel and Walton *(4)*, working in the pharmacological laboratories of the Food and Drug Administration, Washington, D. C., on a study of the coliform bacteria in the intestines of rats, clearly demonstrated the role of *Escherichia coli* and *Aerobacter aerogenes* in conversion of DDT. *p,p'*-DDT was administered to one series of rats by stomach tube and to another by intraperitoneal injection. Feces were collected for 48 hours. From the rats receiving *p,p'*-DDT by stomach tube the major chlorinated pesticide in the feces was TDE with, at most, a trace of DDT. When these rats were sacrificed 48 hours after dosing, the ratio of DDT to TDE in the livers ranged from 1:1 to 3:1. In rats where the gastro-intestinal track was by-passed by intraperitoneal injection, essentially no chlorinated pesticides were found in the feces collected in the first 48 hours, and the livers of these rats had a 24:1 DDT:TDE ratio. In another phase of the investigation cultures of *E. coli* and *A. aerogenes* were maintained for 24 hours at 37 C resulting in the conversion of 35% to 50% of the introduced DDT to TDE. No more than 30% to 55% of the chlorinated pesticide could be accounted for as DDT or TDE, yet no such loss was recorded when DDT was incubated with plain culture medium, indicating that the bacteria had caused the production of other unidentified degradation products.

Stenersen *(5)*, working at Oslo University for the Norwegian Plant Protection Institute, isolated three bacteria, *Serratia marcescens*, *E. coli*, and an unidentified strain, from the excrement of flies. These were grown on bouillon containing C^{14}-labeled DDT *under nitrogen*. "All converted DDT almost completely to TDE (90%) and DDE (5%)."

In the Department of the Interior's Fish Pesticide Laboratory at Denver, Wedemeyer *(6)* chose the facultative anaerobe, *A. aerogenes*, for detailed studies. Grown under anaerobic conditions in trypticase-soy broth containing 5.0 ppm DDT, this bacterium effects up to 80% conversion to TDE. In a refinement of the study, *A. aerogenes* bacteria were disrupted sonically, resulting in a cell-free system. Under these conditions the average conversion to TDE was about 70%, leading Wedemeyer to conclude that reduced cytochrome oxidase is probably the cellular agent in reductive dechlorination of DDT to TDE.

Johnson *(16)* at the Bureau of Commercial Fisheries, Biological Laboratory, has now extended our information on bacterial degradation by studies on the marine bacteria *(Pseudomonas piscicida)*. The uptake of DDT from culture media by this bacteria is very rapid. Within 48 hours, TDE and DDE within the cell walls of the bacteria constitute 25% of the total DDT-complex. Also significant in this study of isotope-labeled DDT is the finding that both bacteria and oysters are capable of metabolizing small fractions of DDT so completely that it becomes part of the metabolic pool and is utilized in the biochemistry of the cells.

Chacko, Lockwood, and Zabik *(7)* at Michigan State University in 1966, demonstrated for the first time the ability of certain aerobic soil fungi (Actinomycetes: *Nocardia erythropolis*, and five species of *Streptomyces*) to convert DDT to TDE. The test organism was cultured for 6 days in a nutrient medium containing 5 to 10 μg/ml DDT resulting in 25% conversion of DDT to TDE in 6 days.

Johnson *et al. (17)* found that 23 out of 27 pathogenic and saprophytic bacteria associated with plants could convert from a trace to better than 50% of *p,p'*-DDT to *p,p'*-TDE in a space of 2 weeks under anaerobic conditions. In the majority of cases, the pace of conversion quickened during the second week.

Clear Lake in California is a large, relatively shallow, warm body of water (41,600 surface acres) with bottom deposits of soft, deep, black ooze. The lake is rather turbid most of the time *(8)*. Miskus, Blair, and Casida *(9)* in 1965 collected a sample of this lake water near Lakeport, introduced ring-labeled C^{14}-DDT at 0.01 ppm and incubated it in a stoppered flask for 7 days at room temperature. In the 1-week time, 70% to 80% of the DDT was converted to TDE. The results were verified by two additional methods. Six additional samples were collected later from various parts of the lake and similarly tested. These samples varied markedly in the amounts of DDT converted to TDE, but the two samples that contained large amounts of plankton converted 83% and 95%, respectively, of DDT to TDE within the week.

To this point, the importance of biological systems in the degradation of DDT has been stressed. There is, however, no agreement that this is the only factor, or even the most important. Ecobichon and Saschenbrecker *(10)*, working at the University of Guelph in Ontario, Canada, repeated the analysis of a frozen sample of avian blood that they had studied 3 weeks before, by mistake. The ratio of DDT to its metabolites TDE and DDE were so different from their original readings that it presented a possible source of error in analytical procedures. They then prepared a single large sample of heparinized chicken blood, introduced an acetone solution of technical DDT at 1.0 ppm, sealed the flask and stored it at —20 C. Each week the sample was removed, quickly thawed, a 2-ml subsample removed for analysis, and the basic sample returned to —20 C. This was repeated for 12 consecutive weeks. Both *p,p'*-DDT and *o,p'*-DDT completely disappeared by the 10th week while DDE and TDE increased in quantity until the 7th week and then in turn began to slowly decrease. At the same time, a plasma sample that contained DDT serving as a control showed no evidence of degradation. With the repeated freezing and thawing the erythrocytes in the blood were hemolyzed, thus exposing the insecticides to high concentrations of free hemoglobin. The authors suggest that tissues and microorganisms which contain large quantities of reduced coenzymes, porphyrins, and other metalloproteins could carry out these steps by simple chemical redox reaction.

Castro *(11)* at the University of California, Riverside, exposed dilute solutions of iron porphyrins (Fe^{++} deuteroporphyrin) to DDT at room temperatures. The porphyrin complex was rapidly oxidized. Castro points out that low-valent iron porphyrin complexes are manifest in all aerobic organisms.

Miskus, *et al. (9)* at the University of California, Berkeley, were also interested in the role of reduced porphyrins. They added C^{14}-labeled DDT to solutions of hemoglobin or of hematin in a Thunberg tube and shook the mixture for 4 hours at room temperature. No conversion took place unless the color remained red, representing the state of reduced porphyrins. By adding sodium dithionite to the hemoglobin mixture the conversion of DDT to TDE ranged from 60% to 75%.

At the Monks Wood Experimental Station in England, Jefferies and Walker *(12)* were studying the acute and chronic toxicity of *p,p'*-DDT to Bengalese finches by feeding caged birds concentrations of pure DDT in their diet. Birds that died, or were sacrificed at different periods, were placed in −11.5 C to −14.5 C refrigeration. The livers of two treated birds were analyzed within 10 minutes of death and the ratio of DDT:TDE was 100:1, a negligible conversion. Thereafter at different intervals, groups of treated birds were withdrawn from refrigeration, dissected, and the livers analyzed. By the 67th day in cold storage, the conversion of DDT to TDE was 1:1 — fairly convincing evidence that cold storage of a little over 2 months permits significant changes in the ratio of DDT with that of its metabolites.

At Mississippi State University, Walley, Ferguson, and Culley *(13)* have attempted to segregate the chemical and bacterial factors as they pertain to the degradation of DDT in the liver *in vitro*. Livers were removed by sterile techniques from newly sacrificed birds, the livers sliced and transferred to cultural vials containing a sterile medium in which 50 μg of purified *p,p'*-DDT had been added. Incubated at 37 C, subsamples showed the presence of TDE and traces of DDE by the end of 24 hours. By 96 hours much of the DDT had been converted to TDE. No bacterial contamination could be demonstrated in the liver cultures at the end of the study. Again, the capability of tissues, independent of living microorganisms, to convert DDT to lower metabolites is indicated. Basically there are three factors responsible for the degradation of *p,p'*-DDT, and the shift in ratio between metabolites: (1) the continuing activity of bacteria and other microorganisms, (2) enzymatic action, and (3) conversion by reduced porphyrins. The activity of bacteria and reduced porphyrins to convert DDT is greatly enhanced by anaerobic conditions — which is quite characteristic of the handling of many samples for residue analysis.

Most programs today that monitor pesticide residues in the environment attempt not only to analyze for DDT, TDE, and DDE, but both the *para-para* and *ortho-para* isomers of all three. The cost of chemical analysis and the time required to make such studies is appreciably greater than simply searching for *p,p'*-DDT. Nevertheless there is considerable merit in the new approach. Not only do the metabolites of DDT have less toxicity for man, domestic animals, and wildlife, but the *ortho-para* isomer of DDT is also less toxic by a factor of 5 to 9 times in tests with rats *(14)*. These six pesticides also differ in their persistence in the environment, the rate of storage in neutral fat, the elimination of residue from fat depots, and the routes by which they are degraded *(15)*.

The problems of monitoring the DDT-complex in the environment begin with the choice of samples. Too often samples are collected an unknown time *after* the death of the organism, when it was the actual metabolites present at the time of death that were important. For example, a fish kill is reported in a stream draining cotton fields on which pesticides have been used. Frequently it is several days to a week before responsible investigators reach the scene to collect samples. At summer temperatures, conversions of the insecticide progress very quickly as some of the foregoing laboratory studies indicate. In other cases the program may make use of parts of game animals contributed by hunters from broad sections of the country. There is considerable lag at seasonal temperatures before these samples can be properly stored or processed. But it can also be the deliberate action of an investigator who eventually collects specimens of eggs "one week past expected hatching date in advanced state of decomposition." There is little point to reporting metabolites in fractional parts per million at this late date if the study is concerned with the possible effect on hatching success of the egg.

However, most samples collected for monitoring pesticide residues correctly reflect the ratio of metabolites at the time: silts from a stream bed, muck from a tidal marsh, water and its suspended organic matter, vegetation, pools of small invertebrates, even blood and fat samples taken by biopsy. But the collector is commonly 4 to 8 hours away from facilities for processing or otherwise storing his samples. Deep freeze is commonly employed for checking any further spoilage of biological samples; however, certain bulky samples such as soil, muck soils, water with suspended organic matter, and vegetation often lack this protection. In fact, some bottom sediments, sealed in metal or glass containers have exploded from gases generated by anaerobic biological action before the sample could be handled by the chemist. Lastly, the field collection commonly is a seasonal matter, and numbers of samples sufficient to keep a residue analysis laboratory busy for a whole year are collected in a matter of weeks. It is a fact that samples have been held 1 to 2 years in crowded chest-type refrigerators awaiting analysis.

LITERATURE CITED

(1) *Hill, David W., and Perry L. McCarty. 1966.* The anaerobic degradation of selected chlorinated hydrocarbon pesticides. Presented: Water Pollut. Contr. Fed. Meeting, Kansas City.

(2) *Cope, O. B., and H. O. Sanders. 1963.* Microorganisms and hydrocarbons. USDI: Fish and Wildlife Circ., Pesticide-Wildlife Studies. 167:27.

(3) *Barker, P. S., and F. O. Morrison. 1965.* The metabolism of TDE by Proteus vulgaris. Canad. J. Zool. 43:652-654.

(4) *Mendel, J. L., and M. S. Walton. 1966.* Conversion of *pp'*-DDT to *pp'*-DDD by intestinal flora of a rat. Science 151(3717):1527-1528.

(5) *Stenersen, J. H. V. 1965.* DDT-metabolism in resistant and susceptible stable flies and in bacteria. Nature (London) 207(4997):660-661.

(6) *Wedemeyer, Gary. 1966.* Dechlorination of DDT by Aerobacter Aerogenes. Science 152(3722):647.

(7) *Chacko, C. I., J. L. Lockwood, and M. Zabik. 1966.* Chlorinated hydrocarbon pesticides: Degradation by microbes. Science 154(3750):893-895.

(8) *Hunt, Eldridge G., and Arthur I. Bischoff. 1960.* Inimical effects on wildlife of periodic DDE applications to Clear Lake. Calif. Fish and Game 46(1): 91-106.

(9) *Miskus, Raymond P., Deanna P. Blair, and John E. Casida. 1965.* Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. J. Agr. Food Chem., 13(5):481-483.

(10) *Ecobichon, D. J., and P. W. Saschenbrecker. 1967.* Dechlorination of DDT in frozen blood. Science 156(3775):663-665.

(11) *Castro, C. E. 1964.* The rapid oxidation iron (Fe^{++}) porphyrins by alkyl halides. A possible mode of intoxication of organisms by alkyl halides. J. Amer. Chem. Soc. 86(11):2310-2311.

(12) *Jefferies, D. L., and C. H. Walker. 1966.* Uptake of *pp'*-DDT and its post mortem breakdown in the avian liver. Nature (London) 212(5061):533-534.

(13) *Walley, W. Wayne, Denzel E. Ferguson, and Dudley D. Culley. 1966.* The toxicity, metabolism, and fate of DDT in certain icterid birds. J. Miss. Acad. Sci. XII:281-300.

(14) *Dale, W. E., M. F. Copeland, G. W. Pearce, and J. W. Miles. 1966.* Concentration of *op'*-DDT in rat brain at various intervals after dosing. Arch. Int. Pharmacodyn. 162(1):40-43.

(15) *Menzie, Calvin M. 1966.* Metabolism of pesticides. USDI Fish and Wildlife Serv., Spec. Sci. Rep. No. 96, 274 p., May.

(16) *Johnson, Robert F. 1967.* Food chain studies. USDI, Bur. Commercial Fish. Circ. 260, p. 9-11.

(17) *Johnson, B. Thomas, Robert N. Goodman, and Herbert S. Goldberg. 1967.* Conversion of DDT to DDD by pathogenic and saprophytic bacteria associated with plants. Science 157(3788):560-561.

Information for Contributors

The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

The publication is distributed principally to scientists and technicians associated with pesticide monitoring, research, and other programs concerned with the fate of pesticides following their application. Additional circulation is maintained for persons with related interests, notably those in the agricultural, chemical manufacturing, and food processing industries; medical and public health workers; and conservationists. Authors are responsible for the accuracy and validity of their data and interpretations, including tables, charts, and references. Accuracy, reliability, and limitations of the sampling and analytical methods employed must be clearly demonstrated through the use of appropriate procedures, such as recovery experiments at appropriate levels, confirmatory tests, internal standards, and interlaboratory checks. The procedure employed should be referenced or outlined in brief form, and crucial points or modifications should be noted. Check or control samples should be employed where possible, and the sensitivity of the method should be given, particularly when very low levels of pesticides are being reported. Specific note should be made regarding correction of data for percent recoveries.

- Preparation of manuscripts should be in conformance to the STYLE MANUAL FOR BIOLOGICAL JOURNALS, American Institute of Biological Sciences, Washington, D. C., and or the STYLE MANUAL of the United States Government Printing Office.

- An abstract (not to exceed 200 words) should accompany each manuscript submitted.

- All material should be submitted in duplicate (original and one carbon) and sent by first-class mail in flat form—not folded or rolled.

- Manuscripts should be typed on $8\frac{1}{2}$ x 11 inch paper with generous margins on all sides, and each page should end with a completed paragraph.

- All copy—including tables, and references, should be double spaced, and all pages should be numbered. The first page of the manuscript must contain authors' full names listed under the title, with affiliations, and addresses footnoted below.

- Charts, illustrations, and tables, properly titled, should be appended at the end of the article with a notation in text to show where they should be inserted.

- Charts should be drawn so the numbers and texts will be legible when considerably reduced for publication. All drawings should be done in black ink on plain white paper.

- Photographs should be made on glossy paper. Details should be clear, but size is not important.

- The "number system" should be used for literature citations in the text. List references alphabetically, giving name of author/s/, year, full title of article, exact name of periodical, volume, and inclusive pages.

Pesticides ordinarily should be identified by common or generic names approved by national scientific societies. The first reference to a particular pesticide should be followed by the chemical or scientific name in parentheses—assigned in accordance with CHEMICAL ABSTRACTS nomenclature. Structural chemical formulas should be used when appropriate. Published data and information require prior approval by the Editorial Advisory Board; however, endorsement of published information by any specific Federal agency is not intended or to be implied. Authors of accepted manuscripts will receive edited typescripts for approval before type is set. After publication, senior authors will be provided with 100 reprints.

Manuscripts are received and reviewed with the understanding that they previously have not been accepted for technical publication elsewhere. If a paper has been given or is intended for presentation at a meeting, or if a significant portion of its contents has been published or submitted for publication elsewhere, notation of such should be provided.

Correspondence on editorial and circulation matters should be addressed to: *Mrs. Sylvia P. O'Rear*, Editorial Manager, PESTICIDES MONITORING JOURNAL, Pesticides Program, National Communicable Disease Center, Atlanta, Georgia 30333.

The *Pesticides Monitoring Journal* is published quarterly under the auspices of the Federal Committee on Pest Control and its Subcommittee on Pesticide Monitoring as a source of information on pesticide levels relative to man and his environment.

The parent committee is composed of representatives of the U. S. Departments of Agriculture, Defense, the Interior, and Health, Education, and Welfare.

The Pesticide Montoring Subcommittee consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Federal Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Federal Water Pollution Control Administration, Food and Drug Administration, Public Health Service, and the Tennessee Valley Authority.

Responsibility for publishing the *Pesticides Monitoring Journal* has been accepted by the Pesticides Program of the Public Health Service.

Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the Pesticide Monitoring Subcommittee which participate in operation of the national pesticides monitoring network, are expected to be principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernment research institutions, both within and without the United States. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the Monitoring Subcommittee. Authors are given the benefit of review comments prior to publication.

Trade names appearing in the *Pesticides Monitoring Journal* are for identification only and do not represent endorsement by any Federal agency.

Address correspondence to:
Mrs. Sylvia P. O'Rear
Editorial Manager
PESTICIDES MONITORING JOURNAL
Pesticides Program
National Communicable Disease Center
Atlanta, Georgia 30333

CONTENTS

Volume 1 December 1967 Number 3

EDITORIAL

Units for Reporting Pesticides

The choice of units for reporting pesticide residues and concentrations is largely arbitrary but is influenced by custom, the magnitude of values commonly measured, and the nature of the environment.

Custom is perhaps the strongest influence in scientific as well as in lay circles. If residues in soils are customarily reported in parts per million, the tendency will be to continue the practice unless convincing reasons are presented to change to other units.

The magnitude of values commonly measured generally dictates the size of the unit selected but not the system. For example, milligrams per kilogram (mg/kg) will serve very well for values in the range of 1 to 1,000 mg, but micrograms per kilogram (μg/kg) would be better if the values are in the range of 0.001 to 0.1 mg which is equivalent to 1 to 100 μg. However, choice of the metric (dimensional) system of milligrams and micrograms per kilogram rather than the nonmetric (dimensionless) system of parts per million and parts per billion is an arbitrary decision based on custom or preference.

It has been pointed out that parts per million can be considered metric or nonmetric. This is true. For simplicity in this discussion, however, milligrams, micrograms, etc., will be considered metric and parts per million, parts per billion, etc., nonmetric.

The nature of the environment for which pesticides are being reported is also a factor in the choice of units. For example, in the water environment, concentrations generally are measured in units from one to two orders of magnitude smaller than those measured in soils, food, and fish and wildlife. Thus, the principal Federal agencies that measure pesticides in water have chosen micrograms per liter (μg/l) as the primary unit for reporting pesticide concentrations. Micrograms per kilogram (μg/kg) is used for concentrations in water-associated sediments. A canvass was made of the Federal agencies represented on the Monitoring and Research Subcommittees of the Federal Committee on Pest Control to ascertain presently used units for reporting pesticide residues and concentrations. Of 12 respondents, 6 preferred using metric units, 3 preferred nonmetric units, and 3 had no strong preference.

Those agencies that prefer nonmetric units have frequent contacts and dealings with nontechnical people who have become accustomed to these units. There is strong reluctance to convert to different units that might cause confusion. This attitude may be valid for the short haul. However, if the United States is ever going to change to metric units for all measurements, it seems clear that both public and private agencies and groups will have to provide examples by getting on the bandwagon.

The Editorial Board of the Journal is not contemplating requiring authors to report pesticide residues and concentrations in any particular system or unit. Authors know, or should know, what units are best suited to the profession and to the reader audience. However, the Board strongly recommends the use of metric units wherever possible. Furthermore, to aid in transition from nonmetric to metric, it will be acceptable practice to report values in metric followed by nonmetric values in parentheses. For example, 200 mg/l (200 ppm). The decision as to this form of expression versus the customary practice will be the author's.

More than one system of units or orders of magnitude in a single system should be avoided in a given paper. It is particularly confusing to use two or more orders of magnitude in a single table. For example, micrograms per liter (μg/l) and milligrams per liter (mg/l) should not be used in the same table. Erroneous impressions are easily formed by the reader in such instances.

Metric (dimensional) units have the advantage of showing actual weights of pesticides, whereas nonmetric (dimensionless) units do not. Knowledge of actual weights of specific pesticides in an environment is significant to many investigators. Nearly all continental European countries and many others throughout the world use metric units.

Views of Journal readers will be welcomed by the Editor. If there is sufficient reader interest in this and other topics related to monitoring pesticides, it may be desirable to include a section in the Journal on "Communications to the Editor."

S. K. Love
Member, Editorial Advisory Board

RESIDUES IN FOOD AND FEED

Chlorinated Pesticide Residues in Fluid Milk and Other Dairy Products in the United States

R. E. Duggan[1]

ABSTRACT

The findings on 12,836 objective samples of milk and dairy products examined by the U. S. Food and Drug Administration from domestic and imported lots during the period July 1, 1963, through June 30, 1966, are reported. A majority of the samples contained pesticide residues. Residues of DDT, DDE, TDE, dieldrin, heptachlor epoxide, BHC, lindane, aldrin, heptachlor, and methoxychlor account for 99.3% of the residues. About 95% of the values were below 0.51 ppm on a fat basis, and 71.5% of the values were below 0.11 ppm on a fat basis. The average level for DDT and its analogs was 0.134 ppm on a fat basis, slightly more than one-tenth the legal tolerance of 1.25 ppm for the combined DDT compounds. The average levels for dieldrin and heptachlor epoxide were 0.042 and 0.036 ppm, fat basis, respectively—slightly more than one-tenth of the current administrative guides for each chemical. The levels and kinds of pesticides are in good agreement with the findings on total diet samples examined during this period.

Introduction

Recently, tolerances were established for DDT and its analogs, singly or combined, at levels of 0.05 ppm in fluid milk and at 1.25 ppm in the fat of other dairy products. Tolerances were requested by a petition submitted by the State of California. Following a review of the petition and evaluation of other data, a Committee appointed by the National Academy of Sciences recommended that these tolerances be established.

In 1957 and 1959, Clifford *et al.*, *(1, 2)* reported results of surveys by the Food and Drug Administration on residues of pesticides in market milk. Since that time, major advances in gas-liquid chromatography and other improvements have been made in the methods of analysis used to determine the kind and quantity of residues in milk. Thus, we do not consider the earlier data comparable to current findings because of significant changes in analytical procedures.

[1] [illegible] Compliance, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20201

The principal purpose of this paper is to report and evaluate the findings on 8,548 samples of fluid milk and 3,598 samples of other dairy products examined by the U. S. Food and Drug Administration between July 1, 1963, and June 30, 1966, within the United States. The findings on 690 samples of manufactured dairy products imported into the United States from 29 countries and examined for pesticide residues are also reported.

Sampling Procedures

Samples of fluid milk and manufactured dairy products were collected nationwide as a part of FDA's surveillance program on pesticide residues in foods. The program predetermined the total number of samples to be collected at each of its 18 District Laboratories but did not specify how many of these were to be "objective" versus "selective." The selection of sampling points and the scheduling of samples also was left to the discretion of the District offices. In the surveillance program, samples classified as "objective" are unknown with respect to suspicion of residue content or actual misuse of pesticide chemicals. Samples collected because of suspected excessive residues are classified as "selective." Only the "objective" samples are included in this report.

Fluid milk samples were collected from bulk tank trucks, bulk storage tanks at milk and dairy product plants, and from stocks of bottled milk. Therefore, each sample represents composites from one or more dairy herds.

The samples of domestic manufactured dairy products (butter, cheese, condensed milk, frozen desserts, and other products) were collected under similar program instructions.

Samples of imported dairy products were collected from shipments at the time the products were offered for entry.

The physical sample was taken after mixing fluid milk, or by removing several portions of solid products, for compositing in the laboratory. Several units of products in containers were collected as a sample. Where codes or batch numbers were used, each batch was sampled separately.

Samples were collected from 45 States in FY 1964, 47 States in FY 1965, and from 42 States in FY 1966. Samples were collected from the District of Columbia each year. No samples were obtained from Alaska, and only one sample was reported from Hawaii.

Analysis

Generally, samples were examined promptly after collection.

All analyses were performed in FDA District Laboratories using multi-residue gas-liquid chromatographic methods. Microcoulometric and electron capture detectors were employed. The official A.O.A.C. method *(3)* to detect multi-residues was used in FY 1965 and 1966. The quantitative sensitivity of 0.25 ppm (fat basis) was based on ½ full-scale deflection (1 x 10^{-9} AFS) for 1 nanogram of aldrin. These procedures are described in detail in FDA's Pesticide Analytical Manual—Volume I *(4)*. Residues above these sensitivity levels were confirmed by thin layer chromatography. Quantitative figures are reported below these sensitivities but were not confirmed by check analysis and are recognized as having reduced accuracy common to all quantitative estimations at the lower ranges of method sensitivity. All FDA laboratories participated in a collaborative study *(5)* of the method using heptachlor epoxide and dieldrin. This study showed an average recovery of 113% for heptachlor epoxide and 95.9% for dieldrin. Standard deviations of ±0.039 ppm at the 0.29 ppm level for heptachlor epoxide and ±0.052 ppm at the 0.26 ppm level for dieldrin were reported. Additional data are being published *(6)* describing the application of this method to other pesticide chemicals. All results are reported on a fat basis, and no correction for recovery has been made.

Results

Although a majority of the samples were collected within milk-producing States, some were not. In order to evaluate the distribution of the sampling, the samples were grouped in Table 1 according to the U.S.D.A. Crop Reporting Divisions for comparison with milk production *(7)*.

A total of 12,836 samples were collected and examined during the 3-year period, distributed by year and product class as shown in Table 2.

Residues were reported in 7,346 (57%) of the total samples examined. More than one pesticide chemical was found in 5,154 samples.

The percent of samples containing residues and multiple residues are shown for each year in Table 3.

TABLE 1.—*Comparison of samples and incidence of residues with production of milk*

Division[1]	Percent Production FY 1966	Total Samples		Percent Distribution of Samples by Fiscal Year		
		Percent Distribution	Percent Containing Residues	1964	1965	1966
North Atlantic	19.3	16.1	74.3	12.8	17.2	20.8
E. North Central	28.8	27.9	30.3	29.3	23.9	29.6
W. North Central	20.9	15.6	48.7	16.4	13.2	17.0
S. Atlantic	7.0	13.4	68.0	12.8	15.9	11.5
S. Central	10.6	15.4	74.2	14.9	17.1	14.2
West	13.3	11.7	77.6	13.8	12.7	6.8

1 U. S. Department of Agriculture, Crop Reporting Divisions.

TABLE 2.—*Distribution by year and product class of 12,836 samples collected and examined during fiscal years 1964-1966*

Samples	Percent of Total Samples		
	1964	1965	1966
DOMESTIC:			
Fluid Milk	28.7	18.6	19.3
Mfd. Dairy Products	14.9	8.4	4.8
IMPORTED:			
Mfd. Dairy Products	2.1	1.3	1.9
TOTAL	45.7	28.3	26.0

TABLE 3.—*Percent of samples, by fiscal year, containing residues and multiple residues*

Samples	Percent Samples with Residues			Percent Samples with Multiple Residues		
	1964	1965	1966	1964	1965	1966
DOMESTIC:						
Fluid Milk	41.0	72.8	69.3	24.9	51.6	50.7
Mfd. Dairy Products	47.8	68.1	62.8	36.2	52.4	42.9
IMPORTED:						
Mfd. Dairy Products	38.0	55.8	60.4	22.4	33.7	45.6

Ten chemicals (DDE, dieldrin, DDT, heptachlor epoxide, TDE, BHC, lindane, aldrin, heptachlor, and methoxychlor) account for 99.3% of the residues. Twenty-three other pesticide chemicals representing 131 residues also were found. However, except for pentachlorophenol found in 20 samples, these were found too infrequently to be considered significant.

Table 4 shows the incidence of the above 10 specific residues in percent of total samples. Since more than one residue is found in many samples, the total exceeds 100%. The factor for number of residues per sample was 1.5 based on all samples and 2.6 based on the positive samples only.

Table 5 shows the percent of residues at arbitrarily selected ranges in levels of residues and is based on the total number of residues of the specific chemical found. The percent of residues in the various ranges was relatively uniform between fluid milk and manufactured dairy products, both domestic and import. There was a definite break between the range 0.11-0.50 ppm and the next higher range of 0.51-1.00 ppm. Ninety-five percent of all residues were below 0.51 ppm, and 71.5% were below 0.11 ppm.

The average pesticide level for each chemical shown in Table 5 includes all samples and was calculated by using the mid-point of each range and the percent of samples falling in the range. The actual average values were used for the range exceeding 2.00 ppm. The standard deviation and 95% confidence limits are shown for each chemical. The large standard deviation is not unexpected because of the large number of negative samples. The large number of negative findings and low values must be considered in using the standard deviation.

Table 6 shows the percent distribution of residues, by year and product class, in different quantitative ranges. This information is shown for total residues, as well as for residues of individual chemicals.

Generally, there are no significant changes in the incidence and relative levels of residues when individual chemicals are considered on an annual or commodity basis.

