PESTICIDE-WILDLIFE STUDIES

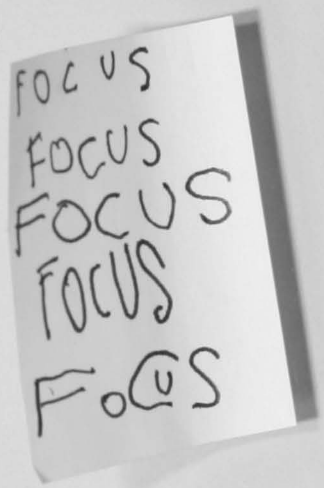
A Review of Fish and Wildlife Service Investigations During 1961 and 1962

UNITED STATES DEPARTMENT OF THE INTERIOR

Stewart L. Udall, Secretary

FISH AND WILDLIFE SERVICE

Circular 167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FOREWORD

The Fish and Wildlife Service is the agency of the Federal government responsible for conservation of wildlife in the United States. In Public Law 85-582, the Service was specifically directed to evaluate the effects of pesticides on wildlife, and to assist in the development of chemicals and techniques to minimize losses.

This report summarizes research findings for 1961 and 1962. Reports published by Service personnel during 1961 and 1962 are reviewed, and progress is reported for research studies underway. Certain publications appearing in 1961 or 1962 that were prepared by other scientists are reviewed also. Separate accounts summarize the commercial fisheries investigations by the Division of Biological Research of the Bureau of Commercial Fisheries; sport fisheries investigations by the Branch of Fishery Research of the Bureau of Sport Fisheries and Wildlife; and wildlife investigations by the Denver and Patuxent Wildlife Research Centers, both of the Branch of Wildlife Research of the Bureau of Sport Fisheries and Wildlife. Also, wildlife investigations of Provinces, States, universities, and the Cooperative Wildlife Research Units of the Branch of Wildlife Research are briefly summarized. Recommendations are made for the use of pesticides that will cause minimum damage to wildlife.

Many people have contributed to this publication. The principal research workers for most projects are identified in the various reports. In addition, the work was facilitated by L. D. Stringer, Acting Chief, Branch of Shellfisheries, Division of Biological Research; P. E. Thompson, Chief, Branch of Fishery Research; D. L. Leedy, Chief, C. E. Carlson, Assistant Chief, E. H. Dustman, Head of the Cooperative Wildlife Research Units, and W. W. Dykstra and J. L. George, Staff Specialists for pesticide-wildlife studies of the Central Office of the Branch of Wildlife Research; and C. S. Williams and J. L. Buckley, Directors of the Denver and Patuxent Wildlife Research Centers, respectively. The report was coordinated and edited by J. L. George.

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PESTICIDE-WILDLIFE STUDIES:

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INTRODUCTION

by

John L. George
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Pesticide use in 1961 as indicated by total sales volume increased about 15 percent over 1960. Sales of organic pesticidal chemicals in the United States totaled $301 million in 1961 (Shepard, et al., 1962). Sales continued to increase in 1962 and totaled $325 million (Agricultural Chemicals, 1963). A large factor in 1961 was a major increase of insecticide sales owing to heavier than usual infestations in some farm areas. Consumption of herbicides, especially those of the selective type for agricultural use and those for lawn care, continued the upward trend in 1961 (Shepard, et al., 1962). Production of synthetic organic pesticidal chemicals in 1961 was made up of about 24 percent fungicides, 20 percent herbicides, and 56 percent insecticides; this compares with about 28, 18, and 54 percent, respectively, in 1960.

At present, about 60 percent of the output of pesticidal chemicals in the United States is used in agriculture. Of the remaining $120 million value, about $50 million is consumed in non-agricultural uses, and about $70 million is exported, largely formulated to some extent before shipping (Shepard, et al., 1962).

DDT production in 1961 was greater than ever before for the fourth successive year. The output of 2,4-D and 2,4,5-T rose in 1961 as it has in each year recently. Also, the toxic aldrin-toxaphene group increased from 87 million pounds in 1959 to 91 million pounds in 1960 to 104 million pounds in 1961. The total volume of synthetic organic pesticidal chemicals produced in 1961 and 1962 was not reported; but the total production of all pesticidal chemicals, including inorganic compounds, was approximately 1 billion pounds, active ingredient, as reported in 1960 (Shepard, et al., 1960). The number of products listed in the 1962 Pesticide Handbook (Frear, 1962) was 9,444, compared with 7,851 in the 1960 Pesticide Handbook (Frear, 1960).

Despite the record production figures of chlorinated hydrocarbons, S. A. Hall (1962) points out that most of the newer products are organophosphorus compounds and indicates this trend is because of superior performance, lower cost, reduced chronic toxicity to warm-blooded animals, lack of residue problems in agricultural products, and lesser problems of insect resistance. He indicates this is true in most industrial nations, with the Russians concentrating on phosphonates.

Carbamates are a new group with many of the advantages listed above for organophosphorus compounds. Despite spectacular successes such as a 2.3 million-pound shipment to save the Egyptian cotton crop, Hall does not think this group will rank with the other major groups of insecticides.

As to new types of pesticides, Hall states that it is entirely feasible to write specifications in advance and screen for and develop compounds to meet those specifications. Two new developments in the experimental stage are chemosterilants, which block effective reproduction of the insect pest, and attractants. Combinations of chemosterilants and attractants are obvious methods of great promise to be explored. Also, notable progress has been made in recent years for the abatement of nuisance pests through repellents which are quite effective and may obviate the need for insecticides in many areas and places.

In the early screening of new chemicals for effects on fish and wildlife, the chemical industry has willingly furnished rather costly samples to all research branches of the Service for such testing as they were able to conduct. This has not only aided the Service in determining the effects of such chemicals at an early date in their development, but also has led to the discovery of some promising compounds which might have use in wildlife management. This aspect of the Service's research needs to be greatly expanded, probably through the development of a screening center.

Dr. Hall states that insecticides will continue as our mainstay in insect control, but believes that biological control through the use of parasites, predators, and pathogens, as well as chemosterilants, holds promise that has not been exploited and will be used to a greater extent than in the past. Dr. B. T. Shaw, Administrator, U. S. Agricultural Research Service, agrees that there are more opportunities in these areas of control than have been used (Shaw, 1962). Development of cultural methods which eliminate pests, or reduce pest damage, and development of strains of plants resistant to pests or disease, are promising aspects of biological control. Some ecologists believe there are neglected opportunities in a diversification approach to crop growing.

During 1962, the Entomological Society of America (D. Hall, 1962) compiled and published figures on acreages of major habitats or land-use categories in conterminous United States which are treated annually with insecticides. A summary of this compilation, in tabular form, follows.

Major uses of land	Acres in category (millions)	Acreage upon which insecticides are applied (millions)	Percentage of category insecticide applied on
Forest lands	640	1.8	.28
Grassland pasture	630	1.6	.25
Desert, swamp, dunes, and wildland	77	2.5	3.24
Water areas	32.6	0	-
Cropland and cropland pasture	457	68.6	15.0
Urban or built-up areas	53	15	28.3
Non-forested parks, wildlife refuges, duck reserves, national defense sites	45	-	-
Total acreage, U. S. (48 States)	1,934.6	89.5	4.62

Of the 457 million acres of cropland and cropland pasture, the more intensively treated areas are:

Crop	Acreage (millions)	Acres insecticides applied on (millions)	Av. no. pounds applied per acre	Percent of category insecticide applied on
Fruits-nuts	2.8	2.3	7.0	80.0
Cotton	15.8	11.9	7.5	75.0
Vegetables	4.1	2.1	3.0	50.0
Grains	216.6	32.5	1.0	15.0
All other	217.6	19.9	.5	9.0

The survey report states that "the total quantity of insecticidal chemical (active ingredients) used in an average year in the United States (minus Alaska and Hawaii) is estimated to be 225,000,000 pounds"--applied on a total acreage of 89,500,000 acres. The more heavily treated agricultural lands and urban areas are described as "less important to wildlife." As a matter of fact, these habitats are very important and the highest population densities of many species are attained on them. Furthermore, the wildlife on these lands includes species that give the greatest esthetic enjoyment to man in his day-to-day living. The figures in the survey report refer to active ingredients of "organic insecticides," "phosphates," "inorganic insecticides," and "botanical insecticides;" the report states that 75 percent of the United States has never been treated by any insecticide and that only about 5 percent is treated each year. This is about as indicated by the Fish and Wildlife Service in past reviews.

Despite the low percent of area treated, contamination of wild forms is widespread. For example, 25 of 26 specimens of bald eagles analyzed by the Service contained DDT; and of 2,300 specimens of birds and mammals from 22 States and 3 Provinces of Canada, 75 percent contained residues when analyzed. Even in remote areas of the world animals have residues. Waterfowl and their eggs in the Far North and resident fish in streams in Alaska were collected on areas far from any known pesticide application, and they contained residues of insecticides, as did the surrounding vegetation. Also, marine fishes from different oceans of the world have been found to contain DDT. Special collections of woodcock from northern and southern portions of its range have been made, and during 1961 and 1962 some 190 of 280 contained heptachlor epoxide when analyzed and 129 of 220 contained DDT.

This almost universal contamination can be explained on biological and physical grounds. Mobile animals can transport residues, and food-chain organisms carried by physical forces transport chemicals. Much pesticidal material never reaches the target area but drifts away. Other proportions of chemicals applied are lost or dissipated through either co-distillation or volatilization to become a part of the atmosphere. Experts have computed that a few grams of a highly stable pesticidal material evenly dispersed in the atmosphere could yield a deposit of several thousand molecules per square foot over the entire world, including the oceans.

To help hit the target area, helicopters are being used more and more in application of agricultural chemicals, especially insecticides. In 1959, helicopters were used in 1.2 percent of total hours flown by aerial applicators; in 1960, this figure had risen to 5.6 percent, and a check of Federal Aviation Agency records indicates "helicopter application is increasing at a faster rate than is fixed wing application" (Moore, 1963). Helicopters permit lower applications of chemicals. For example, it was stated that 17 pounds so applied give the same ground dosages on the target area as 25 pounds applied by fixed wing aircraft. Helicopters are also more versatile and can be used in more variable ground than fixed wing aircraft. From a wildlife standpoint, aerial application is important in that it permits blanket application of rough ground and coverage of large acreages of remote areas in a short period of time. These practices may increase hazard of pesticides to wildlife.

Herbicides are being used more widely. Weed control was practiced on about 85 million acres in cropland in 1961. It is said to be needed on about 140 million acres of row and 230 million acres of drilled crops, 1 billion acres of hay, pasture and range, 30 million acres along railroads and highways, and about 33 million acres of single family homes (Shepard, et al., 1962). Not all brush control is chemical. In Texas, brush control in 1961 was practiced on 1,471,000 acres. On 640,000 of these acres, chemicals were used; on the remainder, mechanical methods (Shepard, et al., 1962).

There has been no overall plotting or totaling of the use of all pesticides; but since 90 million acres are treated one or more times with organic insecticides and 85 million with herbicides, and millions more acres with inorganic chemicals, the total number of acre-treatments is very high. Today, about 1 acre in 12 in conterminous United States is treated at least once each year with a pesticide.

Although several bills were introduced into the 87th Congress, there was no major change in Federal legislation with respect to pesticides as they affect wildlife or restrictions on the use of pesticides or residues during 1961 and 1962. The 1959 act raising the authorized fund limit from $280,000 to $2,565,000 for pesticide-wildlife research has permitted steadily increasing appropriations. For the last fiscal year (fiscal 1963) the appropriations for study of pesticides have been:

Bureau of Commercial Fisheries	$100,000
Bureau of Sport Fisheries and Wildlife:	
Branch of Wildlife Research	537,000
Branch of Fishery Research	215,000
Total	$852,000

There have been several reviews of the problems of pesticide pollution of the environment, and considerable State legislation governing the use of pesticides has been enacted during the past 2 years. The principal reviews have been by the National Academy of Sciences, the Federal Pest Control Review Board, and the Office of Science and Technology. The National Academy of Sciences-National Research Council study was conducted by a committee appointed by the Academy and subcommittees appointed by the parent committee. Two reports have been issued by the Academy, and the third report is being completed. The reports conclude that although there is a pesticide-wildlife problem and further research is needed, the problem is not especially acute. Individual reports of the minority views of the professional wildlife biologists concerned with these reviews were not published in Parts I and II. This accounts, in part, for the delay in Part III in which minority views are given greater emphasis.

The Federal Pest Control Review Board was established through joint action of the Secretaries of the Interior, Agriculture, Defense, and Health, Education, and Welfare during the spring of 1961 and was formally announced on October 4, 1961. In the order establishing the Board it was directed to: Review pest control programs in which there is active participation by Federal agencies for planning and developing of procedures and where there is some responsibility for supervision; and advise the departments and agencies concerning the use of pesticides and other chemicals, especially in cases involving interdepartmental interest and responsibilities, to insure that effective, economical, and safe procedures are followed.

The Department of the Interior is represented by Mr. Robert M. Paul and Mr. Lansing A. Parker. Many meetings, sometimes several in 1 month, have been held by the Board since its formal organization. All Federal pest control programs of the four Departments have been screened and cleared, some tentatively, by the Review Board. At present, the Board is reconsidering all programs, especially those which were given qualified approval initially. Currently the Board is very concerned about residues, and as a result has advised that Federal programs should restrict the use of chlorinated hydrocarbons in those programs which would lead to residues in food crops or contribute to the long-term environmental pollution of areas on which food crops might be raised. The increasing evidence of residues in wild game or fisheries products is another serious aspect of this problem.

A third review was initiated by the Federal Council for Science and Technology and developed by the Life Science's Panel of the President's Science Advisory Committee. These groups report to the President's Science Advisor who is the head of the Office of Science and Technology. The survey has been completed and a report for high level administrative consideration has been prepared. A 25-page report entitled "The Use of Pesticides" was released by President Kennedy on May 15, 1963.

State legislation has been developed in the past 2 years, and at the present time many States have regulations governing the use of pesticides or are developing legislation governing their use. There is no single summary of these laws, but some, such as those in Massachusetts and Connecticut, provide for consideration of wildlife values in making decisions on the need for control and the method of control to be used.

Much of this interest in legislation and review stems from the publication of the articles and book on pesticides and environmental pollution by Miss Rachel Carson. Miss Carson's writings have had considerable impact on the entire problem of pest control. Therefore the difficult problems of residues, toxic chemicals in food, physiological effects of chemicals, and the growing environmental pollution by chemicals have been reappraised by the responsible Federal, State, and private officials. Secretary of the Interior Stewart Udall, on the occasion of the dedication of the new Biochemistry-Wildlife Pathology Laboratory, which was built at the Patuxent Wildlife Research Center during 1961 and 1962, stated: "We owe much to Rachel Carson."

There have been efforts to find safer means of insect control. Many of these will permit control of pests without wildlife loss. In this connection a recent article in Chemical Week (1963) pointed out that two approaches look particularly promising: combining food bait with only tiny amounts of toxicant, and using a sex attractant to draw insects into contact with a chemical, perhaps a chemosterilant, which destroys their ability to reproduce. An example of the former is the use of Mirex, a stomach poison, in a soy-bean oil bait on a corncob grit carrier to kill fire ants. Only 3.4 grams of toxicant in 10 pounds of finished bait per acre has successfully controlled the ant, and tests to date have demonstrated no danger to wildlife as a result of this treatment.

Other experiments with this bait principle are being tried in control of termites and the boll weevil. The latter is particularly imaginative - the effort being to lure the weevil with an extract of cotton flowers to environments in which they will lay eggs which cannot survive. Sex attractants are effective over larger areas and recently have been isolated, identified, and synthesized for the gypsy moth and silkworm. The attractant for the American cockroach has been separated and identified but not synthesized. Sex attractants are quite specific for a given species. Other lures have been developed and used for the Mediterranean fruit fly, melon fly, oriental fruit fly, and European chafer. Once the insects are attracted to a given area it may be possible to bring them into contact with chemosterilants rather than to kill them outright. Biologically, sterilizing individuals is a more effective population depressant than killing them, as sterile individuals can prevent fertile individuals from producing young. Another example of relatively safe insect control is control by disease, particularly Bacillus thuringiensis, which is being produced commercially and is being applied, experimentally at least, on large areas for forest insect control. Recently, combinations of pathogenic bacteria and virus have been tried. At present, the U. S. Department of Agriculture is said to be examining 32 strains of microorganisms and 200 viruses as insect pathogens. In addition to chemosterilants, about 88 antimetabolites which block important metabolic processes other than reproduction are being studied in insect control. Other fields of control include work with desiccating materials. These remove protective layers from the insect and permit the draining of vital body fluids. Some desiccants are marketed commercially. To some extent, antibiotic materials such as cyclohexamide and phytoactin are being used to combat forest diseases which formerly were controlled by the use of insecticides to control the insect vectors or carriers of the diseases or through other major habitat change.

Many studies, therefore, have indicated a need for and an interest in safer means of insect control. In this trend toward elimination of toxicity it should be noted that some compounds are much less toxic than others in the various groups of pesticides. For example, methoxychlor of the chlorinated hydrocarbons has been found to be as effective as DDT in controlling the elm bark beetle and without acute toxicity to wildlife. Malathion is an example of an organophosphorus compound and Sevin of a carbamate which data to date indicate are relatively safe to higher forms of life.

One of the interesting biological developments of the past 2 years has been the suggestion of resistance to pesticides in vertebrates. This was reported independently by several workers in at least two locations, and has been suggested for various populations in the South in unpublished reports. King (1962) reported the possible development of resistant populations through laboratory experimentation with the guppy, Lebistes reticulatus, and the brown trout, Salmo trutta. Later in the year workers at Mississippi State University reported the possibility of resistance in several species of vertebrates: in the mosquito fish, Gambusia affinis (Vinson, et al., 1962); and two species of cricket frogs, Acris (Boyd, et al., 1963). The development of resistant vertebrate populations should, of

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During the period of this review a new survey of the value of wildlife was developed (U. S. D. I., 1961). It substantiated the original survey (U. S. D. I., 1956), and indicated that about $4 billion was being spent on wild forms today by hunters and fisherman in pursuit of their hobby. This tremendously valuable wildlife resource is all the more economically important when one considers the vast aesthetic values of this resource which are not measured in the present survey.

In the management of this resource, chemicals are sometimes used. A recent development has been the production of molluscacides for the control of predators on commercial fisheries forms. Many research projects have been undertaken to find herbicides which control problem weeds and facilitate fish production and utilization. Fish control chemicals have been used increasingly in fishery management of unwanted populations.

The present report indicates that considerable progress is being made in the attainment of knowledge of effects of pesticides on wildlife. However, it is still evident that additional information is needed on effects of sublethal levels of pesticides, particularly in combination with factors of stress such as disease and starvation; effects of pesticides in different habitats, particularly measurements of concentration within the food-chain organisms; the danger of elimination of a local form or extinction of a species with limited numbers and range or specialized habits which might make them particularly susceptible; residue levels in representative wild forms of the various phyla, particularly those thought to be resistant to pesticides because they might be able to store higher levels of toxicant; physiological effects, not only on tissues and organs such as the reproductive organs, but also on processes such as excretion of residues; effects of various chemicals on laboratory populations of representative phylogenetic groups; effects of multiple exposure to several pesticides simultaneously or over a period of time; and effects of control programs on wild populations in areas treated with pesticides, including studies of movement into and out of the area.

Tables are at the end of each section, but all references are listed in one list of references at the end of the publication.

Abbreviations routinely used by research workers in toxicology may not always be clear to non-technical people. These include:

ppm, parts per million, is the number of parts of toxicant per million parts of the substance in question; these may include residues in whole animals, certain tissues of animals or plants, or amount of toxicant in natural or artificial foods. Residues in this publication, unless otherwise noted, are expressed in terms of ppm dry weight. For animal tissues averaging 90 percent water, 1 ppm wet or live weight would average about 10 ppm dry weight. The amount of moisture in a specimen, and therefore the wet weight, varies after death. Therefore, wet weights are most meaningful when taken of live or freshly-killed animals.

ppb, parts per billion, is the number of micrograms per kilogram of the substance in question.

mg/kg, milligrams per kilogram, is the same ratio as ppm but is used to designate the amount of toxicant required per kilogram of body weight of test organism to produce a designated effect, usually the amount necessary to kill 50 percent of the test animals.

μg/g, micrograms per gram, is much the same as mg/kg except that amounts involved, 1/1,000,000 of a gram per gram, are 1/1,000 those of mg/kg, 1/1,000 of a gram per 1,000 grams, but the ratio (ppm) is the same.

μg/l, micrograms per liter, is another expression much the same as the ratio of ppb, as the weight of a liter of water is approximately 1,000,000,000 micrograms, but the weight in micrograms of toxicant per volume of material is given.

LD_{50}, lethal dose 50, is the amount of toxicant lethal to 50 percent of the test animals to which it is administered under the conditions of the experiments. Usually the dosage necessary to kill 50 percent of test animals will vary with time, greater total amounts being needed to kill over longer periods of time. When only one oral administration is made to produce a LD_{50}, it is usually referred to as the single oral LD_{50}.

LC_{50}, lethal concentration 50, is the concentration of toxicant in the environment which kills 50 percent of the test organisms exposed to it. The expression is used by biologists working with aquatic organisms in water contaminated with the toxicant.

ED_{50}, effective dose 50, is the amount of toxicant, usually measured in mg/kg, that produces a designated effect to 50 percent of the population of test organisms receiving the dose. The term is used when the effect of the toxicant is measured in terms other than mortality or lethal effect.

EC_{50}, effective concentration 50, is the concentration of toxicant in the environment which produces a designated effect to 50 percent of the population of test organisms exposed to it.

TLm, median tolerated limit, is the median amount or concentration which produces mortality to 50 percent of the tested population in a given period of time. Commonly used periods in fisheries experiments are 24 hours (24-hour TLm), 48 hours (48-hour TLm), or 96 hours (96-hour TLm).

COMMERCIAL FISHERIES INVESTIGATIONS

by

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Initial pesticide investigations were conducted at four different Bureau laboratories in order to make the most efficient use of available personnel and equipment. During the past 2 years, the studies have been gradually concentrated at the Biological Laboratory at Gulf Breeze, Florida. Much of the early work had to be concerned with development and standardization of testing methods since the response of marine animals to pesticides varies significantly from that of many fresh water forms used as test animals. This phase of the work has been completed insofar as the laboratory studies are concerned, and much of our effort is being directed now towards the standardization of techniques for the field program.

The investigations fall into three categories. Still the most urgent and requiring a majority of the effort is the determination of the acute toxic levels of the more important chemicals now in use or expected to go into production soon.

The second type of investigation involves observations of possible toxicity due to chronic exposure to relatively low concentrations. This work involves few species and only the most common pesticides, since observation periods extend approximately 6 months. Emphasis is placed on possible ill effects during the early growth of the test animals.

The third phase of the program involves the evaluation of important chemicals under field conditions. The objectives are to relate laboratory findings to field results under varying conditions of terrain and weather so that pesticides having minimal effects on commercial fisheries can be identified.

The following employees have been actively concerned with the pesticide research program at some time during the past 2 years: Miles S. Alton, Philip A. Butler, Robert A. Croker, Jack I. Lowe, Roger J. Reed, Alan J. Rick, and Alfred J. Wilson.

Laboratory Studies and Toxicology

The diversity of commercially important marine species has made it necessary to limit the screening program to representative species of the major groups of animals.

Phytoplankton

There has been particular concern that these chemicals might have serious effects on the phytoplankton community in estuarine waters, since these microscopic plant cells form the base of the marine food chain.

Productivity of plankton samples was measured by adding known amounts of the radioisotope, Carbon-14, to the samples. The amount of this carbon utilized by the phytoplankton in a given period of time can be precisely determined and is a measure of their well-being or productivity.

In considering the data reported in table 1A, it should be recognized that the natural phytoplankton community contains a large number of animal forms which feed on the plant cells. Although a majority of the chemicals tested caused a significant decrease in productivity at 1 ppm, at lower concentrations an increase in productivity rate was frequently observed. This was apparently due to the fact that the pesticide was toxic primarily to the animal part of the community.

Cultures of some phytoplankton cells suitable as food for oyster and clam larvae are available in relatively pure condition. The effects of typical pesticides of the different types have been evaluated on two of these plant species. As might be expected, the herbicides are particularly toxic; surprisingly, the insecticide DDT is also very toxic to them (table 2A).

Crustacea

Since many of the pesticides in use today were developed to control terrestrial arthropods, it is natural to suspect that marine crustaceans might be susceptible to the same chemicals. Previous work has shown the post-larvae of brown shrimp and blue crabs to be quite sensitive to the chlorinated hydrocarbon insecticides. Reported here are the results of bioassays to determine the relative toxicities of a variety of pesticides to adult brown shrimp (*Penaeus aztecus*), adult pink shrimp (*Penaeus duorarum*), and juvenile blue crabs (*Callinectes sapidus*).

Acute toxicity tests were conducted by exposing separate groups of shrimp and crabs to several concentrations of each pesticide for a period of 48 hours. All of the shrimp tests and most of the crab tests were conducted in running sea water to which an acetone stock solution of the pesticide was introduced at a continuous rate. Because of their insolubility in acetone and comparatively low toxicities, some of the herbicides and fungicides were tested on crabs in standing water aquaria. Constant-flow systems, in which the test solutions are renewed continually, have the advantage of insuring sufficient oxygen and the desired concentration of pesticide.

