PESTICIDE-WILDLIFE STUDIES, 1963

A Review of Fish and Wildlife Service Investigations During the Calendar Year

UNITED STATES DEPARTMENT OF THE INTERIOR

Stewart L. Udall, Secretary

FISH AND WILDLIFE SERVICE

Circular 199

Created by Act of Congress in 1849, the Department of the Interior is responsible for a wide variety of programs concerned with the management, conservation, and wise development of America's natural resources. For this reason it often is described as a "Department of Conservation."

Through a score of bureaus and offices the Department has responsibility for the use and management of millions of acres of federally owned lands; administers mining and mineral leasing on a sizable area of additional lands; irrigates reclaimed lands in the West; manages giant hydroelectric power systems; administers grazing and forestry programs on federally owned range and commercial forest lands; protects fish and wildlife resources; provides for conservation and development of outdoor recreation opportunities on a nationwide scale; conserves hundreds of vital scenic, historic, and park areas; conducts geologic research and surveys; encourages mineral exploration and conducts mineral research; promotes mine safety; conducts saline water research; administers oil import programs; operates helium plants and the Alaska Railroad; is responsible for the welfare of many thousands of people in the Territories of the United States; and exercises trusteeship for the well-being of additional hundreds of thousands of Indians, Aleuts, and Eskimos, as well as being charged with resource management of millions of acres of Indian-owned lands.

In its assigned function as the Nation's principal natural resource agency, the Department of the Interior bears a special obligation to assure that our expendable resources are conserved, that renewable resources are managed to produce optimum yields, and that all resources contribute their full measure to the progress, prosperity, and security of America, now and in the future.

UNITED STATES DEPARTMENT OF THE INTERIOR, STEWART L. UDALL, SECRETARY
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Fish and Wildlife Service, Clarence F. Pautzke, Commissioner
Bureau of Sport Fisheries and Wildlife, Daniel H. Janzen, Director
Bureau of Commercial Fisheries, Donald L. McKernan, Director

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INTRODUCTION

This is the fourth report on studies of pesticides by Bureaus of the U. S. Department of the Interior. By Public Law 85-582 (August 1, 1958) "the Secretary of the Interior is authorized and directed to undertake comprehensive continuing studies on the effects of insecticides, herbicides, fungicides, and pesticides, upon the fish and wildlife resources of the United States, for the purpose of determining the amounts, percentages, and formulations of such chemicals that are lethal to or injurious to fish and wildlife and the amounts, percentages, mixtures, or formulations that can be used safely, and thereby prevent losses of fish and wildlife from such spraying, dusting, or other treatment."

The preceding reports on these studies are "Bureau of Sport Fisheries and Wildlife Pesticide-Wildlife Review, 1959" (Fish and Wildlife Circular 84), issued in May 1960; "Effects of Pesticides on Fish and Wildlife; a Review of Investigations During 1960" (Fish and Wildlife Circular 143), issued in May 1962; and "Pesticide-Wildlife Studies: A Review of Fish and Wildlife Service Investigations During 1961 and 1962" (Fish and Wildlife Service Circular 167), issued in June 1963.

Pesticide research in the calendar year 1963 included studies of acute and chronic toxicity, development of new methods and improvement of methods of testing and analysis, measurement of pesticide residues in target animals and in their environments, and preapplication and postapplication observations in pest-control programs. At the end of the year, 22 technical papers had been published or were in press; these are listed at the end of this report.

As in the 1962 report, summaries are given of pesticide research by the Bureau of Commerical Fisheries and of pesticide research by the Bureau of Sport Fisheries and Wildlife, including sport fishery studies, wildlife studies at the Denver Wildlife Research Center, wildlife studies at the Patuxent Wildlife Research Center, and wildlife studies by the Cooperative Wildlife Research Units.

HIGHLIGHTS

Here are some highlights of the research findings, with references to the pages of this report where details are given.

In about a month, oysters accumulated DDT residues 70,000 times the amount of DDT in their environment (page 12).

In estuaries with moderate tidal flushing rates, most of the pesticides of inflowing waters will be accumulated in the tissues of resident animals and in the bottom materials (page 14).

Of the tidal marsh animals killed by a DDT test application, 98 percent died within the first 3 weeks (page 14).

After a hemlock looper control spray with Sevin and DDT, no evidence of oyster or clam kill was found (page 15).

Polystream, a mixture of polychlorinated benzenes, seems safest of the compounds tested for controlling predaceous drills on oyster beds (page 18).

Cutthroat trout fed or treated with malathion showed brain cholinesterase inhibition after each exposure (page 31).

Bluegills exposed to the herbicide sodium arsenite accumulated arsenic residues proportional to amount of exposure, and growth and survival were inversely proportional to exposure (page 31).