TABLE 4.—*Frequency of specific chemicals, July 1, 1963 — June 30, 1966*

Pesticide	Percent of Samples Containing Residues			
	Total	Milk	Other Dairy Products: Domestic	Other Dairy Products: Import
DDE	40.6	40.7	41.8	34.3
Dieldrin	27.9	30.0	26.2	17.0
DDT	24.1	23.5	25.3	24.5
Heptachlor epoxide	23.6	25.4	23.2	3.2
TDE	16.9	15.5	20.6	16.4
BHC	8.0	6.7	9.6	15.5
Lindane	5.6	4.9	7.1	5.9
Aldrin	1.4	1.6	0.5	3.9
Heptachlor	0.9	1.0	0.8	0
Methoxychlor	0.7	1.0	0.6	0

DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
Lindane	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethano= naphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane

TABLE 5.—*Percent distribution of residues of specific chemicals in different ranges—3-year total*

Range (ppm) (fat basis)	Percent Distribution of Positive Samples										
	All Residues	DDT	DDE	TDE	Dieldrin	Heptachlor Epoxide	BHC	Lindane	Aldrin	Heptachlor	Methoxychlor
T-0.03	45.5	48.2	43.8	51.6	38.2	36.3	62.1	67.1	84.3	66.1	24.7
0.04-0.10	26.0	23.6	26.6	28.0	28.7	27.8	19.7	20.7	8.4	18.3	17.2
[illegible]	[illegible]	24.0	25.9	17.7	30.0	33.1	16.1	10.4	7.3	14.8	44.1
[illegible]	[illegible]	[illegible]	2.6	1.4	2.6	2.2	1.8	1.4	—	0.9	7.5
[illegible]	[illegible]	0.6	0.7	0.5	0.2	0.5	0.3	0.3	—	—	3.2
[illegible]	[illegible]	[illegible]	0.2	0.1	0.1	0.1	—	—	—	—	2.2
[illegible]	[illegible]	[illegible]	0.2	0.7	0.2	<0.03	—	0.1	—	—	1.1
[illegible]	[illegible]	[illegible]	0.066	0.026	0.042	0.036	0.007	0.004	0.001	0.002	0.001
[illegible]	[illegible]	[illegible]	0.358	0.264	0.138	0.110	0.049	0.042	0.010	0.045	0.013
[illegible]	[illegible]	[illegible]	±0.006	±0.005	±0.002	±0.002	±0.0009	±0.0007	±0.0002	±0.0008	±0.0002

[illegible] averages, standard deviations, and confidence limits.

TABLE 6.—*Percent distribution of residues, by fiscal year and product class, in different quantitative ranges*

Range (ppm) (fat basis)	Percent Distribution of Positive Samples								
	Fluid Milk Domestic			Manufactured Dairy Products Domestic			Manufactured Dairy Products Imported		
	1964	1965	1966	1964	1965	1966	1964	1965	1966
I. ALL RESIDUES									
T-0.03	41.5	44.5	49.7	43.5	48.3	47.8	44.0	35.8	42.9
0.04-0.10	26.6	25.7	24.8	28.7	24.4	26.4	22.9	31.1	25.4
0.11-0.50	27.6	26.2	22.0	26.0	25.3	24.5	27.7	24.3	26.6
0.51-1.00	3.3	2.7	1.8	1.5	1.7	1.1	2.4	3.8	4.3
1.01-1.50	0.5	0.5	0.7	0.2	0.2	0.1	2.4	1.3	0.5
1.51-2.00	0.2	0.1	0.4	0.04	0.05	0.1	—	0.4	—
>2.00	0.3	0.3	0.6	0.04	—	—	0.5	3.4	0.3
II. DDT									
T-0.03	35.1	50.5	60.1	38.1	53.5	57.5	28.2	23.3	35.6
0.04-0.10	34.3	25.0	18.4	27.9	14.9	15.5	33.3	27.9	14.9
0.11-0.50	25.7	20.8	16.5	31.5	28.3	25.9	23.1	34.9	46.0
0.51-1.00	3.2	2.3	2.6	2.0	2.7	1.1	5.1	7.0	3.4
1.01-1.50	0.6	0.5	0.4	0.2	0.6	—	10.3	2.3	—
1.51-2.00	—	0.3	0.8	—	—	—	—	—	—
>2.00	1.2	0.6	1.1	0.2	—	—	—	4.7	—
III. DDE									
T-0.03	43.4	45.2	44.4	42.9	40.8	45.3	54.1	43.9	34.2
0.04-0.10	27.3	25.5	26.0	28.7	26.6	27.5	22.9	29.8	25.2
0.11-0.50	25.8	25.3	24.8	26.2	29.9	24.5	23.0	19.3	32.4
0.51-1.00	2.8	3.3	2.2	2.0	2.3	2.0	—	3.5	7.2
1.01-1.50	0.4	0.4	1.5	—	0.4	0.3	—	1.8	1.1
1.51-2.00	0.1	0.2	0.5	0.2	—	0.3	—	—	—
>2.00	0.1	0.2	0.6	—	—	—	—	1.8	—
IV. TDE									
T-0.03	55.1	52.2	56.1	50.3	44.2	43.3	44.0	50.0	48.9
0.04-0.10	25.3	30.9	25.1	29.4	31.0	31.7	8.0	20.6	27.7
0.11-0.50	17.9	14.1	15.3	18.7	22.9	23.3	40.0	11.8	19.1
0.51-1.00	1.2	1.3	1.1	1.4	1.6	1.7	4.0	2.9	4.3
1.01-1.50	—	0.4	0.9	0.3	0.4	—	4.0	2.9	—
1.51-2.00	0.2	—	0.2	—	—	—	—	2.9	—
>2.00	0.2	1.0	1.3	—	—	—	—	8.8	—
V. DIELDRIN									
T-0.03	37.8	39.2	36.0	37.4	38.3	42.2	51.7	45.9	47.6
0.04-0.10	26.2	25.8	27.8	35.7	28.2	34.7	37.9	43.2	45.2
0.11-0.50	30.2	31.0	33.8	24.8	32.4	23.1	10.3	8.1	4.8
0.51-1.00	5.0	3.7	1.8	1.2	0.8	—	—	2.7	—
1.01-1.50	0.2	0.2	0.2	0.5	—	—	—	—	2.4
1.51-2.00	0.2	0.1	0.1	0.2	0.3	—	—	—	—
>2.00	0.5	—	0.3	0.2	—	—	—	—	—
VI. HEPTACHLOR EPOXIDE									
T-0.03	30.5	28.7	45.0	37.9	53.8	30.0	57.1	33.3	33.3
0.04-0.10	22.9	26.1	37.9	27.9	22.5	39.0	28.6	16.7	33.3
0.11-0.50	40.5	41.1	17.0	33.4	23.4	31.0	14.3	50.0	33.3
0.51-1.00	4.5	3.2	0.2	0.7	0.3	—	—	—	—
1.01-1.50	0.8	0.9	—	—	—	—	—	—	—
1.51-2.00	0.6	—	—	—	—	—	—	—	—
>2.00	0.1	—	—	—	—	—	—	—	—
VII. BHC									
T-0.03	30.2	71.5	85.5	45.0	60.6	72.0	24.1	23.1	29.7
0.04-0.10	28.1	21.1	9.0	20.2	29.1	10.8	10.3	38.5	24.3
0.11-0.50	34.4	7.4	5.1	33.7	7.3	17.2	58.6	33.3	37.8
0.51-1.00	5.2	—	—	1.1	3.0	—	6.9	5.1	8.1
1.01-1.50	2.1	—	0.4	—	—	—	—	—	—
1.51-2.00	—	—	—	—	—	—	—	—	—
>2.00	—	—	—	—	—	—	—	—	—

TABLE 6. *Percent distribution of residues, by fiscal year and product class, in different quantitative ranges*—Continued

RANGE (PPM) (FAT BASIS)	PERCENT DISTRIBUTION OF POSITIVE SAMPLES								
	FLUID MILK DOMESTIC			MANUFACTURED DAIRY PRODUCTS					
				DOMESTIC			IMPORTED		
	1964	1965	1966	1964	1965	1966	1964	1965	1966
VIII. LINDANE									
T-0.03	67.2	66.1	54.4	65.6	84.7	40.0	52.9	28.6	94.1
0.04-0.10	24.5	24.2	10.5	22.9	10.6	20.0	17.6	42.9	—
0.11-0.50	7.5	9.7	26.3	10.2	4.7	20.0	23.5	28.6	5.9
0.51-1.00	0.8	—	7.0	1.3	—	13.3	—	—	—
1.01-1.50	—	—	1.8	—	—	6.7	—	—	—
1.51-2.00	—	—	—	—	—	—	—	—	—
>2.00	—	—	—	—	—	—	5.9	—	—
IX. ALDRIN									
T-0.03	55.6	86.7	81.8	100	100	91.7	—	—	96.3
0.04-0.10	44.4	6.7	8.2	—	—	8.3	—	—	3.7
0.11-0.50	—	6.7	10.0	—	—	—	—	—	—
0.51-1.00	—	—	—	—	—	—	—	—	—
1.01-1.50	—	—	—	—	—	—	—	—	—
1.51-2.00	—	—	—	—	—	—	—	—	—
>2.00	—	—	—	—	—	—	—	—	—
X. HEPTACHLOR									
T-0.03	57.9	55.6	80.0	83.3	100	—	—	—	—
0.04-0.10	20.9	11.1	20.0	8.3	—	100	—	—	—
0.11-0.50	19.4	22.2	—	8.3	—	—	—	—	—
0.51-1.00	—	11.1	—	—	—	—	—	—	—
1.01-1.50	—	—	—	—	—	—	—	—	—
1.51-2.00	—	—	—	—	—	—	—	—	—
>2.00	—	—	—	—	—	—	—	—	—
XI. METHOXYCHLOR									
T-0.03	20.0	26.9	38.7	33.3	100	—	—	—	—
0.04-0.10	68.0	23.1	6.5	33.3	—	40.0	—	—	—
0.11-0.50	8.0	46.2	25.8	33.3	—	60.0	—	—	—
0.51-1.00	4.0	—	16.1	—	—	—	—	—	—
1.01-1.50	—	3.8	3.2	—	—	—	—	—	—
1.51-2.00	—	—	6.5	—	—	—	—	—	—
>2.00	—	—	3.2	—	—	—	—	—	—

Discussion

From Table 1, it is obvious that within the broad geographic sections, the samples were reasonably related to milk production. The relationship was more variable on an individual State basis. In our opinion, there were enough samples collected over a wide geographic range reasonably proportionate to milk production to consider the findings representative of residues in fluid milk and other dairy products during the 3-year period.

Over half of all samples contained one or more residues. [illegible] graphic division was free of residues. The incidence of residues in the East North Central States was [illegible] in most of the country, and the incidence of residues in the adjoining West North Central States [illegible] significantly lower. The incidence of residues in the [illegible] geographic divisions (68.0, 74.2, 7[illegible] and 7[illegible]) was not significantly different.

Although almost half of the samples were collected during FY 1964, the number of samples collected each year was considered large enough to yield significant results.

The data show beyond question that a majority of the milk and other dairy products marketed in the United States contain detectable quantities of one or more pesticide chemicals. Almost half of the lots examined contained more than one pesticide residue.

The incidence of residues reported was lower in FY 1964 than in FY 1965 or 1966. The closer relationship between the 1965 and 1966 findings suggests that either there was a significant increase in residues in milk or the laboratories were more proficient in detecting residues. We believe the latter is the most logical explanation, because 1964 was the initial year of the program and the first year that gas chromatography was in general use. In our opinion, there has been no significant change in the incidence of residues during this period.

The pesticide chemicals shown in Table 3 were found each year and in each commodity grouping, which is not surprising. The order of frequency varies slightly, but not by order of magnitude. DDE and TDE are metabolites of DDT, and their presence in milk is to be expected. The incidence of dieldrin residues in domestic samples is almost double that found in imported products. The incidence of BHC residues in domestic products is about half that found in imported products. The findings on heptachlor and heptachlor epoxide are noteworthy in their very low incidence in imported products and the frequent occurrence of heptachlor epoxide in domestic milk fat. Heptachlor and aldrin are metabolized and normally excreted in the milk as heptachlor epoxide and dieldrin, respectively. The low incidence of heptachlor and aldrin suggests analytical error, external contamination after milking, or incomplete conversion to the epoxide. We are inclined toward the latter two possibilities because of positive findings in several different laboratories in each year and confirmation in the total diet samples.

The 95% confidence ranges for the averages for each chemical shown in Table 5 are rather narrow. Specific attention is directed to the averages of dieldrin and heptachlor epoxide residues. Although each average is equivalent to the average of the individual DDT compounds, the latter are usually considered in combination which makes the averages of dieldrin and heptachlor epoxide about one-third of that resulting from DDT. Considering the sampling program and procedures, in our opinion, the averages are reliable indices of the pesticide residue content of milk and dairy products throughout the United States during this period. They may be useful as baselines to compare future results.

The data were not amenable to consideration of the various combinations of residues in samples. It is well known that the DDT metabolites, DDE and TDE, are most often found in combination with DDT. The relatively high incidence of dieldrin and heptachlor epoxide suggests that either of these two chemicals may often be found in milk fat containing the DDT group.

The percent distribution of residue levels is about the same for each chemical and product when only the samples containing that chemical are considered as shown in Table 6. As expected, deviations from the overall averages become greater as the data are classified in more detail, but the deviations are not great enough to invalidate the general statement. These patterns are typical of residue levels in all food classes. A tendency can be observed toward fewer extreme values, above 0.51 ppm, of the more toxic pesticide chemicals such as dieldrin, heptachlor epoxide, and BHC. It is significant that the percent of values above 0.11 ppm for dieldrin and heptachlor epoxide was substantial and relatively constant, with the exception of dieldrin in imported products as noted above.

Table 7 compares the 3-year average values for the 10 most commonly found residues with the averages found in composites of the dairy portion of 40 total diet samples examined by FDA from April 1964 through June 1966. The total diet samples are collected at the retail level representing a different point in the distribution chain. The results of both investigations are reported on a fat basis, and since processing techniques used in manufacturing dairy products probably do not affect the pesticide residue content of the fat, each should serve as a check on the reliability of the results.

TABLE 7.—*Average levels of pesticide chemicals in dairy products*

(Parts per Million—fat basis)

Pesticide	Objective Samples (3-year average)	Total Diet Samples (2-year average)
DDE	0.066	0.074
Dieldrin	0.042	0.017
DDT	0.042	0.037
Heptachlor epoxide	0.036	0.010
TDE	0.026	0.013
BHC	0.007	0.008
Lindane	0.004	0.005
Aldrin	0.001	0.001
Heptachlor	0.002	—
Methoxychlor	0.001	0.002

These averages are in remarkably good agreement considering the extremes in the number of samples represented. The average levels of dieldrin and heptachlor epoxide found in the total diet samples are lower than in the objective samples. These differences exceed the standard deviation calculated for the total diet samples. No logical explanation for the lack of agreement in these two residues is immediately apparent; a search is being made for the reason. The average level in parts per million does not change the order of magnitude for any pesticide residue.

There are no known approved uses of pesticide chemicals which might result in residues in milk above the legal tolerance levels. Their presence in milk fat results from indirect sources, some of which (air, dust, and drift) are beyond control of the dairyman. Other sources, such as feed, equipment, and direct application to animals, are controllable.

Recently, there has been a reduction in the approved uses and use patterns of some of the more persistent chlorinated organic pesticides. This reduction in use would not be reflected in this report because of the time periods involved.

It is unlikely that the current levels will be reduced or even remain constant without continued specific attention by industry and government to eliminating all controllable sources and maintaining the residue load from uncontrollable sources at a minimum.

No satisfactory system has been designed to identify for sampling only those lots containing unsanctioned or excessive residues. While such a system would be the most effective control, the factors influencing residues change so rapidly and are so complex and interrelated, that it is unlikely such control will be practical in the foreseeable future. There continues to be a need for information as described in this report concerning the character and levels of all pesticide residues being consumed.

Significant monitoring programs at production and distribution centers are capable of identifying problems at early stages. Corrective measures by government and industry for consumer protection are most effective during these early stages. This type of program serves to prevent local situations from spreading into national problems affecting the Nation's health.

Summary and Conclusions

A representative annual sampling of milk and dairy products marketed during FY 1964, 1965, and 1966 shows that DDE, dieldrin, DDT, heptachlor epoxide, TDE, BHC, lindane, aldrin, heptachlor, and methoxychlor account for over 99% of the chlorinated organic residues in milk and dairy products. These chemicals were found in each of the 3 years. Twenty-three other chemicals were found at low levels in 1 or more of the 12,836 samples examined.

Over half of the samples contained residues, and most of these contained more than one pesticide chemical. The incidence of residues in the U.S.D.A. East and West North Central Crop Reporting Divisions was lower than in other portions of the United States.

A substantial majority, 95%, of the residues found were below 0.5 ppm on a fat basis, and 71.5% were below 0.11 ppm.

No substantial annual variations were noted in these observations with respect to fluid milk, domestic manufactured dairy products, and imported dairy products, except for dieldrin, heptachlor epoxide, and BHC in imported dairy products.

The average levels and kinds of pesticide chemicals found in the objective samples are in good agreement with the findings on the dairy portion of total diet samples collected at a different point in the distribution [illegible] and add a measure of confidence to the total diet [illegible] as a whole as a broad index to the quantities [illegible] consumed in the diet.

The [illegible]ted are approximately one-tenth the [illegible] of 1.25 ppm (fat basis) for DDT, DDE, and TDE residues combined. The average levels of dieldrin and heptachlor epoxide are approximately one-tenth of the current administrative determination of 0.3 ppm (fat basis) for excessive residues for each chemical. The averages of the remaining five chemicals are much lower.

The total pesticide content consists, in a majority of samples, of a combination of chemicals. The most probable combinations will include one or more members of the DDT group with dieldrin or heptachlor epoxide.

It is obvious that the total residue content of milk fat should not be permitted to increase since this is the source of 13.6% of the total dietary intake *(8)* of chlorinated organic pesticides. The residue pattern indicates that increases would be accompanied by considerable loss in economic terms and food value through the control mechanisms at city, county, State and Federal levels designed to prevent consumption of dairy products containing excessive residues.

Even though no major nationwide problem is obvious, there have been several instances of considerable concern to specific localities during this period. The effects of these incidents were minimized through the cooperative efforts of all sharing the responsibility for an adequate and safe supply of dairy products. Reductions in the residue content of dairy products can only be made through a general continued and cooperative effort by the dairy industry and all agencies of government.

Acknowledgments

Recognition must be given to the chemists, too numerous to mention as individuals, among the 18 FDA District Laboratories responsible for these analyses and to R. K. Dawson, Division of Program Operations, for his assistance in processing the data.

LITERATURE CITED

(1) *Clifford, Paul A. 1957.* Pesticide residues in fluid market milk. Public Health Rep. 72:729.

(2) *Clifford, Paul A., Jonas L. Bassen, and Paul A. Mills. 1959.* Chlorinated organic pesticide residues in fluid milk. Public Health Rep. 74:1109.

(3) *Association of Official Analytical Chemists.* Changes in official methods of analysis. J. Ass. Offic. Anal. Chem. 49:222 (1966); *ibid.* 50:210 (1967).

(4) *U. S. Department of Health, Education, and Welfare, Food and Drug Administration. 1963. Revised 1964, 1965.* Pesticide analytical manual. Vol. 1.

(5) *Johnson, L. Y. 1965.* Collaborative study of a method for multiple chlorinated pesticide residues in fatty foods. J. Ass. Offic. Agr. Chem. 48:668.

(6) *Wells, Clyde E. December 1967.* Validation study of a method for pesticide residues in foods and animal feeds. J. Ass. Offic. Anal. Chem. (In press).

(7) *U. S. Department of Agriculture. 1966 (issued Feb. 1967).* Annual statistical summary, milk production and dairy products.

(8) *Duggan, R. E. and J. R. Weatherwax. 1967.* Dietary intake of pesticide chemicals. Science 157:1006.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Chlorinated Pesticide Levels in the Eastern Oyster (Crassostrea virginica) From Selected Areas of the South Atlantic and Gulf of Mexico

John C. Bugg, Jr.[1], James E. Higgins[2], and Eric A. Robertson, Jr.[3]

ABSTRACT

Oysters were collected from estuarine areas in South Carolina, Georgia, Florida, Mississippi, Louisiana, and Texas and analyzed for pesticide residues.

Pesticide levels were determined by the electron capture gas chromatography method and were confirmed by thin layer chromatography and dual-column electron capture gas chromatography.

In general, chlorinated pesticides were either not detected or were found at relatively low levels in samples collected from the Atlantic and Gulf Coast areas. Of a total of 133 samples, 94.7% contained 1 or more pesticides; 89.5% contained 2 or more; 81.2% contained 3 or more; 63.9% contained 4 or more; and 31.9% contained 5 or more.

The level of sensitivity for pesticide residues was 0.01 ppm. Some correlation was found between spraying operations and pesticide levels in the oysters.

Purpose

The purpose of this report is to present data on the occurrence of chlorinated pesticides in oysters in selected areas of the South Atlantic and Gulf of Mexico as determined through research conducted at the Gulf Coast Marine Health Sciences Laboratory on the development and evaluation of methodology for the analyses of chemical contaminants and natural toxins in shellfish.

Factual Data

Oysters for this study were obtained from South Carolina, Georgia, Florida, Mississippi, Louisiana, and Texas. The oysters were either collected directly from oyster-growing areas by representatives of the State health and conservation agencies or purchased from oyster dealers who verified the general locations of the sampling sites. The oysters were chilled in ice immediately after collection and then frozen. Frozen shellstock or shucked oysters were shipped to the Research Laboratory in insulated containers with dry ice. Immediately upon arrival, shellstock was shucked and drained of liquor. Samples not analyzed immediately upon receipt were stored at —10 C. No samples were stored over 60 days. At least a pint of shucked oysters was used for each sample. The samples were placed in a blender for 5 minutes after which a homogenized 50-g sample was withdrawn for analysis.

The laboratory methods and techniques used for the analysis of pesticide residues in oysters were essentially those compiled by Barry *et al.* *(1)*. The major deviation from these methods was the utilization of the "perforated" basket centrifuge head as described by Robertson and Tyo *(6)* for separating oyster meats from the extracting solvent.

Quantitative determinations of the residues were initially carried out on a 5% DC-11 column and later on a mixed column containing equal parts by weight of 10% DC-200 (12,500 CSTKS) and 15% QF-1 (10,000 CS) on Gas Chrom Q 60/80 mesh solid support *(2)* with a Tritium-parallel plate electron capture detector. The level of sensitivity was 0.01.

Confirmatory procedures used were thin layer chromatography as described by Kovacs *(5)*, microcoulometry, and dual differential columns as described by Burke and Holswade *(2)*, coupled with a Ni^{63} pin cup electron capture detector and a H^3 parallel plate electron capture detector.

Standard mixtures containing the pesticides were injected into the gas chromatograph each day before any sample injection, as well as during the course of injection of samples for residue determinations. Standards were also injected after any sample yielding significant pesticide residues.

1 Gulf Coast Marine Health Sciences Laboratory, U. S. Public Health Service, Dauphin Island, Ala. 36528.
Present Address: Humble Oil & Refining Company, 909 Jefferson Davis Parkway, New Orleans, La. 70160.
2 Bureau of Commercial Fisheries Exploratory Fishing and Gear Research Base, U. S. Fish and Wildlife Service, Pascagoula, Miss. 39567.
3 Gulf Coast Marine Health Sciences Laboratory, U. S. Public Health Service, Dauphin Island, Ala. 36528.

Results and Discussion

The pesticide levels detected in the 133 oyster samples from South Carolina, Georgia, Florida, Mississippi, Louisiana, and Texas are shown in Appendix I. Of the total number of oyster samples, 126 were found to contain 1 or more chlorinated pesticides. For each pesticide, the number of oyster samples in which the pesticide was detected and the median, low, and high values of pesticide concentration in ppm, as taken from Appendix I, are shown in Table 1.

Table 2 summarizes the results of analyses of all oyster samples. The distribution of specific pesticides in positive samples at different arbitrarily selected residue levels is shown, as well as the number of samples in which the specific pesticide was not detected.

TABLE 1.—*Frequency of chlorinated pesticide residues in oyster samples*

[Period of sampling—Feb. 1, 1964 through Aug. 24, 1966]

Pesticide	No. Samples Examined	No. Samples Positive for Pesticide Shown	Residue (ppm drained weight)		
			Median	Low	High
Aldrin	133	17	0.01	<0.01	0.03
BHC-Lindane	133	55	0.01	<0.01	0.50
Chlordane	132	20	<0.01	<0.01	0.01
DDD	81	81	0.02	<0.01	0.37
DDE	131	123	0.02	<0.01	0.12
p,p'-DDT	131	117	0.02	<0.01	0.22
Dieldrin	115	54	0.01	<0.01	0.03
Endrin	115	27	<0.01	<0.01	0.07
Heptachlor	133	12	<0.01	<0.01	<0.01
Heptachlor epoxide	133	20	<0.01	<0.01	<0.01
Methoxychlor	133	6	0.01	<0.01	<0.01
Toxaphene	133	6	0.08	<0.01	1.00
Trithion [a]	55	0	—	—	—

Aldrin — not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethano-naphthalene
BHC-Lindane — 1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
Chlordane — 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
DDD — 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE — 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
p,p'-DDT — 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
Dieldrin — not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
[illegible] — 1,2,3,4,10,10-hexachloro-6,7 epoxy-1,4,4a,5,6,7,8,8a-[illegible]hydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
[illegible] — [illegible] heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
[illegible] — [illegible] heptachloro-2,3 epoxy-3a,4,7,7a-[illegible] 4,7-methanoindan
[illegible] — [illegible] 2,2-bis(*p*-methoxyphenyl)ethane
[illegible] — [illegible] containing 67% to 69% [illegible]
[illegible] — [illegible] *O,O*-diethyl [illegible]

TABLE 2.—*Distribution of chlorinated pesticides at different residue levels—all oyster samples*

[Period of sampling—Feb. 1, 1964 through Aug. 24, 1966]

Pesticide	No. Samples Examined	Residue Levels in ppm Drained Weight			
		Not Detected	<0.01	0.01-0.05	>0.05
Aldrin	133	116	16	1	0
BHC-Lindane	133	78	27	27	1
Chlordane	132	112	19	1	0
DDD	81	0	2	72	7
DDE	131	8	24	83	16
p,p'-DDT	131	14	17	90	10
Dieldrin	115	61	16	38	0
Endrin	115	88	19	7	1
Heptachlor	133	121	12	0	0
Heptachlor epoxide	133	113	20	0	0
Methoxychlor	133	127	6	0	0
Toxaphene	133	127	2	1	3
Trithion [a]	55	55	0	0	0

As shown in Table 1, the high values of pesticide concentration in all positive oyster samples ranged from <0.01 ppm for heptachlor, heptachlor epoxide, and methoxychlor to 1.00 ppm for toxaphene. The median value of pesticide concentration ranged from <0.01 ppm for aldrin, chlordane, endrin, heptachlor, heptachlor epoxide, and methoxychlor to 0.08 ppm for toxaphene. The low value of pesticide concentration was <0.01 ppm for all pesticides.

Endrin and dieldrin, the more toxic of the chlorinated hydrocarbon pesticides *(4)*, were generally found in low concentrations. Only 27 of 115 oyster samples were positive for endrin, the range being from <0.01 to 0.07 ppm. Fifty-four of 115 oyster samples were positive for dieldrin, the range being from <0.01 to 0.03 ppm. The chlorinated pesticides found most frequently were *p,p'*-DDT and two of its metabolites, DDD and DDE. Although these were found at higher concentrations, generally, than the other chlorinated pesticides, the levels of concentration were still relatively low. Maximum values for *p,p'*-DDT, DDD, and DDE were 0.22 ppm, 0.37 ppm, and 0.12 ppm, respectively.

BHC-lindane was found in 55 of 133 oyster samples, with the high, median, and low values being 0.50 ppm, 0.01 ppm, and <0.01 ppm, respectively. Toxaphene was found in only 6 of 133 oyster samples, with the high, median, and low values being 1.00 ppm, 0.08 ppm, and <0.01 ppm, respectively. The high concentrations of BHC-lindane and toxaphene were found in the same oyster sample collected from growing waters affected by recent application of these pesticides in an adjacent area. Oyster samples taken from these waters at later dates showed successively lower levels of these pesticides, with an eventual decrease to non-detectable levels.

Aldrin, chlordane, heptachlor, heptachlor epoxide, methoxychlor, and Trithion® generally were found infrequently and in very low concentrations.

The results of the laboratory analyses of all oyster samples showed that, in general, chlorinated pesticides were either not detected or were found in relatively low levels in the positive samples. The ranges of the pesticide levels in all oyster samples were generally of the same magnitude as those found by the U. S. Food and Drug Administration in 1964 and 1965 in the analyses of 216 composite samples of 12 major food groups comprising the American food supply. The amounts of the pesticide residues found by the Food and Drug Administration were reported as insignificant from a health standpoint *(3, 7)*.

Conclusions

In general, chlorinated pesticides were either not detected or were found in relatively low levels in the oyster samples collected from the South Atlantic and Gulf of Mexico coastal areas for this study. The data on chlorinated pesticide concentrations in oysters indicate little or no public health hazard at the present time. The occasional occurrence of the higher concentrations of chlorinated pesticides in oysters as found in this study, however, indicates that contamination of shellfish-growing waters with such pesticides does represent a potential problem that should be kept under surveillance.

Acknowledgments

The cooperation and assistance of the South Carolina State Board of Health, Georgia Department of Public Health, Florida State Board of Health, Mississippi Marine Conservation Commission, Louisiana State Board of Health, Louisiana Wild Life and Fisheries Commission, and Texas State Department of Health in the conduct of this study are gratefully acknowledged. The participation of personnel of these State health and conservation agencies included the harvesting and shucking of shellstock, arranging for the procurement of oyster samples from dealers, and handling arrangements for shipment of samples to the Laboratory. These activities are recognized as a significant contribution to this study and are deeply appreciated.

LITERATURE CITED

(1) *Barry, Helen C., J. G. Hundley, and Loren Y. Johnson. 1965.* Pesticide analytical manual, Vol. 1. Revised ed. Food and Drug Admin. U. S. Dep. Health, Educ., and Welfare.

(2) *Burke, J. A. and W. Holswade. 1966.* A gas chromatographic column for pesticide residue analysis: retention times and response data. J. Ass. Offic. Anal. Chem. 49 (2):374-385.

(3) *Duggan, R. E., H. C. Barry, and L. Y. Johnson. 1966.* Pesticide residues in total-diet samples. Science 151 (3706):101-104.

(4) *Kenaga, E. E. 1966.* Pesticide reference standards of the Entomological Society of America. Bull. Entomol. Soc. Amer. 12(2):117-127.

(5) *Kovacs, Martin F., Jr. 1963.* Thin-layer chromatography for chlorinated pesticide residue analysis. J. Ass. Offic. Agr. Chem. 46:884-893.

(6) *Robertson, E. A. and R. M. Tyo. 1966.* Note on improved extraction for chlorinated pesticide residues in oysters J. Ass. Offic. Anal. Chem. 49(3):683-684.

(7) U. S. Food and Drug Administration press release. April 9, 1967.