After exposure to lethal or near-lethal concentrations of toxic materials, crabs and shrimp often remain in a moribund condition for hours, exhibiting only the faintest signs of life. Consequently, the 24- and 48-hour effective concentration (EC_{50}) figures given in tables 3A and 4A are the concentrations of pesticide causing death or loss of equilibrium to 50 percent of the test population. Manufacturers' recommended application rates were used as guides for maximum concentrations tested. Since the maximum concentration tested did not always cause death or loss of equilibrium, an EC_{50} was not calculated in some experiments.

Mollusks

The commercial oyster, *Crassostrea virginica*, and the New England hard clam, *Mercenaria mercenaria*, are the principal mollusks used in the screening program. Incidental observations have been made on other clams and mussels typical of the estuarine habitat.

The acute toxicity of each pesticide was determined by exposing separate groups of small oysters (1"-2") to several concentrations of the chemical for a period of 96 hours. Tests were conducted in running water aquaria to which acetone-stock solutions of the pesticide were introduced at a continuous rate. The reaction of an oyster when sufficiently irritated by contaminated water is to close its shell. The oyster does not feed while closed and therefore does not grow, so relative chemical toxicity is indicated by changes in growth rate. Consequently measurement of shell growth is an objective method for evaluating the effects of pesticides. Average shell growth of oysters in each test concentration was compared with control animals receiving no pesticide to determine median toxicity values. The concentration causing a 50 percent decrease in shell growth in 4 days (96-hour EC_{50}) was obtained by graphical interpolation.

As a group, the chlorinated hydrocarbon insecticides were the most toxic pesticides to oysters. Most of them inhibited shell growth at well below 1.0 part per million (table 5A). Several of the organophosphorus and carbamate insecticides had little or no effect on oyster growth; presumably this is because of their decomposition in water.

Since pesticides are applied at different seasons of the year it is desirable to know the effect of water temperature on the toxicity of pesticides to oysters. Several of the chemicals were tested at both summer and winter temperatures (see duplication in table 5A). Surprisingly, in many cases the calculated EC_{50} values did not differ significantly at summer and winter temperatures. However, at the higher water temperatures some of the chlorinated hydrocarbon insecticides killed oysters at concentrations which at lower winter temperatures only stopped growth. This was probably due to the increased metabolism at summer temperatures which forced the oysters to open and take in the contaminated water.

In order to determine the recovery rate of oysters surviving a short-term exposure to pesticides, experimental oysters showing less growth than the controls at the termination of a screening experiment were transferred to unpolluted running sea water. They were maintained there until their growth rate was restored to the same level as control oysters. This recovery period varied from 1 to 4 weeks and in most cases appeared to be independent of the severity of toxicity. These data indicate that for the chemicals tested, a single field application which might temporarily affect oyster growth would probably not have a lasting effect.

The estuarine habitat is more likely to be subjected to low concentrations of pollutants over relatively long periods of time rather than very high concentrations. Consequently, it is of interest to determine the effect known toxic pesticides have on mollusks under such conditions of chronic exposure.

Very small clams and oysters approaching their first cycle of reproductive activity were selected as test animals. Chemicals tested included aldrin, dieldrin, DDT, toxaphene, malathion, and acetone. Acetone is used in all of the screening program as a solvent, and it was desirable to learn if it caused toxic reactions during a long exposure period. Observations were conducted over a period of 6 months at concentrations approximately 10 percent that of the median effective dose.

In general, there was no decrease in growth rate and in some cases, treated groups grew slightly better than controls. While clams do not reach maturity at this age, the oysters spawned spontaneously during the same periods that controls did. Mortality in all groups was comparable and in many cases was caused by predators (crabs) that grew up in the experimental aquaria. It was noteworthy that all aquaria maintained a high population density of local animals whose swimming larvae came into the tanks naturally with the water supply. More than 15 local species of mollusks, echinoderms, and worms were identified in the test and control aquaria.

At the termination of these experiments, the oysters were screened to determine whether they had acquired any tolerance to the individual pesticides. In no case was such an effect observed.

Fish

Laboratory and field studies have shown many of the commonly used pesticides to be highly toxic to certain species of fresh-water fishes. The data reported here are the results of bioassays to determine the relative toxicity of pesticides to marine species. Because of their abundance in local waters and the commercial importance of mullet, juvenile white mullet (*Mugil curema*) and longnose killifish (*Fundulus similis*) were used as test animals in acute toxicity tests.

Twenty-four- and 48-hour median tolerated limit values, TLm, were obtained by exposing groups of 10 fish to five or more concentrations of each chemical. Unless otherwise noted, the tests were conducted in running sea water aquaria. The toxicity values in table 6A were determined by graphical interpolation of test results.

Many marine fish pass their early growth stages in estuaries, the so-called nursery areas. The effects of chronic low-level pollution are, therefore, of considerable importance.

Three groups of spot (*Leiostomus xanthurus*) approximately 1 inch long were exposed continuously for 3 months to sublethal concentrations of dieldrin (0.1, 0.01, and 0.001 parts per billion) in running sea water. Mortalities were high, 30-37 percent, but not significantly different in control and experimental groups. Neither were there significant differences in the attained mean standard lengths of the different groups. However, some of the experimental fish had axial skeletal distortions that were not apparent in the control group.

Survivors of the experiment were exposed to 2 parts per billion of dieldrin to determine whether earlier low-level exposure had created any resistance to dieldrin. Eighty percent of the experimental fish survived a 24-hour exposure to the dieldrin, while in a group of previously unexposed fish there were no survivors.

Future Considerations

Available data show that various chemicals suitable for the control of similar pests may have important differences in their degree of toxicity to marine animals. The research program's chief objective at present is to determine which pesticides now considered necessary may be expected to cause the least damage to marine resources. It is evident that careful selection of the control agent and method of application can lessen the current pesticide hazards.

Table 1A. Percentage decrease in productivity of natural phytoplankton communities during a 4-hour exposure to a concentration of 1.0 ppm of the indicated pesticide

Pesticide	Percent decrease	Pesticide	Percent decrease
Chlorinated hydrocarbons		Herbicides	
		2,4-D acid	0
Aldrin	84.6	2,4,5-T acid	0
Chlordane	94.0	2,4-D dimethylamine salt	0
DDT	77.2		
Dieldrin	84.8	Diuron	87.4
Endrin	46.0	Eptam	0
Heptachlor	94.4	Fenuron	40.9
Kepone	94.7	MCP amine weed killer (formulation)	0
Lindane	28.5		
Methoxychlor	80.6	Monuron	94.1
Mirex	41.6	Neburon	89.9
Thiodan	86.6	Tillam	23.8
Toxaphene	90.8		
		Fungicides	
Organophosphorus insecticides		Chemagro 2635	85.3
		Dyrene	91.3
ASP-51	29.5	Ferbam	97.0
Bayer 29493 (Baytex)	7.2	Phaltan	31.9
Bayer 25141	0		
Diazinon	6.8		
Dibrom	55.6	Chlorinated acaricides	
Di-Syston	55.2		
Dylox	0		
Ethion	69.0	Sulphonone	12.2
Guthion	0	Tedion	39.0
Imidan	7.7		
Malathion	7.0		
Meta-Systox R	0	Soil fumigants	
Methyl trithion	85.9		
Systox	7.11	Dexon	14.6
		Nemagon	5.0
Carbamates			
Bayer 37344	38.7		
Bayer 39007	0		
Bayer 44646	0		
Sevin	16.8		

Table 2A. Percentage decrease in productivity of unialgal cultures of Dunaliella euchlora and Platymonas sp. and natural plankton communities during a 4-hour exposure to a concentration of 1.0 ppm of the indicated pesticide

Pesticide	Natural community	Dunaliella euchlora	Platymonas sp.
DDT	77.2	0	24.5
Bayer 29493 (Baytex)	7.2	59.8	50.9
Bayer 44646	0	9.1	25.4
Monuron	94.1	97.6	95.9
2,4-D dimethylamine salt	0	0	0
Fermate	97.0	97.7	94.3

Table 3A. Concentration of pesticides in sea water causing mortality or loss of equilibrium in 50 percent of adult shrimp tested

Pesticide		24-hour EC_{50} ppm	48-hour EC_{50} ppm	Mean water temperature °C.
Chlorinated hydrocarbons				
Aldrin	(P)	0.0006	-	26
Chlordane	(B)	0.0064	0.0044	30
DDT	(B)	0.0055	0.001	21
Dieldrin	(B)	0.025	0.0055	17
Endrin	(B)	0.0006	0.0003	15
Heptachlor	(P)	0.0009	0.0003	21
Kepone	(B)	0.70	0.085	30
Lindane	(B)	0.0007	0.0004	30
Methoxychlor	(B)	0.0074	0.006	30
Mirex	(P)	20% mortality at 2.0	1.2	25
Thiodan	(B)	0.0006	0.0004	30
Toxaphene	(B)	0.0066	0.0049	18
Organophosphorus insecticides				
Bayer 29493 (Baytex)	(P)	0.0004	0.00006	30
Diazinon	(B)	0.044	-	31
Dibrom	(P)	0.0055	0.0055	28
Guthion	(B)	0.025	0.0044	31
Malathion	(P)	0.82	0.50	17
Carbamates				
Sevin	(B)	0.0055	0.0025	30
Herbicides				
Esteron 99 (formulation)	(B)	0.55	0.55	30

(P) = pink shrimp used as test animals.
(B) = brown shrimp used as test animals.

Table 4A. Concentration of pesticides in sea water causing mortality or loss of equilibrium in 50 percent of juvenile blue crabs tested

Pesticide	24-hour EC_{50} ppm	48-hour EC_{50} ppm	Mean water temperature °C.
Chlorinated hydrocarbons			
Aldrin	0.05	0.042	28
Chlordane	0.82	0.48	29
DDT	0.01	0.01	21
Dieldrin	0.55	0.44	18
Endrin	0.1	0.025	11
Heptachlor	0.41	0.063	17
Kepone	Irritated at 1.0	20% mortality at 1.0	29
Methoxychlor	0.55	0.55	31
Mirex	Irritated at 2.0	20% mortality at 2.0	31
Thiodan	0.055	0.035	30
Toxaphene	0.46	0.33	19
Organophosphorus insecticides			
Bayer 29493 (Baytex)	0.006	0.004	28
Dibrom	0.33	0.30	28
Guthion	0.55	0.55	27
Malathion	Irritated at 1.0	Irritated at 1.0	30
Carbamates			
Sevin	0.55	0.55	30
Botanicals			
Ryania*	Irritated at 20.0	Irritated at 20.0	24
Fungicides			
Ferbam*	Irritated at 10.0	Irritated at 10.0	19
Phaltan*	Irritated at 25.0	Irritated at 25.0	21

(Continued)

*Tests performed in standing water aquaria.

Table 4A. Concentration of pesticides in sea water causing mortality or loss of equilibrium in 50 percent of juvenile blue crabs tested (continued)

Pesticide	24-hour EC_{50} ppm	48-hour EC_{50} ppm	Mean water temperature °C.
Herbicides			
2,4-D dimethylamine salt	Irritated at 5.0	Irritated at 5.0	25
Eptam*	Irritated at 20.0	Irritated at 20.0	20
Esteron 99* (formulation)	2.9	2.9	24
MCP amine weed killer* (formulation)	Irritated at 5.0	Irritated at 5.0	21
Radapon* (formulation)	No effect at 50.0	No effect at 50.0	24

*Tests performed in standing water aquaria.

Table 5A. Concentration of pesticides in sea water causing a 50 percent decrease in oyster shell growth, EC_{50}

Pesticide	Salinity percent	Mean water temperature °C.	96-hour EC_{50} ppm
Chlorinated hydrocarbons			
Aldrin	27	30	0.025
BHC	27	27	0.36
Chlordane	29	23	0.007
Chlordane	27	29	0.01
DDT	21	17	0.007
DDT	23	30	0.009
Dieldrin	25	22	0.034
Endrin	22	24	0.033
Heptachlor	21	12	0.027
Heptachlor	23	30	0.03
Kepone	19	14	0.057
Kepone	25	31	0.015
Lindane	21	10	43% decrease at 1.0
Lindane	25	30	0.45
Methoxychlor	21	19	0.097
Mirex	17	25	No decrease at 2.0
Thiodan	22	28	0.065
Toxaphene	19	19	0.063
Toxaphene	24	31	0.057
Carbamates			
Bayer 37344	28	23	No decrease at 1.0
Bayer 39007	27	25	No decrease at 1.0
Bayer 44646	27	27	No decrease at 1.0
Sevin	17	20	19% decrease at 2.0
Sevin	27	29	14% decrease at 2.0
Chlorinated acaricides			
Sulphenone	20	18	1.27
Sulphenone	24	31	18% decrease at 2.0
Fungicides			
Ferbam	27	25	0.075

(Continued)

Table 5A. Concentration of pesticides in sea water causing a 50 percent decrease in oyster shell growth, EC_{50} (continued)

Pesticide	Salinity percent	Mean water temperature °C.	96-hour EC_{50} ppm
Herbicides			
2,4-D acid	19	9	No decrease at 2.0
2,4-D acid	23	30	No decrease at 2.0
2,4-D butoxyethanol ester	29	18	3.75
2,4-D dimethylamine salt	28	25	No decrease at 2.0
2,4,5-T acid	20	16	No decrease at 2.0
2,4,5-T acid	27	30	No decrease at 2.0
Eptam	24	29	43% decrease at 5.0
Organophosphorus insecticides			
ASP-51 (NPD)	28	23	0.055
Bayer 29493 (Baytex)	16	22	0.60
Bayer 25141	27	27	20% decrease at 1.0
DDVP	25	30	No decrease at 1.0
Diazinon	28	25	No decrease at 1.0
Dibrom	25	19	0.64
Dibrom	27	30	0.80
Di-Syston	29	20	0.90
Guthion	14	13	No decrease at 1.0
Guthion	28	30	No decrease at 1.0
Imidan	20	15	No decrease at 1.0
Imidan	27	30	No decrease at 1.0
Malathion	14	16	32% decrease at 1.0
Malathion	24	30	No decrease at 1.0
Methyl trithion	20	18	41% decrease at 1.0
Methyl trithion	25	30	0.23
Parathion	18	17	0.85
Systox	13	24	No decrease at 2.0

Table 6A. Concentration of pesticides in sea water causing 50 percent mortality, 24- and 48-hour TLm, to juvenile white mullet (M) and longnose killifish (K)

Pesticide	Kind of fish	24-hour TLm ppm	48-hour TLm ppm	Mean water temperature °C.
Chlorinated hydrocarbons				
Aldrin	M	0.0031	0.0028	28
BHC	M	0.8	0.8	28
Chlordane	M	0.043	0.0055	22
DDT	M	0.0008	0.0004	26
DDT	K	0.0055	0.0055	24
Dieldrin	M	0.0078	0.0071	28
Endrin	M	0.0026	0.0026	29
Endrin	K	0.0003	0.0003	25
Heptachlor	M	0.0048	0.003	26
Kepone	M	0.5	0.055	31
Kepone	K	0.3	0.084	31
Lindane	M	0.03	0.03	16
Lindane	K	0.3	0.24	29
Methoxychlor	M	0.055	0.055	24
Mirex	M	No mortality at 2.0	10% mortality at 2.0	25
Thiodan	M	0.005	0.0006	29
Toxaphene	M	0.0055	0.0055	19
Fungicides				
Ferbam	K*	1.0	0.8	28
Phaltan	M*	1.56	1.56	28
Phaltan	K*	2.5	2.5	29
Herbicides				
2,4-D acid	M*	No effect at 50.0	No effect at 50.0	20
2,4-D propylene glycol butyl ether ester	K*	5.0	4.5	20
2,4-D butoxy-ethanol ester	K*	5.0	5.0	20
Diuron	M*	10.8	6.3	29

(Continued)

*Tests performed in standing water aquaria.

Table 6A. Concentration of pesticides in sea water causing 50 percent mortality, 24- and 48-hour TLm, to juvenile white mullet (M) and longnose killifish (K) (continued)

Pesticide	Kind of Fish	24-hour TLm ppm	48-hour TLm ppm	Mean water temperature °C.
Herbicides				
Eptam	M*	10% mortality at 20.0	10% mortality at 20.0	19
Eptam	K*	Irritated at 20.0	Irritated at 20.0	28
Esteron 99 (formulation)	M*	1.5	1.5	20
Esteron 99 (formulation)	K*	3.5	3.0	19
MCP amine weed killer (formulation)	K*	No effect at 75.0	No effect at 75.0	28
Monuron	M*	20.0	16.3	28
Radapon (formulation)	M*	No effect at 50.0	No effect at 50.0	27
Radapon (formulation)	K*	No effect at 50.0	No effect at 50.0	29
2,4,5-T acid	K*	No effect at 50.0	No effect at 50.0	19
Tillam (formulation)	M*	6.25	6.25	21
Tillam (formulation)	K*	7.78	7.78	29
Carbamates				
Bayer 37344	K	0.55	0.55	16
Sevin	M*	4.25	2.5	24
Sevin	K*	1.75	1.75	28
Organophosphorus insecticides				
Bayer 29493 (Baytex)	M	1.73	1.59	29
Bayer 25141	K	0.085	0.055	17
Diazinon	M	0.25	0.25	29

(Continued)

*Tests performed in standing water aquaria.

Table 6A. Concentration of pesticides in sea water causing 50 percent mortality, 24- and 48-hour TLm, to juvenile white mullet (M) and longnose killifish (K) (continued)

Pesticide	Kind of fish	24-hour TLm ppm	48-hour TLm ppm	Mean water temperature °C.
Organophosphorus insecticides				
Dibrom	M	0.6	0.55	27
Guthion	M	0.0055	0.0055	28
Imidan	M	0.074	0.055	18
Imidan	K	0.062	0.055	26
Malathion	M	0.95	0.57	19
Methyl trithion	K	0.6	0.55	28
Botanicals				
Ryania	M*	No effect at 20.0	No effect at 20.0	21
Ryania	K*	No effect at 20.0	No effect at 20.0	23
Rotenone	M	0.0057	-	31
Acaricides				
Sulphenone	M*	-	11.9	19
Sulphenone	K*	7.5	6.0	28

*Tests performed in standing water aquaria.

SPORT FISHERY INVESTIGATIONS

by

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The Fish-Pesticide Research Laboratory at Denver, Colorado, underwent important changes in 1961 and 1962. One strengthening feature was the increase in size of staff, both at Denver and the outlying stations. New facilities were developed at Marion, Alabama; Tishomingo, Oklahoma; La Crosse, Wisconsin; and Laurel, Maryland. With these advances in personnel and facilities, the Laboratory was able to increase the scope and depth of research on fish and pesticides. During this period increased emphasis was placed on studies of effects of controlled applications in ponds and raceways and on biochemical and physiological investigations in the laboratory. The acquisition of equipment for working with radioisotopes and the completion of arrangements for securing the best of histopathological services through contract have enhanced our potential for reaching an understanding of what pesticides do to fish.

The ensuing summaries highlight our findings in 1961 and 1962. Names in parentheses indicate the principal research worker(s) concerned with each investigation.

Laboratory Studies and Toxicology

Toxicant Tolerance (W. R. Bridges, A. K. Andrews)

During 1961 and 1962 the laboratory conducted many bioassay tests to establish toxicities and tolerances of various chemicals to different species of fish. Tables 1B and 2B present results for some insecticides, herbicides, and fungicides.

Toxicity, Time, and Temperature (W. R. Bridges, A. K. Andrews)

Studies were conducted on the influence of time and temperature on the toxicity of heptachlor, kepone, and malathion to sunfish. Bridges (in press) has reported on the results on heptachlor and kepone, and his abstract is as follows:

> The toxic effects of heptachlor and kepone were measured by the determination of the Median Effective Concentration, or EC_{50} (the concentration required to produce 50 percent mortality of the fish), at each time and temperature tested. EC_{50} values for heptachlor ranged from 0.017 mg/l for 96 hours exposure at 75° F. to 0.092 mg/l for 24 hours exposure at 45° F.; comparable values for kepone were 0.044 mg/l and 0.62 mg/l. The toxicity

of kepone greatly increased with the time of exposure, whereas the influence of increased time on the toxicity of heptachlor was only moderate. Higher temperatures caused a moderate increase in toxic effects of both compounds. (Note: In this abstract, EC_{50} equals LC_{50}).

Table 3B summarizes the measurements of effects of heptachlor on redear sunfish, and table 4B summarizes the data on kepone and redear sunfish.

The work with malathion was done with bluegill sunfish, and the results are presented in table 5B. Time and temperature made a moderate difference on toxicity.

Microorganisms and Hydrocarbons (O. B. Cope, H. O. Sanders)

Some work was done in 1962 to learn of the effects of some species of bacteria on chlorinated hydrocarbons in water. This laboratory is interested in methods of altering the toxicity or the structure of pesticides in nature so that hazards to fish may be reduced. The possibility of degradation of DDT, toxaphene, and endrin was explored in aquaria in the laboratory as a preliminary to possible future investigations.

Five species of bacteria were used. In the work on DDT, it was found that *Micromonospora chalcea*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Corynebacterium pyogenes* appreciably reduced the concentration of the insecticide in the water in 7 to 16 days, while *Pseudomonas stutzeri* apparently did not. Toxaphene was affected by *C. pyogenes* and *M. chalcea* in the preliminary tests performed. Endrin concentrations were moderately reduced by *C. pyogenes*, but not by the other bacteria.

Studies with radioactive DDT, water, and *Corynebacterium pyogenes* indicated that a high proportion 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 DDD and possibly to DDE.

Experimental Field Studies

DDT and Cutthroat Trout (D. A. Allison, B. T. Kallman, C. C. VanValin)

Studies on chronic effects of DDT on cutthroat trout began at Jackson, Wyoming, in December, 1960, and were terminated in August of 1962. In this work, five lots of fish were given DDT once a week in their pelleted diets at a different rate for each lot, five lots were given DDT once a month in bath form, and one lot was furnished no DDT. Samples were withdrawn at intervals for chemical analysis for DDT and its metabolites, and for histological examination. Records were kept on fish size and on day-to-day mortalities in the various lots.

Results of whole-body analyses are presented in table 6B and table 7B. Table 6B shows residues of DDT, DDD, and DDE in the fish fed DDT weekly in the diet, and demonstrates the relatively large amounts stored in the fish fed 3 and 1 mg/kg of body weight. The fish fed the three lowest levels contained the insecticide in amounts not much different than those in the control group. Table 7B shows residue measurements in fish given DDT monthly in baths. It is seen that the fish bathed in the two highest levels stored the most insecticide, and that the lower three levels resulted in stored amounts similar to those in the control fish.

Variation from fish to fish was high in both fed fish and in those given DDT baths.

Mortalities occurring in the different lots of trout because of stresses of various kinds are summarized in table 8B. The data show that the highest day-to-day mortalities took place in the two highest treatment lots of both the fed and the contact fish, with the greatest number of deaths in the lot fed 3 mg/kg of body weight.

Histopathological studies on these fish revealed no pathology attributable to DDT. Microhaematocrit measurements made on the trout throughout the experiment showed no differences between treated and untreated fish.

Differences among lots in average sizes of fish developed during the experiment. Table 9B presents average weights of trout, and shows that fish in lots receiving the greatest amounts of DDT had the greatest average weights. Since the highest mortalities also occurred in the lots receiving the most DDT, it is suggested that selection of less vigorous individuals accounts for the differences in growth. The DDT was probably responsible for mortality in the smaller or weaker fish, leaving the larger or stronger ones to survive and grow at faster rates than the untreated fish.

2,4-D and Bluegill Sunfish (G. H. Wallen)

An experiment to measure chronic effects of 2,4-D on bluegill sunfish in treated ponds was begun at Tishomingo, Oklahoma, in July, 1961. Three ponds were partitioned with polyvinyl chloride sheeting to provide six testing spaces for fish. Each subdivision measured 1/10 acre. One space was used for the untreated control, and each of the others was treated with one of five concentrations of Esteron 99, propylene glycol butyl ether ester of 2,4-D, the treatment concentrations being 10, 5, 1, 0.5, and 0.1 ppm.

The herbicide remained in the ponds near treatment concentrations for about 3 weeks, and all disappeared from the water after 12 weeks.

The control of the aquatic weeds in the treated ponds varied in effectiveness. The pond treated at 10 ppm had 80-100 percent control of Chara, Potamogeton, Najas, Digitaria, Salix, and Typha. Some regrowth of Chara took place after 12 weeks. Death of weeds in other ponds ranged from 0-100 percent, depending upon the kind of weed and the treatment level.

Mortality among fish was 19 percent in the 10 ppm pond in the first week, but there were essentially no deaths in the other ponds. Applications at the same rates in adjacent ponds in 1962 resulted in 100 percent mortality at 10 and 5 ppm. Spawning was delayed for 2 weeks in the 10 ppm pond; all other lots spawned at the normal time. Fry production appeared to be essentially the same in all lots.

Whole-body analyses of fish for 2,4-D were performed, and the results are reported in table 10B. It is seen that residues were found in fish from only the ponds with the two highest concentrations, and in relatively small amounts.

Histopathological examination of sampled bluegill was made by Dr. E. M. Wood (1962, unpublished). Three kinds of pathology were found in the fish, involving the liver, vascular system, and brain. Liver glycogen was markedly depleted, accompanied by shrinkage, irregular staining characteristics, and loss of normal morphology of liver parenchyma cells. Globular deposits suggestive of glycoproteins appeared throughout the vascular systems. A marked stasis and engorgement of the circulatory system of the brain occurred. Surviving fish recovered completely, and after 112 days no pathological changes were seen.

Microhaematocrit readings did not differ among the lots of fish.