APPENDIX I.—*Distribution of residues of specific chlorinated pesticides, by region*

[Period of sampling—Feb. 1, 1964 through Aug. 24, 1966]

	South Carolina	Georgia	Florida	Mississippi	Louisiana	Texas	Total
No. Samples examined	32	22	44	1	20	14	133
No. Samples in which one or more pesticides detected	29	21	44	1	19	12	126
CHLORINATED PESTICIDES (ppm drained weight)							
ALDRIN							
No. positive	(1)	(1)	(8)	(0)	(4)	(3)	(17)
Median	—	—	<0.01	—	<0.01	<0.01	<0.01
Low	—	—	<0.01	—	<0.01	<0.01	<0.01
High	<0.01	<0.01	<0.01	—	<0.01	0.03	0.03
BHC-LINDANE							
No. positive	(14)	(5)	(19)	(0)	(9)	(8)	(55)
Median	0.01	<0.01	<0.01	—	<0.01	0.01	0.01
Low	<0.01	<0.01	<0.01	—	<0.01	<0.01	<0.01
High	0.50	0.01	0.01	—	0.02	0.02	0.50
CHLORDANE							
No. positive	(6)	(4)	(8)	(1)	(1)	(0)	(20)
Median	<0.01	<0.01	<0.01	—	—	—	<0.01
Low	<0.01	<0.01	<0.01	—	—	—	<0.01
High	<0.01	<0.01	<0.01	<0.01	0.01	—	0.01

APPENDIX I. *Distribution of residues of specific pesticides*—Continued

[Period of sampling—Feb. 1, 1964 through Aug. 24, 1966]

CHLORINATED PESTICIDES (ppm drained weight) (Continued)

	South Carolina	Georgia	Florida	Mississippi	Louisiana	Texas	Total
DDD[1]							
No. positive	(11)	(15)	(40)	(1)	(10)	(4)	(81)
Median	0.02	0.02	0.02	—	0.01	0.02	0.02
Low	0.01	0.01	<0.01	—	<0.01	0.01	<0.01
High	0.05	0.04	0.37	0.02	0.07	0.05	0.37
DDE							
No. positive	(29)	(19)	(44)	(1)	(18)	(12)	(123)
Median		0.02	0.02	—	[2]	0.01	0.02
Low		0.01	<0.01	—	<0.01	<0.01	<0.01
High		0.04	0.12	0.02	0.02	0.04	0.12
p,p'-DDT							
No. positive	(25)	(19)	(44)	(1)	(16)	(12)	(117)
Median	0.01	0.02	0.02	—	0.01	0.02	0.02
Low	0.01	<0.01	<0.01	—	<0.01	<0.01	<0.01
High	0.03	0.03	0.22	0.02	0.06	0.07	0.22
DIELDRIN							
No. positive	(11)	(12)	(16)	(1)	(9)	(5)	(54)
Median	0.01	0.01	0.01	—	0.01	0.01	0.01
Low	0.01	<0.01	<0.01	—	<0.01	0.01	<0.01
High	0.02	0.02	0.03	<0.01	0.01	0.03	0.03
ENDRIN							
No. positive	(1)	(6)	(7)	(1)	(8)	(4)	(27)
Median	<0.01	0.01	0.01	—	[2]	[2]	<0.01
Low	<0.01	<0.01	<0.01	—	<0.01	<0.01	<0.01
High	<0.01	0.07	<0.01	<0.01	0.02	0.02	0.07
HEPTACHLOR							
No. positive	(1)	(1)	(5)	(0)	(2)	(3)	(12)
Median	<0.01	—	<0.01	—	<0.01	<0.01	<0.01
Low	<0.01	—	<0.01	—	<0.01	<0.01	<0.01
High	<0.01	<0.01	<0.01	—	<0.01	<0.01	<0.01
HEPTACHLOR EPOXIDE							
No. positive	(2)	(0)	(9)	(0)	(7)	(2)	(20)
Median	<0.01	—	<0.01	—	<0.01	<0.01	<0.01
Low	<0.01	—	<0.01	—	<0.01	<0.01	<0.01
High	<0.01	—	<0.01	—	<0.01	<0.01	<0.01
METHOXYCHLOR							
No. positive	(2)	(1)	(2)	(0)	(1)	(0)	(6)
Median	0.01	—	<0.01	—	—	—	<0.01
Low	<0.01	—	<0.01	—	—	—	<0.01
High	<0.01	<0.01	<0.01	—	<0.01	—	<0.01
TOXAPHENE							
No. positive	(6)	(0)	(0)	(0)	(0)	(0)	(6)
Median	0.08	—	—	—	—	—	0.08
Low	<0.01	—	—	—	—	—	<0.01
High	1.00	—	—	—	—	—	1.00
TRITHION[a]							
No. positive	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Median	—	—	—	—	—	—	—
Low		—	—	—	—	—	—
High		—	—	—	—	—	—

[1] DDD not included in routine analysis until October 1964
[illegible]

Galveston Bay Pesticide Study — Water and Oyster Samples Analyzed for Pesticide Residues Following Mosquito Control Program

Victor L. Casper[1]

ABSTRACT

The purpose of this study was to determine the effect of increased pesticide applications in the Houston area on shellfish and shellfish-growing waters of Galveston Bay.

The study was conducted during the fall of 1964 following a large-scale mosquito control program in the Houston area. Water and oyster samples were collected in September and October 1964, during and after the mosquito control operations. Oyster samples collected in this study were compared to samples collected from April to July 1964, prior to the mosquito operations.

Analyses included determination of levels of BHC-lindane, DDE, DDT, dieldrin, endrin, heptachlor, aldrin, chlordane, heptachlor epoxide, methoxychlor, toxaphene, and Trithion®. Pesticide levels were determined by the use of electron capture gas-liquid chromatography, with thin layer chromatography for confirmation.

Pesticide levels in both water and oysters were low at all times. The data indicate little or no increase in levels due to the control program in Houston.

Purpose

The purpose of this study was to determine the effect of increased pesticide applications in the Houston area on shellfish and shellfish-growing waters of Galveston Bay.

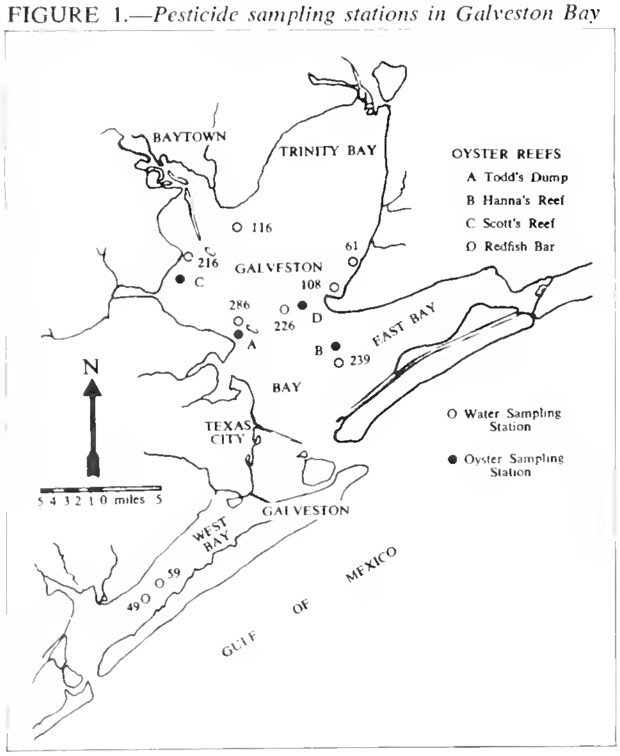
Factual Data

Following an outbreak of equine encephalitis in the Houston area during the summer of 1964, a large-scale mosquito control program was begun which utilized considerable quantities of pesticides, especially malathion, DDT, and BHC. Actual spraying operations began the third week of August. With the increased use of pesticides in the Houston area, the Texas State Department of Health became concerned over potential pesticide contamination of shellfish-growing waters in Galveston Bay. On September 2, officials of the Texas State Department of Health requested the PHS Regional Office and, in turn, the Gulf Coast Marine Health Sciences Laboratory to provide assistance in laboratory analyses of pesticides in water and oysters.

Following discussions between representatives of the Texas State Department of Health and the Laboratory, a sampling program was established for the collection of water and oyster samples in Galveston Bay at locations shown in Figure 1. Sampling activities were begun on September 3 and completed on October 6. 1964.

Water and oyster samples were collected by personnel of the Texas State Department of Health. Water samples were collected in 1-gallon chemically clean glass jugs at nine stations for 5 consecutive weeks. Each week water samples were shipped unrefrigerated to the Gulf Coast Marine Health Sciences Laboratory and

FIGURE 1.—*Pesticide sampling stations in Galveston Bay*



[1] Gulf Coast Marine Health Sciences Laboratory, U. S. Public Health Service, Dauphin Island, Ala. 36528.

TABLE 1.—*Summary of oyster analyses*

[ND = Not detected]

Sampling point	Date of sampling	Pesticides in ppm wet drained weight[1]							
		BHC-Lindane	DDE	DDT	Dieldrin	Endrin	Heptachlor	Aldrin	Others[2]
East Galveston Bay (Lease 357-A)	4/23/64	ND	ND	ND	ND	ND	ND	ND	ND
East Galveston Bay (Miller's Reef)	5/11/64	ND	ND	ND	ND	ND	ND	ND	ND
East Galveston Bay	6/04/64	0.01	0.02	0.01	ND	ND	ND	ND	ND
East Galveston Bay	6/24/64	0.01	0.01	0.01	ND	ND	ND	ND	ND
East Galveston Bay (Blume's Reef)	7/07/64	ND	0.05	0.07[3]	0.01	0.02	ND	0.03	ND
East Galveston Bay (Blume's Reef)	7/18/64	ND	0.05	0.1[3]	0.01	0.01	ND	ND	ND
Galveston Bay (Todd's Dump)	9/03/64	<0.01	0.01	0.01	<0.01	<0.01	ND	ND	ND
Galveston Bay (Hanna's Reef)	9/09/64	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	ND	ND
Galveston Bay (Scott's Reef)	9/14/64	<0.01	<0.01	0.01	<0.01	<0.01	ND	ND	ND
Galveston Bay (Redfish Bar)	9/14/64	<0.01	0.02	0.02	<0.01	<0.01	<0.01	ND	ND

[1] Analysis by gas chromatography with electron capture.
[2] Includes chlordane, heptachlor epoxide, methoxychlor, toxaphene, and Trithion®.
[3] *p,p'*-DDT.

refrigerated until ready for analysis. Following collection, the oysters were shucked into 1-gallon cans aboard the collecting vessel and packed in ice. Upon return to shore, the oysters were frozen, packed in dry ice, and shipped to the Laboratory. After arrival, the oysters were kept frozen until ready for analysis.

Due to difficulty in obtaining oysters during the closed season, only four oyster samples were collected during the study period. However, six oyster samples collected between April 23 and July 18 had been analyzed for chlorinated pesticides in connection with studies at the Laboratory on development of analytical techniques.

The oysters were prepared for analysis by homogenizing 1 pint of the shucked oysters in a blender for 5 minutes after which a 50-g aliquot was withdrawn for analysis. One liter of water was used for the water analysis.

Laboratory examinations were made for residues of the following chlorinated hydrocarbon pesticides: aldrin, BHC-lindane, chlordane, DDE, DDT, endrin, dieldrin, heptachlor, heptachlor epoxide, methoxychlor, and Trithion. Electron capture gas-liquid chromatography was used for both qualitative and quantitative determinations and thin layer chromatography for confirmation of results of oyster analyses. The methods and procedures were those used by the Food and Drug Administration with confirmatory procedures by Kovacs *(2)*.

Since malathion, in addition to chlorinated pesticides, was used for mosquito control operations, studies were initiated to develop analytical techniques for detection of organo-phosphate residues in water samples. Water samples were extracted as described by Iyo *(3)* for organo-phosphate residues, and laboratory analyses were conducted using electron capture gas-liquid chromatography.

Standard mixtures containing the pesticide were injected each day prior to injection of samples, as well as during the course of injection of samples. Additional standards were injected after any samples having a significant pesticide residue.

Findings of chlorinated pesticides were reported at levels of 0.01 ppm and 0.001 ppm or greater for oysters and water, respectively. The sensitivity limit for organophosphates was 0.008 ppm. All positive samples having pesticide concentrations below these levels were reported as "less than." Results of samples in which pesticides were not found are reported as "not detected."

Results and Discussion

Oyster Analyses—The results of pesticide analyses of six oyster samples collected prior to August 1964 and four samples collected between September 3 and 14 from Galveston Bay are shown in Table 1.

Results of analyses of the four oyster samples collected during and following the mosquito control operations showed concentrations of DDE and DDT between <0.01 and 0.02 ppm. All four samples contained trace amounts (<0.01 ppm) of BHC-lindane, dieldrin, and endrin, while heptachlor was found in two of four samples at <0.01 ppm. Those chlorinated pesticides not detected were aldrin, chlordane, heptachlor epoxide, methoxychlor, toxaphene, and Trithion®. Of the six oyster samples collected during the methodology study earlier in the year, four were positive for one or more chlorinated

TABLE 2.—*Summary of water analyses*

[ND = Not detected]

Sampling point	Date of sampling	Pesticides in ppm[1]								
		BHC-Lindane	DDE	DDT	Heptachlor	Heptachlor epoxide	Methoxy-chlor	Chlordane	Others[2]	Organo-phosphates
GALVESTON BAY (Todd's Dump)	9/03/64	<0.001	ND	ND	ND	ND	ND	ND	ND	Not examined[3]
TRINITY BAY (Station 116)	9/08/64	ND	ND	ND	ND	ND	ND	ND	ND	Do.
EAST BAY (Hanna's Reef)	9/09/64	<0.001	<0.001	<0.001	ND	ND	ND	ND	ND	Do.
TRINITY BAY (Station 108)	9/14/64	ND	<0.001	ND	<0.001	ND	ND	ND	ND	Do.
GALVESTON BAY (Station 226)	9/14/64	ND	<0.001	ND	<0.001	ND	ND	ND	ND	Do.
GALVESTON BAY (Scott's Reef)	9/22/64	<0.001	ND	ND	ND	ND	<0.001	ND	ND	ND
TRINITY BAY (Station 61)	9/22/64	ND	ND	ND	ND	<0.001	ND	ND	ND	ND
WEST BAY (Station A-49)	10/06/64	ND	ND	ND	<0.001	ND	ND	<0.001	ND	ND
WEST BAY (Station A-59)	10/06/64	ND	ND	<0.001	<0.001	ND	ND	<0.001	ND	ND

[1] Analysis by gas chromatography with electron capture.
[2] Includes aldrin, dieldrin, endrin, toxaphene, and Trithion®.
[3] Analytical technique under development.

pesticides. Four samples were positive for DDE and DDT with ranges of 0.01-0.05 ppm and 0.01-0.1 ppm, respectively. Concentrations of BHC-lindane, dieldrin, endrin, and aldrin were each detected in one or two of the six samples and ranged from 0.01 to 0.03 ppm. Those pesticides not detected were chlordane, heptachlor epoxide, methoxychlor, toxaphene, and Trithion®.

Water Analyses—Results of the analyses of nine water samples collected during and following the mosquito control operations are shown in Table 2.

Eight of nine samples were positive for one or more chlorinated pesticides, and all pesticide concentrations were <0.001 ppm. No sampling station was found to be positive for more than three pesticides, and no single pesticide appeared dominant. Those chlorinated pesticides not detected were aldrin, dieldrin, endrin, toxaphene, and Trithion®. No organo-phosphates were detected within the limits of the analytical techniques used in this study.

The chemical names of compounds mentioned in this paper are:

BHC-Lindane	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
Chlordane	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane
Toxaphene	chlorinated camphene containing 67% to 69% chlorine
Trithion®	*S*-[(*p*-chlorophenylthio)methyl] *0,0*-diethyl phosphorodithioate
Malathion	diethyl mercaptosuccinate, *S*-ester with *0,0*-dimethyl phosphorodithioate

Conclusions

Pesticide levels in both water and oysters were low at all times, even during and after the period of intense mosquito control activity. There was no indication of elevated pesticide levels because of the mosquito control operations. In fact, the limited data indicate higher levels of DDE and DDT in oysters prior to the beginning of the mosquito control operations.

Acknowledgments

Laboratory analyses of oyster and water samples were performed by Mr. Robert M. Tyo, Mr. E. A. Robertson, Jr., Mr. Lavern C. Walters, and Mr. James E. Higgins. The author would like to express his grateful appreciation to the Texas State Department of Health for its assistance in collection and shipment of samples.

LITERATURE CITED

(1) *Barry, Helen C. and J. G. Hundley. 1963.* Pesticide analytical manual, Vol. 1. Revised ed. Food and Drug Admin., U. S. Dep. Health, Educ., and Welfare.

(2) *Kovacs, Martin F., Jr. 1963.* Thin-layer chromatography for chlorinated pesticide residue analysis. J. Ass. Offic. Agr. Chem. 46:884-893.

(3) *Tyo, R. M. 1965.* Extraction of the organo-phosphate insecticides from water samples for residue analysis. Tech. Rep. Gulf Coast Shellfish Sanit. Res. Center.

Investigation of Effects of Large-Scale Applications of 2,4-D on Aquatic Fauna and Water Quality[1]

Gordon E. Smith and Billy G. Isom

ABSTRACT

In 1966, the Tennessee Valley Authority applied 888 tons of 20% 2,4-D, butoxyethanol ester, granular herbicide to 8,000 acres of Eurasian watermilfoil growths in seven reservoirs, at rates varying from 40 to 100 lb of 2,4-D acid equivalent per acre. Laboratory analyses showed little uptake of 2,4-D by fish, but some by mussels. All mud samples contained 2,4-D, in varying concentrations. Eight of nine water treatment plants sampled showed 2,4-D concentrations of less than 1 ppb. The ninth was the only plant at which 2,4-D was applied directly above the water intake supply, and its highest concentrations were 2 and 1 ppb, respectively. Extensive pre- and post-monitoring data indicate that high application rates of 2,4-D for watermilfoil control on TVA reservoirs have not produced adverse effects on aquatic fauna or water quality.

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.), a submersed aquatic plant, was first introduced into a TVA reservoir about 1953. By 1966, it had spread to seven TVA reservoirs and was posing serious threats to mosquito control, recreation, navigation, and many other water uses (*1*). Plans were made in 1966 to use 20% 2,4-D, butoxyethanol ester (BEE), granular herbicide to treat all known colonies of watermilfoil in Melton Hill, Watts Bar, Chickamauga, Hales Bar, Guntersville, Wheeler, and Wilson Reservoirs. Some 8,000 acres were scheduled for treatment at rates from approximately 40 to 100 lb of 2,4-D acid equivalent per acre. Earlier work had shown watermilfoil to be highly susceptible to 2,4-D while other aquatic organisms were relatively unaffected by it.

From March through December 1966, TVA applied 888 tons of 20% 2,4-D granular herbicide, or 355,200 lb of 2,4-D acid equivalent, to about 8,000 acres of watermilfoil growing in seven reservoirs from Melton Hill in east Tennessee to Wilson Dam in north Alabama.

[1] Division of Health and Safety, Tennessee Valley Authority, Muscle Shoals, Ala. 35660.

These reservoirs are spread over a main-channel distance of 352 river miles. They have 4,000 miles of shoreline, 237,000 water-surface acres, and hold about 4,600,000 acre-feet of water at normal full-pool elevation.

On Watts Bar and Melton Hill Reservoirs, 617 acres of hard-to-kill milfoil were treated at the rate of approximately 100 lb of 2,4-D per acre (Watts Bar, 578 acres; Melton Hill, 39 acres). Previous treatments at a lower rate were unsuccessful in controlling this aquatic plant. The remaining 7,383 acres in the other five reservoirs were treated at the rate of approximately 40 lb of 2,4-D per acre, with Hales Bar and Guntersville receiving most of the treatment. These rates of application are two to five times greater than those used in previous years.

To collect the best possible data on the toxicity of 2,4-D, outside agencies interested in this problem were urged to join TVA in planning and carrying out extensive and intensive monitoring of the watermilfoil control program. Those invited to participate in the cooperative research project were representatives of the U. S. Department of the Interior (Fish and Wildlife Service and Federal Water Pollution Control Administration); U. S. Department of Agriculture (Agricultural Research Service); U. S. Department of Health, Education, and Welfare (Public Health Service); and State agencies of Tennessee and Alabama. Within TVA, the Reservoir Ecology Branch, Water Quality Branch, Fish and Wildlife Branch, and Public Health Engineering Staff joined forces in the study.

Before, during, and after the 1966 large-scale applications of 2,4-D, vast amounts of monitoring data were collected. The purpose of this paper is to summarize some of these data.

Effects of 2,4-D on Insectary Mosquito Larvae

Prior to field monitoring, a simple laboratory experiment was conducted, first, to measure the toxicity of 2,4-D to confined mosquito larvae, and, secondly, to determine if exposure of immature mosquito stages to exceptionally high concentrations of this herbicide would affect reproductive capabilities of the adults. A concentrated solution (69.3% acid equivalent) of butoxyethanol ester of 2,4-D was mixed with ethanol alcohol for use in this experiment in the ratio of 0.45 ml of 2,4-D to 99.55 ml of alcohol. Six plastic cans were used, each containing 1 cu ft of water and about equal numbers of third and fourth instar *Anopheles quadrimaculatus* Say mosquito larvae. Five pans of larvae were treated with the 2,4-D-alcohol solution at the rate of 100 ppm, and one pan was left for control. Each pan contained about 2,000 larvae by sample count and visual estimate.

About two-thirds more of the larvae in the control pan reached the pupal stage than did larvae in each of the five treated pans. Thus, an apparently consistent degree of mortality occurred in the treated larvae; however, some of them remained alive in the 100-ppm 2,4-D solution for as long as 8 days before emerging as adults. From the larvae and pupae which persisted in the five pans of 2,4-D-alcohol solution, 85.7% emerged to the adult stage, while only 79.3% of the pupae from the untreated larvae in the control pan became adult mosquitoes.

Two colonies of mosquitoes were established and maintained in separate insectary cages—one from the treated group and one from the untreated group. Both were carried to the F_2 generation. Adult mosquitoes mated, took blood, and oviposited viable eggs. No difference in hardiness or reproductive ability could be detected.

The rate of treatment in this experiment was, of course, many times the maximum level attainable in the field even immediately following treatment. This test showed that some mosquito larvae can survive 2,4-D exposure even at the staggering rate of 100 ppm.

Analytical Results of 2,4-D Monitoring at Water Treatment Plants

The 2,4-D residue in water was monitored at nine water treatment plants along the Tennessee River system by use of carbon filters *(Davidson, C. M. and K. L. Shalibo. 1967. Analytical results of 2,4-D monitoring at water plants. Unpublished TVA report).* Special filter units were provided by the Federal Water Pollution Control Administration, Athens, Ga., and 72 samples were taken for analysis. Samples were collected at each station prior to 2,4-D application to determine whether 2,4-D was already present in the water. Continuous monitoring began at Watts Bar Dam immediately after the first 2,4-D treatments started. As the treatment operation moved downstream, monitoring began at other stations and continued approximately 2 to 3 weeks at each station following chemical application.

The flow rate through each carbon unit was determined each hour to assure an accurate record of the volume of water filtered. Raw water was passed through the carbon filter unit only when the water plant was operating. After approximately 500 gallons of water had passed through the carbon unit, the unit was removed and replaced with a unit containing clean carbon. The used carbon was removed from its pyrex container and dried—adsorbed 2,4-D was extracted with ethanol at the TVA Water Quality Laboratory in Chattanooga. The extracted sample was then shipped to the Southeast Water Laboratory of the Federal Water Pollution Control Administration in Athens, Ga., and analyzed for 2,4-D using electron capture and microcoulometric gas chromatography. The weight of 2,4-D in the extracted sample divided by the volume of water filtered equaled the concentration of 2,4-D expressed in micrograms per liter or parts per billion.

No recovery studies were performed in this study to determine the rate of adsorption on and desorption from activated charcoal. Accuracy and precision figures for this method can be found in the JAOAC 45:367 (1962). Sensitivity for the liquid-liquid extractions using a 250-ml aliquot of sample is 10 ppb acid equivalent and less than 1 ppb by carbon adsorption. However, work was done in the FWPCA Southeast Water Laboratory to determine 2,4-D degradation after sample collection. Values reported indicate minimums present.

Results showed that samples from eight water treatment plants contained concentrations of less than 1 ppb for both the butoxyethanol ester of 2,4-D and 2,4-D acid. The highest concentrations of 2,4-D were found in raw water samples collected at Scottsboro, Ala., following application. Prior to the 2,4-D application, the Scottsboro water intake had been clogged by watermilfoil, and 2,4-D at the rate of 40 lb per acre was applied directly over the water intake supply. The raw water sample collected during the 3 days immediately following application contained 2 μg/1, and the raw water sample collected 4 to 9 days after herbicidal treatment contained 1 μg/1 of herbicide. Finished water samples from the treatment plant contained <1 μg/1 or no herbicide.

Laboratory detection of the higher levels of 2,4-D at the heavily treated Scottsboro plant lends support to the accuracy of the other tests.

Effects of 2,4-D Upon Aquatic Organisms and Its Persistence in Mud and Water

In 1966 and 1967, the effects of butoxyethanol ester of 2,4-D on aquatic organisms were studied at 21 stations in a 5-acre embayment (Gordon Branch) on Watts Bar Reservoir. Water samples were taken from five stations. In a 275-acre slough above Comer Bridge in Guntersville Reservoir, six stations were selected for study. The Watts Bar test site was treated with a 20% granular material at the rate of 100 lb of 2,4-D acid equivalent per acre, and Guntersville Reservoir was treated at the rate of 40 lb per acre.

The toxic effect of 2,4-D was evaluated by sampling the benthic invertebrate communities of both reservoirs before treatment and at least twice after treatment. Residue analyses of water, fish, plants, mussels, and sediment were used to study diffusion, accumulation, translocation, and/or degradation of 2,4-D.

Since 1960, TVA, in cooperation with State fish and wildlife representatives, has routinely surveyed treatment areas for dead or distressed native fish following herbicidal applications for milfoil control. None have been found. This survey has consisted of visual inspection of water and shoreline before and after treatments. In the 1966-67 studies, TVA fishery biologists, in cooperation with State representatives, set up concurrent monitoring stations on Watts Bar and Guntersville to observe the effects of treatment at 40 and 100 lb per acre on both caged and free-swimming native fish. It was observed that, in general, both concentrations appeared to result in some movement of lake fish out of the treated area. Again, no mortality of native fish in the treated areas of the lakes was found.

In the Watts Bar area, percent mortality of caged fish compared before and after treatment differed significantly at the 5% level of probability. Comparing control and test cages, percent mortality was significantly higher for test cages at 72 and 96 hours of exposure. *(Chance, C. J. 1967. Monitoring tests of fish mortality in connection with TVA reservoir milfoil treatment, 1966. Unpublished TVA report).* However, we do not believe that this mortality was due to 2,4-D alone since the concentration in the water was much below the median tolerance limit for fish.

SAMPLING PROCEDURES

Benthic fauna and mud samples were collected with a 0.9-sq-ft Petersen dredge. Fish were collected with gill nets for 2,4-D analysis. Bluegills *(Lepomis macrochirus)* were placed in test areas before treatment and removed at various time intervals after treatment for analysis for 2,4-D. Mussels, primarily *Elliptio crassidens,* were used to monitor uptake of 2,4-D by these invertebrates. Mussels were placed in the test areas prior to treatment, and some specimens were removed at various times after treatment and analyzed for 2,4-D.

Of the six stations selected for study on Guntersville Reservoir, three were established on the quiet overbank area with little or no current, and three on the old river channel where there was usually a current. Stations referred to as "in" were on the overbank in the embayment. Stations referred to as "out" were on the margin of the channel nearest the embayment. A seventh station referred to as "control" in Table 1, which shows results of 2,4-D analyses, received an unplanned application of 2,4-D and, as a consequence, cannot be considered as a control. The prestudy data were used as the control for analysis of variance.

Fifty assorted frozen samples of plants, animal tissues, and mud from Watts Bar and Guntersville Reservoirs were analyzed for 2,4-D by the C. W. England Laboratories, Washington, D. C. Tissues of fish, mussels, and plants were each ground in a high-speed blender; then samples were removed for analysis. Sensitivity of the chemical test was 0.14 mg/kg as BEE; however, recovery of known standards when added to our samples was from 52% to 72%. *(Wimsatt, J. C. 2,4-D determination in shellfish using GLC. Unpublished method—Nat. Center Urban and Ind. Health, U. S. Public Health Serv., Cincinnati, Ohio).* Thus, there would be a tendency toward underestimating actual concentrations present rather than overestimating. It should be noted that analytical results on one sample each of mud and mussel showing higher concentrations of 2,4-D by this procedure were confirmed by paper chromatography. Values are reported on wet weight basis (Table 1).

2,4-D ANALYSIS

In Watts Bar Reservoir, watermilfoil samples collected after a 24-hour exposure showed 2,4-D concentrations (BEE) up to 8.26 mg/kg. This figure apparently represents the result of active uptake and translocation of 2,4-D, since 1 hour after treatment only 37 μg/l BEE was found in water samples (Table 2). Less than 1 μg/l BEE was present after 8 hours. However, significant concentrations of 2,4-D were found accumulated in mud samples, with detections ranging from 0.14 mg/kg to 58.8 mg/kg BEE.

Two samples of mussels, held in cages for 96 hours in the treated area, showed concentrations of 0.38 mg/kg and 0.70 mg/kg BEE. The ratio of 2,4-D content in water to that in mussels indicates that they concentrated 2,4-D. Eight fish samples (4 species) were negative for 2,4-D. One sample of bluegills *(Lepomis macrochirus),* collected 50 days after the area was treated, contained 0.15 mg/kg BEE (Table 1).

Mussels and clams held in cages for 30 days in the Watts Bar test area showed no ill effects from their incarceration in this environment which had received a massive dose of 2,4-D.

Three fish samples from Guntersville were negative for 2,4-D. Mussels exposed to treated water for 24 and 72 hours on the overbank were positive for 2,4-D. Concentration of BEE in fish ranged from 0.24 mg/kg to 1.12 mg/kg BEE. Mussels held at stations near the channel during the same period were negative for 2,4-D. Mussels exposed 144 hours in Guntersville were negative for 2,4-D; however, one sample exposed 42 days showed 0.20 mg/kg BEE.