<u>DDT in Blackburn Pond</u> (W. R. Bridges, B. J. Kallman, A. K. Andrews)

A ½-acre pond on the Blackburn property near Denver was treated with DDT by our personnel at the relatively low rate of 0.02 ppm in July, 1961, for a study on the breakdown and distribution of the compound in a warm-water pond. Sampling of the existing fish population, rainbow trout and bullheads, and of water, crayfish, aquatic vegetation, and bottom sediments, was carried on after the application of DDT, and the samples were analyzed for residues of the insecticide and its metabolites. Sampling was continued until the termination of the study in November, 1962.

The concentration of DDT in the pond water was at its highest point 30 minutes after treatment. A decline in DDT levels then took place; none could be detected 21 days later. Aquatic vegetation contained 6-30 ppm of DDT plus DDE plus DDD in the first week after treatment and declined to 1 ppm in 65 days. In the bottom mud there was 8.3 ppm of the DDT complex after 24 hours; after the third day the concentrations were 1.5 ppm and lower.

Bullheads and trout contained the greatest amounts of chlorinated hydrocarbon 30-40 days after treatment, with concentrations over 4 ppm. Levels slowly declined after that, averaging 3.5 ppm in samples taken 9 and 10 months after treatment in both species, and 3 ppm in rainbow trout taken 14 months after treatment. Crayfish developed lower DDT residues than did trout, and contained 0.33 ppm after 14 weeks.

The fish and crayfish in Blackburn Pond showed unusually high proportions of DDD (50-60 percent of the total chlorinated hydrocarbons in some fish after 12 months), compared with the pattern seen in other studies.

Organs and tissues of bullheads taken 14 months after treatment showed the following amounts of the DDT complex, in ppm: Brain, 10.1; skeletal muscle, 0.5; gut, 1.9; liver, 0.9; fat, 38.8; ovary, 0.2; and blood, 5.0. DDD was found in relatively great amounts in all tissues and organs except brain and blood.

Effects on Field Populations

Forest Insect Control

Spruce Budworm (W. R. Bridges, A. K. Andrews)

Studies on effects of spruce budworm control were conducted in two areas, on Swan Creek in Montana and on the Pecos River and the Rio La Junta in New Mexico.

In 1960, studies were made on the effects of aerial application of DDT for spruce budworm control in the Gallatin River drainage in Montana. Measurements on a tributary, Swan Creek, showed that a drastic kill of aquatic invertebrates took place, leaving the sprayed portion of the stream essentially devoid of bottom organisms.

In 1961, to learn about the recovery of invertebrates in the same stream sections, DDT was hand-sprayed on the stream at approximately the same rate applied by airplane in 1960. Samples of dead and dying invertebrates drifting in the stream were then collected, as in 1960. Although the 1960 and 1961 treatments were not strictly comparable, it is clear from drift samples that considerable increase in aquatic insect populations took place between the two sprayings. A noteworthy change in population composition was the failure of Baetis to recover as completely as did some other forms, and the development of relatively large numbers of the stonefly, Hastaperla. One hour after application, the 1960 drift sample contained 19,470 organisms, while that in 1961 had 5,533. The 3-hour samples showed 6,029 organisms in 1960 versus 418 in 1961. Thus, the data suggest that the build-up in invertebrate numbers from 1960 to 1961 was substantial, but not to the 1960 levels.

Work in 1961 on the 1960 exposure, as reported by Bridges and Andrews (1961), indicated that: Portions of Swan Creek received at least 0.01 ppm of DDT, but no DDT could be measured in the water after 1 hour; there were no apparent acute effects on fish; rainbow trout accumulated up to 0.1 ppm of DDT after short exposure, regardless of the presence of stream insects in stomachs; rainbow trout acquired a whole-body concentration of 0.1 ppm of DDT by feeding on insects that had been exposed to low concentrations of DDT for 3-6 hours; aquatic insects exposed to low concentrations of DDT for 3 hours averaged 11 ppm of DDT and 14.2 ppm of DDE.

In 1962, the U. S. Forest Service conducted a large-scale spruce budworm control program on the Carson and Santa Fe National Forests in New Mexico. Application rate for 1,000 feet on each side of major streams was ½ pound per acre, but the major portion of the area received 1 pound per acre.

Our study of the effects of the spray on fish and aquatic invertebrates was limited to the Pecos River and the Rio La Junta. Observations of fish in the streams and of fish in live-cars indicated no acute effects. Large numbers of aquatic insects were killed by the DDT spray on the Rio La Junta, even though the greatest amount of DDT detected in the water was only 2.7 ppb.

Elm Spanworm (P. J. Frey)

The U. S. Forest Service sprayed DDT at the rate of ½ pound per acre for the control of elm spanworm in two drainages in north Georgia in 1959 and 1960. Studies of the effects of these spray applications on the aquatic fauna were made by our personnel (Frey, 1961).

In one drainage, the Conasauga Lake area, particular care was taken by the pilots to avoid the waterways, and no important kill of aquatic organisms took place.

In the other drainage, the Brasstown Bald area, routine spray methods were used, and enough DDT reached the water in 1959 to seriously deplete the bottom organisms. Reductions in numbers of bottom-dwelling invertebrates at three stations amounted to 65, 80, and 40 percent. Drift samples showed that these animals were affected almost immediately by the DDT, with a peak of mortality ½ hour after the spray. All groups of invertebrates were injured, and the Tendipedidae, Tabanidae, and Noteridae were completely eliminated. Recovery of animal populations began after a month, and a complete recovery, in terms of total numbers, was indicated after 4 months.

Tent Caterpillar (P. J. Frey)

Our personnel participated in a cooperative study on control of the forest tent caterpillar (Malacasoma disstria) in southwest Alabama in 1961. The Alabama Department of Conservation, Auburn University, the U. S. Forest Service, and the U. S. Public Health Service were the other cooperators in this effort to learn about the feasibility and the effects of using ¼, ½, and 1 pound per acre of DDT in oil to control these moths in gum forests located in swampy terrain.

Plots were established for studies on bluegill and shellcrackers in live-cars placed in strategic locations in and near the spray areas. Live-cars in areas treated at ½ pound per acre sustained from 0-76 percent mortality among the fish. Fish in the live-cars exposed to DDT began showing signs of distress after 8 hours, and continued to die up to 144 hours after treatment. Buffalo fish, longeared sunfish, and bluegill outside of the live-cars were found dead from 1-7 days after treatment.

There was no mortality to fish in areas treated at ¼ pound per acre.

Effects on fish-food organisms were difficult to measure since dead insects appeared in only one plot.

Agricultural Pest Control

Alfalfa Weevil (O. B. Cope, W. R. Bridges)

Methoxychlor was applied to 900 acres of alfalfa at the rate of 1½ pounds per acre for the control of alfalfa weevil at Jackson, Wyoming, in July, 1961. An irrigation canal through the field was the only water area involved, and it was partially dewatered before the spray. The flow near the inlet at spraytime was ¼ cfs.

Heavy mortality of aquatic invertebrates occurred for about 2 hours after the aerial application, and a few dace and a small rainbow were killed. After 3 hours, the contaminated water had apparently flowed through the canal; no further mortality to aquatic life was noted, and a number of unaffected insects and fish were observed.

Studies of deposition of methoxychlor spray showed that from 6.5-141 percent of the intended rate of application reached the ground, with an average of 42.9 percent.

Imported Fire Ant (P. J. Frey)

Observations and measurements on fire ant insecticides in ponds were made in northwest Florida in 1959 and 1960. Heptachlor and aldrin were used in various situations.

Bluegill were found to be very susceptible to heptachlor poisoning, with dead specimens found in all ponds treated with ¼ pound per acre of pelleted heptachlor in a mine pit area. In all instances, the small fish were affected first and most severely, but some adult fish were killed in each pond. Largemouth bass were more resistant; dead bass found were found in only one pond.

The effect of heptachlor on aquatic insects was somewhat erratic. Phantom midges were severely affected immediately. Tendipedid larvae, and the snail, Helisoma, showed little effect from the heptachlor. Mayfly, damselfly, caddisfly, dragonfly, and horsefly immatures were eliminated from the population, but began to reappear after 10 months.

Aldrin at ½ pound per acre eliminated all invertebrates except the midges. Aldrin killed tadpoles readily, in contrast to heptachlor.

Chemical analyses of fish tissues point out the possible greater tolerance of bass and golden shiners to heptachlor, as compared with bluegill. Dead bluegill from treated ponds had 2.8-5.3 ppm of heptachlor, while live bass contained as much as 6.4 ppm, and a golden shiner had 8.3 ppm.

Aquatic Pest Control

Alligatorweed (P. J. Frey)

The control of alligatorweed at Halfway Swamp on the Santee River in South Carolina was studied in 1961. Pelleted silvex was applied by aircraft at 20 pounds of active ingredient per acre. After 1 month, samples showed no depletion of invertebrates on the bottom or on submerged parts of the weed. Changes in fish populations were not apparent. It is difficult to say whether the elimination of the alligatorweed was beneficial or detrimental to fish in the area.

Fish Population Control

Toxaphene in Clayton Lake (B. J. Kallman, C. C. VanValin)

The State of New Mexico treated Clayton Lake with toxaphene in 1959 for the eradication of rough fish. Before the treatment our laboratory was consulted for predictions on the detoxication of toxaphene in the lake. Since there was a dearth of knowledge concerning the circumstances surrounding dissipation of toxaphene in lakes and its rate of uptake and possible decomposition by various components of the lake economy, and since the use of toxaphene is so often accompanied by protracted periods of intoxication, the two agencies engaged in a cooperative research project involving periodic sampling of materials from the lake and the chemical analysis of toxaphene residues in these materials. Kallman, Cope, and Navarre (1962) published these findings.

The fate of toxaphene, applied in three treatments at a total calculated concentration of 0.05 ppm to Clayton Lake, was followed over a period of 1½ years. Water concentrations of toxaphene were higher in leeshore samples than in windward samples for 2 weeks after the application; toxaphene levels then appeared to reach a constant value of 0.001 ppm for at least an additional 250 days. Total body concentrations of toxaphene were determined in trout and bullheads present in the lake during the poisoning and in trout subsequently placed in the lake in live-cars. Trout were more susceptible to toxaphene and accumulated lower body levels than bullheads. Bullheads which showed symptoms of toxaphene poisoning when collected had higher levels than did normal-appearing individuals. No difference in levels was observed in live-car trout collected dead as compared to survivors. Aquatic vegetation accumulated high concentrations of toxaphene; low concentrations were found in sediment samples.

Equipment, Methods, and Techniques

Plastic Film and Herbicides (W. R. Bridges, H. O. Sanders)

The use of plastic films in herbicide research has been important to this laboratory because of the possible utility of plastic bags for test containers and the fabrication of plastic barriers across ponds to contain herbicides during field tests. Bridges and Sanders (in press) have described studies made in the Denver laboratory and have given results of measurements of the permeation and retention of herbicides in water in contact with plastic films.

Tests with various herbicides and polyethylene and saran films showed that some herbicides diffuse through these materials in aqueous situations. Other tests with polyvinyl chloride film showed that it is an effective barrier for propylene glycol butyl ether esters of 2,4-D and for the butoxyethanol ester of silvex.

Analysis of Malathion (B. J. Kallman)

Kallman (1962) described the microdetermination of malathion by photometric means. The method involves the formation of acethydroxamic acid by reaction with alkaline hydroxylamine and subsequent formation of a colored complex with ferric iron at acid pH. The reaction appears to be specific for esterified carboxyl groups; several organophosphorus compounds not possessing such groups do not react similarly when tested.

Mud Sampler (P. J. Frey)

The problem of securing uniformity in the sampling of sediments from stream and lake bottoms was partially solved with the development of a plane-type sampler. Frey (1963) devised a simple piece of equipment which shaves a layer of mud from the bottom, much as a wood plane operates. The device is limited by the consistency of the bottom sediments and by extreme water depths, but has been very useful for sampling in most situations from which samples have been required.

Charcoal Sampler (C. C. VanValin)

The collection, storage, and recovery and analysis of insecticides in natural waters have been a serious problem in the operations of this laboratory because of the breakdown of the insecticides and the adsorption of chemical on container surfaces. VanValin and Kallman (in press) investigated adaptations of existing methods of trapping and recovering chlorinated hydrocarbons on charcoal, and developed an improved technique that has proven valuable in our work. The following abstract of their paper describes their results:

> To replace the time-consuming and often unreliable method of solvent extraction of water samples, a method has been devised which utilizes small quantities of charcoal for the adsorption of pesticides from relatively small quantities

of water. In practice, one-gallon samples of water are shaken with 10 grams of charcoal. The charcoal is filtered off, briefly air-dried and sent to the laboratory for extraction and subsequent analysis. With heptachlor and heptachlor epoxide, recoveries of 72 and 80 percent, respectively, have been achieved in the range of 25 to 500 micrograms, using a paper chromatographic analytical procedure. The recovery of p,p'-DDT has averaged 46 percent. Recoveries for other pesticides remain to be determined.

General

Papers on various aspects of the pesticide problem were presented in 1961 and 1962. George and Cope (1961) described research by the Bureau of Sport Fisheries and Wildlife on wildlife and pesticides. Cope (1961) reported on studies of DDT and fish in the Yellowstone River system. Cope (in press) discussed pesticide-wildlife relations at a pollution symposium at Cincinnati. Cope (1961a) discussed criteria for reporting toxicity tests on fish.

Table 1B. Toxicity expressed as 24-, 48-, or 96-hour LC_{50} of various insecticides to rainbow trout, bluegill, and redear sunfish

Toxicant	Species	Weight or length	Temperature °F.	Estimated LC_{50}, µg per liter 1/		
				24-hour	48-hour	96-hour
Aldrin, Technical	Bluegill	0.6 g.	65	10	6	-
Allied GC 3707, Technical	Bluegill	0.4 g.	75	600	500	-
Allied GC 3707, WP	Rainbow	0.6 g.	65	95	-	-
Allied GC 3707, EC	Rainbow	0.6 g.	65	170	-	-
Allied GC 4072, Technical	Bluegill	0.4 g.	75	3	-	-
DDT,p-p'	Rainbow	2-3 in.	55	18 (18 hrs.)	11 (32 hrs.)	10 (56 hrs.)
DDT,p-p'	Rainbow	2-3 in.	55	10	8	7
DDT,p-p'	Rainbow	0.6 g.	65	10	-	-
DDT,p-p'	Rainbow	0.4 g.	65	6	-	-
DDT,p-p'	Bluegill	1.5 g.	75	5-6	-	-
DDT,p-p'	Bluegill	0.4 g.	75	6	-	-
DDT,p-p'	Redear	3 g.	75	19	15	-
DDT, antiresistant, 25 percent EC	Rainbow	2-3 in.	55	10	-	-
DDT, antiresistant, 25 percent, oil	Rainbow	2-3 in.	55	10	-	-
DDT, antiresistant, 50 percent WP	Rainbow	2-3 in.	55	24	21	16
DDD (TDE), Technical	Rainbow	2-3 in.	55	30	-	-
DDVP, Technical	Rainbow	2-3 in.	55	500	-	-
Dibrom	Rainbow	2-3 in.	55	80 (18 hrs.)	-	-
Dibrom, Technical	Rainbow	2-3 in.	55	70	-	-
Endrin, Technical	Bluegill	0.4 g.	75	0.4	-	-
Endrin, Technical	Bluegill	0.6 g.	75	0.4	-	-

(Continued)

1/ Times of exposure are indicated in parentheses where they deviate from times in column heads.

Table 1B. Toxicity expressed as 24-, 48-, or 96-hour LC_{50} of various insecticides to rainbow trout, bluegill, and redear sunfish (continued)

Toxicant	Species	Weight or length	Temperature °F.	Estimated LC_{50}, µg per liter 1/		
				24-hour	48-hour	96-hour
Fairfield's 279	Rainbow	0.5 g.	55	360	-	-
Fairfield's OT 60-6	Rainbow	0.8 g.	55	100	-	-
Heptachlor, 2 percent granular	Rainbow	2-3 in.	55	150	90	70
Hercules 7175, Technical	Bluegill	0.4 g.	75	40,000	-	-
Hercules 7531, Technical	Bluegill	0.4 g.	75	25,000	-	-
Kelthane, Technical	Rainbow	0.7 g.	55	110	-	-
Kepone	Bluegill	2 in.	65	380 (18 hrs.)	240 (32 hrs.)	110 (56 hrs.)
Malathion, Technical	Rainbow	2-3 in.	55	100	-	-
Malathion, Technical	Redear	3 g.	75	170	100	60
Malathion, Technical	Bluegill	0.4 g.	75	45	35	-
Malathion, Technical	Bluegill	0.6 g.	75	120	-	-
Methyl parathion, Technical	Bluegill	0.6 g.	75	8,500	-	-
MGK's Evergreen	Rainbow	0.4 g.	55	800	-	-
MGK's 6103	Rainbow	0.5 g.	55	150	-	-
MGK's 6243	Rainbow	0.8 g.	55	750	-	-
Methoxychlor, Technical	Rainbow	0.7 g.	55	20	-	-
Phosphamidon	Rainbow	2-3 in.	55	5,000 (18 hrs.)	-	-
Sevin, Technical	Rainbow	2-3 in.	55	3,500	2,000	-
Stam F-34, Technical	Rainbow	2-3 in.	55	-	4,000	-
Zectran	Rainbow	2-3 in.	55	7,000 (18 hrs.)	-	-

1/ Times of exposure are indicated in parentheses where they deviate from times in column heads.

Table 2B. Toxicity expressed as 24-, 48-, or 96-hour LC_{50} of various herbicides, fungicides, and antibiotics to rainbow trout, bluegill, and redear sunfish

Toxicant	Species	Weight or length	Temperature °F.	Estimated LC_{50}, µg per liter[1] 24-hour	48-hour	96-hour
Amchem's silvex	Bluegill	2 in.	65	700 (18 hrs.)	600 (32 hrs.)	-
Amitrol-T	Bluegill	2 in.	65	(No mortality at 10,000 g/l over 100 hrs.)		
Antimycin A.	Rainbow	2-3 in.	55	0.25 (18 hrs.)	-	-
Casoron	Redear	3 g.	65	(No mortality at 20,000 g/l at 48 hrs.)		
Diquat	Rainbow	2-3 in.	55	(No mortality at 10,000 g/l over 100 hrs.)		
Esteron 99	Bluegill	2 in.	65	1,200 (18 hrs.)	-	-
Esteron 99, EC	Bluegill	0.6 g.	75	700	-	-
Fenac, sodium salt	Redear	3 g.	75	(No mortality at 12,000 g/l at 48 hrs.)		
Fenac, sodium salt	Rainbow	0.6 g.	65	10,000	7,500	-
Kurasol	Bluegill	0.6 g.	75	120,000	-	-
Weedar MCP	Bluegill	2 in.	65	(No mortality at 10,000 g/l over 100 hrs.)		

1/ Times of exposure are indicated in parentheses where they deviate from times in column heads.

Table 3B. LC_{50} values in milligrams per liter of heptachlor to redear sunfish

Temperature °F.	LC_{50} values in milligrams per liter				
	6 hours	12 hours	24 hours	48 hours	96 hours
45	-	-	0.092	-	-
55	-	-	0.064	-	-
65	-	-	0.047	-	-
75	0.062	0.046	0.034	0.023	0.017
85	-	-	0.022	-	-

Table 4B. LC_{50} values in milligrams per liter of kepone to redear sunfish

Temperature °F.	LC_{50} values in milligrams per liter				
	6 hours	12 hours	24 hours	48 hours	96 hours
45	5.6	2.8	0.62	0.27	0.14
55	4.4	2.1	0.54	0.21	0.096
65	2.0	0.70	0.34	0.13	0.064
75	1.3	0.50	0.24	0.090	0.044
85	0.68	0.23	0.12	0.051	0.029

Table 5B. LC_{50} values in milligrams per liter of malathion to bluegill

Temperature °F.	LC_{50} values in milligrams per liter				
	6 hours	12 hours	24 hours	48 hours	96 hours
45	1.04	0.52	0.28	0.16	0.087
55	0.80	0.54	0.22	0.11	0.084
65	0.56	0.22	0.135	0.11	0.055
75	0.35	0.20	0.124	0.086	0.040
85	0.16	0.078	0.07	0.044	0.020

Table 6B. Total chlorinated hydrocarbons in parts per million (DDT+DDD+DDE) in whole trout fed DDT. Fish were fed treated food once a week at indicated DDT concentration in mg/kg of body weight

Treatment	Number of weekly feedings								
	1	9	16	27	34	42	49	63	79
	Total chlorinated hydrocarbons in ppm								
None	0.4	0.7	0.5	0.8	0.6	0.5	0.9	1.0	0.4
3/mg/k	0.7	7.0	15.2	-	-	-	-	-	-
1 mg/k	0.8	3.0	5.6	9.2	6.8	-	11.0	11.3	11.8
0.3 mg/k	0.6	0.9	1.4	3.0	2.1	1.4	3.8	3.9	2.8
0.1 mg/k	0.6	0.9	1.4	1.0	1.3	1.2	2.2	2.2	1.1
0.03 mg/k	0.9	0.7	0.8	1.1	0.4	0.8	1.4	1.4	0.6

Table 7B. Total chlorinated hydrocarbons in parts per million (DDT+DDD+DDE) in whole trout bathed in DDT. Fish were treated with an aqueous bath of DDT at the indicated concentration for ½ hour at monthly intervals

Treatment	Days after first bath								
	3	55	111	220	278	335	391	497	612
	Total chlorinated hydrocarbons in ppm								
None	0.4	0.7	0.5	0.75	0.6	0.5	0.9	1.0	0.4
1.0 ppm	1.23	2.03	1.91	4.17	5.9	-	4.5	6.5	4.4
0.3 ppm	1.61	1.85	2.58	3.35	4.4	-	5.9	3.7	5.0
0.1 ppm	0.76	1.37	1.75	2.28	1.7	3.0	3.5	5.7	1.3
0.03 ppm	0.71	0.78	0.86	1.47	0.9	1.3	1.8	1.6	1.2
0.01 ppm	0.58	0.97	0.94	1.17	0.3	0.6	1.2	1.1	1.0

Table 8B. Cumulative mortality of cutthroat trout exposed to DDT at Jackson, Wyoming

Lot	Treatment	Months after first treatment					
		3	5	7	11	15	19
I	None	11	35	50	90	112	143
II	1.0 ppm bath	31	190	286	304	329	412
III	0.3 ppm bath	16	182	280	301	325	405
IV	0.1 ppm bath	20	91	154	186	212	293
V	0.03 ppm bath	16	49	69	113	122	162
VI	0.01 ppm bath	13	49	68	138	154	184
VII	3.0 mg/k in diet	291	419	431	447	508	511
VIII	1.0 mg/k in diet	37	262	303	311	407	454
IX	0.3 mg/k in diet	11	46	74	99	117	190
X	0.1 mg/k in diet	6	28	49	88	109	142
XI	0.03 mg/k in diet	9	39	58	90	132	154

Table 9B. Average weights, in grams, in 11 lots of cutthroat trout treated with DDT at Jackson, Wyoming, from December, 1960 to February, 1962

Lot	DDT treatment	Sampling dates						
		1960	1961					1962
		12-2	2-10	5-6	7-28	10-20	12-16	2-3
I	None	64.3	77.3	101.3	120.7	155.7	174.6	187.7
II	1.0 ppm bath	63.3	81.0	110.7	152.7	202.0	226.7	241.0
III	0.3 ppm bath	63.3	78.3	109.3	142.7	194.3	216.7	227.0
IV	0.1 ppm bath	63.7	76.0	102.3	128.0	166.3	193.0	203.3
V	0.03 ppm bath	63.7	79.3	107.0	127.0	167.0	188.0	200.7
VI	0.01 ppm bath	63.7	80.0	105.7	124.7	166.0	189.3	197.7
VII	3.0 mg/k feed	63.3	78.7	113.7	162.0	213.7	240.0	-
VIII	1.0 mg/k feed	64.3	77.7	100.3	137.7	185.7	202.7	221.7
IX	0.3 mg/k feed	65.0	77.7	102.0	120.0	155.7	172.3	185.3
X	0.1 mg/k feed	63.3	76.7	100.7	121.7	156.7	172.7	186.7
XI	0.03 mg/k feed	63.7	77.7	100.7	121.7	158.3	181.3	194.0

Table 10B. Amounts of 2,4-D, in ppm, measured in whole bodies of bluegill exposed to Esteron 99 on July 4, 1961, at Tishomingo, Oklahoma

Date of sample	Pond 11 treated at 10 ppm	Pond 12 treated at 5 ppm	Pond 21 treated at 1 ppm	Pond 22 treated at 0.5 ppm	Pond 31 treated at 0.1 ppm	Pond 32 Control
July 3, 1961	ND[1/]	ND	ND	ND	ND	ND
July 5, 1961	2.0	1.0	ND	ND	ND	ND
July 7, 1961	1.6	0.3	ND	ND	ND	ND
July 18, 1961	ND	ND	-	-	-	-
August 1, 1961	ND	ND	-	-	-	-
August 29, 1961	ND	ND	-	-	-	-

1/ ND denotes no detectable 2,4-D

WILDLIFE STUDIES, DENVER WILDLIFE RESEARCH CENTER

by

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In 1961 and 1962 considerable progress was made in acquiring and training new personnel and extending physical facilities at Denver for chemical and biological investigations of pesticide effects on resident and migratory wildlife in western United States.

Added facilities include new chemical laboratory space and the acquisition of a liquid scintillation spectrometer allowing the use of radioactive pesticides containing Carbon-14 for evaluating the accuracy of analytical methods, determining the fate and distribution of various pesticides in the animal body, and the isolation and identification of metabolites arising from physiological reactions elicited within the animal to effect the detoxification or elimination of these foreign substances. Thin film chromatographic apparatus, together with infrared spectroscopy, gas chromatography as well as ultra-violet and visual spectroscopy and paper chromatography, are techniques available and currently employed for biochemical research and pesticide residue determinations.