TABLE 1.—*2,4-D Analyses—Watts Bar and Guntersville Reservoirs*

Sample No.	Date Collected	Hours/Days After Treatment	Material	mg/l[1] or mg/kg BEE	Station[2]	Species
			Watts Bar Reservoir			
19	Prestudy	Control	Fish	< 0.14	Control	*Lepomis macrochirus*
25	3-20-66	24 hours	Watermilfoil	< 0.14	Gordon Branch	*Myriophyllum spicatum*
18	3-20-66	24 hours	Watermilfoil	8.26	do.	*Myriophyllum spicatum*
16	3-20-66	24 hours	Watermilfoil	3.36	do.	*Myriophyllum spicatum*
21	3-22-66	72 hours	Fish	< 0.14	do.	*Lepomis macrochirus*
26	3-23-66	96 hours	Mud	56.0	do.	—
27	3-23-66	96 hours	Mussel	0.38	do.	*Assorted mussels*
22	3-23-66	96 hours	Mud	2.8	do.	—
23	3-23-66	96 hours	Mud	0.95	do.	—
15	3-23-66	96 hours	Fish	< 0.14	do.	*Lepomis macrochirus*
8	3-23-66	96 hours	Mussel	0.70	do.	*Elliptio crassidens*
32	4-13-66	24 days	Mud	35.0	do.	—
34	4-13-66	24 days	Mud	0.15	do.	—
33	5-24-66	35 days	Mud	0.14	do.	—
6	5-25-66	50 days	Fish	< 0.14	do.	*Lepomis macrochirus*
10	5-25-66	50 days	Fish	< 0.14	do.	*Lepomis macrochirus*
48	5-25-66	50 days	Fish	< 0.14	do.	*Ictalurus punctatus*
49	5-25-66	50 days	Fish	< 0.14	do.	*Stizostedion canadense*
50	5-25-66	50 days	Fish	0.15	do.	*Lepomis macrochirus*
47	1-17-67	10 months	Fish	< 0.14	do.	*Pomolobus chrysochloris*
43	1-17-67	10 months	Mud	0.24	do.	—
44	1-17-67	10 months	Mud	0.91	do.	—
45	1-17-67	10 months	Mud	0.28	do.	—
46	1-17-67	10 months	Mud	58.8	do.	—
			Guntersville Reservoir			
17	Prestudy	Control	Mussel	< 0.14	Control	*Elliptio crassidens*
2	Prestudy	Control	Asiatic Clams	< 0.14	—	*Corbicula manillensis*
5	May 1966	Control	Mud	0.14	—	—
11	4-06-66	24 hours	Mussel	0.25	In-1	*Elliptio crassidens*
13	4-06-66	24 hours	Mussel	0.24	"Control"	*Elliptio crassidens*
14	4-06-66	24 hours	Mussel	< 0.14	Out-1	*Elliptio crassidens*
30	4-06-66	24 hours	Mussel	1.12	In-3	*Elliptio crassidens*
12	4-08-66	72 hours	Mussel	0.18	"Control"	*Elliptio crassidens*
1	4-08-66	72 hours	Mussel	0.30	In-1	*Elliptio crassidens*
40	4-08-66	72 hours	Mussel	0.98	In-3	*Elliptio crassidens*
41	4-08-66	72 hours	Mussel	1.0	In-2	*Elliptio crassidens*
4	4-11-66	144 hours	Mussel	< 0.14	In-1	*Elliptio crassidens*
24	4-11-66	144 hours	Mussel	< 0.14	In-3	*Elliptio crassidens*
39	4-21-66	15 days	Fish	< 0.14	In-2	*Ictalurus furcatus*
28	4-11-66	144 hours	Mussel	< 0.14	Out-3	*Elliptio crassidens*
20	4-11-66	144 hours	Fish	< 0.14	Out-1	*Lepomis macrochirus*
7	5-17-66	42 days	Mud	< 0.14	Out-1	—
29	5-17-66	42 days	Mud	33.6	In-3	—
31	5-17-66	42 days	Mud	0.14	Out-2	—
3	5-17-66	42 days	Mussel	< 0.14	In-1	*Elliptio crassidens*
9	5-17-66	42 days	Mussel	0.20	In-2	*Elliptio crassidens*
35	1-20-67	9 months	Fish	< 0.14	In-2	*Dorosoma cepedianum*
36	1-20-67	9 months	Mud	0.34	In-2	—
37	1-20-67	9 months	Mud	0.30	Out-2	—
38	1-20-67	9 months	Mud	0.49	In-3	—
42	1-20-67	9 months	Mud	0.30	"Control"	—

[1] The C. W. England Laboratories converted 2,4-D and its esters to the methyl ester for reporting to TVA; however, for comparison with published data on toxicity *(2)*, all data were converted to the BEE equivalent.

2,4-D — 2,4-dichlorophenoxyacetic acid
————————, butoxyethanol ester
————————, methyl ester

[2] In=Inside embayment
Out=Outside in river channel external to embayment

Note: The station labelled "Control" received an unplanned application of 2,4-D and, therefore, cannot be considered a control.

Table 2.—*Analyses of water samples*
WATTS BAR RESERVOIR
Gordon Branch Embayment

Station	Date	Time (EST)	Temp. (°F)	Dissolved Oxygen (mg/l)	pH	Alkalinity: Phenol (mg/l)	Alkalinity: Total (mg/l)	2,4-D: BEE (µg/l)	2,4-D: Acid (µg/l as BEE)
(Surface samples collected prior to 2,4-D application)									
A	3-17-66	0805	55.7	10.0	8.1	0.0	70.1	—	—
B	3-17-66	0825	56.3	10.2	8.6	1.4	35.9	—	—
C	3-17-66	0835	56.7	11.4	7.8	0.0	33.4	—	—
D	3-17-66	0840	56.2	10.9	8.1	0.0	34.9	—	—
E	3-17-66	0855	56.4	11.4	8.2	0.0	34.5	—	—
(Surface samples collected 1 hour after 2,4-D application)									
A	3-18-66	0920	55.4	11.7	8.8	6.0	94.0	<1	<1.45
B	3-19-66	0945	56.1	10.4	7.8	0.0	35.0	1	<1.45
C	3-19-66	0955	57.2	11.4	8.3	0.0	34.0	<1	<1.45
D	3-19-66	1000	53.0	8.8	6.7	0.0	19.5	37	<1.45
E	3-19-66	1020	56.1	11.4	8.4	1.0	32.5	6	<1.45
(Surface samples collected 4 hours after 2,4-D application)									
A	3-18-66	1220	59.0	12.8	9.1	18.0	92.0	<1	<1.45
B	3-19-66	1230	61.6	10.8	7.9	0.0	35.0	6	<1.45
C	3-19-66	1240	59.3	11.5	8.5	2.0	36.0	<1	<1.45
D	3-19-66	1245	62.4	12.6	8.6	3.0	36.0	<1	<1.45
E	3-19-66	1305	56.8	10.4	8.5	1.5	35.0	2	<1.45
(Surface samples collected 8 hours after 2,4-D application)									
A	3-18-66	1550	64.4	11.6	8.7	5.0	91.0	<1	<1.45
B	3-19-66	1510	64.7	10.4	8.2	0.0	36.0	<1	<1.45
C	3-19-66	1515	62.2	11.4	8.5	2.0	38.0	<1	<1.45
D	3-19-66	1520	67.2	10.4	8.6	3.0	35.0	<1	<1.45
E	(Sample missing)								

TABLE 3.—*Analysis of variance testing differences in the mean numbers of* Hexagenia *between pre- and post-treatment of Watts Bar Reservoir with 2,4-D*

Source of Variance	SS	Df	MS	F
Between four time periods	1,421.27	3	473.75	2.15
Between stations	6,155.24	20	307.76	1.38
Error	13,210.05	60	220.17	
Total	20,786.98	83		

Note: F [illegible] 2.76
F [illegible] 1.75

All mud samples taken on the overbank were positive [illegible] 2,4-D [illegible] values ranging from 0.34 to 33.6 mg/kg BEE. [illegible] single mud sample from the stations in the [illegible] had a concentration of BEE (0.30 mg/kg) [illegible] than [illegible] in the prestudy control (0.14 mg/kg BEE) (Table 1).

Water samples [illegible] in Guntersville showed a maximum of 157 [illegible] BEE 1 hour after application (Table 4).

BENTHIC FAUNA

Benthic fauna was sampled at 21 randomly located stations within the 5-acre test area on Watts Bar Reservoir. Principal components of the Watts Bar benthic fauna were mayflies *(Hexagenia)*, midges *(Tendipedidae)*, biting midges *(Heleidae)*, phantom midges *(Chaoborus)*, worms *(Oligochaeta)*, and Asiatic clams *(Corbicula)*.

Submerged aquatic plants made excellent habitats for aquatic insects. Elimination of watermilfoil would be expected to affect the fauna associated with these plants. Vannote *(Unpublished TVA report, 1964. Insect survival in reservoir areas treated with 2,4-D herbicide)* showed that two applications of 2,4-D in Guntersville Reservoir did not depress benthic population densities, but the eradication of watermilfoil eliminated a broad expanse of substrate suitable for colonization by large populations of epiphytic insects such as the immature stages of midges, mayflies, and dragonflies. This was also shown in Watts Bar Reservoir where pre-treatment bottom samples contained Anisoptera, Elmidae, Leptoceridae, and *Caenis*, all of which were absent from 12-month post-treatment samples.

Burrowing mayflies *(Hexagenia)* normally inhabit overbank substrate in TVA reservoirs and are a principal component of the benthic fauna. Their abundance was used as an index of the toxic effects of 2,4-D on benthos. *Hexagenia* and other bottom fauna were sampled in Watts Bar at 21 stations on 4 occasions. Analysis of variance (Table 3) showed no significant change in the abundance of *Hexagenia* in Watts Bar Reservoir between one pre-treatment and three post-treatment sampling periods (post 1 month, 10 months, and 12 months). Density of *Hexagenia* populations was used to evaluate effects of 2,4-D treatment on the benthic community from the six stations sampled in Guntersville Reservoir. Analysis of variance (Table 5) showed no significant change in the mean numbers of *Hexagenia* between one pre-treatment period and two post-treatment periods (post 1 month and 12 months).

SUMMARY

Results of this study may be summarized as follows:

1. Application of 2,4-D herbicide in TVA's watermilfoil control program caused no measurable toxic effect on benthic fauna in Watts Bar and Guntersville Reservoirs.
2. Analysis of variance showed no significant change in the mean numbers of burrowing mayflies *(Hexagenia)* between pre- and post-treatment periods in Watts Bar and Guntersville Reservoirs.
3. Bottom fauna samples indicated watermilfoil constituted the principal substrate inhabited by some invertebrates. Elimination of substrate provided by the watermilfoil resulted in loss of these invertebrates.

TABLE 4.—*Analyses of water samples*
GUNTERSVILLE RESERVOIR
Vicinity of Comer Bridge

Station	Date	Time (CS)	Temp. (°F)	Dissolved Oxygen (mg/l)	pH	Alkalinity Phenol. (mg/l)	Alkalinity Total (mg/l)	2,4-D BEE (μg/l)	2,4-D Acid (μg/l as BEE)
(Surface samples collected prior to 2,4-D application)									
A	3-29-66	1120	57.0	9.2	7.7	0.0	37.0	3	<1.45
B		1110	57.0	—	—	—	—	<0.5	<1.45
C		1101	57.0	—	—	—	—	7	<1.45
D		—	60.0	8.8	7.5	0.0	39.4	7	<1.45
E		—	—	—	—	—	—	6	<1.45
F		1215	59.0	—	—	—	—	7	<1.45
(Surface samples collected 1 hour after 2,4-D application)									
A	4-5-66	0835	56.5	8.8	7.2	0.0	36.0	91	<1.45
B		0845	56.0	8.7	7.6	0.0	41.0	157	<1.45
C		0855	56.3	8.6	7.4	0.0	41.0	36	<1.45
D		0925	56.0	9.0	7.6	0.0	36.0	5	<1.45
E		0910	56.0	8.6	7.6	0.0	38.0	5	<1.45
F		0905	56.0	8.6	7.7	0.0	39.0	3	<1.45
(Surface samples collected 4 hours after 2,4-D application)									
A	4-5-66	—	—	—	—	—	—	—	—
B		—	—	—	—	—	—	—	—
C		—	—	—	—	—	—	—	—
D		1220	57.0	9.2	7.6	0.0	36.0	8	<1.45
E		1230	58.0	9.3	7.6	0.0	37.0	130	<1.45
F		1240	57.5	9.4	7.8	0.0	36.0	19	<1.45
(Surface samples collected 8 hours after 2,4-D application)									
A	4-5-66	1455	56.5	8.6	7.4	0.0	39.0	2	<1.45
B		1505	57.0	8.5	7.5	0.0	39.0	<1	<1.45
C		1515	57.0	8.5	7.5	0.0	39.0	4	<1.45
D		1535	58.0	9.7	7.8	0.0	38.0	18	<1.45
E		1530	58.0	10.1	8.0	0.0	36.0	64	<1.45
F		1525	58.0	9.9	7.9	0.0	38.0	21	<1.45

TABLE 5.—*Analysis of variance testing differences in the mean numbers of* Hexagenia *between pre- and post-treatment of Guntersville Reservoir with 2,4-D*

Source of Variance	SS	Df	MS	F
Between three time periods	62.44	2	31.22	0.60
Between stations	775.33	5	155.07	2.97
Error	519.27	10	51.93	
Total	1,357.04	17		

Note: $F_{2,10,0.95} = 4.1$
$F_{5,10,0.95} = 3.33$

4. There was little uptake of 2,4-D by fish but some by mussels.
5. Significant concentrations of 2,4-D were noted in isolated sediment samples up to 10 months after treatment.

Conclusion

These data indicate that high application rates of 2,4-D for watermilfoil control on TVA reservoirs have not produced adverse effects on aquatic fauna or water quality.

LITERATURE CITED

(1) *Smith, Gordon E., T. F. Hall, Jr., and R. A. Stanley. 1967.* Eurasian watermilfoil in the Tennessee Valley. Weeds 15:(2).

(2) *Mount, D. I. and C. E. Stephan. 1967.* A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2,4-D. Trans. Amer. Fish Soc. 96(2):185-193.

PESTICIDES IN SOIL

Monitoring for Chlorinated Hydrocarbon Pesticides in Soil and Root Crops in the Eastern States in 1965

W. L. Seal[1], L. H. Dawsey[2], and G. E. Cavin[3]

ABSTRACT

Forty-nine fields planted to root crops were sampled in the fall of 1965 to determine levels of chlorinated hydrocarbon pesticide residues in soils and crops. Selection of fields was based on the relatively heavy use of persistent insecticides in prior years. Materials analyzed in the study were soil, potatoes, carrots, and peanut meats. The methods of analysis employed were gas chromatography (electron capture) and thin layer chromatography.

DDT was found in soil in 48 of the 49 fields sampled, ranging from 0.10 to 12.8 ppm, and averaging 2.8 ppm. Residues of DDT were well below the tolerance levels in all crop samples. Dieldrin was present in soil in 28 of the 49 fields sampled, ranging from 0.05 to 0.26 ppm. No dieldrin residues were detected in potato tubers, and residues of the chemical averaged 0.05 ppm in 6 of 19 composite carrot samples. Dieldrin was present in all five composite peanut meat samples, averaging 0.10 ppm. Sampling was too limited, however, to draw any conclusions as to whether this contamination of peanuts is a significant problem. Additional monitoring was conducted in 1966 and 1967.

Introduction

A limited study was conducted in the fall of 1965 in seven Eastern States to determine levels of chlorinated hydrocarbon pesticide residues in food and soil from land treated with persistent pesticides. Potatoes, carrots, and peanuts were selected for this study since these root crops are more readily contaminated by pesticide residues in the soil through adsorption or translocation.

A preliminary survey of appropriate fields was conducted prior to sampling. Selection of fields to be sampled was based on the relatively heavy use of persistent insecticides since 1961, particularly chlorinated hydrocarbons, for the control of insect pests. All of the fields selected were treated with persistent pesticides at least 1 or more years during this period. Of 49 fields selected, 25 were planted to potatoes, 19 to carrots, and 5 to peanuts.

Sampling Methods

Three 1-acre plots were laid out in each field on a stratified random basis. The plots were located in relation to drainage and other topographical features. Fifty soil cores, 2 inches in diameter and 3 inches deep, were taken from within the rows in each 1-acre plot. Potato, carrot, and peanut samples were collected at the same time and place the soil cores were taken.

The soil cores from each plot were placed in a large container and passed through a ¼-inch screen to facilitate mixing. Stones, roots, and other trash that would not pass through the screen were discarded. A new 1-gallon paint can was then filled with the mixed, screened soil and sealed with an airtight lid. Each container was labeled with a field sample number and date. Extreme care was taken to thoroughly clean the sampling equipment after each sample was collected.

The containers of soil and bagged crop samples were stored at room temperature until they could be shipped to the laboratory at Gulfport, Miss.

Analytical Procedures

The sensitivity limits of the analytical procedures used were generally 0.05 ppm for residues in soil and 0.01 ppm for residues in crops.

Three hundred grams of soil were tumbled for 4 hours with 600 ml of a 3:1 mixture of hexane and isopropanol of chromatographic grades. The isopropanol was removed by repeated washing with distilled water and the washed extract was dried by filtration through anhydrous sodium sulfate. A representative aliquot was stored under refrigeration in a sealed glass bottle prior to direct determination of pesticides in the extract by gas chromatography. Another 100-g lot of soil was dried overnight in an oven at 110 C for parallel determination of the moisture contents of the soil at the time of analysis for pesticides.

[1] Plant Pest Control Division, Agricultural Research Service, Hyattsville, Md. [illegible]

[2] Plant Pest Control Division, Agricultural Research Service, Gulfport, Miss. [illegible]

[3] Plant Pest Control Division, Agricultural Research Service, Moorestown, N. J. [illegible]

Preparation of samples and analytical procedures used were the same for carrots and potatoes. Samples were water-washed and air-dried before chopping. A vertical segment of each root or tuber was included in the first sample size reduction which was accomplished in a food chopper. One hundred grams of the pulp was homogenized for 1-2 minutes with 200 ml of chromatographic grade acetonitrile in the 450-ml stainless steel cup of a Lourdes Multimix Homogenizer (MM-1)[4], with about 10 g of Celite added. The solvent and pulp were separated by centrifuging in the cup. The acetonitrile was decanted through a filter paper into a 1-liter flask and held. Another 100-ml portion of acetonitrile was added to the pulp in the cup; the extraction, settling, and filtration were repeated, the second extract being added to the first in the flask. The total volume of acetonitrile was then reduced by evaporation through a Snyder column on a hot plate, to leave a water layer covering the bottom of the flask. One hundred milliliters of hexane was added through the column to the water layer; the hexane was evaporated completely, carrying with it remaining traces of acetonitrile from the water. Another 200-ml portion of hexane was added through the Snyder column to the water layer in the flask, and the contents were refluxed to insure complete solution of the pesticides in hexane. Water and hexane were transferred to a separatory funnel where the water was rejected. The hexane extract was filtered through sodium sulfate, made to 300-ml volume, sealed in a glass bottle, and held under refrigeration until final determination was made by gas chromatography. Cleanup was unnecessary with carrots and potatoes when the initial extraction was made with acetonitrile.

The harvested peanut pods had been air-dried according to farm practice. Shells were removed by hand at the laboratory and discarded. Pesticides which might have passed from the shells to meats in handling were eliminated by rinsing the meats, first with an isopropanol wash, then with a hexane wash, prior to grinding of samples. The washed meats were ground dry in a blender to give a free-flowing meal, from which a 20-g aliquot was weighed. The meal was homogenized with 100 ml of isopropanol in the Lourdes Multimixer (see above). The mixture was washed into a Mason jar with 300 ml of pentane, tumbled for 2 hours, then allowed to settle. The extract was decanted through a filter into a separatory funnel, and the isopropanol was removed by repeated water washes. The peanut oil was eliminated from the pentane extract by means of the acetonitrile partition method. This method consisted of reducing the volume to 50 ml by evaporation of pentane, transferring the extract to a 125-ml separatory funnel, adding an equal volume of acetonitrile (saturated with pentane), equilibrating, settling, and drawing off the acetonitrile into a 250-ml separatory funnel. The pentane in the first separatory was washed two more times with acetonitrile to extract all pesticides from the pentane which was rejected. The combined washings in the second separatory were backwashed with 40 ml of hexane which was rejected, and the acetonitrile was transferred to a 500-ml ₮-jointed flask for evaporation and transfer of pesticides back into fresh hexane. Transfer back to hexane was accomplished using the same procedures as for the carrot extracts.

Upon completion of partitioning for removal of fat, the extract was divided in half, which was equivalent to 10 g of the original peanut meats, and the half-extract was concentrated by evaporation to 5.0-ml volume preparatory to cleanup by column chromatography. The glass chromatographic column, 450 mm x 10 mm ID, resembled an ordinary 50-ml burette with teflon stopcock at the bottom. Prior to operation, the column was filled about halfway with 10 g of Florex absorbent (AARVM 60/100 mesh, activated at 130 C for 16 hours); the absorbent was pre-washed first with 50 ml of 10% ether in hexane, then with 50 ml of hexane. The 5.0 ml of concentrated extract was then passed through the absorbent elution with 150 ml of 10% ether in hexane, which solvent was caught in a 250-ml Kuderna-Danish evaporator at the bottom of the column. The evaporator was fitted with a 15-ml graduated centrifuge tube, which enabled the eluate to be reconcentrated to a 5.0-ml volume after placing the evaporator on a steam bath. This final cleanup sample was sealed by means of a glass stopper in the centrifuge tube, pending determination of pesticides by gas chromatography.

Unknown residues in the above described hexane extracts were determined by injecting 2.5- to 10.0-μliter portions into columns of gas chromatographs followed by interpretation of the tracings made on the record charts, as compared with similar tracings made from injection of known amounts of pesticides. Columns used were as follows:

DC-200,	3%,	on Gas-Chrom-Q	(100-120 mesh)
QF-1,	5%,	on Diatoport-S	(100-120 mesh)
SE-30,	5%,	on Chromosorb-W	(60-80 mesh)
Dow-11,	5%,	on Chromosorb-W	(60-80 mesh)

Chromatographic instruments employed were the Jarrell-Ash 28-730 and the F & M 810, each equipped with two columns and two electron capture detectors. Typical operating conditions for the DC-200 column as installed in the F & M 810 instrument were as follows:

Column:	All glass, 8 feet x 3 mm ID
Gas:	Methane-Argon, at 120 ml/min for inlet pressure of 60 lb

[4] Lourdes Instrument Corp., 656 Montank Ave., Brooklyn, N. Y. 11208.

Sensitivity	5.12 × 10[illegible]	
Temperatures:	Column	180 C
	Detector	210 C
	Sampler	235 C
Chart speed:	15 inches hour	

The other three columns may have been operated under conditions somewhat different from this particular one; however, portions of extract were injected in at least two different columns, sometimes four different columns, to verify the identities of pesticide peaks produced on the charts. When identity of a peak appeared doubtful on two or more charts, further confirmation was obtained by thin layer chromatography methods.

Soil, potatoes, carrots, and peanut meats were analyzed in different groups. Each group was organized with a set of five controls before starting the material through the various analytical steps. The five controls built into each group of samples at the start were as follows: (1) A 9-component pesticide standard, diluted with the extraction solvent, was prepared and bottled for calibrating use in the final gas chromatographic determinations; (2) A portion of the extraction solvent only was carried through all analytical steps to detect pickup of extraneous substance, if any; (3) An extraction solvent fortified with pesticides, same as the first calibrating standard, was carried through the analysis; (4) A composite sample was prepared from portions of each sample in the group to be analyzed; and (5) A composite sample was prepared similar to the fourth control but fortified with the same pesticides as were added to the calibrating standard (first control) and the fortified solvent (third control). The last four controls were carried through all analytical steps for the purpose of determining overall recovery of pesticides, both with solvent alone and with the actual material under analysis.

Recoveries of dieldrin, endrin, heptachlor, and members of the DDT-complex from soils, ranged from 87% to 107% with composite control samples. Soil residues were corrected with appropriate recovery factors applying on individual groups of samples, and also for moisture content of each sample. With potatoes, recovery of the DDT-complex was 134%, dieldrin—120%, endrin—100%, and heptachlor—97%. With carrots, recovery of the DDT-complex was 87%, dieldrin—85%, endrin—[illegible] heptachlor 73%. Efficiency of analysis of [illegible] meats was less than that of soil, potato, and [illegible] inasmuch as cleanup involving partition-[illegible] column treatment was employed. Recov-[illegible] samples of peanuts were 50% for the DDT [illegible] 47% for dieldrin. No controls [illegible] porated in the groups, and the [illegible] certain of the soils, but not the crops [illegible] bench standard. Such residues were reported without [illegible] for losses.

Results and Discussion

DDT was found in the soil in 48 of the 49 fields, averaging 2.8 ppm. Few analyses were above 6 ppm DDT. The highest found were 12.8 and 9.5 ppm in samples from two carrot fields and 7 ppm in one potato field. DDT residues in potatoes and carrots were well below tolerance levels. All carrot samples contained some DDT, however, and DDT residues were found in potato samples from 21 of 25 fields but in extremely low amounts. Residues of DDT in soil in peanut fields averaged 0.3 ppm, and residues in peanut meats averaged 0.05 ppm in samples that contained detectable residues.

Dieldrin was present in soil samples in all 5 peanut fields sampled; 19 of 25 potato fields; and 4 of 19 carrot fields. No measurable residues were found in potatoes, and residues in carrots did not exceed 0.14 ppm. Residues in peanut meats averaged 0.10 ppm.

Treatment histories indicate that aldrin, which converts to dieldrin, was used in prior years on all five of the peanut fields. The most recent treatment of record was on one field where 40 lb of 5% dust or 2 lb of technical aldrin were applied per acre. Residues in peanuts from this field were 0.13 ppm. In another field, some dieldrin residues were found in peanuts even though the last known application of aldrin was in 1953, at the rate of 1.5 lb actual per acre. A study of the limited residue data showed that dieldrin residues in peanut meats were roughly equal to about two-thirds of the residues in the top 3 inches of soil where the crop was grown.

Endrin was found in the soil in about one-fifth of the fields. Residues were not found in crop samples using a method sensitive to 0.01 ppm.

Heptachlor and/or heptachlor epoxide was found in the soil in four potato fields and five carrot fields. No residues were detected in potatoes, but residues averaged 0.07 ppm in two carrot samples.

Endosulfan has been applied to potatoes in the past few years. It is replacing some of the chlorinated hydrocarbons previously used in the control of certain pests. Endosulfan residues averaging 0.46 ppm were found in soil from 23 of the 25 potato fields. No residues were detected in tubers, however. Endosulfan was not used on the peanut fields sampled and on only 1 of the 19 carrot fields.

Summary

DDT ranged from 0.10 to 12.8 ppm in 48 of the 49 fields that were sampled in 1965. Based on the data developed in these studies, it appears that DDT residues in soil in these fields should not result in residues above presently accepted levels for potatoes, carrots, and peanuts.

TABLE 1.—*Pesticide residues in soil and root crops collected in the Eastern States in 1965*

Crop	No. of Fields	Material Sampled	DDT		Dieldrin		Endrin		Heptachlor and/or Heptachlor epoxide		Endosulfan	
			Positive Composite Samples[1]	Residue (ppm) Range & Average	Positive Composite Samples[1]	Residue (ppm) Range & Average	Positive Composite Samples[1]	Residue (ppm) Range & Average	Positive Composite Samples[1]	Residue (ppm) Range & Average	Positive Composite Samples[1]	Residue (ppm) Range & Average
Peanuts	5	Soil	5	0.10-0.71 (0.30)	5	0.08-0.20 (0.15)	0		0		0	
		Meats	3	0.02-0.07 (0.05)	5	0.08-0.13 (0.10)						
Potatoes	25	Soil	24	0.33-7.04 (2.75)	19	0.08-0.20 (0.10)	8	0.08-0.50 (0.27)	4	0.05-0.10 (0.08)	23	0.08-1.17 (0.46)
		Tubers	21	0.01-0.06 (0.02)	0		0		0		0	
Carrots	19	Soil	19	0.49-12.8 (3.67)	4	0.05-0.26 (0.19)	2	0.05-0.10 (0.08)	5	0.06-0.26 (0.16)	1	0.49
		Roots	19	0.20-2.31 (0.85)	6	0.01-0.14 (0.05)	0		2	0.06-0.08 (0.07)	0	

[1] Represents results from analysis of one composite sample per field.

DDT 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane

Dieldrin not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene

Endrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene

Heptachlor 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene

Heptachlor epoxide 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan

Endosulfan 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide

Dieldrin residues were not found in potatoes grown on soil previously treated with aldrin or dieldrin, but preliminary investigations indicate low-level dieldrin residues (0.10 ppm) in peanut meats. The limited sampling that has been done is not sufficient to serve as a basis for any definite conclusion as to whether this is a significant problem. Further testing was conducted in 1966 and 1967 to confirm these results.

Trade names and the names of commercial companies are used in this paper solely for the purpose of providing specific information. Mention of a trade product or manufacturer does not constitute a guarantee or warranty by the U. S. Department of Agriculture or an endorsement by the Department over other products or manufacturers not mentioned.

The PESTICIDES MONITORING JOURNAL welcomes from all sources qualified data and interpretive information which contribute to the understanding and evaluation of pesticides and their residues in relation to man and his environment.

The publication is distributed principally to scientists and technicians associated with pesticide monitoring, research, and other programs concerned with the fate of pesticides following their application. Additional circulation is maintained for persons with related interests, notably those in the agricultural, chemical manufacturing, and food processing industries; medical and public health workers; and conservationists. Authors are responsible for the accuracy and validity of their data and interpretations, including tables, charts, and references. Accuracy, reliability, and limitations of the sampling and analytical methods employed must be clearly demonstrated through the use of appropriate procedures, such as recovery experiments at appropriate levels, confirmatory tests, internal standards, and interlaboratory checks. The procedure employed should be referenced or outlined in brief form, and crucial points or modifications should be noted. Check or control samples should be employed where possible, and the sensitivity of the method should be given, particularly when very low levels of pesticides are being reported. Specific note should be made regarding correction of data for percent recoveries.