The biological laboratory has been equipped with an Auto-technicon and all necessary facilities for the preparation of tissue sections for histological examination to determine possible pathological changes in vital tissues resulting from exposure of wildlife to pesticide applications. This line of investigation is expected to prove invaluable in detecting at an early stage the development of adverse effects in various species due to pesticide action.

Surveillance was continued of large operational applications of pesticides such as those for the control of spruce budworm in forests and the grasshopper on rangelands. By the collection of pre- and post-spray specimens of wildlife resident in the treated area, the accumulation and retention of pesticides acquired by various species were studied by chemical analyses of tissues.

Knowledge of the problem of pesticide residues in nesting waterfowl in the Far North and the apparent transfer of the pesticide to progeny through the egg was extended by the collection of additional specimens for chemical analysis in the summer of 1962.

Studies of the effect of agricultural pest control on wildlife were pursued in the Klamath Basin and on the Tule Lake National Wildlife Refuge in California. This is a situation in which pesticides applied on agricultural lands are transported by irrigation water draining into a "sump irrigation system". In the system pesticides are concentrated in food-chain organisms and fish. Apparently, fish-eating birds such as the pelican acquire acute lethal amounts from the consumption of contaminated fish. Representative samples of the 78 pelicans and 135 other fish-eating birds which were found dead on the Tule Lake National Wildlife Refuge in 1962 are being analyzed for pesticide content at the present time.

A field study to evaluate the effects of an experimental grasshopper control program using a compound which laboratory data indicate would present minimum hazard to wildlife was initiated at the Lostwood National Wildlife Refuge in North Dakota in cooperation with the Branch of Wildlife Refuges, the Plant Pest Control Division of the U. S. Department of Agriculture, and the Union Carbide Corporation. Two plots of 2,000 acres each were selected as an experimental and control area. The experimental plot was sprayed by aircraft in early July, 1962, at the rate of 1 pound of the insecticide Sevin per acre. Careful surveillance of the two areas through October, 1962, indicated little, if any, immediate harm to wildlife. Continued observation of these plots will be maintained in 1963 and possibly in 1964 to determine possible chronic effects. This field investigation is adequately supported by chemical analyses of environmental and wildlife specimens collected at specified intervals.

Accomplishments in the chemical and biochemical field of research resulted in an improved analytical method for the pesticide Sevin capable of detecting and measuring 2 micrograms of the pure compound in most field samples.

A study of the metabolites of DDT established the conversion of DDT in the animal body to TDE and other metabolites, some of which are as yet unreported in the literature.

The details of this research, as well as others not mentioned above, are outlined herein. Names of investigators are listed in parentheses after each project title, biologists being listed first.

Laboratory Studies and Toxicology

Analytical Methods Development (James E. Peterson)

A method suitable for the analysis of Sevin in a large number of sample types has been developed. This method allows detection and estimation of microgram quantities of the insecticide in such diverse materials as spray concentrates, water, soil, vegetation, prepared feeds used in feeding tests, as well as bird and animal tissues. An average sample of animal tissue, for example, will allow as little as a total of 5-10 micrograms to be detected. The method employs paper chromatography and depends upon the formation of 1-naphthol with subsequent production of an intensely colored complex with a diazonium dye coupler. With this method, Sevin and its stable metabolite, 1-naphthol, can be detected and estimated simultaneously.

A "preparative" paper chromatographic method has been developed which allows the quantitative separation of simultaneously occurring chlorinated hydrocarbon insecticides. The method allows quantities sufficiently large for infrared spectrophotometric analysis to be recovered in a high state of purity. This is particularly useful when identification of an unknown material is uncertain due to lack of a reference of the same material.

Determination of Sevin in Field and Laboratory Samples (Lowell C. McEwen; James E. Peterson)

Analyses of samples collected at Trail Creek, Montana; Benton Lake, Montana; Buffalo, Wyoming; Lostwood Refuge, North Dakota; as well as a large number of feeding test samples, have been completed. These samples have included various birds, soil and leaf litter, mice, rabbits, vegetation, and water. The feeding tests involved the use of ring-necked pheasants and mourning doves. The field-collected small birds and mammals yielded no more than trace quantities (usually less than 1 ppm of Sevin and/or 1-naphthol) of residual spray material, indicating that Sevin is readily metabolized and eliminated with little or no storage in the living body.

Being deposited as a solid on the surface of vegetation and dry ground, Sevin was very easily extracted from samples of this type. Analyses yielded widely variant quantities of this material, depending upon rate of application, the nature of the vegetation sprayed, the manner of sample collection, etc. Sevin exposed to sunlight, air, and atmospheric moisture appears quite stable and persistent.

Avian toxicity of Sevin to species tested is quite low. Ring-necked pheasants died only after several days of feeding on Sevin at the rate of 32,000 ppm in the diet, with refusal of food probably being responsible for death. Sevin residues in pheasants that died ranged from 2.6-6.0 ppm in vital organs. Mourning doves showed toxic symptoms at levels of 2,000 ppm in their diet. Whole-body analyses of mourning doves that died ranged from 0.0-0.7 ppm. Evidence to date indicates a negligible chronic toxicity hazard to many wildlife species.

Laboratory Studies on the Metabolic Fate of DDT (William H. Robison, James E. Peterson)

A large number of samples of wildlife species has been collected in areas in which DDT had been applied for the control of various insects. Most of these samples analyzed have shown the presence of original DDT plus two or more other similar related materials, some of which are common to commercial grade DDT. It was considered possible that the presence of certain compounds in residues extracted could be explained by a possible accumulation-rate differential. This idea was rejected, however, when short-term exposures to DDT resulted in the deposition of DDT-related materials in the various tissues often in amounts exceeding the residual DDT and previously reported metabolites. This observation suggested that DDT metabolism could produce materials previously unknown or unreported. Controlled feeding of highly purified p,p'DDT to rats was carried out in March, 1962, to determine if the in vivo conversion of DDT to TDE (DDD, Rhothane) as well as DDE and DDA could, in reality, occur. The analyses of liver, kidney, and blood samples from these test animals contained TDE, indicating a partial conversion of DDT to TDE takes place in vivo. Subsequent feeding of TDE in a manner identical to that used for DDT allowed isolation and identification of three additional materials. This has allowed an expanded but still incomplete knowledge of the steps through which DDT passes during detoxication and elimination of DDT from the warm-blooded animal body. A paper describing findings of these tests has been prepared and submitted to a scientific journal for publication.

Laboratory Studies of Pesticide Using Carbon-14 Labeled Compounds (Mitchell G. Sheldon, Paul Johnson; William H. Robison, James E. Peterson)

The recent acquisition of a liquid scintillation counting instrument has allowed the initiation of controlled feeding studies on captive and laboratory animals using Carbon-14 labeled pesticides. The use of radioactive pesticides will allow more detailed studies of such things as metabolic fate, routes of elimination, points of storage, and principal target sites of various pesticide materials.

In order to determine the feasibility of employing an autoradiographic method to locate labeled material in thin sections of tissue, rats were administered radioactive 2,4-D. Tissues from these animals will be sectioned and exposed to film strips for varying periods of time.

Canada Geese: Feeding Test With C-14 Tagged 2,4-D (Mitchell G. Sheldon; William H. Robison, Richard A. Wilson, Milton H. Mohn)

Through the cooperation of the Colorado Game and Fish Department, 50 young-of-the-year Canada geese were trapped at Two Buttes Reservoir, Colorado, and transported to the Center. Food consumption, adjustment to confinement, and problems of captivity were investigated. Samples of this flock were also analyzed for chlorinated hydrocarbon residues; others have been placed on controlled feeding tests using C-14 tagged sodium salt of 2,4-D to determine toxicant deposition and effects. Tests are still in progress.

Pesticidal Residues in Waterbirds Collected in the Field (Mitchell G. Sheldon; James E. Peterson, Milton H. Mohn, Richard A. Wilson)

Seventeen species of waterbirds or their eggs were collected during 1961 and 1962 for pesticide analysis. Field collections were made in Canada near Yellowknife, N. W. T., north of Great Slave Lake; parts of Colorado, Texas, Utah, Minnesota, Nebraska, and Wyoming. Residue determinations are shown in table 1C. All residues are expressed in parts per million on a "wet" weight basis.

A total of 17 ducks of various species and 4 clutches of 24 scaup eggs were collected near Yellowknife, N. W. T., Canada. Chemical analysis showed that 61.1 percent of the collected waterfowl and all clutches of eggs contained insecticide residues. The 13 adult ducks averaged 0.3 ppm of DDT and metabolites, with a range of 0.0 to 1.0 ppm; the 4 immature ducks averaged 0.4 ppm, with a range of 0.0 to 0.9 ppm; and the 24 eggs averaged 2.2 ppm, with a range of 1.3 to 4.0 ppm.

It is interesting to note among the Canadian samples that the eggs contained more DDT and metabolites than any duck collected, and that the immatures averaged more DDT and related compounds than did the adults, with 1 adult duck having slightly higher residues than the immatures. It is postulated that adult ducks were exposed to the pesticides on migration and wintering areas and passed the residues along to their progeny, as all specimens were collected more than 500 miles north of known insecticide applications.

Additional Canadian samples collected during 1962 consist of duck families, individual ducks, snails, and vegetation. Analyses of these specimens will further clarify the nature of contamination in the Far North, but data from these specimens are not yet available.

A paucity of samples prevents the drawing of conclusions, but it is interesting to note in table 1C that the specimens collected at Two Buttes were the lesser Canada goose which nests in the Canadian Far North beyond man's influence, and other specimens were of the greater Canada goose which exists all year near man. Residues were found only in a single lesser Canada goose, but in all but 1 of the greater Canada goose and its eggs. The lone specimen of the northern sub-species with residues might have been contaminated while in captivity at the Center, as it was the only specimen held captive. It was held captive for a period of several months.

Cottontail Rabbit: Feeding Test (Richard E. Pillmore, James O. Keith, Lowell C. McEwen; Milton H. Mohn, Richard A. Wilson, George H. Ise)

Wild cottontail rabbits trapped near the Center were fed p,p'DDT. Purified rather than technical grade DDT was used to exclude the possibility of DDE or TDE (DDD or Rhothane) being introduced in the diet. Pesticide residues were determined for various tissues and organs by a paper chromatographic technique. It was hoped that the residue information would help in assessing harmful effects of DDT applications on wildlife even when no direct or immediate effects could be observed.

Coburn and Treichler (1946) reported that 2 of 4 cottontail rabbits given an acute oral dose of 2,000 mg/kg DDT survived and that 1 of 3 given a 2,500 mg/kg dose also survived.

One cottontail rabbit (*Sylvilagus floridanus*) was administered an acute oral dose of 2,000 mg/kg p,p'DDT as a suspension in 1 percent gum Arabic by stomach tube. There was a loss of weight and appetite, but no other symptoms were observed during the 8 days after treatment. The animal was then sacrificed. Analyses of tissues and organs of this rabbit in parts per million on a fresh weight basis are as follows:

	DDT	TDE
Fat	480	120
Muscle	3	1.5
Heart	30	5
Liver	0	10*
Kidneys	1,250	66
Brain	4	1
Testes	40	8
Adrenals (dried)	1,780**	889**
Dried excrement	21,700	1,100

*Plus 30 ppm of undetermined chlorinated hydrocarbons.
**Residues on fresh weight basis would be about 1/3 of these values.

Coburn and Treichler (1946) reported that 3 of 4 cottontails on a diet containing 0.4 percent DDT died within 3 weeks. In the present tests 4 cottontails (3 Sylvilagus audubonii and 1 Sylvilagus floridanus) were placed on a diet containing this same concentration of p,p'DDT; and 3 of the 4 rabbits died - a male after 4, a female after 10, and a male after 18 days - on the treated diet. The surviving female rabbit (Sylvilagus audubonii) was sacrificed on the 21st day. None of the rabbits on the treated diet was fat, but all controls on an untreated diet were fat. All test rabbits exhibited tremors during the test. The lone survivor exhibited tremors which began on the 4th day and subsided after 8 to 10 days; while in the other 3 the tremors continued until the death of the rabbit.

The range of residues found in the tissues and organs of the 4 adult rabbits, and of the 2 live young produced by each of the 2 female rabbits during the test, are reported in parts per million. The newborn young were killed by the mother on the day of birth.

	DDT	TDE
Muscle	5-32	2-12
Heart	23-100	6-34
Liver	0-240	69-240
Kidneys	4.5-50	15-50
Brain	21-72	7-14
Testes	30	12
Newborn young	67-380	33-51

Concerning the cottontail which survived to the termination of the test, there were three possibly significant observations on the residue data: First, considerable amounts of the DDT and TDE residues were found in the newborn young indicating that transferral to the fetus is a means of eliminating the pesticide; second, this rabbit contained the least amount of residue in the brain tissue, which might be associated with the fact that tremors had subsided prior to the time of sacrifice; and third, the liver of this rabbit contained no detectable amount of DDT and the least total residue.

Four other cottontails (2 Sylvilagus floridanus and 2 Sylvilagus audubonii) were fed on a diet containing .02 percent p,p'DDT over a period of 70 days. None showed any symptoms during the test period. The ranges of residues on a fresh weight basis in parts per million were:

	DDT	TDE
Fat	40-96	0-<0.5
Muscle	<0.5-4	1
Heart	<0.1-6	0-<0.5
Liver	0-<0.5	2-10
Kidney	0-5	0-2
Brain	<0.5	0
Testes (3 rabbits)	2-6	<0.5

Of possible significance are the relative absence of TDE as a residue in the fat, the relative absence of DDT in the liver, and the fact that DDT at this lower level of intake was accumulated in the testes to the same extent that it was in any other tissue except fat. DDE residues in slight amounts were found in some of the tissues in the above groups of cottontails but have been omitted from these data.

In a more recent test, cottontails (Sylvilagus audubonii) were paired and sacrificed at intervals of 1 week, 2 weeks, 3 weeks, 4 weeks, and 8 weeks on a diet containing 0.1 percent p,p'DDT. Some weight was lost by most of the rabbits on the treated diet, but rabbits on the untreated diet also lost weight. Fat was absent or present in relatively small amounts in female rabbits as compared to males, and pesticidal residues in the livers of the females tended to be higher than in males. There was no consistent pattern of residues with respect to time on test and total amounts of DDT ingested. The residues found in the livers on the basis of fresh tissue weight are reported in parts per million in table 2C.

Dale, et al. (1962) reported a possible relationship between the concentration of DDT and DDE (no mention of TDE) residues in the brain and the tremors and other symptoms resulting from exposure of rats to DDT. It is of some interest to note that a residue of 30 ppm in the brain of 1 rabbit after 3 weeks on a diet containing only 0.1 percent DDT was about the same as or larger than that found in 2 rabbits on the diet containing 0.4 percent DDT, 21 ppm and 27 ppm.

Radeleff and Bushland (1960) state that chemical analyses usually confuse rather than aid in diagnosis of insecticide poisoning, and cite the fact that higher residues may be found in animals surviving exposure to chlorinated hydrocarbons than in those which succumb to the effect.

Our feeding tests indicate that chemical analysis of the liver for determination of the ratio between the amounts of DDT and TDE present may have some value in the diagnosis of DDT mortality. DDT was absent in the liver of the surviving rabbit on the 0.4 percent diet of DDT and this fact may be significant to its survival, considering the factor of repair or adaptation noted in the livers of DDT-fed rabbits examined histopathologically by Nelson, et al. (1944). It is interesting to note that the only possible source of TDE was from the purified DDT.

Ring-necked Pheasants: Toxicity and Tissue Residues of Aldrin and Sevin (Lowell C. McEwen; James E. Peterson, Milton H. Mohn, George H. Ise)

Male pheasants were fed aldrin and Sevin added to the diet at the following levels:

Aldrin	4 ppm	Sevin	500 ppm
Aldrin	16 ppm	Sevin	2,000 ppm
Aldrin	64 ppm	Sevin	8,000 ppm
Aldrin	256 ppm	Sevin	32,000 ppm

One bird was fed at each level on each pesticide, and an additional bird was fed untreated food. The birds were held in separate cages in order to measure individual consumption of the treated food. Feeding of these diets was continued until the death of the particular bird or until sacrifice after 198 days on experimental or control diet.

Individual tissues from each bird were analyzed for aldrin, dieldrin (epoxide of aldrin), or Sevin residues. No aldrin residues were found in these birds. Table 3C shows amount of pesticide ingested, survival in days, and residues found in tissues. Dieldrin residues in the birds fed aldrin were relatively high in the 2 birds that died but were low in the bird that was sacrificed after 198 days. The latter bird had a greater total intake of aldrin, but the rate of intake was so low that the bird evidently was able to eliminate the pesticide rapidly enough to prevent accumulation to any great degree. In the 2 pheasants that died of aldrin ingestion, all tissues analyzed contained dieldrin. Sevin was added to the diet at much higher levels than aldrin because of its known lower toxicity. The birds fed Sevin ingested massive total doses during the course of the experiment but only 1 bird died. The other 3 were sacrificed after 198 days in apparent good health. Residues of Sevin in the tissues were irregular with only the bird that succumbed having Sevin in measurable amounts in all tissues analyzed.

Mule Deer: Feeding Test (Richard E. Pillmore; James E. Peterson, Richard A. Wilson, Milton H. Mohn)

A 5-year-old female mule deer which had been in captivity at the Denver Federal Center since 1957 was placed on a diet containing p,p'DDT in February, 1961. The daily administered dose of 6 mg/kg was contained in 352 grams of feed. In addition to the treated food, supplemental untreated food and alfalfa hay were given. The test extended over 67 days; but as treated food was not offered on weekends and holidays, the deer received the treated food for only 47 days. During the course of the test the weight of this deer increased from 129 to 135 pounds, then decreased to 126 pounds when the test was terminated and the deer was sacrificed. Chemical analyses of tissues and organs on a natural weight basis are reported in parts per million (ppm) as follows:

	DDT	TDE
Fat	50	30
Muscle	0.5	0.2
Heart	0.4	0.7
Liver	0	2.0
Kidneys	<0.5	<0.5
Brain	<0.5	<0.5
Spleen	<0.5	<0.5
Lymph nodes (mediastinal)	3.8	1.8
Thymus gland	5.6	4.1
Ovaries	<0.5	8.0
Adrenals	0	4.5
Pancreas	1.3	0.6

Trace amounts of DDT and TDE were found in some tissues and organs of a deer on the untreated diet, but even in the fat the amounts were less than 0.5 ppm.

Toxicity and Residues of Aldrin and Sevin in Mourning Doves (Lowell C. McEwen; Milton H. Mohn, James E. Peterson, George H. Ise)

Wild-trapped mourning doves were held in individual cages and fed pelleted grain with aldrin or Sevin incorporated into the pellets. Additional doves were fed untreated pellets.

Toxicity of the insecticides, amounts eaten by the birds, and residues in the birds following death or sacrifice are shown in table 4C. Birds fed Sevin at relatively high levels showed little or no Sevin residues in their tissues. All birds fed aldrin contained residues of dieldrin, the epoxide of aldrin. The much greater toxicity of aldrin in comparison with Sevin is apparent in table 4C. One dove survived 309 days on a diet containing 500 ppm of Sevin and was then sacrificed. A diet of 256 ppm aldrin was fatal in 9 days and lower doses of this chemical were also fatal.

Enclosure Studies

Wildlife Hazards of Endrin Used as a Rodenticide in Alfalfa (James O. Keith)

Alfalfa is a major crop in the southern Sacramento Valley, and fields are consistently plagued with irruptions of meadow mice. In late 1961 and early 1962 tests were made by personnel of the Center, Shell Chemical Company, and California Department of Agriculture, in cooperation with county officials and land owners, to determine the efficiency of endrin for mouse control in dormant alfalfa. Dosages ranged up to 0.8 pound per acre. Since the Valley is an important wintering area for large concentrations of waterfowl, and alfalfa is one of the few sources of green feed available to these birds during winter, they probably would be exposed to the endrin. Also, pheasants are abundant on the area and could be affected. Therefore, wildlife biologists studied fields treated at 0.8 pound per acre to determine wildlife effects.

Treated fields were observed to determine if treatments caused wildlife mortality. Several animals were found dead or dying on small test plots shortly after endrin was applied. Four dead cackling geese, a dying house cat, a dead jack rabbit, a dead killdeer, and a dead long-eared owl were found on or near one treated area of 30 acres. A dying pheasant, a dead pheasant, and a dead cackling goose were found on other treated plots.

In cooperation with the California Department of Fish and Game, geese, ducks, and pheasants were held on treated fields. Eight cackling geese, 7 pintails, 7 American widgeon, and 10 pheasants were placed in cages on a field that had been treated with 0.8 pound of endrin per acre. Within 1 week 4 geese, 2 pintails, and 1 widgeon had died, while birds held under similar conditions on untreated fields survived. Pheasants were apparently unaffected after 3 days exposure, at which time dogs upset their cages and the birds escaped. Endrin residues on alfalfa during this test ranged between 23 and 120 parts per million.

Table 5C shows residues of pesticides found in geese examined. Amounts of endrin in tissues of birds surviving experimental field exposure were about the same as amounts in both wild and captive birds dying on the plot.

Laboratory tests to determine amounts of endrin, in single and repeated dosages, necessary to cause mortality in cackling geese, white-fronted geese, and widgeon showed that endrin apparently is equally toxic to these three species. All birds given single oral doses of 5 milligrams or more of endrin per kilogram of body weight died within 3 hours. All birds given 2.5 mg/kg survived a 9-10 day observation period. Pure endrin in a corn oil solution was encapsulated in gelatin and force-fed for these tests. The acute oral LD_{50} of endrin to these species is probably between 2.5 and 5.0 mg/kg when administered in this manner. Rudd and Genelly (1956) found the LD_{50} for adult female pheasants to be between 3.6 and 5.6 mg/kg.

In a test of the effects of repeated doses scheduled over a 5-day period, widgeon given daily doses of 1.0, 2.0, and 2.5 mg/kg died before the 5th day. However, 1 widgeon given 1.5 mg/kg daily survived the 5-day trial. These results suggest that daily feedings resulting in the daily intake of 1.0 mg/kg of endrin would be hazardous, if not fatal, to most widgeon. With endrin residues of about 20 ppm on alfalfa, a 500-gram widgeon that each day ate 25 grams of alfalfa, a modest part of the daily diet, would daily ingest 1.0 mg/kg of endrin.

<u>Captive Mallards Exposed to Insecticides in Artificial Ponds</u> (James O. Keith; Richard A. Wilson, George H. Ise)

Many areas used by waterfowl receive repeated treatments each year for the control of mosquitoes. Little is known of the effects of these insecticides on waterfowl and waterfowl food organisms. Tests in California were begun in 1962, in cooperation with the Kern Mosquito Abatement District and the University of California at Riverside, to observe direct effects on waterfowl of the field use of certain organophosphorus insecticides. Formulations tested are among those showing most promise for future mosquito control operations in California. Alternate insecticides are needed, as mosquitoes are becoming increasingly resistant to materials now in use.

In these tests an attempt was made to simulate field exposure of mallards to insecticides as used in control applications. In the San Joaquin Valley private duck clubs are the aquatic areas most often treated for mosquito suppression. These areas are usually treated five to seven times during August, September, and October. Duck clubs offer resting areas for waterfowl, but birds usually feed elsewhere. In the experimental ponds, therefore, supplemental feed available to birds was not treated with insecticides. Birds in ponds were not sprayed directly as it is doubtful such exposure would exist in the field.

Twelve shallow ponds, each about 1/32 of an acre in size, were fenced to hold pinioned birds. Narrow dikes between ponds support a lush growth of weeds and grasses. Six applications of the insecticides parathion, Baytex, SD-7438, Guthion, and Sumithion were made to water and vegetation in ten ponds between September 12 and November 12, 1962. Each material was applied to two ponds so that treatments were replicated; two ponds served as untreated controls. All materials were emulsifiable concentrates applied at the rate of 0.4 pound per acre, which is about four times the rate to be recommended for mosquito control operations.

Five mallards, including both males and females, are being held in each of the ten treated ponds, and 3 birds are present in each of the two control ponds. Birds were hand-reared after artificial incubation of eggs in Jamesway Incubators. The eggs were collected in 1962 during the nesting season at Tule Lake National Wildlife Refuge.

Ducks on test have shown normal growth and development, are active and apparently healthy. No further treatments are planned for this test, but birds will be maintained on ponds until next summer to enable an evaluation of long-term effects, including any influence of exposure on nesting success next spring.

Effects of Field Treatments

<u>Forest Insect Control</u> (Robert B. Finley, Jr., Richard E. Pillmore; James E. Peterson, Richard A. Wilson, Milton H. Mohn, George H. Ise)

<u>Spruce Budworm</u>

With the cooperation of the Montana Game and Fish Department, collections of 4 mule deer were made during June and July, 1961, on the Gallatin National Forest which was treated with DDT in July of 1960 at a rate of 1 pound per acre except along streams where the rate was reduced to ½ pound per acre. Chemical analysis of various tissues including fat from these 4 deer revealed only trace amounts (less than

0.5 ppm) of DDT or related chemical residues present. These results indicate that residues resulting from DDT applied for spruce budworm control are largely eliminated within a year of the application. Residues in the fat of deer collected 30 and 60 days after DDT application computed on a "fresh" tissue weight basis in parts per million were as follows:

	DDT	TDE
30 days after	19.2	4.0
30 days after	24.0	3.0
60 days after	19.0	1.9
60 days after*	12.0	3.0

*Collected in area treated with only ½ pound DDT per acre.