Preparation of manuscripts should be in conformance to the STYLE MANUAL FOR BIOLOGICAL JOURNALS, American Institute of Biological Sciences, Washington, D. C., and/or the STYLE MANUAL of the United States Government Printing Office.

An abstract (not to exceed 200 words) should accompany each manuscript submitted.

All material should be submitted in duplicate (original and one carbon) and sent by first-class mail in flat form—not folded or rolled.

Manuscripts should be typed on 8½ x 11 inch paper with generous margins on all sides, and each page should end with a completed paragraph.

All copy, including tables and references, should be double-spaced, and all pages should be numbered. The first page of the manuscript must contain authors' full names listed under the title, with affiliations, and addresses footnoted below.

Charts, illustrations, and tables, properly titled, should be appended at the end of the article with a notation in text to show where they should be inserted.

Charts should be drawn so the numbers and texts will be legible when considerably reduced for publication. All drawings should be done in black ink on plain white paper.

Photographs should be made on glossy paper. Details should be clear, but size is not important.

The "number system" should be used for literature citations in the text. List references alphabetically, giving name of author/s/, year, full title of article, exact name of periodical, volume, and inclusive pages.

Pesticides ordinarily should be identified by common or generic names approved by national scientific societies. The first reference to a particular pesticide should be followed by the chemical or scientific name in parentheses—assigned in accordance with CHEMICAL ABSTRACTS nomenclature. Structural chemical formulas should be used when appropriate. Published data and information require prior approval by the Editorial Advisory Board; however, endorsement of published information by any specific Federal agency is not intended or to be implied. Authors of accepted manuscripts will receive edited typescripts for approval before type is set. After publication, senior authors will be provided with 100 reprints.

Manuscripts are received and reviewed with the understanding that they previously have not been accepted for technical publication elsewhere. If a paper has been given or is intended for presentation at a meeting, or if a significant portion of its contents has been published or submitted for publication elsewhere, notation of such should be provided.

Correspondence on editorial and circulation matters should be addressed to: *Mrs. Sylvia P. O'Rear*, Editorial Manager, PESTICIDES MONITORING JOURNAL, Pesticides Program, National Communicable Disease Center, Atlanta, Georgia 30333.

The *Pesticides Monitoring Journal* is published quarterly under the auspices of the Federal Committee on Pest Control and its Subcommittee on Pesticide Monitoring as a source of information on pesticide levels relative to man and his environment.

The parent committee is composed of representatives of the U. S. Departments of Agriculture, Defense, the Interior, and Health, Education, and Welfare.

The Pesticide Montoring Subcommittee consists of representatives of the Agricultural Research Service, Consumer and Marketing Service, Federal Extension Service, Forest Service, Department of Defense, Fish and Wildlife Service, Geological Survey, Federal Water Pollution Control Administration, Food and Drug Administration, Public Health Service, and the Tennessee Valley Authority.

Responsibility for publishing the *Pesticides Monitoring Journal* has been accepted by the Pesticides Program of the Public Health Service.

Pesticide monitoring activities of the Federal Government, particularly in those agencies represented on the Pesticide Monitoring Subcommittee which participate in operation of the national pesticides monitoring network, are expected to be principal sources of data and interpretive articles. However, pertinent data *in summarized form*, together with interpretive discussions, are invited from both Federal and non-Federal sources, including those associated with State and community monitoring programs, universities, hospitals, and nongovernment research institutions, both within and without the United States. Results of studies in which monitoring data play a major or minor role or serve as support for research investigation also are welcome; however, the *Journal* is not intended as a primary medium for the publication of basic research. Manuscripts received for publication are reviewed by an Editorial Advisory Board established by the Monitoring Subcommittee. Authors are given the benefit of review comments prior to publication.

Address correspondence to:
Mrs. Sylvia P. O'Rear
Editorial Manager
PESTICIDES MONITORING JOURNAL
Pesticides Program
National Communicable Disease Center
Atlanta, Georgia 30333

CONTENTS

Volume 1 March 1968 Number 4

EDITORIAL

The increasing number of pesticide monitoring programs magnifies the difficulty in evaluating the results of individual studies and of comparing them with earlier studies.

The list of factors contributing to the problem is long and includes such items as differences in experimental design; lack of adequate experimental controls; insufficient knowledge concerning the chemical characteristics of pesticides; differences in sample collection, handling, and storage; variations in efficiency of cleanup procedures; differences in sensitivity of chemical analytical procedures; use of chemicals of inadequate purity as controls; technician variation; and so on.

Each could quite properly serve as the subject of an editorial. However, the present discussion is limited to the uncertainty introduced into pesticide monitoring data by the use of analytical methods of unknown reliability and the difficulty in comparing results on similar systems when different cleanup and analytical methods are used. Certainly no one is against progress, and changes in methodology to improve sensitivity, resolution, or recovery are necessary. However, constant alteration of methodology must lead to confusion. Ideally, a sensitive, reliable, and reproducible analytical procedure should be adopted for each substrate studied. The analytical procedure should be standardized and fully evaluated in order to serve as a reference in evaluating future modifications or entirely new procedures. This is equally important for sampling techniques, cleanup procedures, and instrumental analysis, including interpretation of tracing. Procedures used for closely related substrates should then be compared.

There are those who would argue that such standardization is not necessary because the same technique does not work equally well for every laboratory; and that, therefore, each laboratory should use its best technique. This, of course, is just what has been happening; hence the current difficulties. Such an approach is characterized by the statement, "We use the .... procedure but with certain modifications." Sometimes it seems everyone has his own set of modifications!

Development of the gas chromatograph and of the electron capture and other detectors has been a boon to pesticide residue chemistry. This chemical specialty has long been an art; it is time to add standardization and make it a science.

Anne R. Yobs
Member, Editorial Advisory Board

RESIDUES IN FOOD AND FEED

Pesticide Residues in Vegetable Oil Seeds, Oils, and By-Products

R. E. Duggan[1]

Earlier reports (*1-3*) have discussed, in terms of broad food categories, the pesticide residue data obtained by the Food and Drug Administration in surveillance and monitoring programs conducted from July 1, 1963 through June 30, 1966. The findings on fluid milk and other dairy products have been reported in considerable detail (*4*).

The principal purpose of this paper is to report and evaluate the findings on samples of products derived from oil seed crops. Since these crops constitute an important segment of the Nation's food supply, pesticide residues incurred in their production are of substantial importance. Direct additions of these pesticide residues to man's diet may occur from consumption of these crops which have been treated with pesticides in their production. Indirect additions to man's diet from these crops may occur from the use of by-products in the production of milk, meat, and poultry; and some tolerances have been established on this basis. Additionally, man may receive residues by consuming foods contaminated through drift and runoff and through crop rotation—for example, the planting of soybeans in areas previously treated for cotton production. It may be impossible currently to measure the relative effects of the various factors influencing the incidence and levels of pesticide residues in food. However, there is a need to establish, with a reasonable degree of certainty, the major factors making up the total residue content of the food chain.

Portions of several major program divisions, such as raw agricultural products, processed animal feeds, vegetable oils, and processed foods, have been excerpted for this report.

[1] Office of Associate Commissioner for Compliance, Food, and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20204

Sampling Procedures

Samples were collected on a nationwide basis as a part of the Food and Drug Administration's surveillance program carried out in 18 District offices. Samples collected in surveillance programs are classified as "objective" if "unknown" with respect to the possibility of excessive residue content or actual misuse of pesticide chemicals. The selection of sampling points and scheduling of samples was left to the discretion of the 18 District offices.

Specific lots were sampled (*5*) for analysis by taking several portions from the lot. The portions were combined for analysis.

Laboratory Analysis

Generally, samples were examined promptly after collection. All analyses were made in FDA District Laboratories by multiple residue gas-liquid chromatographic methods. All of the laboratories concerned participated in method validation studies reported by Johnson (*8*), Krause (*9*), Gaul (*10*), and Wells (*11*). Electron capture and microcoulometric detectors were employed. The methods used during this investigation were basically those which have become official A.O.A.C. procedures (*6*); the detailed procedures employed are described in the FDA *Pesticide Analytical Manual*, Vol. I (*7*). Quantitative sensitivity limits for gas chromatographic analysis, readily attainable on most products in normal laboratory operations, were based on ½ full-scale deflections (1×10^{-9} Amperes Full-Scale) for 1 ng of aldrin and, for program purposes, were established at 0.05 ppm for raw agriculture products, and at 0.25 ppm (fat basis) for fatty foods. Confirmatory analyses by thin layer chromatography were made when results exceeded these figures. Quantitative results below these levels were reported but were not confirmed by check analysis, and are recognized as having reduced accuracy limitations common to all quantitative estimations at the lower ranges of method sensitivity.

Recoveries, in general, range between 80% and 110% for most pesticide residues and commodities. No corrections for recovery have been made, and the values are reported on an "as is" basis.

Results

The data are not amenable to evaluation on a geographic or production basis. Inspection of the raw data indicates that samples were reasonably well distributed according to major program divisions among the 18 District offices.

A total of 1,230 residues of 20 pesticide chemicals were reported in 641 positive samples of the 2,389 samples of raw products, meal, crude oil, refined oil, and oleomargarine. DDT and its analogues (DDE and TDE), dieldrin, lindane, toxaphene, endrin, BHC, and chlordane account for 95% of the residues found in oil seeds, 96% of the residues in oil seed meals, 98% of the residues in crude oils, and 95% of the residues in refined oils. Malathion residues were found only in the raw cottonseed, peanuts, and soybeans. Aldrin and heptachlor epoxide were found too infrequently to be considered significant. Seven other pesticide chemicals were found in one or two samples.

Table 1 shows the percent of residues at arbitrarily selected ranges in the raw products, meals, crude oil, refined oil, and oleomargarine based on the total number of instances in which residues were found. Most of the residues were found at low levels: 94.5% of the values were below 0.51 ppm, and 75.5% of the values were below 0.11 ppm. This general pattern is observed regardless of the commodity, product, or individual pesticide chemical involved.

Tables 2-5 summarize the incidence and average levels of specific pesticide chemicals found in soybeans, peanuts, cottonseed, corn, and products derived from the raw commodity. Although corn is not generally classified as an oil seed, all available data have been included since the production of corn oil is substantial. The samples represent grain corn generally and are not confined to that used for the production of corn oil.

During the period covered in this report, 53 objective samples of oleomargarine were collected as shown in Table 6. Of these samples, 18.9% contained DDT, 5.7% contained TDE, 7.5% contained DDE, and two samples (3.8%) contained BHC.

The number of objective samples examined in some product classes, such as refined peanut and cottonseed oil, are too few to be considered representative of the surveillance period involved and therefore have limited usefulness.

The average level for each pesticide residue in Tables 2-6 includes all samples and was calculated by using the midpoint of each range and the percent of samples falling in the range. The actual values were used for those residues exceeding 2 ppm.

No significant trends were observed on an annual basis where the number of samples was large enough for consideration of trends.

Discussion

Legal tolerances have been established for some of the chemicals found in the raw agricultural product as follows:

	Residues in PPM			
	Cotton-seed	Soybeans	Peanuts	Grain Corn
DDT	4	1.5	7	
Dieldrin		zero		zero
Toxaphene	5	2	7	
Endrin	zero			
Chlordane			3	

Except for the positive findings of dieldrin in soybeans, there were no samples containing pesticide chemicals exceeding the tolerance level. Over 60% of the residues commonly present are not sanctioned by tolerances in the raw agricultural product.

The kinds, incidence, and levels of pesticide residues in soybeans, cottonseed, peanuts, and corn are quite similar. Although the tabular data indicate higher average levels of all residues except endrin in cottonseed, the small number of cottonseed samples involved does not permit a high degree of reliability in this observation.

It must be recognized that peanuts and corn may be eaten unprocessed and that processing procedures would significantly change the pesticide residue content of the product. Cottonseed and soybeans, on the other hand, generally undergo processing into other products before consumption.

Table 7 compares the pesticide chemicals found in the various oil seeds and oil seed products, including oleomargarine. Data from the "Oils, Fats, and Shortening" portion of the Total Diet studies also are included in this table for comparison.

Average residue values are higher in crude oils than in the raw products or meals. The average levels of pesticide residues in soybeans, cottonseed, and peanuts are quite low when compared to the established tolerances; for example, the average of 0.15 ppm DDT compared to the 4 ppm tolerance on cottonseed, or 0.03 ppm DDT compared to the 7 ppm tolerance on peanuts.

The incidence of residues within various quantitative ranges shows that 78% of the values on oil seed are below 0.11 ppm, and 98% are below 0.51 ppm. The incidence of residues in grain corn at these levels was 96% and 100%, respectively.

The residue content of oil seed meals is important because of the general use of such products in animal feed. The average levels of pesticide residues in oil seed meals or cakes are low. The distribution of residues within various quantitative ranges shows that 87% of all residues were below 0.11 ppm, and 96.5% were below 0.51 ppm.

As expected, the average values of residues in crude oils are much higher than in the other products. Since the results for oil seeds are reported on an "as is" basis, the higher values for the oil content of the product under examination must be considered. The incidence of residues within various quantitative ranges shows that 61% of the values were below 0.11 ppm, and 28% were between 0.11 ppm and 0.50 ppm.

After refining, the average levels of residues are substantially lowered and are similar to the average values found in oleomargarine.

The incidence of residues in the various quantitative ranges shows 56% of the values below 0.11 ppm, and 31% between 0.11 ppm and 0.50 ppm. However, no values in excess of 1.50 ppm were reported in refined oils compared to 1.8% of the values in crude oils.

Except for endrin, residues of the pesticide chemicals most commonly found in the raw product were also found in the refined oil at considerably lower levels. Endrin was not found in refined oils. Only residues of the DDT compounds and BHC were found in oleomargarine.

The average levels found in these samples of refined oils are somewhat higher than those found in the 70 composites of oils, fats, and shortening from the total diet samples examined during the period June 1964 through April 1967. Residues of toxaphene and chlordane were not reported in the total diet composites. The oils, fats, and shortening composite is prepared from salad dressings, mayonnaise, salad oil, shortening, and peanut butter.

Summary and Conclusions

Residues of DDT and its analogues (TDE and DDE), dieldrin, lindane, toxaphene, endrin, BHC, and chlordane were frequently found in vegetable oil seeds and products.

Residues of other pesticide chemicals were not found with sufficient frequency to be considered significant.

None of the residues found in oil seeds exceeded the tolerances where finite tolerances have been established. Endrin residues were found in cottonseed, for which the established tolerance is zero. Dieldrin residues were found in soybeans and grain corn which have an established tolerance of zero. Over 60% of the residues found were not sanctioned by tolerances in the raw agricultural product.

Chlorinated organic pesticide residues are relatively high in the crude oil. Significantly lower values were found in the refined oils and in the oil seed meals and cakes.

While the residue levels found in these samples indicate that oil seeds and products do not present a serious problem, it is obvious that, when such residues are present—whether from approved applications, misuse, or unavoidable sources—the finished product will prob-

TABLE 1.—*Distribution of residues, by product, in different quantitative ranges*

[T = <0.001 PPM]

Level (PPM)	Percent of Positive Samples				
	Seed	Meal	Crude Oil	Refined Oil	Oleomargarine
T-0.03	65.2	71.4	43.0	33.3	68.4
0.04-0.10	17.8	15.1	17.7	23.1	10.5
0.11-0.50	15.8	10.1	28.5	23.1	21.1
0.51-1.00	1.0	2.1	6.6	7.7	
1.01-1.50	0.2	0.8	2.4	12.8	
1.51-2.00		0.4	0.5		
>2.00			1.3		

TABLE 2.—*Incidence of specific pesticide residues in soybean products*

[T = <0.001 PPM; — = Not detected]

	Percent of Samples Containing Specific Pesticides and Average Level (PPM) of Each Pesticide			
	Soybeans	Crude Oil	Meal (Cake)	Refined Oil
DDT	9.6 (0.006)	16.3 (0.015)	7.0 (0.005)	13.0 (0.003)
TDE	0.7 (T)	7.1 (0.006)	1.4 (T)	—
DDE	2.7 (T)	6.1 (0.002)	3.5 (0.001)	—
Dieldrin	8.0 (0.002)	17.3 (0.013)	1.4 (T)	4.3 (T)
Lindane	2.2 (T)	2.0 (T)	4.9 (0.001)	—
Toxaphene	8.0 (0.004)	4.1 (0.024)	—	4.3 (T)
Endrin	9.8 (0.008)	6.1 (0.013)	0.7 (T)	—
BHC	2.9 (T)	11.2 (0.004)	0.7 (T)	—
Chlordane	0.9 (T)	—	0.7 (T)	—
Total Number of Samples	550	98	143	23
Percent w residues	26.7	34.7	14.0	17.4

ably contain a portion of the pesticide chemical. When these residues are added to other unsanctioned additions in the total diet, they may eventually reach a total level that will have an impact on the existing tolerances for residues on raw agricultural products generally.

TABLE 3.—*Incidence of specific pesticide residues in peanut products*

[T = <0.001 PPM; — = Not detected]

	Percent of Samples Containing Specific Pesticides and Average Level (PPM) of Each Pesticide			
	Nuts	Crude Oil	Meal (Cake)	Refined Oil
DDT	13.5 (0.025)	66.7 (0.466)	45.2 (0.140)	20 (0.060)
TDE	1.7 (T)	41.7 (0.128)	22.6 (0.026)	20 (0.007)
DDE	9.6 (0.001)	58.3 (0.909)	38.7 (0.025)	20 (0.032)
Dieldrin	8.5 (0.008)	22.2 (0.017)	22.6 (0.006)	20 (0.007)
Lindane	2.8 (0.002)	5.6 (0.001)	3.2 (T)	—
Toxaphene	1.7 (0.006)	2.8 (0.008)	—	—
Endrin	1.1 (T)	2.8 (0.008)	—	—
BHC	3.4 (0.007)	8.3 (0.002)	12.9 (0.006)	10 (0.002)
Total Number of Samples	177	36	31	10
Percent w/Residues	26.6	75.0	61.3	33.3

TABLE 4.—*Incidence of specific pesticide residues in cottonseed products*

[T = <0.001 PPM; — = Not detected]

	Percent of Samples Containing Specific Pesticides and Average Level (PPM) of Each Pesticide			
	Seed	Crude Oil	Meal (Cake)	Refined Oil
DDT	69.5 (0.154)	29.2 (0.077)	28.5 (0.028)	12.2 (0.024)
TDE	13.0 (0.029)	35.4 (0.093)	12.9 (0.003)	17.1 (0.016)
DDE	13.0 (0.005)	15.0 (0.012)	16.1 (0.005)	12.2 (0.010)
Dieldrin	4.3 (0.015)	2.7 (T)	1.6 (T)	2.4 (T)
Lindane	4.3 (0.003)	8.4 (0.008)	8.6 (0.003)	2.4 (0.018)
Toxaphene	30.4 (0.023)	1.3 (0.010)	1.1 (0.003)	12.2 (0.140)
BHC	8.7 (0.017)	2.7 (0.004)	4.8 (0.008)	—
Chlordane	8.7 (0.004)	2.2 (0.017)	6.5 (0.012)	2.4 (0.007)
Total Number of Samples	23	226	186	41
Percent w/Residues	78.3	53.5	39.8	41.5

TABLE 5.—*Incidence of specific pesticide residues in corn products*

[T = <0.001 PPM; — = Not detected]

	Percent of Samples Containing Specific Pesticides and Average Level (PPM) of Each Pesticide		
	Grain	Crude Oil	Refined Oil
DDT	5.7 (0.007)	14.8 (0.067)	12.5 (0.038)
TDE	1.2 (0.003)	11.1 (0.060)	12.5 (0.002)
DDE	2.9 (T)	11.1 (0.016)	—
Dieldrin	4.4 (0.001)	11.1 (0.013)	—
Lindane	2.6 (T)	—	—
Toxaphene	—	—	—
Endrin	0.1 (T)	—	—
Chlordane	—	3.7 (0.080)	—
Total Number of Samples	819	27	8
Percent w/Residues	13.4	25.9	25.0

TABLE 6.—*Incidence of specific residues in oleomargarine*

[T = <0.001 PPM]

	Percent Samples Containing Pesticides	Average Level (PPM)
DDT	18.9	0.026
TDE	5.7	0.002
DDE	7.5	0.001
BHC	3.8	T

Total Number of Samples: 53
Percent with Residues: 18.9

The chemical names of compounds mentioned in this paper are:

DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Lindane	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer
Toxaphene	chlorinated camphene containing 67% to 69% chlorine
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
Chlordane	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
Malathion	diethyl mercaptosuccinate, *S*-ester, with *0,0*-di=methyl phosphorodithioate
Aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan

TABLE 7.—*Summary: Average levels of chlorinated pesticide residues in vegetable oil seeds and products—fiscal years 1964-66*

[T = <0.001 PPM, — = Not detected]

Sample	Raw Product	Crude Oil	Meal or Cake	Refined Oil	Oleo-margarine	Total Diet Composites [1]
			Parts per Million			
			DDT			
Soybean	0.006	0.015	0.005	0.003		
Cottonseed	0.154	0.077	0.028	0.024		
Peanut	0.025	0.466	0.140	0.060		
Corn	0.007	0.067	—	0.038		
TOTAL	0.015	0.097	0.023	0.022	0.026	0.006
			TDE			
Soybean	T	0.006	T	—		
Cottonseed	0.029	0.093	0.003	0.016		
Peanut	T	0.128	0.026	0.007		
Corn	0.003	0.060	—	0.002		
TOTAL	0.001	0.072	0.005	0.010	0.002	0.008
			DDE			
Soybean	T	0.002	0.001	—		
Cottonseed	0.005	0.012	0.005	0.010		
Peanut	0.001	0.090	0.025	0.032		
Corn	T	0.016	—	—		
TOTAL	0.003	0.016	0.005	0.010	0.001	0.004
			DIELDRIN			
Soybean	0.002	0.013	T	T		
Cottonseed	0.015	T	T	T		
Peanut	0.008	0.017	0.006	0.007		
Corn	0.001	0.013	—	—		
TOTAL	0.004	0.006	T	T	—	0.001
			LINDANE			
Soybean	T	T	0.001	—		
Cottonseed	0.003	0.008	0.003	0.018		
Peanut	0.002	0.001	T	—		
Corn	T	—	—	—		
TOTAL	T	0.005	0.003	0.01	—	T
			TOXAPHENE			
Soybean	0.004	0.024	—	T		
Cottonseed	0.023	0.010	0.003	0.140		
Peanut	0.006	0.008	—	—		
Corn	—	—	—	—		
TOTAL	0.017	0.015	0.002	0.078	—	—
			ENDRIN			
Soybean	0.008	0.013	T	—		
Cottonseed	—	—	—	—		
Peanut	T	0.008	—	—		
Corn	T	—	—	—		
TOTAL	0.006	0.004	T	—	—	T
			BHC			
Soybean	T	0.004	T	—		
Cottonseed	0.017	0.004	0.008	—		
Peanut	0.007	0.002	0.006	0.002		
Corn	—	—	—	—		
TOTAL	0.003	0.003	0.004	T	T	0.001
			CHLORDANE			
Soybean	T	—	T	—		
Cottonseed	0.004	0.017	0.012	0.007		
Peanut		—	—	—		
Corn		0.080	—	—		
TOTAL	T	0.017	0.006	0.004	—	—

[1] Includes salad dressing, salad oil, mayonnaise, shortening, and peanut butter (74 composites, 6-64-4-67).

Acknowledgments

Recognition must be given to the chemists, too numerous to mention as individuals, among the 18 FDA District Laboratories responsible for these analyses and to R. K. Dawson, Division of Program Operations, for his assistance in processing the data.

LITERATURE CITED

(*1*) *Duggan, R. E. and Keith Dawson. 1967.* Pesticides—A report on residues in food. FDA Papers 1:4.

(*2*) *Duggan, R. E. 1967.* Pesticide residues in food. Conference on Biological Effects of Pesticides in Mammalian Systems. N. Y. Acad. of Sci. May 1967.

(*3*) *Duggan, R. E. and J. R. Weatherwax. 1967.* Dietary intake of pesticide chemicals. Science 157:1007-1010.

(*4*) *Duggan, R. E. 1967.* Chlorinated pesticide residues in fluid milk and other dairy products in the United States. Pesticides Monit. J. 1(3):2-8.

(*5*) *Duggan, R. E. and F. J. McFarland. 1967.* Residues in food and feed — Assessments include raw food and feed commodities, market basket items prepared for consumption, meat samples taken at slaughter. Pesticides Monit. J. 1(1):1-5.

(*6*) *Association of Official Analytical Chemists.* Changes in official methods of analysis. J. Ass. Offic. Anal. Chem. 49:222(1966); *ibid.* 50:210-214(1967).

(*7*) *Barry, Helen C., Joyce G. Hundley, and Lore:: Y. Johnson. 1963 (Revised 1964, 1965).* Pesticide analytical manual. Food and Drug Admin., U. S. Dep. Health, Educ., and Welfare, Washington, D. C. 20204.

(*8*) *Johnson, L. Y. 1965.* Collaborative study of a method for multiple chlorinated pesticide residues in fatty foods. J. Ass. Offic. Agr. Chem. 48:668-675.

(*9*) *Krause, R. T. 1966.* Collaborative study of a method for multiple chlorinated pesticide residues in nonfatty vegetables. J. Ass. Offic. Anal. Chem. 49:460-463.

(*10*) *Gaul, J. 1966.* Collaborative study of a method for multiple chlorinated pesticide residues in leafy and cole-type vegetables. J. Ass. Offic. Anal. Chem. 49:463-467.

(*11*) *Wells, C. E. 1967.* Validation study of a method for pesticide residues in foods and animal feeds. J. Ass. Offic. Anal. Chem. 50:1205-1215.

(*12*) *U. S. Department of Agriculture. Agricultural Research Service and Forest Service. 1967.* Agriculture Handbook No. 331. Suggested guide for the use of insecticides to control insects affecting crops, livestock, households, stored products, forests, and forest products.

Investigation of Lead Residues on Growing Fruits and Vegetables[1]

Abram Kleinman

ABSTRACT

An investigation was made of the extent of lead residues on crops grown near heavily traveled highways. Analyses are presented of 132 samples of a variety of fruits and vegetables from four areas of the country. Lead residues are compared with distance from the highway, traffic load, and the period of exposure to these conditions.

Possible contamination of growing food crops and crop-growing areas by lead deposited from the atmosphere has been a matter of concern for several years. Chow and Johnstone (*2*) have estimated that an accumulation of 10 mg of lead per square meter has been deposited over the northern hemisphere since the advent of anti-knock gasolines. Warren (*4*) has reported the presence of lead in roadside vegetation. Cannon and Bowles (*1*) have presented data correlating the amount of lead found in grasses with prevailing wind direction and distance from highways.

The possible accumulation of excessive lead residues on food crops grown near heavily traveled highways raised the question of a possible hazard to public health. The purpose of this investigation was to determine the extent of such accumulation on growing crops.

Four FDA field districts[2] participated in the investigation. A total of 132 samples of a variety of mature fruits and vegetables were collected and analyzed for lead. Samples collected ranged from 4 to 15 lb. All samples were examined without washing or peeling. The smaller samples were ground and mixed in entirety; the larger samples were reduced to about 1 kg, then composited, ground, and mixed. Appropriate aliquots (25 to 200 g) were analyzed by the official A.O.A.C. dithizone spectrophotometric procedure (*3*). Reported recoveries of added lead in recovery experiments ranged from 70% to 100%. The results are summarized in Table 1.

In attempting to relate lead content of the crop to exposure to automobile exhaust, three parameters considered were distance from traffic, traffic load, and period of exposure to the air.

The distance of the crop from the roadway was coded as noted in footnote 1 of Table 1. The three distance codes and the number of samples in each code are shown for each product. The distribution of samples by distance code is as follows:

Code No.	*No. of Samples*
1	49
2	32
3	51

In reporting the second factor, traffic load, there was a lack of uniformity among the districts. One district arbitrarily classified the load as heavy, medium, or light without defining the terms. The other districts named the adjacent highway, indicating whether it was a U.S. highway, turnpike, State highway, or local road. One district reported the number of vehicles passing in a 10-minute period. This factor is shown in Table 1 as heavy, medium, or light. U.S. highways and turnpikes have been arbitrarily classified as heavy; State highways as medium; and local roads as light. Where more than one designation is shown, it means that the samples in that group were distributed accordingly. A breakdown of samples by traffic load factor for distance Codes 1 and 3 is shown in Table 2.

The growth period (exposure period) was not determined during sample collection. The values shown in Table 1 represent approximate periods which relate to

[1] Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Los Angeles, Calif. 90015

[2] Atlanta, Cincinnati, Los Angeles, Philadelphia

California, and may be subject to considerable variation depending on local climate. They are submitted for informational purposes only.

In an attempt to determine whether the above factors influenced the lead burden on the crops, attention was focused primarily on the distance from traffic. A statistical comparison of the averages for distance Code 1 and distance Code 3 was performed, using the "t" test for comparison of averages (*5*). These groups were selected because they contained approximately equal numbers of observations and showed a 10- to 100-fold difference in distance from traffic. Possible sources of bias would, of course, be present due to unequal distribution of different types of crops and unequal distribution of the traffic load factor between the two groups. In addition, two observations (one in each group) were outliers, namely, melons showing 0.71 ppm lead and collards showing 0.90 ppm lead. Both of these outlying values were rejected on the basis of Chauvenet's criterion (*6*).

The resulting distribution by traffic load is shown in Table 2. Table 3 shows the distribution of the two distance codes by fruit or vegetable group.

Although total balance for fruit or vegetable group and traffic load factors is not perfect, it was felt that there was some basis for a valid comparison of the two distance groups. The results of the "t" test for comparison of the averages are shown in Table 4.

Discussion

The value of "t" calculated from the data exceeds the critical value at the 1% level of significance for the "one-tailed" distribution of this statistic. The "one-tailed" distribution of "t" would be the proper one to use if we seek the answer to the question, "Is the lead content of crops growing adjacent to traffic *greater* than that of crops at further distances?" The data suggest that such a difference may exist. However, this conclusion must be viewed in the light that other possible sources of lead have been ignored, i.e., pesticides that contain lead, lead accumulation in the soil, and the possible sources of bias mentioned earlier.

LITERATURE CITED

(*1*) *Cannon, H. L. and J. M. Bowles. 1962.* Contamination of vegetation by tetraethyl lead. Science 137:765-766.

(*2*) *Chow, T. J. and M. S. Johnstone. 1965.* Lead isotopes in gasoline and aerosols of Los Angeles Basin, Calif. Science 147:502-503.

(*3*) *Association of Official Agricultural Chemists. 1965.* Official methods of analysis, 10th ed., Section 24.041, *et seq.*

(*4*) *Warren, H. V. 1961.* Some aspects of the relationship between health and geology. Canad. J. Public Health 52:157.

(*5*) *Youden, W. J. 1951.* Statistical methods for chemists. John Wiley & Sons, Inc., New York.

(*6*) *Young, H. D. 1962.* Statistical treatment of experimental data. McGraw-Hill Book Co., New York.

TABLE 1.—*Lead content of fruits and vegetables correlated with distance from traffic and traffic load*

Product	Distance from Traffic [1]	Number of Samples	Traffic Load [2]	Growth Period (Weeks) [3]	Lead Content (PPM)	
					Average	Range
Grapefruit	1	2	M	13	0.08	0.06—0.09
	2	1	H	13	0.03	—
	3	8	H,M,L,	13	0.02	0.01—0.05
Oranges	1	7	M,L,	13	0.09	0.03—0.22
	2	4	H,L	13	0.08	0.03—0.16
	3	2	M	13	0.12	0.11—0.13
Lemons	1	4	H,M	12-16	0.15	0.13—0.17
	2	2	H,L	12-16	0.18	0.11—0.25
	3	1	M	12-16	0.01	—
Cantaloupe or Honey Dew	1	9	L	7-8	0.16	0.01—0.71
	2	1	M	7-8	0.02	—
Strawberries	1	3	H,M,L	22	0.10	0.04—0.14
	3	5	H,L	22	0.15	0.07—0.16
Peaches	3	1	M	—	0.00	—
Collards	1	2	H,M	12	0.29	0.28—0.30
	2	4	H,M	12	0.21	0.15—0.30
	3	1	M	12	0.90	—
Lettuce	1	5	M,L	12-14	0.10	0.05—0.22
	2	1	M	12-14	0.26	—
	3	5	H,M,L	12-14	0.06	0.03—0.07
Endive	3	3	M,L	12	0.05	0.02—0.10
Spinach	2	2	L	28	0.27	0.19—0.35
Broccoli	1	1	M	8-12	0.02	—
	2	5	H,M,L	8-12	0.30	0.05—0.65
	3	4	H,M,L,	8-12	0.02	0.00—0.03

TABLE 1.—*Lead content of fruits and vegetables correlated with distance from traffic and traffic load*—Continued

Product	Distance from Traffic [1]	Number of Samples	Traffic Load [2]	Growth Period (Weeks) [3]	Lead Content (PPM) Average	Lead Content (PPM) Range
Cabbage	1	4	M,L	8-27	0.03	0.00—0.04
	2	1	H	8-27	0.00	—
	3	3	H,M,L	8-27	0.02	0.00—0.04
Turnip Greens	3	1	H	18	0.31	—
Rape	3	1	H	13	0.25	—
Tomatoes	1	2	H	12	0.03	0.00—0.05
	2	2	M	12	0.05	0.04—0.05
	3	7	H,M,L	12	0.05	0.01—0.07
Squash	3	1	M	7-8	0.00	—
Pole Beans	2	1	L	12	0.12	—
Green Beans	3	1	M	12	0.00	—
Potatoes	1	2	M	10	0.02	0.01—0.02
	3	1	M	10	0.01	—
Carrots	1	5	M,L	12	0.09	0.03—0.16
	2	2	M,L	12	0.03	0.02—0.03
	3	3	M,L	12	0.03	0.00—0.05
Radishes	2	1	L	8	0.06	—
Celery	1	2	M	26-28	0.14	0.09—0.18
	2	4	M	26-28	0.12	0.07—0.15
	3	2	M	26-28	0.16	0.05—0.26
Cauliflower	2	1	L	8-12	0.03	—
	3	1	H	8-12	0.03	—
Asparagus	1	1	M	16	0.00	—

[1] Distance from traffic coded as follows: 1 = 0 to 25 yds; 2 = 25 to 250 yds, 3 = above 250 yds.

[2] H = Heavy; M = Medium; L = Light.

[3] These are general estimates related primarily to California; they represent time of total exposure to air.

TABLE 2.—*Distribution of samples by traffic load for distance Codes 1 and 3* [1]

Traffic Load	No. of Samples: Distance Code 1	No. of Samples: Distance Code 3
Heavy	5	18
Medium	21	18
Light	22	14

[1] Does not include two outlying values rejected on basis of Chauvenet's criterion.

TABLE 3.—*Distribution of samples by fruit or vegetable group for distance Codes 1 and 3* [1]

Fruit or Vegetable Group	No. of Samples: Distance Code 1	No. of Samples: Distance Code 3
[illegible]	13	11
[illegible]	8	0
Other Fruits	3	6
Leafy Vegetables	12	17
Vine Vegetables	2	9
Root Vegetables	7	4
Other Vegetables	3	3

[1] Does not include two outlying values rejected on basis of Chauvenet's criterion.

TABLE 4.—*Comparison of averages for distance Codes 1 and 3*

	Distance Code 1	Distance Code 3
Average (ppm Pb)	0.0910	0.0562
Variance (S^2)	0.00510	0.00434
Standard deviation of difference between averages	0.0139	
F (comparison of variances)	1.17	
The Statistical Test		
"t" (test statistic)	2.51	
Degrees of freedom	96	
Critical value of "t" at 1% level of significance for 60 D.F.	2.39	
("one-tailed" distribution)		

Pesticide Residues in Total Diet Samples (III)

R. J. Martin[1] and R. E. Duggan[2]

ABSTRACT

Pesticide residue levels detected in ready-to-eat foods remained at low levels during the third year of the total diet study. Samples were collected from 30 markets in 29 different cities.

Population of cities ranged from less than 50,000 to 1,000,000 or more. Averages and ranges of pesticides commonly found are reported for the period June 1966—April 1967 by region and food class. Pesticides found infrequently also are reported for this period by region and food class.

The study of pesticide residues in ready-to-eat foods, conducted by the Food and Drug Administration from June 1964 through April 1966, has been described in earlier reports (*1*). This report covers the period June 1966 through April 1967. Tabular data are included comparable to that reported for the previous years. No changes were made in the sampling and compositing procedures given in the "Food and Feed Section" of the *Pesticides Monitoring Journal* (*2*) which describes the National Pesticide Monitoring Program. Earlier reports (*3,4*) discuss data collected from June 1964 through April 1965 and June 1965 through April 1966, respectively.

Samples were collected from 30 markets in 29 different cities. Population of cities ranged from less than 50,000 to 1,000,000 or more. The samples were analyzed for the presence of chlorinated hydrocarbons, organic phosphates, chlorophenoxy acids, bromides, arsenic, amitrole (3-amino-1,2,4-triazole), carbarbyl (Sevin®), and dithiocarbamate residues.

Quantitative values reported for both chlorinated and organic phosphorus compounds were obtained by either electron capture or thermionic gas-liquid chromatography. Confirmation was made by thin layer chromatography and/or microcoulometric gas-liquid chromatography. This procedure determines chlorinated compounds at a sensitivity (quantitative) of 0.003 ppm and organic phosphorus compounds at 0.05 ppm. Each composite was also tested for chlorophenoxy acids and esters at a sensitivity of 0.02 ppm; for amitrole at a sensitivity of 0.05 ppm; for dithiocarbamates, calculated as zineb (zinc ethylene-1, 2-bisdithiocarbamate) at a sensitivity of 0.2 ppm; for carbaryl at a sensitivity of 0.2 ppm; for bromides at a sensitivity of 0.5 ppm; and for arsenic as As_2O_3 at a sensitivity of 0.1 ppm.

All methods used in these studies are described in the FDA *Pesticide Analytical Manual*, Vol. I and II (*5*). Recoveries of specific pesticide chemicals vary within product classes, usually within a range of 85% to 115% at these levels. No correction was made for recovery.

RESULTS

A total of 997 residues were detected during this current reporting period. There was no significant change in the levels, frequency, or types of residues found from those in the past.

Twenty-nine different residues were found in the samples in 1967. The frequency of the residues is shown in

[1] Field Scientific Coordination Branch, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204.
[2] Office of Associate Commissioner for Compliance, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D.C. 20204.

Table 1. The most common residues, maximum levels of those residues, and residues reported less frequently are discussed below for each class.

DAIRY PRODUCTS: Thirteen chlorinated organic pesticides in varying combinations were detected in 27 of 30 composites. The most common, and their maximum values on a fat basis, were: DDE (0.30 ppm); DDT (0.14 ppm); dieldrin (0.08 ppm); heptachlor epoxide (0.03 ppm); TDE (0.18 ppm); and BHC (0.05 ppm). Also present were aldrin, heptachlor, lindane, methoxychlor, 2,4,5-T, 2,4-D, PCP, Kelthane®, and arsenic (As_2O_3). Bromides were found (0.5 ppm to 21.3 ppm) in 28 of 30 composites.

MEAT, FISH, AND POULTRY: Ten chlorinated organic pesticides were present in varying quantities in 29 of 30 composites. DDT, DDE, TDE, heptachlor epoxide, dieldrin, and BHC were the most common, with maximum values of 0.882 ppm, 0.755 ppm, 0.69 ppm, 0.105 ppm, 0.120 ppm, and 0.06 ppm, respectively, on a fat basis. Aldrin, lindane, PCP, and phorate were also present. Bromides were detected (0.8 to 47.2 ppm) in 27 of 30 composites; Arsenic (As_2O_3) was detected 9 times at values ranging from 0.1 to 0.5 ppm; and 2,4,5-T was detected in 1 composite.

GRAIN AND CEREAL PRODUCTS: Nine chlorinated organic pesticides were found in 28 of 30 composites with the most common being lindane, DDT, and dieldrin, at maximum values of 0.171 ppm, 0.02 ppm, and 0.011 ppm, respectively. DDE, BHC, heptachlor epoxide, aldrin, PCP, and TDE also were present. Bromides were detected (0.5 ppm to 47 ppm) in 28 of 30 composites. Eight composites contained malathion, with a maximum value of 0.13 ppm. Arsenic (As_2O_3) and carbaryl also were present.

POTATOES: The most common pesticides found were DDT and DDE at maximum values of 0.03 ppm and 0.02 ppm, respectively. These 2 were detected in 12 of 30 composites. Other chlorinated organic pesticides present were dieldrin, CIPC, lindane, TDE, and PCP. Endrin was detected in 5 of 30 composites at a maximum value of 0.01 ppm. Bromides were found in 25 of 30 composites. Values ranged from 0.3 ppm to 57.2 ppm.

LEAFY VEGETABLES: DDT, DDE, and TDE with maximum values of 0.058 ppm, 0.02 ppm, and 0.045 ppm, respectively, were detected in 22 of 30 composites. Aldrin, BHC, chlordane, dieldrin, endrin, and lindane were also present. Parathion was found in 3 of 30 composites, with a maximum value of 0.04 ppm. Methyl parathion, endosulfan, and arsenic (As_2O_3) were each detected 1 time. Dithiocarbamates (calculated as zineb) were found twice at 0.44 and 0.8 ppm levels. Bromides were detected in 24 of the 30 composites.

LEGUME VEGETABLES: DDE, TDE, and DDT were found in 8 of 30 composites, with maximum values of 0.01 ppm, 0.05 ppm, and 0.062 ppm, respectively. Aldrin, chlordane, and lindane were also present. Arsenic (As_2O_3) was detected twice, with a maximum value of 0.18 ppm. Bromides were found (0.5 ppm to 19 ppm) in 22 of the 30 composites.

ROOT VEGETABLES: TDE, DDT, and DDE were detected in 8 of the 30 composites at maximum values of 0.02 ppm, 0.04 ppm, and 0.01 ppm, respectively. Endrin was detected in 1 composite. Carbaryl and dithiocarbamates (calculated as zineb) were each detected 1 time with values of 0.05 ppm and 0.32 ppm, respectively. Bromides were detected (0.3 ppm to 20.5 ppm) in 26 of the 30 composites. Arsenic (As_2O_3) was detected 3 times with a maximum value of 0.16 ppm.

GARDEN FRUITS: A total of 8 chlorinated organic residues were detected in 27 of the 30 composites. DDT, TDE, and DDE were the most common with maximum values of 0.19 ppm, 0.02 ppm, and 0.04 ppm, respectively. Dieldrin, lindane, aldrin, heptachlor epoxide, and TCNB also were present. Diazinon, carbaryl, and parathion were all detected 1 time with values of 0.003 ppm, 0.10 ppm, and 0.014 ppm, respectively. Bromides were detected (1.1 ppm to 12 ppm) in 28 of the 30 composites.

FRUITS: Ten chlorinated organic residues were found in 25 of the 30 composites. DDT, DDE, Kelthane®, TDE, and aldrin were found most frequently with maximum values of 0.09 ppm, 0.04 ppm, 0.23 ppm, 0.025 ppm, and 0.015 ppm, respectively. Methoxychlor, lindane, BHC, heptachlor epoxide, and dieldrin were also present. Ethion was detected 3 times with a maximum value of 0.054 ppm. Arsenic (As_2O_3) occurred 3 times varying from 0.1 to 0.2 ppm. Carbaryl was detected 1 time, at a level of 0.22 ppm. Bromides were detected 24 times (0.6 ppm to 34.1 ppm) in 30 composites.

OILS, FATS, AND SHORTENING: A total of 7 chlorinated organic residues were detected in 21 of the 30 composites. DDE, DDT, and TDE were the most common, with maximum values of 0.03 ppm, 0.023 ppm, and 0.04 ppm, respectively. Dieldrin, BHC, lindane, and PCP were also present. Bromides were detected (0.7 ppm to 49.1 ppm) in 25 of 30 composites. Malathion was detected 4 times, ranging from trace to 0.062 ppm. Diazinon and ethion were each detected 1 time. Arsenic (As_2O_3) occurred twice at a 0.1 ppm level.

TABLE 1.—*Number of composites where pesticide residues were found and ranges in the amounts (June 1966 - April 1967)*

Pesticide	No. composites with residue	No. of positive composites with residues below sensitivity level [1]	Range at and above sensitivity level (ppm)
BROMIDES	301	3	0.5-57.2
DDT 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	145	6	0.003-0.882
DDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene	123	13	0.003-0.755
TDE 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	112	13	0.003-0.69
DIELDRIN not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	58	7	0.003-0.12
LINDANE 1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer	49	14	0.003-0.374
ARSENIC (As_2O_3)	33	8	0.1-0.40
BHC 1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers	34	4	0.003-0.06
HEPTACHLOR EPOXIDE 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan	33	4	0.005-0.17
KELTHANE® 4,4′-dichloro-*a*-(trichloromethyl) benzhydrol	20	0	0.019-0.23
ALDRIN not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene	14	3	0.003-0.03
MALATHION diethyl mercaptosuccinate, *S*-ester with *0,0*-dimethyl phosphorodithioate	14	4	0.05-0.19
PCP pentachlorophenol	11	2	0.007-0.043
ENDRIN 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene	7	2	0.003-0.05
2,4-D 2,4-dichlorophenoxyacetic acid	6	2	0.027-0.08
PARATHION *0,0*-diethyl *0-p*-nitrophenyl phosphorothioate	5	4	0.093
CARBARYL 1-naphthyl methylcarbamate	4	2	0.22-0.34
ETHION *0,0,0′,0′*-tetraethyl-*S,S′*-methylene bisphosphorodithioate	4	2	0.054-0.25
METHOXYCHLOR 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane	3	1	0.004-0.09
DITHIOCARBAMATES	3	0	0.32-0.8
DIAZINON *0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	2	2	
CHLORDANE 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane	2	0	0.005-0.02
HEPTACHLOR 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	1	0	0.02
CIPC isopropyl *N*-(3-chlorophenyl)carbamate	1	0	0.11
2,4,5-T 2,4,5-trichlorophenoxyacetic acid	2	1	0.19
TCNB 1,2,4,5-tetrachloro-3-nitrobenzene	1	0	0.004
ENDOSULFAN 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	1	0	0.003
METHYL PARATHION *0,0*-dimethyl *0-p*-nitrophenyl phosphorothioate	1	1	
PHORATE *0,0*-diethyl *S*-(ethylthio)methyl phosphorodithioate	1	1	

[1] Pesticide chemicals capable of being detected by the specified analytical methodology may be confirmed qualitatively but are not quantifiable when they are present at concentrations below the sensitivity level.

SUGARS AND ADJUNCTS: Eight chlorinated organic residues were detected in 14 of the 30 composites. 2,4-D was found in 5 composites with a maximum value of 0.08 ppm; DDE and DDT were each found 3 times with maximum values of 0.02 ppm and 0.002 ppm, respectively. Kelthane®, aldrin, lindane, TDE, and PCP were also present. Malathion was detected in 1 composite at 0.07 ppm. Arsenic (As_2O_3) was detected 4 times with a maximum value of 0.15 ppm. Bromides were detected (1.1 ppm to 42.9 ppm) in 25 of 30 composites.

BEVERAGES. Two chlorinated organic residues were detected in 2 composites. PCP at 0.021 ppm was found in 1 composite and a trace of lindane was reported in the other. Bromides were found in 19 composites. Concentrations ranged from 0.5 ppm to 14.7 ppm. Malathion was detected 1 time at a level of 0.19 ppm. Arsenic (As_2O_3) occurred 1 time at a level of 0.25 ppm.

Values include naturally occurring bromides as well as residues from pesticide treatment. The quantitative sensitivity limits were established for the study in 299 of the 360 composite samples. This incidence is 83.1% and does not differ significantly from the 76.8% incidence found in the 1965-1966 results. A total of 4.2% of the residues exceeded 25 ppm while an incidence of 3.8% was reported for 1964-1965.

The data obtained during the third year of the study are reported in more detail in Table 2a, where the findings are arranged by food class and region. Similar information is given in Table 2b for pesticide residues found infrequently (less than five detections per commodity class). The data are reported in the same format used for the earlier period (3) for ease of comparison. Trace amounts, < 0.001 ppm, are not included in the averages. Where no average value is given, the results of individual composites are shown. In these tabulations, as in the earlier report, the bromide and arsenic values are reported on an "as is" basis for three food classes: Dairy Products (I), Meat, Fish, and Poultry (II); and Oils, Fats, and Shortening (X), even though the earlier tabulations indicated a "fat basis."

Discussion

The presence of chlorinated organic residues was confirmed in 221 of the 360 composites examined (62.3%) for this class of chemicals. This percentage of incidence is not significantly different from the 1965-66 data (53.8%). Organic phosphorus compounds were found in 25 composites; 27 detections were reported for 1965-66. Relatively few carbaryl values have been reported during the entire study, and the majority of these were reported by Kansas City; however, Boston, Los Angeles, and Baltimore have also reported positive findings. Carbaryl was detected (0.05-0.34 ppm) in 4 composite samples for the current year. Each composite was analyzed by thin layer chromatography. Positive results were confirmed and quantitated spectrophotometrically (5). In considering the carbaryl values, it must be recognized that at the lower limits of sensitivity of the method, 0.1 to 0.2 ppm, the accuracy of the method is reduced.

For example, carbaryl and other chemicals found infrequently in less than 1% of the composites, cannot be considered as a regular component of residues in the diet.

Chlorophenoxy acids were found in 8 composites for the current year; 13 residues were reported for 1965-66. Dithiocarbamates (calculated as zineb) were found in 3 composites. No detections were reported for 1965-66, while 4 values were reported for 1964-65.

Samples have been analyzed for the presence of amitrole since initiation of the program, but no residues have been detected. Levels and kinds of residues for this period remain in the same order of magnitude as those reported in the earlier studies. The frequency has not changed significantly as a whole or within each food class.

On the basis of these data, we can reasonably conclude that there is no significant difference in the dietary intake among the three reporting periods of this study.

Acknowledgment

The authors gratefully acknowledge the analytical work from the FDA Laboratories in Baltimore, Md.; Boston, Mass.; Kansas City, Mo.; Los Angeles, Calif.; and Minneapolis, Minn.

LITERATURE CITED

(1) *Duggan, R. E., H. C. Barry, and L. Y. Johnson. 1966.* Pesticide residues in total diet samples. Science 151:101-104.

(2) *Duggan, R. E. and F. J. McFarland. 1967.* Assessments include raw food and feed commodities, market basket items prepared for consumption, meat samples taken at slaughter. Pesticides Monit. J. 1(1):1.

(3) *Duggan, R. E., H. C. Barry, and L. Y. Johnson. 1967.* Pesticide residues in total diet samples (II). Pesticides Monit. J. 1(2):2-12.

(4) *Duggan, R. E. and J. R. Weatherwax. 1967.* Dietary intake of pesticide chemicals. Science 157:1006-1007.

(5) *Barry, H. C., J. G. Hundley, and L. Y. Johnson.* Pesticide analytical manual. Vol. I and II., Food and Drug Admin., U. S. Dep. Health, Educ., and Welfare.

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1966-April 1967)*

[T = Trace<0.001 ppm]

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
I. DAIRY PRODUCTS (8-13% fat) [1] Residues in Parts Per Million—Fat Basis					
DDT					
Average	0.033	0.023	0.109	0.029	0.070
Positive Composites					
Number	4	5	3	4	5
Range	0.023-0.05	0.015-0.03	0.056-0.14	0.012-0.050	T-0.11
DDE					
Average	0.029	0.017	0.203	0.021	0.03
Positive Composites					
Number	5	5	5	4	5
Range	0.025-0.038	0.006-0.042	0.019-0.30	0.01-0.026	0.011-0.050
TDE					
Average	0.018	0.011	0.013	0.027	0.026
Positive Composites					
Number	2	4	3	5	3
Range	0.017-0.020	0.006-0.02	0.06-0.18	0.009-0.04	0.012-0.04
DIELDRIN					
Average	0.038	0.027	0.025	0.028	0.035
Positive Composites					
Number	3	5	5	3	3
Range	0.013-0.08	0.008-0.05	0.008-0.051	0.02-0.035	0.007-0.080
HEPTACHLOR EPOXIDE					
Average	0.015	0.012	0.012	0.01	0.013
Positive Composites					
Number	4	3	2	2	5
Range	0.008-0.020	T-0.012	0.007-0.017	0.01-0.013	0.005-0.030
BHC					
Average	0.025	0.029			0.027
Positive Composites					
Number	4	3			5
Range	0.015-0.050	0.020-0.039	0	0	0.016-0.040
TOTAL BROMIDES					
Average	9.2	6.3	3.6	5.6	4.7
Positive Composites					
Number	6	6	6	6	4
Range	0.8-21.3	1.3-8.4	1.2-9.0	0.7-10.0	0.5-14.3
II. MEAT, FISH, AND POULTRY (17-23% fat) [1] Residues in Parts Per Million—Fat Basis					
DDT					
Average	0.370	0.177	0.139	0.107	0.152
Positive Composites					
Number	5	6	6	5	5
Range	0.12-0.882	0.051-0.40	0.095-0.20	0.01-0.160	0.081-0.221
DDE					
Average	0.266	0.123	0.341	0.066	0.064
Positive Composites					
Number	6	6	6	5	5
Range	0.07-0.459	0.03-0.166	0.08-0.755	0.011-0.110	0.04-0.100
TDE					
Average	0.251	0.105	0.089	0.052	0.051
Positive Composites					
Number	6	6	6	6	4
Range	0.04-0.69	0.022-0.28	0.05-0.15	0.004-0.10	0.014-0.09
DIELDRIN					
Average		0.059	0.013	0.015	0.023
Positive Composites					
Number	1	6	4	2	3
Range	0.061	0.016-0.120	0.008-0.02	0.01-0.02	0.008-0.04
HEPTACHLOR EPOXIDE					
Average	0.068	0.019	0.033		0.011
Positive Composites					
Number	3	4	2	0	4
Range	0.05-0.105	0.009-0.033	0.006-0.06		0.006-0.02
BHC					
Average	0.040	0.021	0.025		0.011
Positive Composites					
Number	2	6	3	0	4
Range	0.020-0.06	0.010-0.030	0.006-0.05		0.007-0.02

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1966 - April 1967)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
			II (a). MEAT, FISH, AND POULTRY (17-23% fat) [1]—(Continued) Residues in Parts Per Million—Fat Basis		
ARSENIC (As_2O_3)					
Average	0.23		0.22		
Positive Composites					
Number	4	1	4	0	0
Range	0.1-0.33	0.5	0.1-0.38		
TOTAL BROMIDES					
Average	11.5	6.9	5.1	20.6	4.2
Positive Composites					
Number	5	6	5	6	5
Range	6.9-20.2	2.9-15.0	1.3-14.0	7.9-47.2	0.8-5.4
			III. GRAIN AND CEREAL [1] Residues in Parts Per Million		
DDT					
Average	0.015	0.007	0.005	0.009	
Positive Composites					
Number	3	2	4	4	0
Range	T-0.02	0.006-0.007	0.003-0.007	0.005-0.012	
DIELDRIN					
Average	0.005	0.006	0.006		
Positive Composites					
Number	2	4	2	1	0
Range	T-0.005	T-0.008	0.005-0.006	0.011	
LINDANE					
Average	0.004	0.060	0.003		0.009
Positive Composites					
Number	5	5	6	1	5
Range	0.003-0.005	0.003-0.171	T-0.005	0.003	0.006-0.011
MALATHION					
Average		0.038		0.115	
Positive Composites					
Number	0	4	1	2	1
Range		0.015-0.05	0.009	0.099-0.13	0.063
TOTAL BROMIDES					
Average	22.5	19.3	9.9	18.0	14.8
Positive Composites					
Number	6	6	6	6	4
Range	15.7-30.2	5.3-47.0	5.1-15.4	0.5-40.2	10.9-17.6
			IV. POTATOES [1] Residues in Parts Per Million		
DDT					
Average	0.005		0.003		
Positive Composites					
Number	4	0	2	1	1
Range	0.004-0.01		0.003	0.005	0.03
DDE					
Average	0.004		0.004		
Positive Composites					
Number	4	1	2	1	1
Range	T-0.004	0.006	T-0.004	0.009	0.02
ENDRIN					
Average			0.003		
Positive Composites					
Number	2	0	2	0	1
Range	T-0.01		0.002-0.004		0.005
TOTAL BROMIDES					
Average	8.1	6.6	7.7	19.2	4.5
Positive Composites					
Number	6	5	4	6	4
Range	1.1-17.6	1.9-22	3.6-14.3	5.7-57.2	0.3-9.5
			V. LEAFY VEGETABLES [1] Residues in Parts Per Million		
DDT					
Average	0.015	0.019	0.039	0.025	0.033
Positive Composites					
Number	6	3	5	4	2
Range	T-0.02	0.011-0.028	0.005-0.058	0.003-0.056	0.025-0.04

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1966 - April 1967)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
V. LEAFY VEGETABLES [1]—(Continued) Residues in Parts Per Million					
DDE					
Average	0.011		0.008		
Positive Composites					
Number	2	0	5	1	1
Range	0.002-0.02		0.007-0.009	0.004	0.01
TDE					
Average	0.010		0.015		
Positive Composites					
Number	2	1	5	1	1
Range	0.009-0.011	T	0.003-0.045	0.006	0.01
TOTAL BROMIDES					
Average	5.6	3.7	1.4	4.4	1.5
Positive Composites					
Number	6	6	3	5	4
Range	2.4-9.5	0.8-10	0.9-2.6	1-6.7	0.7-1.9
VI. LEGUME VEGETABLES [1] Residues in Parts Per Million					
DDT					
Average			0.033		
Positive Composites					
Number	1	0	2	1	0
Range	0.031		0.003-0.062	0.04	
TDE					
Average		0.004	0.029		
Positive Composites					
Number	0	2	3	0	0
Range		T-0.004	0.006-0.05		
DDE					
Average			0.004		
Positive Composites					
Number	1	2	3	0	0
Range	T	T-0.01	0.003-0.005		
TOTAL BROMIDES					
Average	7.6	5.4	3.5	5.2	1.8
Positive Composites					
Number	6	4	4	4	4
Range	1.6-13.8	0.5-19	1.1-7.8	1.7-9.6	0.8-3.0
VII. ROOT VEGETABLES [1] Residues in Parts Per Million					
DDT					
Average		0.02		0.022	
Positive Composites					
Number	0	2	0	2	0
Range		0.01-0.02		0.003-0.04	
DDE					
Average			0.003	0.007	
Positive Composites					
Number	0	1	2	3	1
Range		0.01	0.003	0.005-0.009	0.004
TDE					
Average			0.005		
Positive Composites					
Number	0	1	2	2	1
Range		0.02	0.005	0.004-0.01	0.005
TOTAL BROMIDES					
Average	5.7	3.3	3.2	5.4	1.8
Positive Composites					
Number	6	6	4	6	4
Range	0.5-20.5	0.9-8.5	0.4-8.8	0.3-13.3	0.9-2.7
VIII. GARDEN FRUITS [1] Residues in Parts Per Million					
DDT					
Average	0.082	0.038	0.059	0.052	0.021
Positive Composites					
Number	3	4	6	4	3
Range	0.059-0.12	0.016-0.054	0.006-0.19	0.018-0.092	0.007-0.035

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
VIII. GARDEN FRUITS[1]—(Continued) Residues in Parts Per Million					
DDE					
Average		0.03	0.018	0.006	0.017
Positive Composites					
Number	0	2	3	2	3
Range		0.02-0.04	0.003-0.03	0.003-0.01	T-0.03
TDE					
Average		0.033	0.008	0.008	0.008
Positive Composites					
Number	0	5	4	3	3
Range		0.008-0.089	0.004-0.013	0.002-0.02	T-0.01
DIELDRIN					
Average			0.002		
Positive Composites					
Number	1	1	2	0	1
Range	0.003	0.003	0.001-0.003		0.011
LINDANE					
Average	0.002	0.027			
Positive Composites					
Number	3	2	1	0	0
Range	T-0.002	0.004-0.049	0.002		
TOTAL BROMIDES					
Average	6.4	2.4	4.7	5.2	1.7
Positive Composites					
Number	6	6	6	6	4
Range	1.8-11.7	1.1-2.9	1.1-12.0	2.0-9.4	1.1-3.1
IX. FRUITS[1] Residues in Parts Per Million					
DDT					
Average	0.035	0.01	0.059	0.01	0.009
Positive Composites					
Number	5	2	3	2	2
Range	0.005-0.08	0.01-0.011	T-0.09	0.01	0.009
DDE					
Average	0.005	0.04	0.003		0.005
Positive Composites					
Number	3	2	5	0	2
Range	T-0.005	T-0.04	0.001-0.007		0.003-0.006
TDE					
Average	0.013	0.008	0.004	0.013	0.008
Positive Composites					
Number	3	2	3	2	2
Range	T-0.025	T-0.008	0.003-0.005	0.006-0.02	0.005-0.01
ALDRIN					
Average				0.003	0.008
Positive Composites					
Number	1	0	0	2	3
Range	0.008			0.002-0.003	0.004-0.015
KELTHANE®					
Average	0.121	0.067	0.046		0.068
Positive Composites					
Number	5	5	5	1	2
Range	0.06-0.23	0.019-0.133	0.022-0.10	0.02	0.035-0.10
TOTAL BROMIDES					
Average	3.1	4.3	5.7	11.9	4.4
Positive Composites					
Number	5	6	4	6	3
Range	0.8-5.5	0.9-17.0	0.6-13.0	3.7-34.1	2.1-5.8
X. OILS, FATS, AND SHORTENING[1] Residues in Parts Per Million					
DDT					
Average			0.009	0.007	0.013
Positive Composites					
Number	1	0	2	4	4
Range	0.021		0.007-0.01	0.006-0.01	T-0.023
DDE					
Average			0.02	0.006	0.011
Positive Composites					
Number	1	1	2	4	5
Range	1	0.005	0.01-0.03	T-0.01	T-0.02

TABLE 2a.—*Levels of pesticide residues commonly found—by food class and region (June 1966 - April 1967)*—Continued

Pesticide	Boston	Kansas City	Los Angeles	Baltimore	Minneapolis
X (a). OILS, FATS, AND SHORTENING [1]—(Continued) Residues in Parts Per Million					
TDE					
Average			0.023	0.003	0.016
Positive Composites					
Number	1	1	2	2	4
Range	0.04	0.01	0.015-0.03	0.002-0.004	T-0.02
TOTAL BROMIDES					
Average	5.7	6.8	4.6	28.9	6.7
Positive Composites					
Number	6	6	4	5	4
Range	0.7-10.9	1.9-24.0	3.7-6.6	2.7-49.1	3.5-11.2
XI. SUGARS AND ADJUNCTS [1] Residues in Parts Per Million					
2, 4-D					
Average					0.033
Positive Composites					
Number	0	1	1	0	3
Range		0.01	0.08		0.016-0.05
TOTAL BROMIDES					
Average	9.9	9.7	8.7	17.9	10.2
Positive Composites					
Number	6	5	3	6	5
Range	6.6-14.2	4.3-16.7	5.7-13.2	7.2-42.9	1.1-25.4
XII. BEVERAGES [1] Residues in Parts Per Million					
TOTAL BROMIDES					
Average	9.0	0.54	3.5	4.0	3.2
Positive Composites					
Number	6	4	2	5	2
Range	1.6-14.7	0.5-1.1	1.7-4.4	1.1-8.8	1.7-4.7

[1] Six composite samples examined at each of the five sampling sites: Boston, Kansas City, Los Angeles, Baltimore, and Minneapolis.