All of the above deer were free to move into adjacent areas which were not sprayed with DDT; therefore there is no assurance that these residues represent the maximum which might be expected from a 1 pound per acre rate of application. Tissues other than fat contained much lower residues. With the exception of liver tissues which contained up to 0.5 ppm of DDT and 4 ppm of TDE, none of the other tissues (including brain, kidney, skeletal muscle, and testes) contained more than 0.5 ppm DDT or 1 ppm TDE. No DDE was detected in the deer samples; however, DDE was found in some birds (table 6C).

During 1962 approximately 400,000 acres in New Mexico, 400,000 acres in Montana, and 50,000 acres in Colorado were scheduled for spruce budworm control. DDT was applied at ½ pound per acre in Montana, and at 1 pound per acre (except along streams where ½ pound was used) in Colorado and New Mexico. In Colorado, a test application of malathion was made at ½ and ¼ pound per acre, respectively, to two plots of approximately 100 acres each.

Efforts to secure 30 and 60 day post-spray samples from deer for comparison with the information on residues already obtained from Montana were largely unsuccessful because of weather and low numbers of deer. Samples were obtained from hunter-killed deer and elk (3 to 4 months after application) in both New Mexico and Colorado for residue analysis.

A field kit designed for detecting cholinesterase inhibition in humans from exposure to organophosphorus insecticides was given a trial on small mammals before, and about 6 and 24 hours after the malathion application. No cholinesterase inhibition following the application was detected in the small mammals tested, which were mostly chipmunks (*Eutamias* sp.). However, when lethal doses of malathion and parathion were administered to deer mice (*Peromyscus maniculatus*), cholinesterase inhibition was detected with this field kit (Biological Test Products Corporation, Roselle, New Jersey). Effectiveness of the kit depends upon measuring the time for a pH change while heating at a constant temperature 0.02 ml of whole blood, 0.8 ml acetylcholine iodide, and 0.8 ml bromothymol blue solution. It was possible to make this test in the field while running small mammal trap lines. It remains to be determined if such a field test is a practical means of studying field effects of the organophosphorus insecticides.

Malathion Treatment of Lodgepole Pine Needle Miner (James O. Keith)

In 1961 the National Park Service treated 4,912 acres of lodgepole pine in Yosemite National Park with malathion to control an infestation of lodgepole pine needle miner. This control was determined to be necessary to protect forest stands in areas receiving heavy recreational use. One aerial application was made of 1 pound of malathion in 10 gallons of diesel oil per acre, using helicopters. The Park Service requested the help of the Bureau of Sport Fisheries and Wildlife in determining the effect of malathion on wildlife in treated areas.

Bird counts and small mammal trapping records were used to determine the reactions of bird and mammal populations to malathion applications. Eight bird-count routes were selected, four in the spray area designated S-1 through S-4, and four in a check area designated C-1 through C-4. Eight small mammal traplines were also established and given similar designations. To measure the abundance of passerine birds on study areas, birds were counted for 1 hour along established routes. Three counts were taken along each route during each period of measurement. To assess the abundance of small mammals, snap traps were set at each of 25 stations on each trapline for 3 consecutive nights during each measurement period. Records of animal abundance were obtained in July, 1961, before malathion applications to the treated area and in August, 1961, and July, 1962, 6 weeks and 1 year, respectively, after treatment. Bird counts were obtained in the same periods on both areas and, in addition, on the spray area immediately after malathion applications in July, 1961.

Table 7C shows the average number of birds seen per hour on routes before and after treatment. In this study it is assumed that changes in animal numbers recorded on the check area are the same as those that would have occurred on the spray area if malathion had not been applied. Any differences in changes on the spray area are, therefore, attributed to treatment. Whereas numbers of birds seen in July and August, 1961, were about the same on the check area, the average count on the spray area decreased from 34 in July, to 18 in August, indicating a reduction of about 47 percent due to treatment of the area with malathion. By July, 1962, however, bird numbers had increased on the spray area and on some routes exceeded numbers seen before treatment.

No bird mortality was seen or reported after treatment of the spray area. Bird counts taken on the spray area immediately after treatment indicate that no reduction in bird numbers occurred during the period when malathion would be most available. Changes in bird numbers on the spray area took place during the several weeks after treatment and may be due to movements of birds into and out of the area in response to changes in the numbers of insects available for food.

Catches of rodents were variable but totals decreased on the check area between July and August, 1961, and between August, 1961, and July, 1962 (table 8C). On the spray area the catch increased between July and August, 1961, but by July, 1962, was lower than the catch in July, 1961, before treatment. It appears that the malathion application had little effect on rodents.

Additional work is needed to discover the indirect ways in which malathion may have influenced the abundance of birds and mammals on the treated areas. These effects were not evident 1 year later. Malathion gave good control of needle miner.

Agricultural Pest Control

Crop Insects in the Klamath Basin (James O. Keith; Richard A. Wilson, George H. Ise)

Mortality of fish-eating birds, first noticed in 1960, recurred during the summers of 1961 and 1962 at Tule Lake National Wildlife Refuge. Numbers of dead birds found in the field are given in table 9C.

Death of birds was apparently due to pesticide poisoning. All dead birds analyzed contained unusually high residues of DDT and toxaphene (U. S. D. I., 1962) and no other possible cause of death has been determined. The use of toxaphene in 1958 and 1960 on farmlands of the Refuge and the finding of DDT and toxaphene residues ranging from about 0.1-8.0 parts per million in fish collected at the Refuge also suggest a relation between agricultural pesticides and the fish-eating bird mortality.

Birds affected are all migratory. Originally it was assumed that exposure to DDT was occurring at other sites used during migration, since there has been little use of this insecticide in the Klamath Basin in recent years. In 1962, however, water entering the Refuge from adjacent farmlands was sampled at weekly intervals and analyzed for pesticides. Water samples were filtered, and residues in both the filtered water and the sediments and organic materials removed from the water were determined. Results show that DDT was present in most samples, with amounts ranging between 0 and 1.0 part per billion in the filtrate and between 6.5 and 60.0 parts per million in the suspended material. These low amounts of DDT that enter the Refuge seem to be concentrated, in a manner not yet understood, in fish, and ultimately in tissues of fish-eating birds.

Work in 1961 and 1962 at Tule Lake Refuge was designed to determine the entrance, dispersal, and persistence of pesticides in marsh habitats on the Refuge. Samples of water entering the Refuge, and of water, vegetation, invertebrates, fish, and sediments at permanent stations in the marsh, were collected periodically. In 1962, samples at these stations were obtained in April, June, August, and October. Residue analyses have not been completed.

Range Pest Control

Grasshopper Control: Sevin (Lowell C. McEwen, James Macdonald, Robert B. Finley, Jr.; James E. Peterson, Milton H. Mohn, George H. Ise)

A study to determine the effects on wildlife of a grasshopper insecticide possessing relatively low toxicity to wildlife in laboratory tests was initiated at Lostwood National Wildlife Refuge in North Dakota. Cooperators in this investigation are the Research and Refuge branches of the Bureau of Sport Fisheries and Wildlife, the Plant Pest Control Division of the Agricultural Research Service, and the Union Carbide Corporation.

Two study areas of 2,000 acres each were selected on the Refuge. The areas are glaciated, rolling, grasslands dotted with numerous aspen groves and shallow pot-holes. Pre-spray census work and other sampling was begun in 1961. In May, June, and early July, 1962, further pre-spray surveys were carried out. On July 9 and 10, 1962, one area was sprayed with Sevin, a carbamate insecticide, at the rate of 1 pound of active material in 1 gallon of water per acre. The other area was left unsprayed for comparison.

Post-spray investigations through October, 1962, indicated no conspicuous effects on any bird or mammal species on the sprayed area. No sick or dead animals were found in searches with dogs or during general activity on the area. Individual grouse and duck broods were observed before and after the spray and appeared to develop normally. Systematic census of sharp-tailed grouse, ducks, and songbirds indicated no significant differences between the sprayed and unsprayed areas or between pre- and post-spray censuses of both areas. Small mammals in pot-hole edge habitat declined in numbers, but this may have been due to other factors.

Bird and mammal census results are summarized in tables 10C and 11C. All census routes and trap lines were permanently marked so that successive counts and trapping could be done on the same routes and at the same stations. Upland bird census routes were each about 4 miles long and the observer counted all birds within 100 feet on either side as he walked. Pot-hole routes each consisted of five pot-holes of various sizes and the observer counted all birds on the water and within 100 feet of the shore line. Two upland and two pot-hole bird census routes were run on each area. Each bird census began shortly before sunrise and was 2 hours in duration. Sherman live-traps were used for small mammal trapping. Each trap line consisted of 25 stations with two traps at each station. Distance between stations was about 50 feet.

Various types of samples were collected for chemical analysis both before and after the spray; soil, vegetation, pot-hole water, insects, other invertebrates, small mammals, songbirds, and game animals. Only part of these have been analyzed to date. Pre-spray animal samples completed have contained no Sevin. All post-spray animal samples analyzed have contained less than 6 ppm of Sevin. Most contained only trace amounts. Several environmental samples, both organic and inorganic, were also checked for chlorinated hydrocarbons. Almost all had small residues, usually DDE, even though there has been no known spraying of chlorinated hydrocarbons on the Refuge. The source of the chlorinated hydrocarbon contamination may have been drift from spraying of private land in the region.

A low grasshopper population on the study areas, less than 1 per square yard, made it difficult to assess effectiveness of the spray for grasshopper control. This differed from the typical operational grasshopper control program, of course. Another difference was the rather heavy vegetative cover on the study areas. Grasshopper problems are more likely to occur on more sparsely vegetated rangeland. The grass and shrub cover at Lostwood may have reduced the effectiveness of the insecticide. Total kill of all insect species on the sprayed area, based on pre- and post-spray net sweeps in the various vegetative types, was estimated at 50-60 percent.

Invertebrate populations in the pot-holes showed little decline in abundance following the spray application. Most pot-holes are too shallow to support fish. The aquatic invertebrate populations in the pot-holes are of importance as food for ducklings, however. Pre- and post-spray measurements of total weight of aquatic invertebrates indicated only a 15 percent decrease after the spray.

Follow-up surveys will be made on the study areas in 1963 and periodically in following years to determine long-range effects of the spray, if any.

Grasshopper Control: Aldrin, Dieldrin, and Sevin (Robert B. Finley, Jr., Richard E. Pillmore; James E. Peterson, Milton H. Mohn, Richard A. Wilson, George H. Ise)

About 10 miles west of Sheridan, Wyoming, aldrin was used at 2 ounces per acre for grasshopper control on 7,850 acres of rangeland. No direct or immediate effects were observed by wildlife biologists. A month after this application several wildlife samples were collected. Chemical analyses of visceral and subcutaneous fat from a mature buck mule deer revealed 3.3 and 2.6 ppm of dieldrin, respectively, while fat from a lactating doe mule deer contained 1.2 ppm dieldrin (aldrin is usually converted to dieldrin in animal tissues). A composite sample consisting of 5 grams each of heart, liver, kidney, brain, and lean meat of a white-tailed jackrabbit contained 3 ppm dieldrin, while a 25-gram whole-body aliquot of a skinned and ground-up sharptail grouse contained 0.6 ppm dieldrin. All tissue residues are expressed as parts per million on a "wet" weight basis.

From Montana, tissue samples of 2 mule deer and 1 antelope were submitted to the Center for analysis. These were collected from Rosebud County by the Montana Game and Fish Department about a month after a grasshopper control program, where aldrin was applied at 2 ounces per acre. The fat from the antelope contained 3 ppm dieldrin, but no dieldrin was detected in either of the mule deer, though the Montana Game and Fish Department considered the deer to be residents within the treated area.

Over 3,000 acres on the Strand Ranch in Choteau County, Montana, were treated with dieldrin at ½ ounce per acre, and on the adjacent 2,000 acres of National Forest Land 2 ounces per acre of aldrin were used. Tissues of mule deer, a white-tailed jackrabbit, and 5 sharp-tailed grouse collected from the Strand Ranch a month or more after the application were analyzed and the dieldrin present ranged from 0 to less than 0.2 ppm.

On the Benton Lake National Wildlife Refuge, Sevin was used to control grasshoppers at ½ pound per acre in 2 gallons of water. Possible casualties found which might be attributed to this program consisted of 3 young deer mice (Peromyscus) found dead within 24 hours after the spray application. Mice were found on analysis to contain 2 ppm of Sevin. In addition, a meadow mouse and a deer mouse were found dead amongst the pile of Sevin containers and these contained 4.7 ppm of Sevin. A prairie horned lark and a chestnut-collared longspur were shot for analysis and no Sevin was detected in the horned lark, while the longspur which was observed feeding on the affected grasshoppers contained 3 ppm of Sevin. The analysis of these animals for Sevin included the intestinal tract and contents.

Harvester Ant: 1961 Field Tests with 1080 (Robert B. Finley, Jr., James Macdonald, Mitchell G. Sheldon, Richard E. Pillmore; Vernon Perry)

Pesticide-wildlife biologists of the Denver Wildlife Research Center participated with entomologists of New Mexico State University and range managers of the Bureau of Land Management in field tests of compound "1080", kepone, and heptachlor for control of the harvester ant. Because of the high toxicity of "1080" it was desirable to learn whether the bait containing it was picked up by birds and mammals and whether the amount of bait consumed contained enough "1080" to cause casualties.

Tests were conducted on four 100-acre plots on the Ake Ranch, Catron County, New Mexico, on May 5. The bait employed was finely-cracked corn with 1 part "1080" to 750 parts by weight of bait material. It was applied by airplane at the rate of 5 pounds of bait per acre. The test plots were on a high semi-arid plain with only sparse vegetative cover and small numbers of mammals and birds. Only horned larks were moderately numerous. The only casualty found on the "1080" plot after the application was a dead spotted ground squirrel.

Three exposure pens 4 x 8 feet in size were erected on the "1080" plot. Six mourning doves were trapped and placed in one of the pens, 7 cowbirds and blackbirds in a second pen, and 4 kangaroo rats in the third pen. Clean, untreated grain and water were available in each pen, in addition to the sparse deposit of poison bait from the aerial application. None of the doves died. Four of the cowbirds and 3 kangaroo rats died. All survivors were released 3 days after the bait application. Chemical analysis of the casualties revealed "1080" in 2 of the 4 cowbirds and 2 of the 3 kangaroo rats.

In preparation for the field test on August 24, 1961, the bait to be applied to one of the plots was labeled with an inert tracer of aluminum powder amounting to 0.1 percent aluminum. This was applied to a plot being treated with kepone bait at the rate of 3 pounds per acre. The presence of detectable amounts of aluminum powder in 2 deer mice, 5 of 8 horned larks, and 3 of 4 vesper sparrows collected 1 or 2 days after application of the labeled bait clearly showed that the bait was being eaten by small birds and mammals.

Following application of "1080"-treated corn bait at 5 pounds per acre, 1 horned lark and 7 pocket mice were found dead on the study plot; and "1080" was detected by chemical analysis of a combined sample of 5 of the pocket mice, but not detected in the horned lark.

Results from the first summer's work indicate that: the corn bait is attractive to several species of wildlife; and that some birds and rodents consumed lethal amounts of the treated bait. Any large-scale use of "1080" in this formulation would therefore expose many wildlife species to poisoning hazards. No evidence of adverse effects to the mourning dove was obtained from these preliminary findings.

Harvester Ant: 1962 Field Tests with 1080, Kepone, and Mirex
(Robert B. Finley, Jr., James Macdonald, Mitchell G. Sheldon, Richard E. Pillmore; Vernon Perry)

Larger scale field tests were conducted in July, 1962, south and east of Datil, New Mexico. Aerial applications were made on five 640-acre plots. One plot was treated with compound "1080", one with kepone, and three with Mirex. A formulation consisting of 15 percent soy-bean oil and about 85 percent ground corncob grits as an inert carrier was used for all three toxicants. Principal attention was given to the "1080" plot because of the potential hazard to wildlife indicated by the 1961 tests.

The "1080" was mixed with corncob grits in the ratio of 1 part of "1080" to 750 parts of bait. It was planned to apply the treated bait to 640 acres at the rate of 2 pounds of bait per acre. However, the plot received highly erratic aerial coverage; about 100 acres received a dosage of approximately 5 pounds per acre and the remaining acreage received "1080" at irregular lesser rates of coverage. In addition, Mirex bait was applied on the west side of this plot, contrary to plan.

No sick or dead birds or other vertebrates were found on the plot after a total of 12 manhours of searching. General observations did not detect any change in species composition, abundance, or activity after application of the "1080" bait.

As an aid to measure bait acceptance, aluminum powder was mixed with the "1080" corncob grits bait in the amount of 11 grams of aluminum to 50 pounds of bait, or 0.05 percent. In the period between a few hours and 3 days after bait application, 8 mourning doves were shot near a windmill in the kepone plot and about ¼ mile from the edge of the "1080" plot. Later examination of digestive tracts of these doves under low power microscope revealed no aluminum particles and only one particle of corncob grit in the gizzard of 1 dove. Doves from three adjacent test plots came to the same windmill for water. Although all three plots received the same corncob grit bait only the "1080" plot received aluminum powder.

An 8' x 8' exposure pen was erected on the part of the "1080" plot that received more than double the intended 2 pounds of bait per acre. Three live doves were placed in this pen prior to the treatment of the plot. Water and a handful of supplemental grain feed were provided. One dove was sacrificed a day after bait application and the other 2 doves were found dead in the pen 3 days after application. All of the penned doves contained at least a few particles of both corncob grits and aluminum, none in large amounts. Therefore, the limited data indicate that the free-ranging doves picked up very little of the bait, but that confined birds on a scanty diet did eat the bait and this may have caused death within 3 days.

One of the three Mirex plots was adjacent to the "1080" plot in the pinyon-juniper woodland and was treated with 2½ pounds of 0.15 percent Mirex in corncob grits bait. Five days after treatment this plot was searched for 1 hour without finding any wildlife casualties. No changes were noted in bird abundance or activity. The other two 640-acre Mirex plots, which received 1 pound and 5 pounds of the same 0.15 percent bait, were located in the center of the San Augustin Plain south of Datil, where the vegetation was short grass and four-winged saltbush. Bait distribution on much of the 1-pound plot was erratic because of difficulty in calibrating the release mechanism in the aircraft. Coverage on the 5-pound plot was good. This plot was searched for casualties 1 and 2 days after treatment for a total of 4 manhours. No sick or dead vertebrate animals were seen. Abundance and activity of birds and lizards were about the same as before treatment.

One horned lark and 1 longspur were shot on the 1-pound Mirex plot. The horned lark had some particles of corncob grit bait in the gizzard, but the longspur had none.

The kepone plot was searched for 1 hour on the day after treatment; no casualties were seen.

The 1962 observations indicated that the corncob grit bait is less attractive to doves and other wildlife than finely-cracked corn, and that the amounts of Mirex applied under these conditions were not sufficient to cause wildlife losses.

Other Field Studies

Movements of White Pelicans (James O. Keith)

Field work is currently needed to appraise the hazards of pesticides to migratory waterfowl and other waterbirds. It is becoming increasingly evident, especially in the semi-arid western United States, that many rivers, sumps, and impoundments are being contaminated with pesticides carried in waste agricultural waters. In contaminated aquatic habitats pesticides are known to be accumulated and concentrated in food-chain organisms (Hunt and Bischoff, 1960). Birds that use aquatic habitats and especially those that feed on aquatic organisms may, therefore, be subjected to repeated exposure to pesticides during their annual migrations.

To gain an understanding of this problem it is desirable to work with a bird whose habits are simple and easily documented, and one that is in other ways adapted to investigations of this type. The white pelican appears to meet these requirements. Pesticide poisoning was the suspected cause of death to large numbers of these birds at several sites during 1960, 1961, and 1962. Many birds have probably been exposed to pesticides in amounts sufficient to influence general health and reproduction. Pelicans use only limited areas and eat a simple diet of fish. Finally, population counts and evaluations of nesting success should be relatively easy to obtain while birds are in nesting colonies.

The logical beginning for such a study of pelicans is to determine areas used by birds during migration, the periods of use, and the use by various social aggregations of birds within the population. Such information is essential to understand the potential exposure of birds to pesticide contamination and the effects of such exposure on numbers and breeding success in individual breeding colonies.

In 1962, work with white pelicans was undertaken in cooperation with the Branch of Wildlife Refuges at four nesting colonies in the Pacific Flyway to determine the movements of these birds and the pesticide contamination in habitats used by the birds. Adult and young pelicans, pelican eggs, and fish fed to young birds were collected for residue analysis. Young flightless birds were banded and color-marked at nesting colonies as indicated below:

Colony	Number banded	Number color-marked	Color of dye
Pyramid Lake Refuge	520	167	purple
Upper Klamath Refuge	100	0	-
Lower Klamath Refuge	263	103	pink
Clear Lake Refuge	357	142	blue-green
Total	1,240	412	

Band returns and sight records of color-marked birds have so far indicated that at least some young pelicans disperse widely in several directions soon after attaining the ability to fly. Birds marked at Lower Klamath Refuge in July, for instance, had been seen by September in California, Oregon, Idaho, and Utah. Records also suggest that the colonies formed during the nesting season do not persist as separate social aggregations during the year.

Miscellaneous Control Measures

Aldrin Contamination of Lakes at the Rocky Mountain Arsenal (Mitchell G. Sheldon, Robert B. Finley, Jr.; Milton H. Mohn, George H. Ise)

Insecticide contamination of lakes at the Rocky Mountain Arsenal continued to kill some waterfowl during 1961 and 1962. Losses were light compared to past years due, in part, to the lakes in question starting the 1961 fall and winter seasons full of water. No duck fatalities were reported during the fall and winter of 1961, although the wintering population was the largest observed in recent years. Divers, consisting of lesser scaup and some redheads, made up the bulk of the wintering birds, with counts in excess of 10,000 made by the Arsenal's game manager. Dabbling species reappeared in early spring.

Duck casualties began to appear as soon as the upper lake was drained, exposing the mud bottom. This took place about mid-March, and approximately 100 dead ducks of several species were counted on March 28, 1962. This strongly indicates that the toxic material is still present and death occurs with the exposure of mud flats. A sample of 8 dead ducks was taken in proportion to the species found that could be recognized as fresh carcasses. Two samples of each bird were prepared, with one consisting of a liver-kidney composite and the other of the brain. Residue analyses showed a range of 3.3 to 33 ppm of dieldrin in composites of the liver-kidney samples, and from 2.0 to 20 ppm of dieldrin in the brain tissue, as shown in table 12C. A sample of snails and 1 tiger salamander was also collected. A whole-body aliquot of the salamander revealed 14.0 ppm dieldrin, 0.1 ppm aldrin, and 0.1 ppm DDE. Snail tissue extracted from the shells and composited into a sample had 57 ppm dieldrin and 5 ppm aldrin.

It would appear from 1961 and previous data, that mortality could be reduced greatly through proper management practices. These would consist of maintenance of adequate water levels in the lakes and harrassment of the duck's during any period of mud exposure in order to disperse the population to adjoining and possibly uncontaminated areas.

Table 1C. Residues of DDT and related compounds found in waterbirds or their eggs by area. All residues are expressed in parts per million on a wet weight basis. Analysis was by paper chromatography

Area and species	Number of specimens			Amount of DDT and metabolites in specimens with residues		
	Total	Without residues	With residues	Av.	Min.	Max.
CANADA						
Yellowknife, N.W.T.						
Mallard	9	7	2	0.5	0.1	0.8
Pintail	4	3	1	1.0	1.0	1.0
Baldpate	3	1	2	0.2	0.1	0.2
Scaup	1	0	1	0.0	0.0	0.0
Scaup: clutches of eggs (24)	4	0	4	2.2	1.3	4.0
COLORADO						
Denver area						
Redhead	2	1	1	4.0	4.0	4.0
Scaup	4	1	3	2.9	2.1	3.3
Shoveller	2	0	2	0.6	<0.1	1.0
Green-winged teal	1	0	1	<0.1	<0.1	<0.1
Canada goose	5	1	4	1.4	0.5	2.1
Canada goose egg	1	0	1	<0.1	<0.1	<0.1
San Luis Valley						
Mallard	9	0	9	2.2	0.3	4.9
Two Buttes Reservoir						
Canada goose	20	19	1	10.6	10.6	10.6
Snow goose	1	1	0	0.0	0.0	0.0

(Continued)

Table 1C. Residues of DDT and related compounds found in waterbirds or their eggs by area. All residues are expressed in parts per million on a wet weight basis. Analysis was by paper chromatography (continued)

Area and species	Number of specimens			Amount of DDT and metabolites in specimens with residues		
	Total	Without residues	With residues	Av.	Min.	Max.
Yampa River area						
Canada goose eggs	6	0	6	0.7	0.4	1.0
TEXAS						
Gulf Coast						
Mallard	7	3	4	0.4	0.2	0.8
Black duck	4	4	0	0.0	0.0	0.0
Pintail	3	0	3	0.2	<0.2	0.3
Shoveller	4	2	2	0.4	0.3	<0.5
Blue-winged teal	2	1	1	0.2	<0.2	<0.2
Green-winged teal	1	0	1	0.3	0.3	0.3
Snow goose	1	1	0	0.0	0.0	0.0
Blue goose	8	3	5	0.2	<0.1	<0.2
Cormorant	3	1	2	8.5	4.3	12.7
Gull	2	0	2	22.5	13.0	32.0
King rail	2	0	2	1.1	0.6	1.5
King rail: clutch of eggs (5)	1	0	1	0.7	0.7	0.7
Coot: clutch of eggs (4)	1	1	0	0.0	0.0	0.0
MINNESOTA						
Round Lake						
Canvasback	2	0	2	2.8	<0.5	5.1
Redhead	1	0	1	< 0.5	<0.5	< 0.5
Scaup	1	0	1	< 0.5	<0.5	< 0.5

(Continued)

Table 1C. Residues of DDT and related compounds found in waterbirds or their eggs by area. All residues are expressed in parts per million on a wet weight basis. Analysis was by paper chromatography (continued)

Area and species	Number of specimens			Amount of DDT and metabolites in specimens with residues		
	Total	Without residues	With residues	Av.	Min.	Max.
NEBRASKA						
Platte River area						
Canvasback	2	2	0	0.0	0.0	0.0
WYOMING						
Cheyenne						
Swan	1	1	0	0.0	0.0	0.0

Table 2C. Residues in liver of cottontails (Sylvilagus audubonii) after various invervals on a diet containing 0.1 percent of p,p'DDT

Exposure	Males			Females		
	DDT	DDD	DDE	DDT	DDD	DDE
1 week	0.6 ppm	7.5 ppm	1.2 ppm	3.1 ppm	11.6 ppm	1.5 ppm
2 weeks	0.6	6.8	1.0	0.4	25	1.3
3 weeks	1.9	60	1.9	2.6	67	2.2
4 weeks	0	13	<0.5	12	48	3.4
8 weeks	<0.5	43	<0.5	0	30	2.3

Table 3C. Insecticide eaten, survival of birds in days, and insecticide residues in tissues in male ring-necked pheasants fed diets with aldrin or Sevin incorporated into the feed. One bird was fed at each level.

Test data	Dieldrin[1/] residues in ppm ("fresh" tissue weight basis)							
	Brain	Heart	Kidneys	Liver	Muscle	Fat	Testes	Remainder
Fed aldrin @ 4 ppm Total aldrin eaten - 36 mg/kg Survival - 198 days[2/]	0	0	0	0	1.4	1.0	0.1	0.7
Fed aldrin @ 16 ppm Total aldrin eaten - 23 mg/kg Survival - 43+ days	Bird escaped during feeding test							
Fed aldrin @ 64 ppm Total aldrin eaten - 19 mg/kg Survived - 15 days	18.0	13.0	18.0	115.2	3.5	None present	3/	14.4
Fed aldrin @ 256 ppm Total aldrin eaten - 12 mg/kg Survival - 8 days	10.0	7.0	3/	14.0	1.3	None present	3/	1.6
	Sevin residues in ppm ("fresh" tissue weight basis)							
Fed Sevin @ 500 ppm Total Sevin eaten - 3,866 mg/kg Survival - 198 days[2/]	3/	3/	3/	0	3/	0	6.0	0
Fed Sevin @ 2,000 ppm Total Sevin eaten - 16,579 mg/kg Survival - 198 days[2/]	3/	3/	3/	0	3/	0	3.0	0
Fed Sevin @ 8,000 ppm Total Sevin eaten - 50,129 mg/kg Survival - 198 days[2/]	2.6	3/	3/	Trace	3/	200.0	Trace	Trace
Fed Sevin @ 32,000 ppm Total Sevin eaten - 4,856 mg/kg Survival - 18 days	1.7	1.3	0.3	4.0	3/	None present	3/	31.0

1/ Dieldrin is the epoxide of aldrin.
2/ Sacrificed after 198 days.
3/ Not analyzed or lost in chemical analysis procedures.

Table 4C. Mortality and tissue residues in mourning doves fed pelleted grain with aldrin and Sevin incorporated into the pellets

Number of birds	Diet treatment	Total insecticide eaten mg/kg	Survival period days	Tissue residues1/
1	Aldrin 1 ppm	27	309 2/	Dieldrin 3/ 4 ppm
1	Aldrin 4 ppm	93	215	Dieldrin 3/ 24 ppm
1	Aldrin 16 ppm	158	98	Dieldrin 3/ 12 ppm
1	Aldrin 64 ppm	60	10	Dieldrin 3/ 24 ppm
1	Aldrin 256 ppm	72	9	Dieldrin 3/ 48 ppm
1	Sevin 500 ppm	14,057	309 2/	Sevin 0 ppm
1	Sevin 2,000 ppm	20,705	81	Sevin 0 ppm
1	Sevin 8,000 ppm	2,540	11	Sevin 0.1 ppm
1	Sevin 32,000 ppm	3,962	8	Sevin 0.7 ppm
2	Untreated	0	309 2/	Dieldrin 3/ 0 ppm
2	Untreated	0	309 2/	Sevin 0 ppm

1/ Residues are given in parts per million of whole body weight.
2/ These birds were sacrificed after 309 days.
3/ Dieldrin is the epoxide of aldrin.

Table 5C. Residue analyses of 12 cackling geese exposed to endrin on a plot treated for mouse control

History of geese	Residues1/ (ppm)	
	Endrin	DDE
Wild birds found dead	2.4	1.8
	2.4	0.0
	2.0	0.0
	1.8	0.0
Captive birds dying after exposure in cages	4.1	0.0
	2.0	0.0
	2.0	0.0
	1.7	0.0
Captive birds surviving exposure in cages	2.4	0.0
	2.0	0.0
	1.0	0.0
	0.8	0.0

1/ Based on "wet" weight of a composite sample of heart, liver, brain, kidney, and muscle.

Table 6C. Residues found in birds collected about 1 month after DDT was applied for spruce budworm control on the Gallatin National Forest in Montana. Residues are expressed in parts per million on a "fresh" weight basis.

Bird	DDT	DDE	DDD	Remarks
Robin	2.0	4.8	Trace	From Unit 14, ½ lb./acre DDT
Robin	2.4	9.6	2.0	From Unit 14, ½ lb./acre DDT
Robin	3.8	7.7	2.4	From Unit 22, 1 lb./acre DDT
Western tanager	2.4	2.1	1.2	From Unit 14, ½ lb./acre DDT
Western tanager	3.0	-	3.0	From Unit 14, ½ lb./acre DDT
Western tanager	2.5	-	2.5	From Unit 14, ½ lb./acre DDT
Audubon's warbler	8.0	15.0	None	From Unit 14, ½ lb./acre DDT
Blue grouse	None	1.6	None	From edge of Unit 12

Table 7C. Average numbers of birds seen in the spray and check areas before and after malathion application at Yosemite National Park

Routes	Average number of birds seen per hour			
	Pre-treatment	Post-treatment		
	July 1961	July 1961 1/	August 1961	July 1962
Spray area				
S-1	26	28	20	23
S-2	60	58	27	94
S-3	26	23	10	14
S-4	26	22	16	45
Average	34	33	18	44
Check area				
C-1	52	-	43	29
C-2	42	-	47	22
C-3	17	-	18	17
C-4	36	-	34	37
Average	37	-	36	26

1/ Records obtained on spray area within 96 hours after treatment of routes.

Table 8C. Rodents captured on spray and check areas before and after malathion application at Yosemite National Park

Species	Total catch on 4 traplines		
	Pre-treatment	Post-treatment	
	July 1961	August 1961	July 1962
Spray area			
Deer mice	29	54	1
Chipmunks	22	31	23
Golden-mantled ground squirrel	1	6	1
Total	52	91	25
Check area			
Deer mice	46	40	15
Chipmunks	23	10	9
Golden-mantled ground squirrel	6	2	0
Total	75	52	24

Table 9C. Numbers of dead fish-eating birds found at Tule Lake Refuge in 1960, 1961, and 1962

Species	Year		
	1960	1961	1962
White pelicans	156	203	78
Egrets	85	41	27
Western grebes	12	80	82
Great blue herons	23	0	21
Gulls	34	21	5
Night herons	12	3	0
Cormorants	5	1	0
Eared grebes	0	5	0
Forester terns	0	5	0
Total	327	359	213

Table 10C. Bird counts (average number seen per hour) on a 2,000-acre area sprayed with 1 pound per acre of Sevin and on a similar unsprayed area at Lostwood National Wildlife Refuge, North Dakota

Period	Number of birds per hour			
	Sprayed area		Unsprayed area	
	Upland	Pot-hole	Upland	Pot-hole
Pre-spray				
July 1961	100	92	110	130
October 1961	5	98	9	123
June-July 1962	87	139	83	116
Post-spray				
July-August 1962	83	127	82	110
September 1962	37	119	35	134

Table 11C. Small mammals (average number of initial captures per 100 trap nights) on a 2,000-acre area sprayed with 1 pound per acre of Sevin and on a similar unsprayed area at Lostwood National Wildlife Refuge, North Dakota

Period	Captures*			
	Sprayed area		Unsprayed area	
	Upland	Pot-hole	Upland	Pot-hole
Pre-spray				
July 1961	5	9	12	6
October 1961	3	1	8	3
June-July 1962	5	2	7	3
Post-spray				
July-August 1962	8	7	11	5
September 1962	24	2	23	11

*Initial captures per 100 trap nights.

Table 12C. Chlorinated hydrocarbon residues in waterfowl found dead at the Rocky Mountain Arsenal, Colorado. Residues are expressed in parts per million on a "wet" weight basis.

Species	Kidney and liver		Brain	
	Dieldrin	DDE	Dieldrin	DDE
Shoveller	22.0	0.9	10.0	0.1
Shoveller	11.0	-	20.0	0.1
Green-winged teal	20.0	0.1	9.0	-
Redhead	3.3	-	2.0	-
Lesser scaup	14.0	-	7.7	-
Lesser scaup	22.0	-	13.3	3.3
Lesser scaup	11.0	-	10.6	2.1
Lesser scaup	33.0	-	16.0	3.2

WILDLIFE STUDIES, PATUXENT WILDLIFE RESEARCH CENTER

1961-1962

by

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Pesticide research at Patuxent had two principal objectives. One was to appraise new or accepted chemicals to which wildlife might be exposed. This objective was approached through tests of many chemicals on captive birds of selected wild species. Both chronic and acute tests were used. The long-term results of adding small amounts of pesticides to the diet were studied through measurements of growth and reproduction. Chemical analyses for residues in wild and experimental animals also contributed to the evaluation of chemicals, for persistence and accumulation in the body are especially dangerous traits of many chemicals.

The other objective was to study major problems that arose from field applications of pesticides. These problems were approached by the combination of methods that seemed most appropriate. The methods often involved both chemical and biological techniques in the field as well as in the laboratory. Enclosure studies that bridged the gap between field and laboratory studies became an important part of the program. Serious efforts were devoted to devising and testing better field and laboratory methods. The kinetics of pesticides--the relation of rates of assimilation and excretion to toxic action and residue levels--was given increased consideration in interpreting results and in planning research.

Results given here are, for the most part, in summary form, and some are compiled from research in progress, so that final tabulations may differ. For these reasons, the findings should not be quoted in technical publications without first communicating with the responsible investigator, who is designated for most studies.

Evaluation of Chemicals

Feeding tests designed to measure the effects of pesticides on mortality, growth, and reproduction of selected wild species were continued and expanded in 1961 and 1962. Tests of both herbicidal and insecticidal chemicals were included. These tests are a key part of the pesticides program in that they provide a relatively rapid method of detecting potential hazard, and hence are a way to obtain early information on the many new chemicals as they are introduced. This portion of the program is under the direction of James B. DeWitt with assistance from Harold Doty, James Spann, and Clyde Vance.

Bobwhite quail, ring-necked pheasants, and mallard ducks were used as subjects in the principal testing program. Tests also were made on certain other species, particularly red-winged blackbirds, cowbirds, grackles, and starlings. In 1962, experimentation was begun to determine the suitability of coturnix quail for use in the toxicological studies.

Bobwhite, Pheasants, and Mallards

More than 500 toxicological tests involving 42 pesticides were conducted at the Patuxent Wildlife Research Center during 1961 and 1962. Objectives of these tests were to determine: (a) quantities of test compounds producing acute poisoning and death of at least 50 percent of test animals within 10 days, (b) quantities producing chronic poisoning resulting in 50 percent mortality between the 10th and 100th days of the test period, (c) maximum quantities permitting survival for 100 or more days, and (d) effects of sublethal exposure upon growth and/or reproduction. Data from these tests are combined with results of earlier studies in table 1D.

Results of preliminary tests indicated that Zytron is moderately toxic to adult quail, but that the other herbicides tested have relatively low orders of toxicity. Amiben, casoron, diphenamid, and diphenatrile in diets did not result in as much as 50 percent mortality in any of the tests and body weights apparently were not affected. Effects of these compounds on reproduction were not determined. Amitrole, Amitrole-T, dacthal, dalapon, MCPA, and chlorinated phenoxy herbicides also had relatively low orders of toxicity, but sublethal exposures to these compounds had marked inhibitory effects upon reproduction. Quantities producing these inhibitory effects ranged from less than 10 to approximately 50 percent of the estimated lethal dose (table 3D).

Similar inhibitory effects upon reproduction resulted from feeding of diets containing 50 or 100 ppm of kepone prior to and during the breeding season. This lack of reproductive success was accompanied by changes in the feather pigmentation of males. Plumage of male quail fed at these levels resembled that of adult females, and male pheasants and ducks did not exhibit the usual characteristic coloration. The effects of kepone upon plumage and reproduction appeared to be reversible, and normal coloration and increased reproductive success began to appear within 60 to 90 days after the removal of kepone from the diets.

Blackbirds, Cowbirds, Grackles, and Starlings

Tests upon these species were initiated to obtain measures of relative susceptibility to different toxicants, and of the quantities stored in tissues following varying degrees of exposure. All test specimens were wild-trapped birds that had been adjusted to cage conditions prior to being placed on test. All tests were terminated at the end of 30 days.

Results of these studies are given in table 2D. Starlings appeared to be more susceptible than the other species to the effects of chlorinated compounds.

Measurement of Pesticidal Residues

Chemical analyses of pesticidal residues are made as part of specific research programs both in the field and laboratory. Often the determinations are basic to an understanding of the problems and results. Some analyses are made to learn the extent of contamination of wild animals in the field. Among field-collected specimens from all sources, residues of DDT, heptachlor, dieldrin, or toxaphene were found in approximately 68 percent of 1,794 specimens analyzed at Patuxent during 1961 and 1962. Data for DDT, heptachlor, and dieldrin are shown in tables 4D and 5D.

This work is under the direction of James B. DeWitt, with assistance from chemists Vyto A. Adomaitis, Calvin M. Menzie, and William L. Reichel.

Effects of Pesticides on Woodcock Populations

Woodcock populations are in a particularly critical position in relation to pesticidal treatments for several reasons. First, a major proportion of the continental population winters in the Gulf States, in the same area where the fire ant control program is underway. Second, woodcock food consists in large part of earthworms, which can accumulate considerable amounts of chlorinated hydrocarbon insecticides without themselves being killed. Third, woodcock undergo periods of food shortage and cold on the wintering grounds, on their early migration northward, and on the breeding grounds. In the northern areas they are exposed further to a variety of chemicals applied to areas where they feed.

Several interrelated studies have been directed toward understanding the hazards of pesticides to woodcock. Several of these were completed in 1961-1962, some by experiments conducted in those years and some by analyzing data of experiments conducted earlier.

Capsule Dosage Tests

A series of experiments was conducted at Amherst, Massachusetts, under the direction of W. G. Sheldon in 1959 and at the Patuxent Wildlife Research Center by W. H. Stickel in 1960. A manuscript draft, "Effects on Woodcock of Capsule Dosages of Heptachlor, Dieldrin, and DDT," was prepared in 1962. The response of woodcock to the toxicants varied markedly depending on condition of the birds, as shown by weight changes since capture. With underweight birds, nearly all woodcock died at heptachlor dosage levels well below those at which nearly all birds of normal weight lived. Similarly, birds in good condition could scarcely be killed with oral doses of DDT, even in massive quantities. Dieldrin was more toxic than heptachlor to two groups of underweight birds. The study also showed that large portions of the capsule dosages passed quickly through the digestive tract without being assimilated. It was concluded that other methods of appraising the field effects of toxicants on woodcock had to be sought. This conclusion led to further studies.

As prerequisites to the capsule-dosage studies, methods of care of woodcock in captivity had to be developed. A draft manuscript, "Care of Woodcock in Captivity," was prepared in 1962. Another manuscript, "Some Diseases and Parasites of Captive Woodcock," was prepared as a result of studies made on these same birds by Louis N. Locke.

Feeding Experiments with Contaminated Earthworms

The second, and more critical, experimental studies were made in Louisiana in 1961 by W. H. Stickel, with woodcock caught on their winter feeding grounds. The purpose of these experiments was to measure the mortality effects of heptachlor presented in the food. Since woodcock accept only living earthworms, this entailed preliminary experiments in which methods were developed for contaminating worms. Woodcock were fed worms containing heptachlor residues in two concentrations; control birds were fed untreated worms.

Fifty percent of the birds (6 of 12) that fed on worms containing an average of 3 ppm (parts per million) of heptachlor residues died within 34 days. Four more birds died by the 51st day, and the two remaining were killed for chemical analysis. Worms collected in areas treated with 2 pounds per acre of heptachlor often contain more toxicant than the worms fed to the experimental birds.

Birds fed worms with an average residue content of about 0.65 ppm did not die during 60 days on full rations. The residue content of birds fed at this lower rate was of the same magnitude as that found in wild-caught birds in the same area. Birds fed untreated worms for an average of 98 days contained less toxicant in their tissues than did wild-caught birds, indicating loss of heptachlor residues with time.

Results of this study, including mortality under normal feeding and starvation rations of treated and untreated food, residue content, food consumption, and weight changes have been prepared in manuscript draft: "Effects on Woodcock of Feeding on Worms Contaminated with Heptachlor." Development of this study also entailed consideration of the effects of pesticides on earthworms. One aspect of this work resulted in a manuscript, "Effects of Chemicals on Earthworms: A Review of the Literature," by S. P. Davey.

Wing Survey

A woodcock wing survey was initiated during the 1959-1960 hunting season, with the view that age ratios shown by the wings might make it possible to detect any marked changes in reproductive success. Weighted age ratios shown by the wings received in 3 years (approximately 30,000 wings in all) were very similar: 1.8 immatures per adult female in 1959-1960 and 1960-1961 and 1.9 in 1961-1962. Sex ratios also were remarkably uniform each year. Although it seems likely that drastic changes in woodcock breeding success would have been noted, one cannot say that no changes occurred, for there was considerable variation in age and sex ratios shown by wings sent from different States and Provinces.

A manuscript has been prepared giving detailed results and discussion of the first 3 years' study, "Results of the 1959, 1960, and 1961 Woodcock Wing Collection Survey," by F. W. Martin, A. D. Geis, and W. H. Stickel. In the second year of the survey, F. W. Martin developed a new color-pattern method for determining age from wings. This method permits very rapid reading of age without microscopic examination of feathers. Further, it is applicable to late-season birds, which the microscopic method was not, and thus permits wing study of the wintering population. The manuscript describing this method is entitled, "Age and Sex Determination of Woodcock from Wings."

Residues in Field-Collected Woodcock

Woodcock have been collected from both southern and northern areas for determination of heptachlor residues; the first collections were made in 1958, and additional collections were made in subsequent years. Some analyses also have been made to determine DDT residues. The numbers analyzed in 1961 and 1962, proportions that contained residues, and average residue content are shown in table 4D. This study has been under the direction of James B. DeWitt. Summary and analysis of the data are underway.

Effects of DDT on Bald Eagles

People concerned with birds have feared for years that our national emblem, the bald eagle, is becoming increasingly scarce in the United States and that its reproductive success is low. Information on numbers, breeding success, and population trends is now being sought through surveys made annually by the National Audubon Society with the cooperation of the Fish and Wildlife Service.

According to the most recent report of these surveys (Sprunt and Cunningham, 1962), only 3,807 eagles were found in the January 1962 survey, which included all States other than Alaska. Immatures constituted 24 percent of the population. The 1962 breeding survey provided data of 515 nests, of which 257 were in Florida. Overall nesting success was 44 percent and at least 228 young eagles were added to the population. But no nests were reported for New England south of Maine, and nesting success was exceedingly poor in the central Atlantic States. In New Jersey, one nest in six produced young. In Maryland, not one of the 15 nests under observation was successful. In Virginia, two of 18 nests succeeded.

The marked lack of breeding success in coastal areas that are treated frequently for mosquito control has led some conservationists to suspect that since fish are a major item in the eagle's diet, eagle reproduction has been inhibited by DDT residues accumulated by feeding on contaminated fish. Other workers have felt that a more probable explanation was intensified human disturbance, for few areas of the East Coast are now free of houses and motor boats.

As the first step in resolving this question, the Fish and Wildlife Service asked to have all bald eagles that were found dead sent to the Patuxent Wildlife Research Center for DDT analysis. Analyses, reported below, quickly proved that some eagles did contain many parts per million of DDT. But what did such residues mean in relation to eagle mortality? What degree of contamination of fish was necessary to produce such residues? These questions required experimentation.

Alaskan Experimental Studies

The necessary tests were begun at Petersburg, Alaska, in the winter of 1961-1962. Alaska was selected because eagles were still abundant there and because DDT levels were still low in Alaskan animals. Availability of the facilities of the experimental fur farm operated by the University of Alaska made Petersburg the best place for the work.

Sheltered perches for 10 eagles were built. Eagles were captured near Haines, Alaska, by men of the Service's Alaskan Regional Office. They were fed ground fish supplemented with vitamins. DDT dissolved in edible oil was mixed with the feed. Two of the birds were fed clean food; both lived through the 112-day experiment. Two were fed 10 ppm (parts per million) of DDT, 2 were fed 160 ppm, 2 were fed 800 ppm, and 2 were fed 4,000 ppm. The objectives were to find approximately where the lethal level lay, to learn what residues occurred in birds killed by DDT, and to learn how rapidly residues accumulated in eagles feeding on fish containing relatively small amounts (10 ppm) of DDT, such as could occur in nature.

The birds on 4,000 ppm soon died, and a replacement quickly died at the same level. Birds on 800 ppm died a little later. One of the birds on 160 ppm lived throughout the experiment, but the other died after severe tremors. It is likely, therefore, that the lethal level is of the general magnitude of 160 ppm.

As a wild eagle would rarely if ever obtain food contaminated at a level approaching 160 ppm, it seems that an eagle in the wild would die of DDT poisoning only if its system accumulated DDT for long periods. But over long periods the bird would also be excreting DDT or its metabolites, and excretion might well come into balance with intake. It therefore became necessary to learn about the rates of assimilation and excretion of DDT at low levels.

Unfortunately, one of the experimental eagles on 10 ppm languished and died early in the test and the other escaped near the end of the test. Thus no data were gained on residue buildup at low levels. Work was resumed in 1962-1963, with the aim of studying accumulation and loss of DDT in eagles fed 10 ppm of DDT. This work was in early stages at the time of writing and results will not be known before late 1963.

Concentrations of DDT in livers of the experimental birds were as follows: Untreated (fed no DDT), 0.4 to 1.0 ppm (parts per million); fed 10 ppm, 40.5 ppm; fed 160 ppm, 43.3 and 43.8 ppm; fed 800 ppm, 57.8 and 280 ppm; fed 4,000 ppm, 390 and 714 ppm.

Residues in Field-Collected Eagles

Twenty-five of the 26 bald eagles examined contained residues of DDT in amounts ranging from traces in one or more organs up to amounts comparable to those found in experimentally dosed birds that died of DDT poisoning. Three eagle eggs, taken from two unsuccessful nests following abnormally long incubation periods, also contained relatively high concentrations of DDT. An interim report concerning the eagle studies was published (DeWitt and Buckley, 1962).

Effects of Heptachlor on Bobwhite and Songbirds

Aerial applications of heptachlor to large portions of the Southeast in the range of the imported fire ant were initiated in 1957-1958, with treatments at the rate of 2 pounds per acre. Rate recommendations subsequently were reduced to 1¼ pounds per acre, and toward the end of 1960, recommendations were changed to ½ pound per acre, to be applied in two treatments of ¼ pound each. Although many extensive treatments in 1962 were made with a new chemical, Mirex, hundreds of thousands of acres in Louisiana alone were planned for heptachlor treatment at the rate of ½ pound per acre in the winter and spring of 1962-1963.

Enclosure Studies of Bobwhite

Experimental data on effects of heptachlor on bobwhite were obtained from enclosure studies conducted at Patuxent under the direction of James B. DeWitt. Adult birds were confined in 20 x 50-foot wire-covered pens enclosing areas where the ground had been treated with the insecticide. Application rates were those which have been used for control of imported fire ants: 2.0 pounds per acre (6 pens); 1.25 pounds per acre (6 pens); and 0.25 pound per acre (12 pens). Eight other pens remained untreated, and served as controls or check areas. One pair of birds was placed in each pen immediately after application of the insecticide. When mortality occurred in any pen, the surviving member of the pair was sacrificed, and a new pair was placed in the pen.

Results of these tests are given in table 6D. Mortality occurred in 20 of the 22 pairs placed in pens treated with 2.0 pounds per acre, and the survival times for these birds were approximately equal to those of birds fed diets containing 100 or 200 ppm of heptachlor. The two surviving hens produced a total of 7 chicks. Sixteen pairs were started in pens treated with 1.25 pounds per acre, but only four pairs survived. Times of death in these pens were approximately equal to those of birds fed 50 or 100 ppm. All 4 of the surviving hens produced eggs, and 19 chicks were hatched from the two successful nests.

Mortality rates and survival times for birds in pens treated with 0.25 pound per acre were similar to those of quail fed diets containing 10 or 25 ppm of heptachlor. One bird was killed by a predator, and six of the remaining 18 pairs survived. All of the surviving hens produced eggs, and 24 chicks were hatched in the three successful nests.

Eleven pairs were started in the control pens, but 1 bird was killed by a predator. Five of the remaining 10 pairs survived, and produced 21 chicks from the three successful nests.