Note: Bromide and arsenic values are reported on an "as is" basis for Dairy Products; Meat, Fish, and Poultry; and Oils, Fats, and Shortening.

TABLE 2b.—*Pesticides found infrequently—by food class and region (June 1966 - April 1967)*

[T = Trace <0.001 ppm]

Pesticide	District	No. Composites	Amount (PPM)
I (a). DAIRY PRODUCTS (8-13% fat) [1] Residues in Parts Per Million—Fat Basis			
ALDRIN	Baltimore	1	0.017
LINDANE	Kansas City	1	0.09
METHOXYCHLOR	Minneapolis	1	0.09
	Kansas City	1	0.06
PCP	Boston	1	0.043
	Kansas City	1	0.02
	Baltimore	1	0.01
2,4,5-T	Boston	1	0.19
HEPTACHLOR	Kansas City	1	0.02
ARSENIC	Kansas City	1	0.3
(As_2O_3)	Boston	1	0.2
KELTHANE®	Los Angeles	1	0.03
2,4-D	Minneapolis	1	0.027
II (a). MEAT, FISH, AND POULTRY (17-23% fat) [1] Residues in Parts Per Million—Fat Basis			
ALDRIN	Baltimore	1	0.028
PCP	Kansas City	1	T
	Los Angeles	1	0.02
II. MEAT, FISH, AND POULTRY (17-23% fat) [1]—(Continued) Residues in Parts Per Million—Fat Basis			
LINDANE	Los Angeles	1	0.03
	Kansas City	2	0.012,0.374
	Boston	1	0.014
	Minneapolis	1	0.010
PHORATE	Boston	1	0.010
2,4,5-T	Boston	1	0.003
III (a). GRAIN AND CEREAL [1] Residues in Parts Per Million			
ALDRIN	Boston	1	0.01
BHC	Boston	1	0.015
CARBARYL	Kansas City	1	0.34
HEPTACHLOR EPOXIDE	Los Angeles	1	0.001
PCP	Baltimore	1	0.01
	Minneapolis	1	0.007
ARSENIC	Boston	2	0.10,0.12
(As_2O_3)	Los Angeles	1	0.10
DDE	Boston	1	0.003
	Kansas City	1	0.003

TABLE 2b. *Pesticides found infrequently—by food class and region (June 1966 - April 1967)*—Continued

PESTICIDE	DISTRICT	NO. COMPOSITES	AMOUNT (PPM)
III (a). GRAIN AND CEREAL [1]—(Continued) Residues in Parts Per Million			
TDE	Los Angeles	1	0.001
	Kansas City	1	0.012
	Los Angeles	2	0.001,0.004
IV (a). POTATOES [1] Residues in Parts Per Million			
CIPC	Kansas City	1	0.11
LINDANE	Boston	1	T
	Los Angeles	1	0.002
TDE	Los Angeles	3	0.001,0.002,0.005
	Boston	1	T
ARSENIC	Boston	2	0.10,0.14
(As_2O_3)	Kansas City	1	0.2
PCP	Boston	1	T
DIELDRIN	Baltimore	1	0.006
	Boston	1	0.006
	Los Angeles	1	0.002
V (a). LEAFY VEGETABLES [1] Residues in Parts Per Million			
BHC	Boston	3	T,T,0.002
	Los Angeles	1	0.004
DIELDRIN	Kansas City	1	0.003
	Boston	1	T
	Los Angeles	1	0.001
DITHIO-CARBAMATES	Kansas City	1	0.8
	Baltimore	1	0.44
ENDRIN	Los Angeles	1	0.05
LINDANE	Boston	1	0.007
	Minneapolis	1	T
	Kansas City	2	0.002,0.005
PARATHION	Boston	1	0.016
	Kansas City	2	0.03,0.04
ENDOSULFAN	Los Angeles	1	0.003
CHLORDANE	Boston	1	0.02
ALDRIN	Boston	1	0.008
ARSENIC (As_2O_3)	Kansas City	1	0.4
METHYL PARATHION	Kansas City	1	0.01
VI (a). LEGUME VEGETABLES [1] Residues in Parts Per Million			
ALDRIN	Boston	1	T
LINDANE	Boston	1	T
ARSENIC (As_2O_3)	Boston	2	0.18,0.1
CHLORDANE	Boston	1	0.005
VII (a). ROOT VEGETABLES [1] Residues in Parts Per Million			
[illegible]	Baltimore	1	0.05
[illegible]	Los Angeles	1	0.003
[illegible]	Boston	2	0.16,0.11
	Los Angeles	1	0.10
[illegible] CARBAMATES	Baltimore	1	0.32
VIII (a). GARDEN FRUITS [1] Residues in Parts Per Million			
ALDRIN	Boston	2	0.004,T
CARBARYL	Kansas City	1	0.10
DIAZINON	Boston	1	0.003
VIII (a). GARDEN FRUITS [1]—(Continued) Residues in Parts Per Million			
HEPTACHLOR EPOXIDE	Boston	2	0.001,0.01
PARATHION	Kansas City	1	0.014
	Minneapolis	1	0.093
TCNB	Boston	1	0.004
IX (a). FRUITS [1] Residues in Parts Per Million			
CARBARYL	Kansas City	1	0.22
DIELDRIN	Boston	1	T
ETHION	Boston	1	0.024
	Kansas City	1	0.054
	Minneapolis	1	0.03
LINDANE	Kansas City	1	0.002
ARSENIC	Boston	1	0.1
(As_2O_3)	Kansas City	1	0.2
	Los Angeles	1	0.1
BHC	Los Angeles	1	0.002
METHOXYCHLOR	Boston	1	T
HEPTACHLOR EPOXIDE	Boston	1	T
X (a). OILS, FATS, AND SHORTENING [1] Residues in Parts Per Million			
BHC	Boston	1	0.06
DIELDRIN	Kansas City	2	0.003,0.004
LINDANE	Los Angeles	1	0.007
	Baltimore	1	0.004
	Minneapolis	1	0.012
MALATHION	Boston	1	T
	Kansas City	1	0.02
	Minneapolis	1	0.052
	Baltimore	1	0.062
PCP	Baltimore	1	0.02
ARSENIC	Boston	1	0.1
(As_2O_3)	Los Angeles	1	0.1
ETHION	Boston	1	0.25
DIAZINON	Boston	1	T
XI (a). SUGARS AND ADJUNCTS [1] Residues in Parts Per Million			
ALDRIN	Baltimore	1	0.003
DDT	Los Angeles	1	0.002
	Boston	2	T,T,
DDE	Boston	1	T
	Minneapolis	1	T
	Baltimore	1	0.02
LINDANE	Los Angeles	1	T
	Boston	2	T,T
TDE	Minneapolis	1	0.02
ARSENIC	Boston	3	0.1,0.15,0.10
(As_2O_3)	Los Angeles	1	0.1
PCP	Kansas City	1	0.01
MALATHION	Baltimore	1	0.07
KELTHANE ®	Boston	1	0.015
XII (a). BEVERAGES [1] Residues in Parts Per Million			
LINDANE	Boston	1	T
PCP	Boston	1	0.021
ARSENIC (As_2O_3)	Boston	1	0.25
MALATHION	Baltimore	1	0.19

[1] Six composite samples collected at each of the five sampling sites: Boston, Kansas City, Los Angeles, Baltimore, and Minneapolis.

NOTE: Bromide and arsenic values are reported on an "as is" basis for Dairy Products, Meat, Fish, and Poultry; and Oils, Fats, and Shortenings.

RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Pesticide Monitoring of the Aquatic Biota at the Tule Lake National Wildlife Refuge[1]

Patrick J. Godsil and William C. Johnson

ABSTRACT

Because of pesticide poisoning of fish-eating birds, the Federal Water Pollution Control Administration established a water quality monitoring program at the Tule Lake and Lower Klamath Lake Wildlife Refuges in 1964. Over a 2-year period, samples of water, suspended material, submerged aquatic plants, clams, and fish were collected and analyzed for chlorinated hydrocarbon pesticides. Results are reported from a typical station in the Tule Lake National Wildlife Refuge.

Compounds DDE, DDD, DDT, chlordane, and endrin were found regularly in samples of both water and biota. Water contained a maximum of 0.100 ppb of endrin in 1965, while tui chubs, Siphateles bicolor, *accumulated a maximum of 198 ppb during the same year. Concentrations of endrin in the other strata of the biota were distributed between these extremes of the food chain.*

The occurrence of endrin was directly associated with contaminated irrigation return water supplying the Refuge lakes. As the concentrations of studied pesticides increased in the drainage water, the biota also became contaminated. However, at the end of the season, as the concentrations decreased in the water, the biota was cleansed. Concentrations in both water and biota returned to or near analytical sensitivity (water—0.007 ppb; biota—4 ppb) between growing seasons.

Introduction

During the early 1960's, unusually large numbers of fish-eating birds died at the Tule Lake and Lower Klamath Lake National Wildlife Refuges (Fig.1). Researchers of the U.S. Fish and Wildlife Service (5) concluded that these deaths were caused by pesticide poisoning resulting from the use of toxaphene and DDT for pest control on agricultural lands within and surrounding the Refuges. It was recognized that agricultural drainage, carrying pesticides through the extensive irrigation system supplying water to the Refuges, presented a hazard to the wildlife. Consequently, at the request of local groups and governmental agencies, the Federal Water Pollution Control Administration initiated the Klamath Basin Study. Its purpose was to investigate water pollution problems associated with the use of agricultural chemicals on lands of the Lost River system draining into the Tule Lake and Lower Klamath Lake Refuges. Specific objectives of the study were to: (a) measure and identify pollutants responsible for the wildfowl mortalities and, (b) determine the relationships between land and water use and the pollutants.

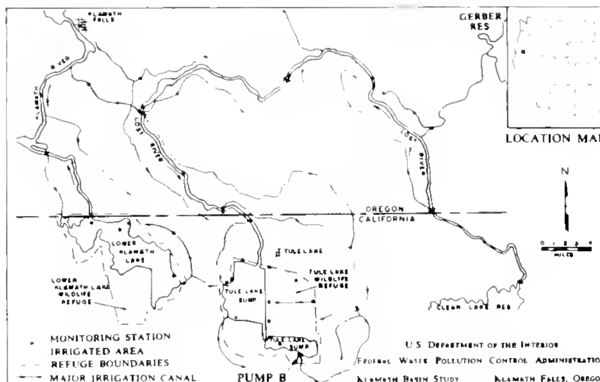


FIGURE 1.—*Lost River Irrigation System*

An intensive monitoring program was initiated in April 1965 to obtain data on the occurrence of chlorinated hydrocarbon pesticides at various locations within the Lost River system. Monitoring stations were carefully selected in and around the Wildlife Refuges. This paper presents data showing the occurrence and fate of pes-

[1] Klamath Basin Study, Federal Water Pollution Control Administration, Department of the Interior, 2261 South Sixth Street, Klamath Falls, Oreg. 97601.

ticide contaminants in various aquatic strata at a selected representative station in the Tule Lake National Wildlife Refuge.

Approximately 156,000 acres of land, irrigated principally for the production of potatoes, grain, and pasture grasses, lie upstream of the Wildlife Refuges (Fig. 1). Irrigation supply water is used and reused throughout this land before being discharged into the wetland sumps of the Refuge. Such reuse creates a pollutant buildup as water moves through the system. For purposes of this paper, data obtained at drainage Pump B, discharging into the Tule Lake sump, were chosen to depict qualitative findings of the Project's pesticide analyses. Pump B represents water that is used throughout the irrigation system. Model studies indicate that this water could be recycled on irrigated lands a maximum of 5.2 times (*8*). Consequently, this station quantitatively represents water which contains high levels of pesticides relative to levels found at other stations in the study area.

Sampling Procedures

Monitoring stations were established at all significant inflows and outflows to and from the Tule Lake and Lower Klamath Lake Refuge areas. At Pump B, as with the other stations, samples of water, suspended material, submerged aquatic plants, clams, and fish were collected for pesticide analysis.

Grab water samples were collected in two 1-gallon glass bottles. From each bottle, 1.5 liters were combined for an analysis which was performed within 48 hours. Suspended material (plankton, small vegetative fibers, suspended solids) was collected by pumping 100 to 150 cubic feet of water through a 295-micron mesh net. These samples, each weighing from 3 to 12 g, were frozen while awaiting analysis. The aquatic plants (attached algae and vascular) were obtained by raking or handpicking. Pondweed (*Potamogeton* sp.) comprised the majority of the vascular plants collected, but significant amounts of watermilfoil (*Myriophyllum* sp.) and small amounts of other submerged aquatic plants were also sampled. *Cladophora* sp. was the predominate attached alga collected. These plants were frozen in 1-lb aliquots and stored for analysis. Native clams (*Gonidea* sp.) ranging in length from 3 to 5 inches, were collected using an Ekman dredge or a hand rake. Five shucked clams were homogenized together and frozen prior to analysis. Fish were collected using electric fishing gear or rotenone. Approximately 90% of these fish were tui chubs (*Siphateles bicolor*) while the others were blue chubs (*Siphateles gila*). All samples of suspended materials, aquatic plants, clams, and fish were wrapped in aluminum foil in the field to prevent contamination.

In addition to sampling the natural biota, an *in situ* study was made using largemouth bass (*Micropterus salmoides*) and clams. These organisms were held in separate submerged cages at the sampling station. Bass were used because they can withstand confinement and have been used extensively for pesticide bioassays. Tui chubs were difficult to cage as they soon died from disease. A baseline pesticide content was determined before starting the *in situ* study. Sufficient numbers of both bass and clams were caged to allow sampling of the exposed populations throughout the agricultural season. Bass were held and sampled in this manner for as long as 209 days.

All fish samples were prepared for analysis by homogenizing whole fish in a blender and then freezing them for storage. Wild chub samples consisted of 5 to 20 individuals ranging in length from 2 to 7 inches, while from 3 to 5 bass, 5 to 7 inches long, were sacrificed at each increment of the cage study. No pathological examinations were made of their internal organs, as all fish appeared to be in good health at the time of sampling.

Laboratory Analysis

Chemical analyses for chlorinated hydrocarbon pesticides provided individual results for DDE, DDT, DDD, toxaphene, heptachlor, heptachlor epoxide, chlordane, dieldrin, and endrin. However, the results of analyses for these compounds in water and tissue samples showed the compounds DDE, DDD, DDT, chlordane, and endrin to be dominant. Other compounds either were not present or were present in very small amounts—i.e., <0.002 ppb in water and <4 ppb in tissue—and therefore were not included in this report.

All analyses were conducted by the Klamath Basin Study laboratory utilizing gas chromatographic techniques in conjunction with a microcoulometric titrating system employing a silver cell for chlorinated hydrocarbon detection. The cleanup procedures for sample extracts were modifications of those presented by Mills (*4*). Following cleanup, an equivalent of 1.5 kg of water sample and 1 to 25 g of the other samples were injected into the chromatograph. Identity of specific pesticides was confirmed by the use of several different columns. The various columns used are as follows:

- 3% Dow-200 on acid washed 60-80 mesh Chromosorb P, ¼″ × 4-6′
- Mixed column containing approx. equal parts of, first, 5% FS-1265 and, second, 3% Dow-200 on acid washed 60-80 mesh Chromosorb P, ¼″ × 6′
- 3% OV-17 on 60-80 mesh Gas Chrom Q, ¼″ × 6′

Sensitivity of the analytical results differs for each type of sample analyzed and for each type of compound detected. Also, varying quantities of material for a specific sample and changes in instrument response add to the variances of analytical sensitivity. The sensitivity levels shown in Fig. 2 represent these variances. Table 1 shows present analytical sensitivities based on the instrument's normal operating capability.

Reproducibility of results was determined from statistical analysis of duplicate analyses of field samples. Approximately 1 out of 40 samples was selected for duplicate analysis. Table 2 shows the results for all duplicate analyses as calculated by the following two methods: (a) student's t-distribution for paired observations at the 95% confidence interval (*3*), and (b) average percent deviation within laboratory (*6*). The results for a particular sample and compound are based on N samples with a mean value of $\bar{X}$. Deviations from the mean are expressed as a percent.

FIGURE 2.—*Occurrence of endrin in water and biota at pump B.*

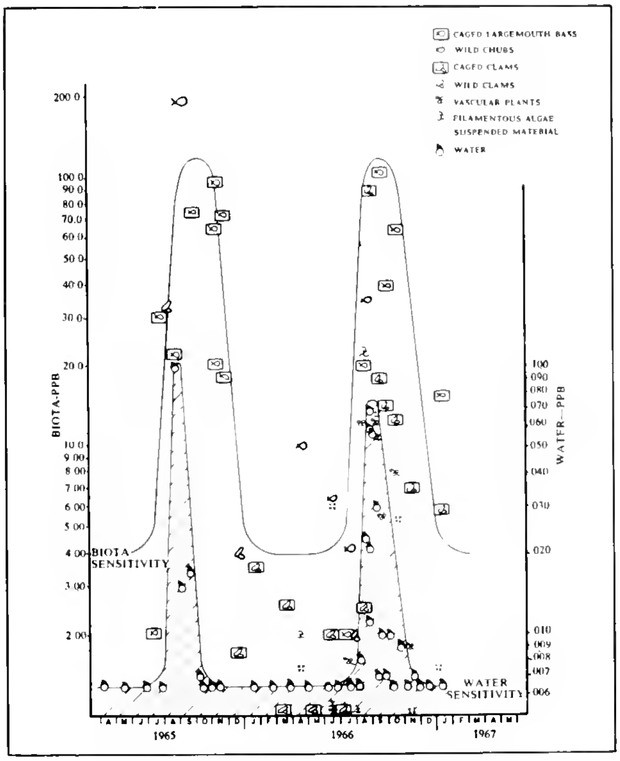


TABLE 1.—*Sensitivity of analytical results*

	Sample Size	DDE, DDD, Endrin (PPB)	DDT, Chlordane (PPB)
Instrument	—	5 ng	15 ng
Water	1.5 kg	0.003	0.010
Fish	2.5 g	2.0	5.0
Clams Vascular Plants Filamentous Algae Suspended Material	10.0 g	0.5	1.5

TABLE 2.—*Reproducibility of analytical results*

	Fish (conc. <100.0 PPB)				Water (conc. <0.100 PPB)			
	N	$\bar{X}$	Student's t-Distribution	Average Deviation	N	$\bar{X}$	Student's t-Distribution	Average Deviation
DDE	23	31.4	14.2	36.3	4	0.015	53.4	26.6
DDD	13	26.2	15.3	39.9	—	—	—	—
DDT	15	29.4	27.2	36.9	—	—	—	—
Chlordane	21	28.5	48.3	34.2	—	—	—	—
Dieldrin	17	10.0	63.6	67.0	—	—	—	—
Endrin	18	13.8	62.8	56.5	6 [1](7	0.031 0.049	25.8 34.7	25.8 30.6)

[1] Includes values >0.100 ppb.

No corrections of results were made for percent recoveries which averaged 80 and ranged from 71 to 95 for the reported compounds.

Results

Pesticides found at Pump B in the various aquatic strata are directly related to the control of insects and other pests plaguing the agricultural industry in and around the Wildlife Refuge. These infestations require the application of thousands of pounds of chemicals. For example, during the 1966 growing season, approximately 14,000 lb of endrin were applied on the study area at a rate of 1.6 lb/acre/year. Portions of these chemicals leach or fall directly into the drainage canals and, as in the case of Pump B, are subsequently discharged into the Wildlife Refuge.

Endrin has predominated in all analytical results, due principally to its abundant usage and persistence in drainage water after application. This, combined with its acute toxicity (*1,2*) singles out endrin as the most hazardous chlorinated hydrocarbon pesticide to wildlife in the Klamath Basin.

The occurrence and fate of endrin in the aquatic strata located in the Tule Lake sump at Pump B during 1965 and 1966 are shown in Fig. 2. Most significant is the increase and subsequent decrease of endrin in all levels of the biota during the main growing season (generally from May through September). If a hydrograph of the discharge from Pump B were shown, it would describe a rise from zero in April to a peak in August, and a fall again to zero in September. Correspondingly, the various aquatic strata become contaminated, the contaminants increase to peak concentrations and fall to near or below levels of sensitivity. Also emphasized, by the generalized curves, is the relationship of the occurrence of endrin in water to the subsequent contamination of the biota.

For the 2 years shown, the level of peak contamination is generally the same for both water and fish samples. Water contained a maximum of 0.100 ppb in 1965 and 0.069 ppb in 1966, while captive fish accumulated a maximum of 97 ppb and 107 ppb of endrin, respectively. Other strata of the biota were distributed between these extremes of the food chain. Although a lesser number of samples were taken during the off season, periodic analyses revealed concentrations near or below the laboratory's low sensitivity levels.

The days of exposure for caged bass and clams are emphasized in Fig. 3 and 4, respectively. For the series of bass and clams which were successfully maintained over an entire irrigation season the rise and fall of pesticide levels are again shown. These studies seem to indicate that the accumulation of endrin is dependent on the time of initial immersion. The greatest accumulation for all series of bass and clams occurred beginning in August of both 1965 and 1966. This rise reflects increased endrin concentrations in water due to July agricultural applications of endrin formulations.

Results of the analyses of water, aquatic biota, and caged bass and clam samples taken at Pump B are shown in Tables 3, 4, and 5, respectively. These tables show results of analyses for other dominant chlorinated hydrocarbon pesticides in addition to data for endrin.

Conclusions and Discussion

Keeping in mind that the occurrence of chlorinated hydrocarbons at Pump B is typical of similar drainage flows in the Lost River system, we conclude that:

1. Agricultural practices in the Lost River system cause chlorinated hydrocarbon pesticide contamination of irrigation return water and the associated biota. This fact, in addition to the reported effects of long-term exposures of wildlife to low concentrations of pesticides (7), indicates the hazards to wildlife, both immediate and long-range, which must be considered. These effects stress the need for water management programs which eliminate, or at least alleviate, the hazards from such contamination. Although no wildlife mortalities due to pesticide poisoning have been reported in the Klamath Basin Refuges in recent years, this does not mean that detrimental effects are not present. The continuing role of researchers is to evaluate these effects in the laboratory and eventually in the field. Until all the answers are found, responsible water users and water pollution control agencies must develop management plans that minimize the occurrence of hazardous materials discharged from agricultural lands.

FIGURE 3.—*Days of exposure — caged bass*

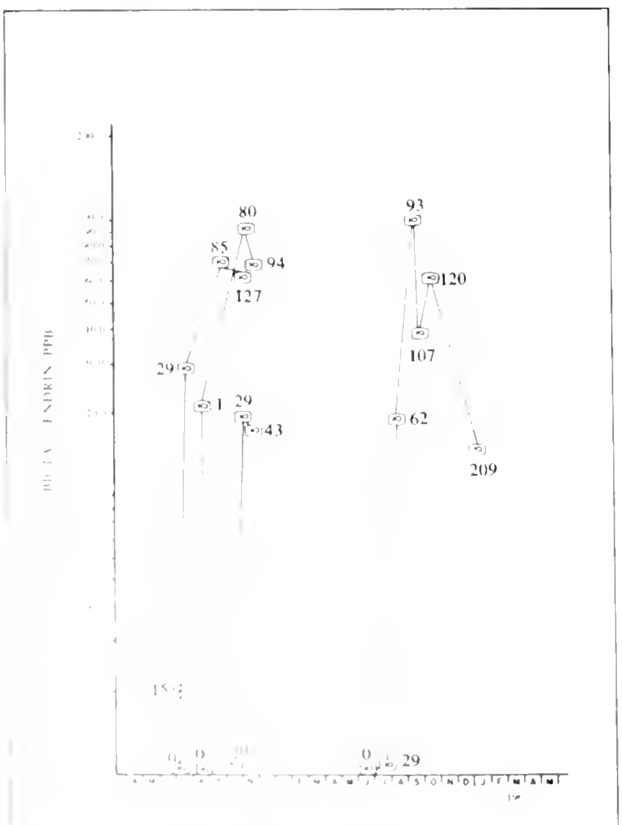
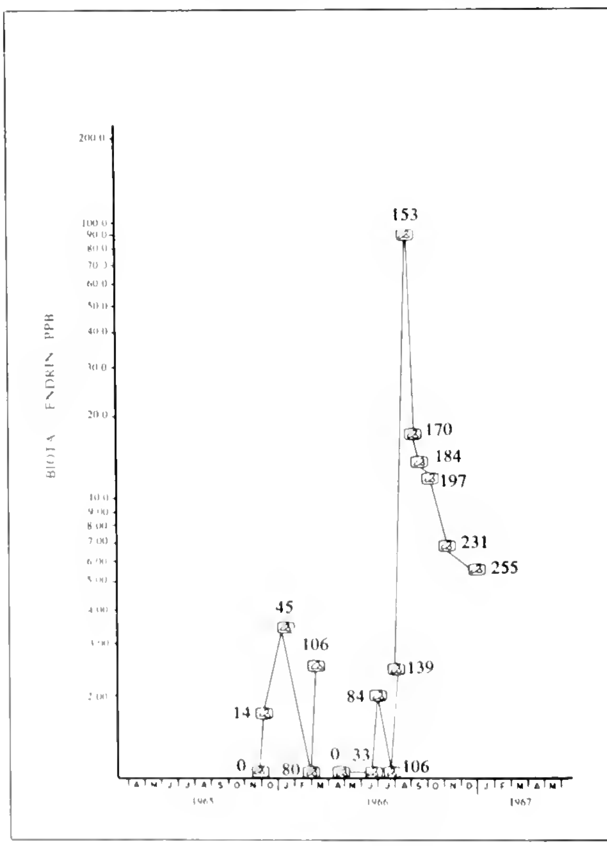


FIGURE 4.—*Days of exposure — caged clams*



2. Concentrations of pesticides in water and biota of the Lost River system increase to peak values during the summer growing season, then decrease to near or below levels of laboratory sensitivity after the close of the season.
   Most important, this fact demonstrates that short-term pesticide contamination of an aquatic environment does not establish permanent residual concentrations of pesticides in the various strata. The dilution effect of irrigation water applications after final pesticide treatment is certainly the cause of this beneficial cleansing. Consequently, flushing with uncontaminated water is a means of controlling pesticide levels in the natural food chain of fish-eating wildfowl.
3. From year to year the concentration of studied pesticides in the aquatic strata of the Lost River system was no greater than the previous year's peak level.

The above conclusions bring out the point that, in agricultural areas with only a short summer growing season, the levels of contamination in the aquatic environment are governed by the seasonal variations, thereby limiting accumulations to the seasonal peaks. For the agricultural community within the Lost River Basin, this fact tempers one of their primary anxieties concerning pesticide usage. At the same time that the wildfowl mortalities were occurring in the Refuge, considerable national attention was focused on the effects of residual pesticides on the Nation's wildlife. Naturally, the first reaction was one of concern that runoff from the irrigated lands in the basin might be causing a continuing buildup of pesticide concentrations in the National Wildlife Refuges which would result in continued mortalities in the migratory bird population. Under present land practices, this continued accumulation is not occurring in the aquatic biota of the basin.