Toxicological Studies and Residue Determinations

Tests with heptachlor and heptachlor epoxide made at Patuxent are summarized in tables 1D and 2D. Results of residue analyses are shown in table 4D. For discussion of experiments, see pages 74-76.

An account of the heptachlor residue content of quail and other animals collected or found dead on treated and untreated land was published (Rosene, et al., 1962).

Bobwhite Populations in Georgia

Quail populations in heptachlor-treated areas in Georgia and on an untreated area in Alabama have been studied by whistling cock counts and covey counts each year since 1958. The work has been conducted by Walter Rosene, who reported early results (Rosene, 1958). Field work was discontinued after covey counts were made in early 1962. On one tract, treated at the rate of 2 pounds per acre in 1957-1958, the quail counts were very low after treatment and increased gradually throughout the study. Populations in the untreated area in Alabama remained essentially constant. Portions of another area were treated with different amounts of heptachlor at various dates, and certain portions remained untreated. On this area, sharp initial population decline and subsequent gradual increase have been followed, with particular consideration of the relationships between treated and untreated portions. Statistical analyses of the data and preparation of a report for publication are well underway.

Songbirds in Alabama

Effects on birds of a 2-pound per acre application of heptachlor were studied for 2 years on a 2,400-acre cattle farm in Alabama. In 1962, data from this study were assembled in a report of 198 pages and 22 tables, "Mortality and Repopulation of Birds Following a Field Application of Heptachlor," by Paul A. Stewart. This report shows that there was heavy mortality of birds of many species, and that all individuals of some species were lost. Many of the missing birds were found dead. Reproductive success of birds on the treated area was lower than that on the control area. Certain species did not succeed in reestablishing their populations on the treated area by the end of the study.

Birds in Arkansas

Effects of ¼-pound applications of heptachlor in Union County were studied by University of Arkansas students under the direction of Dr. Douglas James. Bird population studies and systematic searches for dead animals were made on two treated and two untreated areas. The study was initiated and supported by the Bureau. A report is being prepared by Dr. James.

Effects of Mirex on Wildlife

In 1962, Mirex (Compound GC 1283) became the chemical most generally used for control of fire ants. Some extensive acreages were treated with two applications of ¼-pound of heptachlor per acre, and various industrial or commercial areas were treated with 1¼- or 2-pound applications of heptachlor. Mirex, however, was used on more extensive areas and was expected to gain even wider use in the future.

Mirex, an analog of kepone, is applied at the rate of only 4 grams per acre. It is dissolved in soy-bean oil and is distributed on ground corn cobs. Ants are attracted to this oily bait. Some highly successful examples of control are reported by the U. S. Department of Agriculture.

Laboratory Studies

In studies made at Patuxent, Mirex had no apparent effects upon reproduction or secondary sex characteristics of quail or pheasants. The compound has a relatively low order of toxicity to these species, and both young and adult birds survived for extended periods when fed diets containing 200 ppm. Feeding at higher levels (500 to 5,000 ppm) resulted in the appearance of typical symptoms of chlorinated hydrocarbon poisoning, including tremors, loss of coordination, and death within 48 or 72 hours after the onset of these symptoms.

Field Studies

Little wildlife damage in the field was anticipated, because of the low toxicity and low rate of application of the compound. Mirex bait is applied blanket-wise, however, over woods, waters, and fields, so it was desirable to see what actually happened in the field.

The study method selected consisted of systematic, periodic observations over large areas of animal-rich habitats. Observers searched for dead animals and for any other indications of damage to animal populations or alterations of behavior. This approach was followed by Clark Webster and Enos Mellinger, Branch of Refuges, near Savannah, Georgia; by Ralph Andrews, near Baton Rouge, Louisiana; and by Walter Rosene, Jr., in northeastern Mississippi.

None of these studies revealed any wildlife damage that could be attributed to Mirex. Vertebrate populations appeared to remain normal in all respects. As a more rigorous test, experiments are underway in which bobwhite quail are held on ground treated at far heavier levels of Mirex than are used operationally. Preliminary reports from Dr. Maurice Baker, who is conducting this work at Auburn University, indicated that quail held on heavily treated land suffered no visible damage during the first month and that the experiment would be continued.

Effects on Birds and Small Mammals of DDT Used for Elm Spanworm Control

The U. S. Forest Service, faced with an outbreak of elm spanworm, Ennomos subsignarius (Hbn.), that was threatening to kill many trees of the deciduous forest at the Coweeta Hydrological Station, North Carolina, decided to make an aerial application of 1 pound of DDT per acre in oil solution. The U. S. Fish and Wildlife Service was invited to study the effects on wildlife. Teams of men led by Frank M. Johnson and James S. Lindzey trapped and marked songbirds and small mammals before treatment, which was in late May of 1961, then a week after treatment, and again a month after treatment. Two treated areas and two untreated areas were studied. Birds were taken in grids of mist nets, marked, and released in 3-day study periods. Small mammals were taken in lines of live traps, marked, and released. Each trap was open for 3 days, but was closed on alternate days, so each trapping period was of 6 days.

No mortality that could be attributed to the treatment was detected. Results were clear in the small mammal work, for as large a proportion of marked mice (Peromyscus) was recaptured in treated as in untreated areas. Data for birds were less satisfactory, for although 797 birds of 43 species were caught, recaptures were too few to provide good comparative data.

Deaths of birds and mammals would not have been expected from 1 pound per acre of DDT, and in the present study the amount reaching the ground was considerably less than 1 pound per acre at most spots, as shown by oil-sensitive dye-cards. The chief values of the study, therefore, were in affording opportunity to make practical tests of certain study methods and in obtaining animals for residue analysis. Chemical analyses are underway.

Effects of Pesticides Applied to Aquatic Areas

Herbicides Used for Control of Eurasian Watermilfoil

Studies were initiated in cooperation with personnel of the Chesapeake Biological Laboratory of the University of Maryland Natural Resources Institute and the Virginia Institute of Marine Science. In first tests, granular formulations of 2,4-D esters and salt were applied experimentally for control of Eurasian watermilfoil in Chesapeake Bay, the Potomac River, and tributaries at rates of 10-120 pounds acid equivalent per acre in plots ranging from 1/10 to 6 acres in size. In one instance the dying vegetation in a plot treated with 60 pounds per acre in an area with poor water circulation caused an anaerobic condition that killed oysters, crabs, and fish confined in cages, and free-living clams, worms, and other invertebrate bottom life. A later effort to create an anaerobic condition for study of the factors contributing to this situation was unsuccessful.

DDT Used for Mosquito Control

An agreement was entered into with the University of Minnesota to study the effect of DDT applications for mosquito control on the aquatic life of waterfowl ponds. Bioassays showed no detectable DDT in aquatic invertebrates or vegetation collected in late September from ponds treated in early spring at the rate of approximately 1 pound of chemical per acre. Small quantities of DDT were recovered in the bottom mud. Radioactive DDT is being introduced into mud samples to determine the best methods of recovery.

Methods of Measuring Effects of Pesticides on Wildlife

Measurement of changes in animal populations is extremely difficult; some of the generally accepted methods are largely untested. The necessity of assigning causes to observed changes, as in the evaluation of pesticidal treatments, makes the problem even more severe. Several studies in 1961 and 1962 were directed toward an understanding of the meaning of measurement methods or development of new methods.

Carcass Disappearance Rates

Consideration of the implications of finding carcasses in the field was begun in studies in Alabama and Texas (Rosene and Lay, 1963). Subsequently, quail disappearance rates were studied at Patuxent by James Cross. In all three areas, all carcasses were at least partially eaten within 48 hours. The proportions completely gone within 4 days were 90 percent of 21 carcasses at Patuxent, 47 percent of 30 carcasses in Alabama, and 13 percent of 30 carcasses in Texas. Judging from the distances carcasses could be seen, and the distances a man could walk in a day of searching, Rosene and Lay concluded that failure to find dead quail is poor evidence that no quail died and that finding even a small number of dead quail may suggest considerable mortality.

Carcass Search Technique

Other studies at Patuxent have been aimed at appraisal of the carcass-search technique as a practical field method. The first exploratory trial showed that a high efficiency of search was possible in relatively small areas with ample time. A human searcher found all the birds in the section of hedge-border searched and 75 percent of those in the field. A man and dog team searched immediately thereafter and found 81 percent of all birds present in field and borders, but in a much shorter time. Two further studies, experimentally designed, showed great variation among individual searchers in their ability to find small dead birds. In one set of four independent searches, one biologist found more than three-fourths of the carcasses present, whereas the other found less than one-third. In another search, involving 36 persons, individual success again varied greatly, but the overall success was 60 percent.

Small-Mammal Census with Live Traps

Two extensive field tests were made of the reliability of standard census techniques in detecting losses in wild populations. The first test involved live trapping Peromyscus populations. It was conducted by Don W. Hayne and Mary F. Myers. Four areas of 4 acres each were trapped simultaneously, using grids of live traps. One-third of the mice were then removed from two of the areas and trapping was continued on all four areas as before. Estimates from the comparative data indicated a mortality of 37 percent in the areas from which mice were removed, surprisingly close to the real value of 33 percent. This demonstrated that population changes in Peromyscus, such as the changes caused by insecticide applications or rodent control operations, can be measured quite accurately by standard mark-and-recovery methods that employ live traps on a grid. Peromyscus is especially suitable for such work because of its dependable trap response. It is not likely that many other kinds of small mammals would yield equally clear-cut results. Fortunately, Peromyscus is the most abundant mouse in many habitats and is one of the chief mammals involved in problems of forest reseeding.

Bird Census by Two Methods

The second test was a detailed quantitative comparison of the territorial-male census (Williams method) and the mist-net census, in which mortality up to 50 percent was imposed on netted birds of common species to see how well and how long this mortality was reflected in censuses of both types. C. S. Robbins conducted the study with assistance from others, especially James Cross and David Bridge. The study was made on a 100-acre tract of moist deciduous forest at the Patuxent Wildlife Research Center in the summer of 1962. Data have not been completely analyzed, but preliminary tabulations show that the Williams method detected a loss, but estimated it at only about one-fourth of the actual reduction. Repopulation occurred so rapidly that it masked the reduction. This phenomenon is just one of several variables that make it extremely difficult to obtain close measurements of changes in bird populations.

Table 1D. Quantities of pesticides causing 50 percent mortality of bobwhite, ring-necked pheasants, and mallards

Mg/kg eaten indicates the average amount of toxicant that had been consumed (milligrams of toxicant per kilogram of bird) by the time 50 percent of the birds had died; ppm indicates parts per million of toxicant in the diet; the symbol < means "less than"; the symbol > means "greater than", and in the body of the table indicates that the dietary concentrations and total toxicant consumption so marked did not produce 50 percent mortality in the test period.

Compound	Test period (days)	Bobwhite				Ring-necked pheasants				Mallards			
		Young		Adult		Young		Adult		Young		Adult	
		ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten
Aldrin	<10	10	4.5	10	3.5	10	6.5	50	4.3	100	190	1000	105
	<100	2	9.5	2	6.5	5	11.5	5	18	50	540	200	285
Amiben	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	>1000	>7800	-	-	-	-	-	-	-	-
Amitrole	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	5000	26000	5000	200000	5000	35500	>5000	>13600	5000	15750	5000	>80000
Amitrole-T	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	>5000	>78000	>5000	>51500	5000	16100	>1500	>12500	>2500	>17500	>2500	>13565
Bayer 22408	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	200	3265	-	-	-	-	-	-	-	-	-	-
Bayer 22684	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	200	1920	-	-	-	-	-	-	-	-	-	-
Bayer 25141	<10	25	263	25	16	50	160	-	-	-	-	-	-
	<100	5	42	5	64	-	-	-	-	-	-	-	-
Bayer 29493	<10	25	24	-	-	200	400	-	-	100	120	2500	2
	<100	10	29	25	76	50	410	-	-	>10	>140	500	85
Bayer 38920	<10	250	150	-	-	100	270	-	-	250	320	-	-
	<100	100	300	-	-	25	155	-	-	100	900	1000	780
BHC	<10	1000	400	-	-	250	150	-	-	500	480	-	-
	<100	100	950	100	230	>100	>1200	>100	>800	-	-	2500	1850
Casoron	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	>1000	>9000	-	-	-	-	-	-	-	-
Ceresan	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	100	190	-	-	100	1700	-	-	50	80
Chlordane	<10	250	700	1000	90	500	550	-	-	-	-	-	-
	<100	100	500	250	730	50	170	200	340	-	-	-	-
Chlorobenzilate	<10	-	-	-	-	5000	7100	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Chlorthion	<10	5000	1000	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Co-Ral	<10	100	90	-	-	500	650	1000	160	250	65	-	-
	<100	10	75	>50	>600	200	1700	250	250	50	385	-	-
2,4-D acetamide	<10	2500	1825	-	-	1000	3000	-	-	2500	3400	-	-
	<100	-	-	>2500	>37600	-	-	>2500	16000	>500	>5700	>2500	>35700
2,4-D dimethyl amine salt	<10	5000	8250	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	5000	19780	>5000	>6500	2500	22100	-	-
2,4-D butoxy-ethanol ester	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	5000	37900	5000	40760	5000	29430	-	-	5000	1485	>5000	>33000
Dacthal	<10	5000	5500	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	5000	35775
Dalapon	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	5000	39566	>5000	>69500	-	-	>5000	>40000	5000	15100	>2500	>26000

(continued)

ring-necked pheasants, and mallards (Continued)

Compound	Test period (days)	Bobwhite				Ring-necked pheasants				Mallards			
		Young		Adult		Young		Adult		Young		Adult	
		ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten
DDT	<10	1000	1480	2500	880	1000	2600	1000	225	500	1630	-	-
	<100	400	3200	1000	1000	100	650	>100	>570	>200	>4250	1000	>3800
DDVP	<10	5000	1700	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Delnav	<10	100	250	-	-	-	-	-	-	-	-	-	-
	<100	-	-	500	2140	5000	15400	>1000	>5500	-	-	-	-
Dexon	<10	250	475	-	-	2500	140	-	-	-	-	-	-
	<100	-	-	-	-	1000	160	-	-	-	-	-	-
Diazinon	<10	100	360	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Dibrom	<10	250	640	1250	420	5000	10670	-	-	5000	6750	-	-
	<100	>100	>1100	>100	>1000	1000	2300	>5000	>12000	2500	15800	-	-
Dieldrin	<10	20	10	50	4.2	100	90	-	-	100	40	-	-
	<100	50	47	10	40	5	35	50	75	-	-	50	420
Dimethoate	<10	100	665	250	95	200	585	-	-	-	-	-	-
	<100	50	180	>25	>250	50	425	5000	2875	-	-	5000	340
Diphenamid	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	>1000	>9600	-	-	-	-	-	-	-	-
Dipterex	<10	750	425	-	-	5000	2500	-	-	-	-	-	-
	<100	250	215	>100	>4000	1000	2680	>100	>560	-	-	-	-
Diphenatrile	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	>1000	>8400	-	-	-	-	-	-	-	-
Disyston	<10	1000	800	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Dyrene	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	>5000	>63000	>5000	>83000	>5000	>5000	-	-	-	-	-	-
Endrin	<10	10	12	10	1	5	3	50	7	25	25	-	-
	<100	5	8	2	10	-	-	5	21	-	-	-	-
EPN	<10	200	220	-	-	-	-	-	-	-	-	-	-
	<100	100	1200	-	-	-	-	-	-	-	-	-	-
GC-3707	<10	1000	1600	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Guthion	<10	-	-	-	-	5000	2000	-	-	-	-	-	-
	<100	250	5500	>500	>5000	1000	6600	>50	>350	-	-	-	-
Heptachlor	<10	50	45	200	55	100	305	>500	>200	200	590	1000	200
	<100	>10	>160	25	90	50	140	100	180	100	425	100	410
Heptachlor epoxide	<10	50	55	-	-	-	-	-	-	-	-	-	-
	<100	5	40	10	110	-	-	-	-	-	-	-	-
Kelthane	<10	1000	2060	-	-	2000	1750	-	-	-	-	-	-
	<100	250	410	-	-	-	-	-	-	-	-	-	-
Kepone	<10	400	460	500	225	500	1290	500	130	200	620	-	-
	<100	100	1600	250	250	100	615	150	590	100	850	200	435

(continued)

Table 1D. Quantities of pesticides causing 50 percent mortality of bobwhite, ring-necked pheasants, and mallards (Continued)

Compound	Test period (days)	Bobwhite				Ring-necked pheasants				Mallards			
		Young		Adult		Young		Adult		Young		Adult	
		ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/k eate
Lindane	<10	500	1070	-	-	250	175	-	-	500	415	2500	100
	<100	200	930	1000	1050	>100	>1800	>100	>630	-	-	-	-
Malathion	<10	1000	780	-	-	1000	550	-	-	-	-	-	-
	<100	100	400	250	5400	-	-	>500	>2800	-	-	-	-
MCPA	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	5000	19200	5000	47150	-	-	>5000	>85000	5000	1070	-	-
Methoxychlor	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	5000	15000	1000	3500	>2500	>23000	>2500	>15000	-	-	-	-
Mirex	<10	1500	4900	>5000	>5400	500	350	-	-	-	-	-	-
	<100	300	1550	500	2050	100	540	>300	>2300	1000	10400	-	-
Parathion	<10	100	145	-	-	-	-	-	-	-	-	-	-
	<100	50	140	-	-	25	3	-	-	-	-	-	-
Perthane	<10	5000	9200	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Phosdrin	<10	-	-	1000	90	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Phosphamidon	<10	5	4	50	6	500	315	250	90	500	430	>200	>1
	<100	1	3	10	80	-	-	>200	>270	>100	>2125	100	2
Rhothane	<10	2500	2350	5000	260	500	1100	5000	380	5000	1530	-	-
	<100	1000	6000	2500	2830	100	700	1000	470	1000	14800	2500	75
Semesan	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	>500	>10
Sevin	<10	>5000	>30000	-	-	-	-	-	-	-	-	-	-
	<100	2500	10850	>1000	>13700	5000	20550	>5000	>31600	-	-	-	-
Strobane	<10	500	225	-	-	-	-	-	-	-	-	-	-
	<100	200	400	500	3600	500	1600	>300	2150	-	-	-	-
Systox	<10	1000	680	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Thimet	<10	200	830	-	-	500	350	>100	>100	-	-	-	-
	<100	-	-	-	-	-	-	-	-	-	-	-	-
Thiodan	<10	300	270	-	-	500	620	-	-	1000	200	>5000	>7
	<100	100	380	>250	>2600	>300	>1400	1000	850	-	-	1000	3
Toxaphene	<10	1000	500	-	-	500	300	-	-	1000	2000	-	-
	<100	250	1500	>250	>2100	100	450	>100	>600	-	-	-	-
2,4,5-TP butoxy-	<10	5000	9350	-	-	-	-	-	-	-	-	-	-
ethanol ester	<100	-	-	-	-	5000	9240	-	-	5000	21000	5000	416
Zectran	<10	250	100	-	-	1000	1470	-	-	-	-	-	-
	<100	-	-	-	-	500	7000	-	-	-	-	2500	3
Zytron	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	1000	680	-	-	-	-	-	-	-	-
Lead nitrate	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	>500	>10000	>50	>1
Sodium arsenite	<10	-	-	-	-	-	-	-	-	-	-	-	-
	<100	-	-	-	-	-	-	-	-	>100	>1600		

cowbird, common grackle, and starling1/

Compound	Test period (days)	Blackbirds		Cowbirds		Grackles		Starlings	
		ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten	ppm in diet	mg/kg eaten
Aldrin	<10	-	-	100	140	-	-	-	-
	<30	-	-	-	-	-	-	-	-
Chlordane	<10	-	-	500	850	-	-	-	-
	<30	-	-	-	-	-	-	-	-
Co-Ral	<10	25	55	25	35	-	-	50	120
	<30	10	35	10	50	-	-	25	80
DDT	<10	1000	1700	-	-	-	-	-	-
	<30	500	700	-	-	-	-	-	-
Dibrom	<10	1000	1250	250	520	250	540	250	600
	<30	250	200	-	-	-	-	-	-
Dieldrin	<10	-	-	50	100	100	60	25	45
	<30	-	-	5	25	10	55	5	35
Dimethoate	<10	-	-	200	70	-	-	-	-
	<30	-	-	-	-	-	-	-	-
Heptachlor	<10	100	480	-	-	100	140	10	30
	<30	25	140	50	330	25	180	5	35
Kepone	<10	200	210	-	-	-	-	-	-
	<30	50	135	-	-	-	-	-	-
Phosphamidon	<10	50	20	25	12	-	-	10	30
	<30	10	20	10	25	-	-	-	-
Rhothane	<10	-	-	5000	3460	-	-	-	-
	<30	-	-	-	-	-	-	-	-
Thiodan	<10	-	-	1000	1200	-	-	-	-
	<30	-	-	-	-	-	-	-	-
Zectran	<10	-	-	2500	2050	-	-	-	-
	<30	-	-	-	-	-	-	-	-

1/ See headnote in table 1D.

Table 3D. Effects of pesticides on survival and reproduction of bobwhite, ring-necked pheasants, and mallards. Summary of tests at Patuxent during 1961 and 1962.

Compound	Level in diet (ppm)	Survival, percent (100 days)						Chicks per hen		
		Bobwhite		Pheasants		Mallards		Bob-white	Pheasants	Mal-lards
		Young	Adult	Young	Adult	Young	Adult			
[Controls]	-	75.3	97.0	79.7	92.0	89.4	92.0	29.1	10.7	12.4
Amitrole	5000	20.0	85.0	37.0	90.0	12.0	100.0	0.25	-	0
	2500	-	-	-	-	-	55.0	-	-	0
	1000	-	-	-	-	96.0	100.0	-	-	0
Amitrole-T	5000	52.0	89.0	0	-	-	-	-	-	-
	2500	-	-	-	-	-	75.0	-	-	0
	1000	-	-	-	-	76.0	83.0	-	-	0
Dacthal	5000	0	-	-	-	-	75.0	-	-	-
Dalapon	5000	40.0	95.0	-	79.0	24.0	-	42.0	6.3	-
	2500	-	-	-	-	-	85.0	-	-	2.6
	1000	-	-	-	-	68.0	94.0	-	-	4.7
2,4-D acetamide	5000	0	-	12.0	-	-	-	-	-	-
	2500	28.0	100.0	0	89.0	0	100.0	2.3	5.1	0
	1000	-	-	0	-	-	95.0	-	-	1.3
	500	-	-	-	-	89.0	100.0	-	-	10.7
2,4-D dimethyl amine salt	5000	0	-	0	-	-	-	-	-	-
	2500	-	-	-	-	-	35.0	-	-	0
	1250	-	-	-	-	-	95.0	-	-	0
	500	-	-	-	-	76.0	100.0	-	-	5.[illegible]
2,4-D butoxy-ethanol ester	5000	32.0	35.0	8.0	-	45.0	92.0	-	-	0
	2500	-	-	-	-	55.0	81.0	-	-	0
	1000	-	-	-	-	88.0	95.0	-	-	1.[illegible]
MCPA	5000	24.0	69.0	-	100.0	0	-	-	2.9	-
2,4,5-TP butoxy-ethanol ester	5000	12.0	-	8.0	-	0	42.0	-	-	0
	2500	-	-	-	-	47.0	25.0	-	-	0
	1000	-	-	-	-	88.0	100.0	-	-	5.[illegible]

during 1961 and 1962

Entire carcass, exclusive of skin, feathers, fur, feet, and gastrointestinal tract, analyzed. Minus indicates no detectable residues by methods employed. Averages based on all specimens analyzed, counting minus as zero.

Species	Toxicant								
	Heptachlor epoxide			Dieldrin			DDT		
	No. Analyzed		ppm	No. Analyzed		ppm	No. Analyzed		ppm
	Minus	Plus	(Average)	Minus	Plus	(Average)	Minus	Plus	(Average)
Birds									
Blackbird, red-winged	4	17	4.1	-	-	-	-	-	-
Bluebird	1	1	2.9	0	2	2.5	-	-	-
Bunting, indigo	-	-	-	-	-	-	0	4	7.6
Cardinal	5	4	3.3	1	4	4.7	0	2	7.9
Catbird	-	-	-	-	-	-	0	1	21.1
Chickadee	0	1	1.2	-	-	-	-	-	-
Cowbird, brown-headed	5	8	2.3	-	-	-	-	-	-
Creeper, brown	-	-	-	0	1	9.4	-	-	-
Crow	1	0	0	0	1	4.4	-	-	-
Dove, mourning	11	17	2.2	-	-	-	-	-	-
Duck, fulvous tree	-	-	-	0	1	Trace	-	-	-
Duck, blue-winged teal	1	0	0	-	-	-	-	-	-
Flicker	-	-	-	1	0	0	-	-	-
Flycatcher, Acadian	-	-	-	-	-	-	0	1	6.7
Flycatcher, crested	-	-	-	-	-	-	0	1	14
Flycatcher, yellow-bellied	-	-	-	-	-	-	0	1	26.4
Grackle	0	1	8.1	-	-	-	-	-	-
Hawk, sparrow	0	1	15	-	-	-	-	-	-
Jay, blue	1	3	1	0	11	3.6	-	-	-
Junco	0	2	3.8	6	19	12.6	-	-	-
Killdeer	1	3	3.7	1	0	0	-	-	-
Kingbird	1	0	0	-	-	-	-	-	-
Martin, purple	1	0	0	-	-	-	-	-	-
Meadowlark, eastern	6	15	2.7	0	1	2	-	-	-
Mockingbird	3	1	9.8	1	0	0	-	-	-
Ovenbird	-	-	-	-	-	-	1	10	4.3

(Continued)

Table 4D. Pesticide residues in field specimens found dead or collected and analyzed at Patuxent during 1961 and 1962 (continued)

Species	Toxicant								
	Heptachlor epoxide			Dieldrin			DDT		
	No. Analyzed		ppm	No. Analyzed		ppm	No. Analyzed		ppm
	Minus	Plus	(Average)	Minus	Plus	(Average)	Minus	Plus	(Average)
Owl, barred	1	0	0	-	-	-	-	-	-
Owl, screech	0	1	6.8	0	1	2.5	-	-	-
Pigeon	-	-	-	1	0	0	-	-	-
Pipit	3	2	1.2	-	-	-	-	-	-
Quail, bobwhite	11	40	2.1	7	17	1.2	-	-	-
Rail, king	0	1	<1	-	-	-	-	-	-
Rail, sora	0	1	1.6	-	-	-	-	-	-
Rail, Virginia	0	2	7.6	-	-	-	-	-	-
Robin	10	16	4.2	19	5	2.7	-	-	-
Shrike, loggerhead	0	1	<1	-	-	-	-	-	-
Snipe, common	5	13	4.1	1	2	2.9	0	4	9.4
Sparrow, chipping	1	0	0	1	0	0	-	-	-
Sparrow, English	6	10	7.1	1	9	10	-	-	-
Sparrow, field	1	0	0	2	1	<4	-	-	-
Sparrow, savannah	3	2	8.9	-	-	-	-	-	-
Sparrow, song	2	4	<2	-	-	-	-	-	-
Sparrow, swamp	2	0	0	-	-	-	-	-	-
Sparrow, vesper	1	4	5.9	-	-	-	-	-	-
Sparrow, white-throated	2	11	6.8	6	7	12	-	-	-
Starling	0	1	Trace	1	1	6.6	-	-	-
Tanager, scarlet	-	-	-	-	-	-	0	4	9.4
Thrasher, brown	4	9	4	2	7	2.9	-	-	-
Thrush, Louisiana water	-	-	-	-	-	-	0	1	12.7
Thrush, Swainson's	-	-	-	0	1	3.7	-	-	-
Thrush, wood	-	-	-	-	-	-	0	7	7.7
Titmouse, tufted	-	-	-	-	-	-	0	1	10
Towhee, rufous-sided	1	4	3.4	1	0	0	6	32	7.3
Veery	-	-	-	-	-	-	0	2	8.1
Vireo, solitary	-	-	-	-	-	-	0	2	13.5
Vireo, red-eyed	-	-	-	-	-	-	0	2	3

(Continued)

during 1961 and 1962 (continued)

Species	Toxicant: Heptachlor epoxide No. Analyzed Minus	Heptachlor epoxide No. Analyzed Plus	Heptachlor epoxide ppm (Average)	Dieldrin No. Analyzed Minus	Dieldrin No. Analyzed Plus	Dieldrin ppm (Average)	DDT No. Analyzed Minus	DDT No. Analyzed Plus	DDT ppm (Average)
Warbler	2	1	18	-	-	-	-	-	-
Warbler, black-and-white	-	-	-	-	-	-	1	3	5.1
Warbler, black-throated blue	-	-	-	-	-	-	0	2	21.5
Warbler, Canada	-	-	-	-	-	-	0	2	13.4
Warbler, chestnut-sided	-	-	-	-	-	-	0	2	11.3
Warbler, hooded	-	-	-	-	-	-	0	3	17.3
Warbler, Kentucky	-	-	-	-	-	-	0	2	13.1
Warbler, myrtle	1	1	Trace	-	-	-	1	1	4.8
Warbler, worm-eating	-	-	-	-	-	-	0	1	8
Woodcock	90	190	1.6	-	-	-	91	129	1.7
Woodpecker, red-bellied	2	1	3.9	-	-	-	-	-	-
Woodpecker, red-headed	1	0	0	-	-	-	-	-	-
Wren, Carolina	0	1	<4						
<u>Mammals</u>									
Bat, brown	-	-	-	-	-	-	0	1	21.1
Bat, hoary	-	-	-	-	-	-	0	1	22
Mouse, cotton	1	2	5.1	-	-	-	-	-	-
Mouse, deer	-	-	-	1	5	13.9	-	-	-
Mouse, fulvous harvest	8	9	6.4	0	10	8.4	-	-	-
Mouse, harvest	2	8	20.5	-	-	-	-	-	-
Mouse, house	4	4	4.7	-	-	-	-	-	-
Mouse, oldfield	0	3	3.5	-	-	-	-	-	-
Mouse, white-footed	4	5	4.9	-	-	-	-	-	-
Opossum	1	0	0	-	-	-	-	-	-
Rabbit, cottontail	0	5	1.2	0	4	1.4	-	-	-
Rat, cotton	7	23	2.9	1	0	0	-	-	-
Rat, rice	4	15	3.2	-	-	-	-	-	-
Vole, pine	6	12	2	1	0	0	-	-	-

(Continued)

Table 4D. Pesticide residues in field specimens found dead or collected and analyzed at Patuxent during 1961 and 1962 (continued)

Species	Toxicant								
	Heptachlor epoxide			Dieldrin			DDT		
	No. Analyzed		ppm	No. Analyzed		ppm	No. Analyzed		ppm
	Minus	Plus	(Average)	Minus	Plus	(Average)	Minus	Plus	(Average)
Amphibians									
Bullfrog	-	-	-	1	0	0	-	-	-
Frog, leopard	-	-	-	1	1	Trace	-	-	-
Frog, unidentified	1	0	0	-	-	-	-	-	-
Reptiles									
Snake, black racer	1	0	0	-	-	-	-	-	-
Snake, hognose	0	1	2	-	-	-	-	-	-
Snake, ribbon	2	0	0	1	0	0	-	-	-
Turtle, red-eared	1	1	172	-	-	-	-	-	-
Fish									
Bass, largemouth	-	-	-	3	0	0			
Bluegill	5	3	4.5	-	-	-	-	-	-
Catfish	0	2	0.5	1	2	7.1	-	-	-
Chub, S. E. Creek	-	-	-	0	1	Trace	-	-	-
Pickerel, chain	-	-	-	0	1	11.4	-	-	-
Miscellaneous									
Crayfish	0	1	1.6	-	-	-	-	-	-
Crickets	2	1	3.4	-	-	-	-	-	-
Earthworms	2	5	2	-	-	-	-	-	-
Millipede	-	-	-	1	1	<4	-	-	-

Table 5D. Pesticide residues in tissues of field specimens analyzed at Patuxent during 1961 and 1962

Species	Toxicant	Tissues Analyzed														
		Liver			Kidney			Heart			Muscle			Brain		
		No. Analyzed		ppm	No. Analyzed		ppm	No. Analyzed		ppm	No. Analyzed		ppm	No. Analyzed		ppm
		Minus	Plus	(Av.)	Minus	Plus	(Av.)	Minus	Plus	(Av.)	Minus	Plus	(Av.)	Minus	Plus	(Av.)
Birds	DDT															
Duck, mallard		2	0	0	1	1	6.4	2	0	0	-	-	-	1	0	0
Eagle, bald		1	25	9.6	3	20	13.9	1	23	9.7	1	22	8.0	5	6	21.6
Eagle, golden		1	1	2.5	1	1	2.2	0	2	4.0	0	2	1.6	1	0	0
Mammals	Heptachlor epoxide															
Rabbit, cottontail		5	15	5.7	9	5	11.0	14	1	11.0	-	-	-	4	2	17.3
Rabbit, swamp		0	2	6.3	2	0	0	1	1	6.1	-	-	-	-	-	-
Raccoon		2	3	4.1	0	3	3.7	1	2	3.0	-	-	-	2	0	0
Rat, Norway		8	3	7.1	6	5	55.0	9	2	54.0	-	-	-	-	-	-
Skunk, spotted		0	1	3.1	0	1	19.0	1	0	0	-	-	-	-	-	-
Skunk, striped		0	3	3.0	1	1	18.7	2	1	Tr.	-	-	-	1	1	18.0
Squirrel, fox		1	0	0	1	0	0	1	0	0	-	-	-	1	0	0
Squirrel, gray		0	2	29.0	0	1	Tr.	-	-	-	-	-	-	-	-	-
Reptiles																
Snake, coachwhip		0	1	9.1	-	-	-	1	0	0	-	-	-	-	-	-

Table 6D. Mortality and reproduction of bobwhite confined in 20' x 50' pens treated with granular heptachlor[1/]

Experimental details	Treatment (pounds per acre)			
	0	0.25	1.25	2.0
A. Birds placed in pens on May 2 or 7.				
No. pairs started	8	12	6	9
No. pairs in which mortality occurred[2/]	4	8	6	9
Total mortality	4/16	8/24	8/12	12/18
Average days survival	65.5	18.3	7.5	6.4
No. hens producing eggs	6	4	0	0
No. hens dying during reproduction period	3	0	-	-
No. hens abandoning nests	1	1	-	-
No. successful nests	2	3	-	-
Total chicks produced	16	24	-	-
B. Birds placed in pens on May 17 or 31.				
No. pairs started	1[3/]	6[3/]	9	10
No. pairs in which mortality occurred	-	4	6	9
Total mortality	-	4/10	6/18	9/20
Average days survival	-	87.5	23.2	18.9
No. hens producing eggs	-	4	3	1
No. hens dying during reproduction period	-	3	0	0
No. hens abandoning nests	-	1	2	0
No. successful nests	-	0	1	1
Total chicks produced	-	0	12	3
C. Birds placed in pens on June 8 or 27.				
No. pairs started	2	1	1	3
No. pairs in which mortality occurred	1	0	0	2
Total mortality	1/4	0	0	2/6
Average days survival	3	-	-	15.0
No. hens producing eggs	1	1	1	1
No. hens dying during reproduction period	0	0	0	0
No. hens abandoning nests	0	1	0	0
No. successful nests	1	0	1	1
Total chicks produced	5	0	7	4

1/ Treatment applied May 2, 1962. Observation period ended September 15, 1962.
2/ Test in any pen terminated on date one or both birds died. Survivors sacrificed for chemical analysis.
3/ Bird killed by predator.

PESTICIDE-WILDLIFE STUDIES BY STATE, PROVINCIAL, OR UNIVERSITY PERSONNEL, AND BY THE COOPERATIVE WILDLIFE RESEARCH UNITS

by

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Survey of the Investigations

During 1962 a questionnaire was mailed to State, Provincial, and University personnel asking them to give a summary of their research in pesticide-wildlife relations. This survey was part of an international canvass conducted by the Committee on the Ecological Effects of Chemical Control of the International Union for the Conservation of Nature and Natural Resources. Members of the IUCN committee are from England, Holland, France, Switzerland, Russia, and the United States, and each made a survey of the pertinent ecological research on pesticides in his region. The U. S. representative included Canada and the United States in his survey, but Mexico, Central and South America were not included. Nor was Africa and much of Asia included in the initial canvass of projects.

Replies were received from every State and Province, and details were given on 161 projects. These will be summarized in a special publication later this year, and will be assembled in briefer form with the results of the other countries.

Cooperative Wildlife Research Units have been established at 18 land-grant colleges or universities with the support of State fish and game departments of the State concerned, the university or college at which the Unit is housed, the Wildlife Management Institute, and the Bureau of Sport Fisheries and Wildlife. The Units are training centers as well as research centers, and most of the studies serve as thesis projects by graduate students working under the direction of the Unit Leaders or faculty members of the university. Pesticide research is centered at four Units: in Alabama, Louisiana, Massachusetts, and Ohio. Names of personnel associated with the projects are shown in parentheses.

Alabama Cooperative Wildlife Research Unit

Effects on Bobwhite and Coturnix Quail of a Kepone-peanut Butter Bait Used for Fire Ant Control (Maurice F. Baker, Edward P. Hill, III)

The objectives of this study were to determine whether or not quail would die or exhibit acute or chronic symptoms from ingestion of kepone-killed insects; the approximate amount of chemical which would produce these effects; whether kepone-killed imported fire ants (*Solenopsis* saevissima richteri Forel) could be fed to quail in sufficient numbers to cause chronic or acute effects within a short period of time; and if quail would eat kepone-peanut butter bait, and if so, whether symptoms and/or mortality would occur as a result of this ingestion. An abbreviated version of the abstract from Mr. Hill's thesis follows:

> During the summers of 1961 and 1962, eight tests were conducted at Auburn University, Auburn, Alabama, in which kepone-killed house crickets (Acheta domesticus L.) and kepone-peanut butter bait were fed to bobwhite (Colinus virginianus L.) and coturnix (Coturnix coturnix japonica Temminch and Schlegel) quail. In addition, unpoisoned imported fire ants were fed to bobwhite quail. Daily observations were made to determine the effects of the feedings.
>
> In four tests, kepone-killed crickets fed to bobwhite and coturnix quail did not produce visible symptoms of toxicity during a 2-week period, although the minimum daily intake of kepone exceeded that known to inhibit reproduction in bobwhite.
>
> In four other tests, kepone-peanut butter baits fed to bobwhite and coturnix quail caused characteristic toxic symptoms, and a high mortality. Quail hesitated to eat bait with acetone as a solvent for blending kepone into peanut butter, but readily ate baits in which kepone was dissolved in peanut oil and then mixed with the peanut butter. Adult quail seemed less affected than juvenile quail and apparently have a greater ability to recover from the more severe effects of the chemical.
>
> It was impractical, in the procedures attempted, to obtain sufficient kepone-killed fire ants for secondary poisoning tests. Juvenile quail, in group feedings, consumed freezer-killed fire ants, whereas adult quail, feeding individually, were reluctant to eat them. Neither juvenile nor adult quail would eat live imported fire ants under conditions of the experiment.

Louisiana Cooperative Wildlife Research Unit

The Fulvous Tree Duck (Dendrocygna bicolor): Its Distribution, Abundance, and Relation to Agriculture in Louisiana (Robert B. McCartney, Leslie L. Glasgow)

As part of this project, feeding experiments with captive ducks were conducted to gather information on the effects of aldrin- and panogen-treated rice seed on fulvous tree ducks. All data have been gathered and analyzed, and the dissertation is almost complete. Results will be published on completion of the study.

Massachusetts Cooperative Wildlife Research Unit

Effects of DDT and Sevin on Populations of Vertebrates and Invertebrates on Three Areas in Massachusetts (Frederic W. Davis, A. D. Rhodes, William G. Sheldon, F. Greeley, L. Bartlett, D. Snyder, and the Massachusetts Audubon Society)

This study is an attempt to determine the relative hazards of alternative pesticides, formulations, rates and times of application, and to aid in the development of materials and methods which will provide adequate pest control under conditions of minimum damage to wildlife resources. Effects of applications of DDT and Sevin on certain bird species in a forest community have been studied for the past 3 years. Experiments in the field and on caged birds have been completed and will be made available by the Unit as soon as possible.

Measurement of the General Body Burden of DDT in Rufous-sided Towhees in Sprayed and Unsprayed Areas at Different Intervals Throughout the Breeding Season (Frederic W. Davis, William G. Sheldon, F. Greeley, L. M. Bartlett, D. Snyder)

Cage experiments were designed to determine the kinetics of DDT, the proportion of chemical which passes into the birds from the gut, and the amount of the chemical lost by detoxification and excretion. Experiments have been tabulated and results summarized. Data will be released by the Unit in the near future.

Ohio Cooperative Wildlife Research Unit

The Effects of Field Applications of Endrin at Sublethal Levels on the Reproductive Capability of the Meadow Vole (Microtus pennsylvanicus) (D. Snyder, Tony J. Peterle, E. E. Good)

The objectives of this study are to determine if the reproductive capability of meadow voles is altered when areas are sprayed with sublethal quantities of endrin, and if so, to determine how and in what stage or stages of the reproductive cycle the changes occur, the quantity of recoverable endrin residues accumulated in mice from the sprayed areas, how much endrin was actually ingested by these mice, and to correlate the reproductive data from the animals of each study area with the application rate of endrin, the amount of endrin ingested, and the amount of endrin residues recovered.

Progress has been made to attain the above objectives, but results are not yet ready for reporting. Collaborators in the study are the Bureau of Sport Fisheries and Wildlife, Velsicol Chemical Corporation, Department of Zoology and Entomology of Ohio State University, and Ohio Division of Wildlife.

<u>New Tracer Techniques for Evaluating the Effects of an Insecticide (Malathion) on the Ecology of a Forest Fauna</u> (Robert H. Giles, Jr., Tony J. Peterle)

The objectives of this study are to determine as completely as possible the faunal ecology of several small adjoining forested watersheds; to determine the effects of an aerial application of the insecticide malathion on a selected watershed under study; and to develop techniques for the field study of the radioisotope-labeled insecticide malathion that will allow the location of the insecticide and its metabolites in lethal and sublethal quantities in the ecosystem and within organisms, the detection of movement of insecticide by animals in space and time, the determination of the effects of the insecticide on species interactions, e.g., predator-prey relationships, and the study of water-soil-insecticide relations within the watersheds.

Progress toward the attainment of the outlined objectives has been made but is not at a reportable state. Collaborators in the study are the Atomic Energy Commission and Central States Forest Experiment Station.

RECOMMENDATIONS FOR MINIMIZING DANGERS OF PEST CONTROL AND PESTICIDES TO FISH AND WILDLIFE

by

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Some of the more important recommendations for minimizing dangers of pest control and pesticides to fish and wildlife are summarized below.

1. Be sure there is a real need for pesticide use.

2. Discuss the possible hazards to wildlife with biologists prior to pesticidal use. Usually assistance in the evaluation of local hazards arising from pesticide use may be obtained from State conservation departments. Representatives of the U. S. Department of Agriculture, U. S. Public Health Service, State agricultural or public health services, and the U. S. Fish and Wildlife Service also advise on the probable side effects of pesticide use. This liaison should be established as early as possible, preferably before any major control program is undertaken.

3. Be especially careful to avoid treatment of any significant portion of the range of a rare species or one with specialized habits which make it particularly vulnerable to pesticides except after consultation with responsible conservation officials.

4. Treat the minimum necessary area. When large blocks are treated leave blocks, strips, or headwaters untreated whenever possible to facilitate repopulation of treated areas if wildlife damage occurs. Be especially careful to avoid direct treatment of streams and other waterbodies when applications are for upland pests; and consider the danger of runoff or erosion contaminating drainage areas.

5. Select the chemical or pest control method that will be the least dangerous to fish and wildlife but still control the target pest. Judge the danger of a chemical both by its toxicity and by its disappearance rate. Avoid chemicals that tend to accumulate in the habitat. In recent years, pesticides and pest control methods with varying degrees of toxicity and hazard have been developed. Selection of chemicals offering little danger to wildlife can be guided to some extent by the relative toxicities as reported in laboratory studies. These are summarized in tabular form in table 2.

6. Use a method of application, carrier, or formulation which will minimize wildlife contact with the chemicals. Emulsions are more toxic

in water environments than oil solutions or suspensions. Granules often concentrate the effect of the toxicant in one stratum.

7. Use no more chemical than is absolutely necessary. Be sure no areas receive a double dose. Follow label instructions as to dosage and number of treatments.

8. Plan the time of treatment to reduce hazards. Try to avoid the main spring migration, nesting periods of birds, or other period of principal use of the area by wildlife.

9. Consider the possibility of using repellents or scare devices to move mobile wildlife from the treated area during the period of maximum toxicity or hazard.

10. Physical or biological control methods should be considered as they have a definite advantage over chemical methods in that they usually are very specific and reduce the target pest populations without loss of beneficial populations. Planned integration of chemical and biological control methods have already been applied successfully. Use of selective larvicides which will permit survival of mosquito-feeding fish is an important consideration in nuisance abatement programs.

11. The kinds of animals apt to be affected also should be considered. Susceptibility to different pesticides varies greatly among different animal groups, species, and even among individuals of the same species. Therefore, it is impossible to predict specific effects applicable to all situations, and the material included in the following tables is a generalization which is based on the available information. Table 1 gives the basic effects of dosages of DDT to certain categories of animal life, since effects of DDT are better known than most chemicals. These effects are more predictable on upland birds and mammals than aquatic organisms such as fish and crustaceans, because DDT is readily adsorbed by organic particles in water and this may greatly modify the effect of the toxicant.

Table 1. Acute effects of single applications of DDT in oil solution on invertebrates and crustaceans. Adapted from DeWitt and George (U. S. D. I., 1960)

Pounds DDT per acre	Crustaceans	Fish	Amphibians	Reptiles	Birds	Mammals
0.1	++	++	-	-	-	-
0.2	++	+++	+	+	-	-
1.0	+++	+++	++	++	+	-
2.0	+++	+++	++	++	+	-
5.0	+++	+++	+++	+++	+++	+

-No immediate apparent effect
+Some kill
++Moderate kill
+++Heavy kill

The toxicities of other chemicals as compared to DDT (equals 1) are listed in table 2. By considering the dosage rate, the relative toxicity, and the probable effects as determined by studies of DDT, it is possible to predict the probable effects of a given application. However, toxicity of pesticides as determined in the laboratory varies with species, and even the sex and age of individuals of a given species. Also, results vary with such factors as temperature or time of year, general health and vigor of the test animals, and length of the tests. Laboratory appraisals, therefore, need substantiation in field tests before they can be considered dependable indicators of field hazards, and the ratios listed are to be considered as only approximate. Unless otherwise noted, data used to determine relative toxicities are based on amounts necessary to kill 50 percent of the animals in tests of acute toxicity (usually single oral dose) for rats; chronic toxicity (10 to 100 days) for birds; and 96-hour tests for fish.

Table 2. Relative toxicities of commonly used pesticides with DDT equal to 1[1/]

Compound	Rats	Bobwhite	Pheasants	Mallards	Bluegills
Aldrin	2	300	60	9	1
Baytex	0.5	100	100	14	0.1 T
BHC	ca. 0.2	3	0.5	3	0.1
Ceresan	-	11	2	25	-
Chlordane	0.2	6	4	-	0.7
Co-Ral	0.5-2	45	0.4	25	0.1
Dalapon	0.1	0.1	-	0.3	0.1
DDT	1	1	1	1	1
DDVP	2	0.4	-	-	0.1 T
2,4-D derivatives	0.2	ca. 0.5	ca. 0.5	ca. 0.5	0.1
2,4,5-T derivatives	ca. 0.4	0.2	0.1	0.2	0.1
Dibrom	0.3	3	0.3	0.2	0.1 T
Dieldrin	2	70	19	40	2
Dimethoate	0.5	18	2	-	-
Dipterex	0.2	15	0.2	-	0.1
Endrin	3-23	400	900	65	12-27
Heptachlor	0.9	20	5	3	0.8
Kepone	0.9	2	1	3	0.2
Lindane	0.9	3	0.4	4	0.2
Malathion	0.1	8	5	-	0.2
Methoxychlor	0.1	0.2	0.1	-	0.3
Mirex (GC-1283)	0.1*	2	1	0.4	0.1
Parathion	23	25	200	-	0.2
Phosdrin	17	-	-	-	0.1 T
Phosphamidon	7	1100	8	4	0.1 T
Pyrethrum	0.1	-	-	-	1 T
Rotenone	0.9	-	1 H	-	ca. 0.3
Ryania	0.2	-	-	-	-
Sevin	0.2	0.3	0.1	-	0.1 T*
Systox	15	2	-	-	0.1
TDE (Rhothane, DDD)	0.1	0.5	0.8	1	0.1
TEPP	57	-	-	-	0.1
Toxaphene	2	2	1	8	5

1/ Unless otherwise noted, toxicities to rats are based on data summarized by D. E. H. Frear in the Pesticide Index or from the Handbook of Toxicology; toxicities to bobwhite, ring-necked pheasants, and mallards are based on studies of chronic toxicity of the compound in food as shown by the amount causing a 50 percent mortality of laboratory birds at the Patuxent Wildlife Research Center; toxicities to fish are based on concentrations in water which cause 50 percent mortality of test fish in 96 hours (or for trout, 24 hours) as determined by the Fish-Pesticide Laboratory of the Fish and Wildlife Service and by Dr. Clarence Tarzwell of the U. S. Public Health Service.

Symbols used: *Preliminary data
H - data from Handbook of Toxicology
T - rainbow trout; data on bluegills not available

12. Disposal of pesticides. The disposal of pesticides is always a difficult procedure and in all cases will involve an area of judgment. In the event professional chemists are available, they will be of immeasurable value in advising on the disposition of pesticidal chemicals that are unwanted. Local sanitation departments will also offer helpful advice. The following are suggestions to minimize danger of long-term or other side effects:

In the event large packages of pesticides are to be disposed of and they are in their original container and unopened, they should be returned to the wholesaler or sold as surplus. Opened packages or odd lots may be disposed of as follows:

Liquid organic chemicals should be burned in a hot flame. They may be squirted into an oil burner or diluted with kerosene and burned in the open. Care should be taken to avoid smouldering fires which might volatilize rather than burn the chemicals.

Wettable powders are often broken down by the addition of lime. Therefore, adding lime and putting them in a public dump of the sanitary landfill type is recommended.

Many inorganic materials do not decompose readily and will persist for years. If they are insoluble, they can be dumped into a sanitary landfill-type dump. If they are soluble, they should be put in a suitable container and then carried to the dump.

Empty pesticide containers should be burned or carried to the dumping ground. Spray equipment should not be cleaned at creeks or waterways.

The use of a sanitary landfill-type dump is recommended because the materials would be covered with earth and the area would always be known and marked as a dumping ground and its use accordingly restricted. In the event odd portions of an unmarked area are used as a dumping ground, there is always the possibility that these areas might be dug up or otherwise exposed when the area is used for some future purpose.

Many of the suggestions on disposal reflect the thinking of personnel of the Pesticide Chemicals Branch, U. S. Department of Agriculture.

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