TABLE 3.—*Pumb B water analyses*

[— = Results below analytical sensitivity]

Date Collected	Residues—PPB			
	DDE	DDD/DDT[1]	Chlordane	Endrin
4/01/65	0.006	—	—	—
5/14/65	—	—	—	—
6/22/65	0.005	—	—	—
7/22/65	0.010	—	—	—
8/11/65	0.010	—	—	0.100
8/27/65	0.003	—	—	0.015
9/10/65	0.007	—	—	0.017
10/01/65	0.003	—	—	0.007
10/11/65	0.007	—	0.010	—
10/20/65	0.003	—	—	—
11/09/65	0.010	0.010	0.013	—
1/18/66	0.003	—	0.013	—
2/17/66	0.003	—	—	—
3/22/66	0.003	—	0.010	—
4/26/66	—	—	0.010	—
6/07/66	0.027	0.027/0.027	0.100	—
6/22/66	—	—	0.013	—
7/06/66	0.003	—	0.010	—
7/20/66	0.003	—	0.012	—
7/28/66	—	—	—	—
8/04/66	—	—	0.013	—
8/05/66	—	—	—	—
8/09/66	—	—	—	0.008
8/11/66	—	—	—	—
8/18/66	—	—	—	0.023
8/20/66	—	—	—	0.011
8/22/66	—	—	—	0.021
8/24/66	—	—	—	0.069
8/26/66	—	—	—	0.057
8/29/66	—	—	—	0.056
9/02/66	—	—	—	0.030
9/07/66	—	—	—	0.007
9/13/66	—	—	—	0.010
9/22/66	—	—	—	0.007
9/29/66	—	—	—	0.010
10/04/66	0.003	—	—	—
10/19/66	0.006	0.002/0.013	—	0.009
11/04/66	0.004	0.017	0.051	—
11/18/66	0.003	—	—	—
11/25/66	0.003	0.007	0.017	0.007
11/30/66	—	—	—	—
12/14/66	—	0.017	—	—
1/11/67	—	—	—	—
2/06/67	0.003	0 /0.010	0.017	—

[1] Single values represent a total response, i.e., where DDD and DDT could not be separated.

TABLE 4.—*Chlorinated hydrocarbon pesticides contained in the biota at Pump B*

[— = Results below analytical sensitivity]

Date Collected	Residues—PPB			
	DDE	DDD/DDT[1]	Chlordane	Endrin
Suspended Material				
4/20/66	—	0.75	3.0	1.5
6/22/66	—	—	67.0	6.0
7/22/66	1.7	10.0	6.0	1.3
8/22/66	6.6	4.0	6.0	57.7
9/13/66	—	4.0	8.0	13.0
10/26/66	1.0	0.7/2.0	1.5	5.3
11/16/66	—	—	8.5	—
1/06/67	1.5	3.3/12.0	14.7	1.5
Vascular Plants				
6/22/66	1.0	1.0	5.0	—
7/22/66	—	—	2.0	1.6
8/22/66	1.0	2.0	2.0	12.2
9/13/66	—	—	1.5	12.5
9/29/66	0.8	1.2	—	4.8
10/20/66	1.0	10.0	6.0	8.0
11/16/66	0.6	0.7	2.6	1.8
Algae				
4/20/66	0.5	0.75	2.0	2.0
6/22/66	2.0	3.0	50.0	—
7/22/66	—	—	—	—
8/22/66	0.8	0.4	1.7	22.3
9/13/66	1.3	1.3	13.5	10.8
Chubs				
8/27/65	45.0	17.0	—	198.0
4/20/66	26.0	12.0	24.0	10.0
6/22/66	14.0	10.0	10.0	6.0
7/22/66	6.2	9.6	8.0	4.0
8/22/66	2.5	2.5	—	30.5
Clams				
8/10/65	4.0	4.0	3.0	34.0
12/28/65	4.0	3.0	4.5	4.0
7/22/66	4.8	4.8	12.0	2.0

[1] Single values represent a total response, i.e., where DDD and DDT could not be separated.

TABLE 5. *Chlorinated hydrocarbon pesticides in largemouth bass and clams held in cages at Pump B*

[— Results below analytical sensitivity]

Date Collected	Days Exposed	Number Collected	Residues, PPB: DDE	DDD DDT [1]	Chlordane	Endrin
			Largemouth Bass			
GROUP I						
7 01 65	0	5	27	10	—	—
7 16 65	15	5	32	18	—	2
7 30 65	29	5	37	14	—	31.5
9 24 65	85	2	38	23	—	74
11 05 65	127	1	19	16	15	65
GROUP II						
8 17 65	0	5	27	6	—	2.5
8 18 65	1	5	25	50	—	20.2
11 05 65	80	1	22	13	13	97
11 19 65	94	3	26	15	20	72
GROUP III						
10 07 65	0	5	34	19	11	6.5
11 05 65	29	5	38	14	17	20
11 19 65	43	2	14	17	43	19
GROUP IV						
6 15 66	0	5	16	16	8	—
7 14 66	29	5	37.5	31	33	2
8 16 66	62	4	11	11	—	20
9 16 66	93	5	12.5	9.5/6.5	—	107
9 30 66	107	5	12.5	10/10	7.5	40
10 13 66	120	5	12	10/8.8	8	65
1 10 67	209	2	21.2	8.9/7.3	—	15.3
			Clams			
GROUP I						
12 14 65	0	5	2.5	4	25	—
12 28 65	14	4	2	2	8	1.7
1 28 66	45	3	2.5	5	5	3.5
3 04 66	80	3	2.25	2	6	—
3 30 66	106	2	1	1	—	2.5
GROUP II						
3 30 66	0	5	1	—	—	—
5 02 66	33	5	1.5	3	4	—
6 22 66	84	5	2	2	4	2
7 14 66	106	5	2	4	3	—
8 16 66	139	4	—	—	2	2.5
8 30 66	153	5	0.75	—	—	90
9 16 66	170	5	—	—	3	18
9 30 66	184	5	—	—	6	14
10 13 66	197	5	3	2	—	12.6
11 16 66	231	5	6.3	2.0/3.0	3	7
1 10 67	255	5	2.4	2.3	5.1	5.9

[1] Single values represent a total response, i.e., where DDD and DDT could not be separated.

The chemical names of compounds mentioned in this paper are:

DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
Chlordane	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan
Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Toxaphene	chlorinated camphene containing 67% to 69% chlorine

Acknowledgments

The authors wish to acknowledge Messrs. Robert E. White and Gerald L. Muth who were, successively, in charge of laboratory operations and are responsible for the analytical results presented. We also wish to thank the many staff members of the Klamath Basin Study for their efforts in providing the reported subject material.

LITERATURE CITED

(1) *Henderson, C., Q. H. Pickering, and C. M. Tarzwell. 1959.* Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Trans. Amer. Fish Soc. 88:23-32.

(2) *Katz, M. 1961.* Acute toxicity of some organic insecticides to three species of salmonoids and to the threespine stickleback. Trans. Amer. Fish Soc. 90:264-268.

(3) *Li, J. C. R. 1957.* Introduction to statistical inference. Edwards Brothers, Inc. Ann Arbor, Mich.

(4) *Mills, P. E. 1959.* Detection and semiquantitative estimation of chlorinated organic pesticides in food by paper chromatography. J. Ass. of Agr. Chem. 42:734.

(5) *Pillmore, R. E. 1961.* Pesticide investigations of the 1960 mortality of fish-eating birds on Klamath Basin wildlife refuges. U. S. Fish Wildlife Serv., Wildlife Res. Lab. 12 p.

(6) *Robert A. Taft Sanitary Engineering Center. 1966.* Water Pesticides No. 1, Study Number 24. Public Health Serv. Publication No. 999-WP-39. 64 p.

(7) *Rudd, R. L. 1964.* Pesticides and the living landscape. Univ. of Wis. Press, Madison, Wis. 320 p.

(8) *Woods, Philip C. and Gerald T. Orlob. 1963.* The Lost River system — a water quality management investigation. Water Resources Center Contrib. No. 68, Univ. of Calif., Berkeley, Calif. 54 p.

Chlorinated Pesticide Residues in an Aquatic Environment Located Adjacent to a Commercial Orchard

R. J. Moubry[1], J. M. Helm[2], and G. R. Myrdal[1]

ABSTRACT

Samples of water, silt, bottom organic debris, bottom organisms, and fish were collected from an aquatic environment located adjacent to a commercial orchard. Residue data obtained from the analysis of these samples are presented. The results obtained indicate that contamination of the environment studied was minimal.

Introduction

Pesticides, principally the chlorinated hydrocarbons, have been used extensively in Wisconsin orchards in the production of fruit for market. In 1966, an exploratory investigation was conducted by the Wisconsin Department of Natural Resources to evaluate the effects of such pesticide usage on the aquatic environment of streams located in the drainage area of these orchards.

Knights Creek, located in Dunn County, Wis., was selected as the site of this investigation. The upstream area of this creek branches to the north and to the south. A commercial orchard is located on top of a hill at the confluence of these two branches traversing along the base of the hill. Accurate records of pesticide usage were unavailable, but it was ascertained that 150 acres of the orchard had been treated with endrin for rodent control at a rate of approximately 1 lb/acre actual in the fall of 1963, 1964, and 1965. During this same 3-year period, approximately 100 lb actual of dieldrin also had been used each year in foliar treatment of the entire orchard (195 acres), and, during the period 1955 to 1962, approximately 50 lb actual of dieldrin had been applied yearly to this orchard. Many other types of pesticides, including DDT, also had been used in this orchard, but the total amounts applied were not determined.

[1] Wisconsin Department of Agriculture, General Laboratory Division, Bureau of Chemistry, 4702 University Ave., Madison, Wis. 53702.
[2] Wisconsin Department of Natural Resources, Division of Resource Development, Bureau of Water Resources, 421 State Office Building, Madison, Wis. 53702.

Sampling Methods

Sampling stations were established in the north and south branches of Knights Creek, at the confluence of the two branches, and in a control area located in a tributary of the north branch. On March 8, 1966, samples of silt, bottom organic debris, and bottom organisms were taken at each sampling station with the aid of a dredge which collected stream bottom material to a depth of 3 to 4 inches. The organisms and organic debris were then removed from the material, and 1-quart portions each of the separated organic matter and remaining silt from each of the sampling stations were taken for analysis. Bottom organisms were first separated by species; however, in some instances, difficulty in obtaining a sufficient quantity of individual species necessitated the compositing of different organism species into a single sample. Bottom organism samples were then held in a formaldehyde solution.

A 5-quart sample of runoff ground water entering the stream was collected at each of the sampling station areas on June 1, 1966, either during or immediately after a heavy rain storm. Due to a heavy turf surrounding this stream, these water samples were to all appearances devoid of silt.

The fish samples were collected on August 24, 1966, by means of an electro-fishing apparatus. Fish were unavailable in the control area at the time of sampling.

Analytical Methods

The samples of silt were air-dried to approximately 15% moisture and sieved. The material which did not pass through a No. 8 sieve was discarded. The sieved silt samples were extracted by the hexane-acetone procedure (*1*), and the silt extracts were then cleaned up with Florisil (*2*). The debris samples were ground, mixed, and extracted by the acetonitrile-water extraction procedure (3). A portion of each homogenous sample of silt and debris was taken for moisture determination. Analysis was made on the "as is" basis. The dry weight residue results were obtained by calculation, using the percent moisture obtained from each sample.

The bottom organism samples, submitted in formaldehyde solutions, were drained. Each of the formaldehyde solutions was then analyzed for chlorinated hydrocarbon pesticide residues and interferent gas chromatographic peaks. None were found. Some of the bottom organisms (caddis fly larvae) were incased in a sand covering. These were removed and discarded prior to grinding. The drained and deceased organisms were ground, extracted, and cleaned up (2). The sample size used for analysis ranged from 8 to 10 g. The results obtained were reported on the drained weight basis.

The fish collected from each sampling station were pooled by species. The number of fish composited into each sample is shown in Table 3. The fish samples were ground as received. The ground samples included head, tail, scales, and viscera. The samples were extracted and cleaned up (2), with results being reported on the extracted fat basis. The percentage of fat in the samples was determined and reported.

The water samples (4,800 ml each) were extracted three times with redistilled hexane. The extracts were concentrated and cleaned up with Florisil.

Determination of the amount of pesticide residues present in the samples was by electron capture gas-liquid chromatography. The instrument used was a Jarrell-Ash, Model 28-710, gas chromatograph. The column packing systems used were 10% DC-200 on Anakrom ABS, and a mixed bed column consisting of nine parts 10% DC-200 and five parts 10% QFl on Gas Chrom Q.

The sample size, final volume of sample extract, and amount injected into GLC were adjusted to provide a sensitivity of 0.001 ppm dieldrin for the silt, organic material, bottom organisms, and fish tissue. The level of detection for the water samples was 25 ppt of dieldrin. Inasmuch as this was an exploratory survey, recovery studies were not conducted in conjunction with analysis of these samples. Recovery studies are run at periodic intervals in the laboratory to insure reliable analysis and are in the range of 90%. Due to the minimal amount of residue detected in the majority of these samples, confirmation of the residue detected was restricted to multiple GLC column technique. The data presented are the results obtained using the methodology specified.

Discussion

The results obtained are presented in Tables 1 through 4. No residues were detected in the orchard runoff water entering the stream on the date these samples were collected. No detectable DDT or its analogues were present in the silt and debris samples. Low levels of DDT and its analogues were detected in the bottom organisms. The DDT and dieldrin residues detected in the brook trout were at the same general level as those detected in the same and similar species collected and analyzed in a recent State-wide residue-in-fish survey (4). Although low-level endrin residues were detected in the silt, organic matter, and bottom organisms, none were detected in the fish samples. Evaluation of the results obtained in this limited investigation indicates that the pesticide usage in the orchard has not significantly contaminated the aquatic environment of this adjacent creek.

The chemical names of compounds mentioned in this paper are:

Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethano=naphthalene
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene

Acknowledgments

The authors wish to acknowledge the assistance of R. Smith (Warden) and L. Frankenburger (Biologist) of the Division of Conservation, who assisted in the collection of samples, and L. Lueschow of the Division of Resource Development, who performed the identification of invertebrate organisms.

LITERATURE CITED

(*1*) *Lichtenstein, E. P., G. R. Myrdal, and K. R. Schulz. 1964.* Effect of formulation and mode of application of aldrin on the loss of aldrin and its epoxide from soils and their translocation into carrots. J. Econ. Entomol. 57:133-136.

(*2*) *Barry, Helen C., Joyce G. Hundley, and Loren Y. Johnson.* Pesticide Analytical Manual Vol. 1, 2.21 (A), U. S. Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D. C. 20204.

(*3*) *Ibid.*, 2.21(B).

(*4*) *Kleinert, S. J., P. E. Degurse, T. L. Wirth, and L. C. Hall. 1967.* DDT and dieldrin residues found in Wisconsin fishes from the survey of 1966. Preliminary Report, Research Report No. 23 (Fisheries), Wisconsin Conservation Department, Research and Planning Division, Madison, Wis.

TABLE 1.—*Chlorinated hydrocarbon pesticide residues detected in silt and debris samples*

[— = None detected]

Site	Residues in PPM—Dry Weight Basis			
	Silt Samples		Debris Samples	
	Endrin	Dieldrin	Endrin	Dieldrin
Control	—	—	—	0.004
North Branch	0.003	—	0.025	0.006
South Branch	0.002	—	0.011	0.002
Confluence	0.013	0.005	0.014	0.002

TABLE 2.—*Chlorinated hydrocarbon pesticide residues detected in bottom organism samples*

Site	Sample	Residues in PPM—Whole Weight Basis					
		DDE	DDD	DDT	DDT and Analogues	Dieldrin	Endrin
Control	Caddis Fly Larvae (*Limnephilus rhombicus*)	0.014	0.009	0.010	0.033	0.002	—
	Organism Composite [1]	0.013	0.008	0.012	0.033	0.001	—
North Branch	Alder Fly Larvae (*Sialis* sp.)	0.005	0.003	0.008	0.016	0.013	0.009
	Fresh-Water Shrimp (*Gammarus* sp.)	0.010	0.007	0.012	0.029	0.003	0.025
	Caddis Fly Larvae (*Limnephilus rhombicus*)	0.006	0.007	0.011	0.024	0.002	0.003
South Branch	Organism Composite [2]	0.007	0.011	0.016	0.034	0.002	0.004
Confluence	Fresh-Water Shrimp (*Gammarus* sp.)	0.009	0.007	0.015	0.031	0.013	0.013

[1] Consisted of *Gammarus, Agapetus, Protoptila*, caddis pupae, Dytiscidae, *Atherix variegata*, immature stone flies, *Procladius*, Hydrobaeninae, and *Calopsectra*.

[2] Consisted of *Gammarus, Sialis, Isoperla bilineata, Protoptila*, caddis pupae, *Agapetus, Cheumatopsyche*, aquatic earthworm, *Tipula*, Tabanidae, *Procladius*, immature Coleoptera, Hydrobaeninae, *Limnephilus rhombicus*, and *Potomyia*.

TABLE 3.—*Chlorinated hydrocarbon pesticide residues detected in fish samples*

Site	Sample	No. of Fish	Percent Fat	Residues in PPM						
				Fat Basis					Whole Weight Basis	
				DDE	DDD	DDT	DDT and Analogues	Dieldrin	DDT and Analogues	Dieldrin
North Branch	Brook Trout (*Salvelinus fontinalis*)	2	4.0	1.41	1.04	1.42	3.87	0.26	0.155	0.014
	Northern Creek Chubs (*Semotilus atromaculatus*)	24	3.8	1.02	0.67	0.12	1.81	0.34	0.069	0.013
	Muddlers (*Cottus bairdi*)	17	2.4	0.65	0.45	1.47	2.58	0.69	0.062	0.017
South Branch	Brook Trout (*Salvelinus fontinalis*)	4	4.6	0.83	0.56	1.15	2.54	0.18	0.168	0.008
	Northern Creek Chubs (*Semotilus atromaculatus*)	33	3.6	1.00	0.53	0.59	2.12	0.17	0.076	0.006
	Muddlers (*Cottus bairdi*)	16	2.4	0.57	0.47	1.54	2.58	0.31	0.062	0.007
Confluence	Brook Trout (*Salvelinus fontinalis*)	1	4.6	0.29	0.26	0.37	0.92	0.21	0.042	0.010
	Northern Creek Chubs (*Semotilus atromaculatus*)	6	2.6	1.53	0.63	0.20	2.36	0.31	0.061	0.008
	Black Nosed Dace (*Rhinichthys atratulus*)	10	6.0	1.92	0.78	0.10	2.80	None	0.168	None
	Muddlers (*Cottus bairdi*)	4	2.2	0.55	0.40	0.54	1.53	0.41	0.034	0.009

Note: No endrin residues were detected in these samples.

TABLE 4.—*Results of analyses of rain runoff water for chlorinated hydrocarbon pesticide residues*

Site	Ml H_2O Extracted	Pesticide Residues [1]
Control	4800	None detected
North Branch	4800	Do.
South Branch	4800	Do.
Confluence	4800	Do.

[1] Minimum level of detection was 25 ppt of dieldrin.

PESTICIDES IN SOIL

Monitoring the Effects of the 1963-64 Japanese Beetle Control Program on Soil, Water, and Silt in the Battle Creek Area of Michigan

J. E. Fahey[1], J. W. Butcher[2], and M. E. Turner[3]

ABSTRACT

The 1963-64 Japanese beetle control program in Battle Creek, Mich., was monitored by Michigan State University and the Agricultural Research Service, U. S. Department of Agriculture. Soil, water, and silt samples were obtained after treatment of infested areas with 20 lb of 10% granular dieldrin per acre. Dieldrin was present in only 3 of 22 pre-treatment soil samples. It averaged 1.25 ppm in soil samples collected on November 23, 1963, just after treatment, and 1.39 ppm on June 25, 1964. No detectable residues of dieldrin were present in water after treatment, and residues in silt were low, absent, or inconclusive due to interferences.

Introduction

The 1963-64 Japanese beetle (*Popillia japonica* Newman) control program in the Battle Creek area of Michigan was cooperatively undertaken by the Entomology Research Division and the Plant Pest Control Division, Agricultural Research Service, U.S. Department of Agriculture; the Plant Industry Division, Michigan Department of Agriculture; and the Entomology Department, Michigan State University. The area treated consists of 12,601 acres. Dieldrin was used at the rate of 2 lb technical per acre (10% dieldrin at 20 lb granular per acre). The objectives of the program were to treat the city of Battle Creek and surrounding suburban area and kill as many beetles as possible. The program started on October 27, 1963, and ended April 5, 1964. No operations took place from December 14, 1963 to March 30, 1964. The applications were made with ground equipment, including two buffalo turbines and two Skibee spreaders mounted on pickup trucks, and hand operated Seymour seedcasters.

[1] [illegible] Purdue University, formerly in Charge [illegible] Pesticide Chemicals Research Branch, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture.
[2] Department of Entomology, Michigan State University.
[3] Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture.

Precautions were taken wherever possible to prevent contamination and hazardous residues. Special care was taken to avoid getting dieldrin into lakes, rivers, and creeks. Only small sections of shoreline were treated between rains. Great care was taken also to keep the insecticide off sidewalks, streets, driveways, etc. Feeding dishes for pets, sand boxes, and bird baths were turned over or covered with sections of tarpaulin before treatment. Several pastures, small hayfields, and garden areas with sensitive crops were bypassed in compliance with label recommendations for dieldrin.

The monitoring program was conducted by the Entomology Department, Michigan State University; and the Pesticide Chemicals Research Branch, Entomology Research Division, Agricultural Research Service. The work by Michigan State University was supported by contracts with the Plant Pest Control Division, ARS.

All collections were made by or under the direction of Dr. J. W. Butcher, and all residue analyses were performed by or under the direction of Jack E. Fahey.

A preliminary survey of the occurrence and distribution of chlorinated hydrocarbon insecticide residues in soil from Battle Creek, Mich., was reported by Fahey, Butcher, and Murphy in 1965 (*1*). They found dieldrin in only 17 of 227 samples. The dieldrin residues found ranged from 0.06 to 2.2 ppm. Only one sample contained more than 1.0 ppm of dieldrin.

Collection of Samples

Prior to the start of control operations, twenty 1- by 3-inch soil cores were collected from sod and twenty 1- by 3-inch cores from garden or shrub-planted (cultivated) areas in one city lot per 40 acres. The lot chosen for sampling was always on the extreme southwest corner of each 40 acres. If, for any reason, the sample could not be obtained at the preselected point, the collector sampled the closest accessible lot.

The sod and cultivated soil samples were packaged and analyzed separately.

Similar soil samples were obtained at every tenth point as soon as possible after treatment—in November 1963—and again in June 1964.

Water samples were collected from 13 points in ponds, creeks, and the Kalamazoo River. Collection points were established throughout the entire treatment area in order to detect residues that might be washed off the treated soil surface into the major drainage pathways. A 1-gallon sample of water was collected from preselected points before treatment on October 31 and November 2, and after treatment on December 19, 1963, and March 23, 1964.

Silt samples were collected from the streams and ponds at the water collection points on October 31 and November 2 (pre-treatment) and on December 19, 1963 (after treatment). Collections were discontinued after the first post-treatment sample.

Preparation of Samples for Residue Analysis

SOIL AND SILT

Recovery of Residue: Silt samples were filtered, dried, and ground before analysis. Soil samples were sieved and dried. Aliquots were weighed and 10% moisture added. The samples were then stripped with a 2:1 mixture of hexane and isopropyl alcohol, using 2 ml per gram of soil (or silt). The alcohol was removed by washing with water; the hexane was dried over sodium sulfate.

Cleanup: A 40-ml aliquot was reduced to 10 ml and chromatographed on a 4:1 magnesia celite column (as used in colorimetric analysis).

Analysis: Suitable aliquots of the cleaned residue solution were injected into a Jarrell-Ash gas-liquid chromatograph, electron capture detector.

Critical Temperatures:	Oven	175 C
	Injector	235 C
	Splitter	210 C
	Detector	200 C

Column: ¼″ × 4′ aluminum
2% SE 30 on Anakrom ABS

Results of analyses were qualitatively verified by thin layer chromatography.

WATER

Residue Recovery: Water samples of approximately 2 liters were extracted with 200 ml normal hexane for 5 minutes. The hexane extract was dried over sodium sulfate.

Analysis: Analyses were made by gas-liquid chromatography, using the same instrument and conditions as for soil analyses.

Results of the Analysis

Table 1 lists dieldrin residues recovered from pre- and post-treatment soil samples collected at Battle Creek. The number of dieldrin granules visible in four 1-square-foot soil surface counts per collection point are given along with ppm dieldrin residues recovered from the same points before and after treatment. Table 1 also shows other chlorinated hydrocarbon residues found in pre-treatment samples.

Table 2 shows the results of analysis of pre- and post-treatment water and silt samples. There were no verifiable residues detected in any of the post-treatment water samples. Because of the low residues found in post-treatment silt samples and interferences in analysis, the silt sampling was discontinued after one sampling.

Summary and Conclusions

Analysis of soil, water, and silt samples from the Battle Creek treatment area was carried out. The findings may be summarized as follows:

Substantial, although not uniform, residues of heptachlor, chlordane, BHC, DDE, or *p,p′-o,p′*-DDT were present in virtually all samples taken from turf and cultivated plots throughout the city of Battle Creek before treatment. Only three pre-treatment samples contained measurable dieldrin residues.

Counts of dieldrin granule distribution and levels of dieldrin residues in soil after treatment showed that coverage was almost complete and probably adequate for control. The soil samples collected on November 23, 1963, contained an average of 1.25 ppm of dieldrin while those collected June 25, 1964, contained an average of 1.39 ppm of dieldrin.

No detectable residues were present in water on the dates sampled after treatment.

Dieldrin residues in streambed or pond silt were low, absent, or inconclusive due to interferences.

Michigan Agricultural Experiment Station Publication No. 4267

Acknowledgment

Grateful acknowledgment is made to Mr. Calvin Corley, U.S. Department of Agriculture, Entomology Research Division, Pesticide Chemicals Research Branch, Beltsville, Md., for assistance in the analytical work.

LITERATURE CITED

(*1*) *Fahey, J. E., J. W. Butcher, and R. T. Murphy. 1965.* Chlorinated hydrocarbon insecticide residues in soil of urban areas, Battle Creek, Mich. J. Econ. Entomol. 58:1026-27.

TABLE 1.—*Residues of chlorinated hydrocarbon insecticides in soil samples from Battle Creek, Mich.*

Site	Type [1]	Dieldrin Granule Count [2]	Pesticide Residues in Soil Samples								
			Pre-Treatment							Post-Treatment Dieldrin	
			BHC	DDT	o,p'-DDT	p,p'-DDT	Chlordane	Heptachlor [3]	Dieldrin	11/23/63	6/25/64
1	T	10-17-15-18	—	.03	—	.11	.20	—	—	[4] 0.84	3.30
2	C		—	.03	—	.17	.10	—	—	[4] 0.61	0.15
3	T	6-14-0-2	—	.03	—	1.60	.10	—	—	0.35	1.35
4	C		—	—	—	.21	—	—	—	0.58	0.34
5	T	21-8-12-6	—	—	—	—	—	—	—	3.06	3.00
6	C		—	—	—	.07	—	—	—	0.73	4.40
7	T	3-2-3-0	—	.31	.04	.51	—	—	—	0.86	1.50
8	C		—	.05	.04	.22	—	—	—	1.84	1.20
9	T	19-18-5-10	.20	.20	.30	1.30	—	—	—	8.63	3.10
10	T	5-2-3-3	—	—	—	.15	.10	—	0.1	0.28	3.00
11	C		—	—	—	—	—	—	—	2.33	3.00
12	T	0-0-0-0	.10	—	—	.80	120.0	1.6	—	0.06	0.13
13	C		—	.14	.20	2.10	0.5	—	—	0.37	0.13
14	T	3-18-11-10	—	—	—	.07	0.13	—	—	1.01	1.00
15	C		—	—	—	.08	—	—	—	2.21	2.70
16	T	2-3-1-2	—	—	—	—	—	—	—	0.03	0.26
17	T	12-8-6-26	—	—	—	.07	—	—	—	0.65	0.30
18	C		—	.03	.04	.30	—	—	—	—	—
19	T	21-7-2-3	—	—	—	.08	.10	—	—	1.26	0.14
20	C		—	—	—	.07	.13	—	—	0.58	0.15
21	T	3-3-1-7	.02	—	—	—	—	—	0.07	0.04	0.01
22	C		—	—	—	.25	—	—	0.07	0.05	0.05

[1] T = Turf sample; C = Cultivated soil
[2] Granules found in a unit of space treated.
[3] Includes heptachlor epoxide.
[4] Sampled 11/11/63 instead of 11/23/63.

The chemical names of compounds mentioned in this paper are:

Dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers
Chlordane	1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane
o,p'-DDT, *p,p'*-DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan

TABLE 2.—*Dieldrin residues in water and silt from ponds and streams in Battle Creek, Mich.*

[— = No samples collected]

Sample No.	Collection Point	Dieldrin Residues (PPM)						
		Water				Silt		
		Pre-Treatment		Post-Treatment		Pre-Treatment		Post-Treatment
		Date	Amt	12-19-63	3-23-64	Date	Amt	12-19-63
1	Irving Park Pond, Upper side	11-2	<.0001	—	<.0001	11-2	<.001	—
2	Sperry Creek, bank of Horseshoe Bend	11-2	<.0001	—	<.0001	11-2	.008	—
3	Kalamazoo River, E. of bridge	10-31	.0002	<.0001	<.0001	10-31	[1] <.001	<.001
4	Holmer Creek at West River Rd	11-2	.0055	<.0001	<.0001	11-2	<.001	.002
5	Waubum Creek	—	—	<.0001	<.0001	—	—	.003
6	Below Junction Kalamazoo and B. C. Rivers	10-31	.0002	<.0001	<.0001	10-31	[1] <.001	.008
7	Kalamazoo River at Country Club		<.0001	<.0001	<.0001		.009	<.001
8	Harper Creek, before Kalamazoo Rd.	10-31	<.0001	<.0001	<.0001	10-31	.004	<.001
9	Goguac Lake, 116 Fern	10-31	.0002	—	<.0001	10-31	.010	—
10	(a) Goguac Lake boathouse	10-31	0006	—	<.0001			
	(b) Vince Island by Stone boathouse					10-31	.008	—
11	Kalamazoo River, W. of bridge	10-31	.0006	<.0001	<.0001	10-31	[1] <.001	<.001
12	Battle Creek, Elm St.	11-2	.0003	<.0001	<.0001	11-2	.006	<.001
13	(a) Battle Creek, West Pony Ave.	11-2	.0003	<.001	<.0001			
	(b) Small Elm area at bend in river					11-2	.11	.030

[1] Interferences made analysis impossible.