Adult redear sunfish exposed to the herbicide Kuron developed pathological conditions in livers and testes (page 33).

Bluegills fed heptachlor had better growth in low-treatment and control ponds than in high-dosage tests (page 34).

Rainbow trout fed different diets for 26 months at nine hatcheries all contained DDT and its products (page 35).

Methods for determination of Kuron and Mirex in fish are described (page 35).

A number of improvements were made in techniques for measuring pesticide residues in birds and mammals (pages 45 and 93).

TDE (DDD Rhothane) was demonstrated to be a metabolite of DDT; four other previously unidentified metabolites were detected, "DDMU," "DDMS," "DDNU," and "DDOH" (page 45).

A diet containing 1,000 ppm of 2,4-D fed to Canada geese for 230 days caused a general, progressive "disorganization" of cellular structure in liver and kidney (page 47).

Baytex and parathion were found to be much more toxic to mallard ducks than other organophosphate insecticides evaluated for use in mosquito control (page 50).

Malathion was found to have little immediate effect on birds and mammals (page 52).

Studies in the Klamath basin in California show the persistent nature of DDT and its degradation products in marshes (page 54).

Phosphamidon sprayed at the rate of 1 pound per acre caused some mortality and toxication among birds, including grouse (page 56).

One percent zinc phosphide on oat groats, distributed for meadow mouse control, killed wild geese after the treated field was burned (page 59).

Acute and chronic toxicities of pesticidal chemicals to quail, pheasants, and mallards were determined by pen-feeding (page 78).

Detectable DDT residues were found in 55 of 56 bald eagle specimens collected from 20 States and 2 Canadian Provinces (page 79).

Bobwhite quail and songbirds declined substantially on areas in Georgia treated with heptachlor for fire ant control (page 80).

Malathion application of 1 pound per acre in an aqueous solution did not cause significant harm to farm wildlife in Michigan; in experimental studies, pheasants survived dosage of 10 pounds of malathion per acre (page 83).

DDT and its metabolites have been found in a large percentage of herring gulls collected in the Green Bay area (Wisconsin) of Lake Michigan (page 87).

The number of birds experimentally removed from a field population was substantially greater than estimated by field counts (page 89).

Carcasses of small animals seldom persist for long periods in the field, but may be found if searches are made at intervals of 2 or 3 days after mortality begins (page 92).

Endrin applied by sprayer to bluegrass meadows at rates of 0.9 to 2.0 pounds per acre reduced meadow vole populations by 71 to 95 percent; no deaths were caused by an application of 0.6 pound per acre (page 117).

Much was learned about the techniques of using radioactive labels on insecticides to trace their movement in the environment and their accumulation by plants and animals (page 119).

Appreciable amounts of DDT were accumulated in towhees, a ground bird, following treatment of experimental areas with the pesticide in Massachusetts (page 121).

It is evident that considerable progress is being made by the Service in acquiring knowledge about the effects of pesticides on wildlife. Additional information is needed on the many effects of pesticides

on numerous species of fish and wildlife and on their habitats, particularly with respect to concentrations within food-chain organisms. Other subjects requiring particular attention are effects of sublethal levels, accumulation of residues in tissues and vital organs, and significance of these residues on survival and reproduction.

ABBREVIATIONS USED

EC_{50} median effective concentration - the concentration of toxicant in the environment which produces a designated effect on 50 percent of the organisms exposed to it.

ED_{50} median effective dose - the amount of toxicant (usually measured in mg/kg) that produces a designated effect to 50 percent of the population of organisms receiving the dose.

LC_{50} median lethal concentration - the concentration of toxicant in the environment which kills 50 percent of the organisms exposed to it.

LD_{50} median lethal dose in amount of toxicant lethal to 50 percent of the animals to which it is administered under the conditions of the experiments.

mg/kg milligrams per kilogram

ppb parts per billion

ppm parts per million

TLm median tolerated limit - the concentration which produces mortality to 50 percent of the tested population in a given period of time.

µg/g micrograms per gram.

µg/l micrograms per liter.

COMMERCIAL FISHERY INVESTIGATIONS

by

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In 1959 the Bureau of Commercial Fisheries began pesticide research as a program integrated with projects underway at four laboratories where special equipment and research personnel were available. Since that time, headquarters for the research program and the laboratory studies have been at the Biological Laboratory, Gulf Breeze, Fla. Field studies continue in areas which have acute problems or unique facilities.

The objective of the research is to help maintain at its optimum level the production of wholesome and economically useful marine plant and animal products. We need to learn how to protect and preserve the marine environment from the possibly adverse effects of agricultural chemicals. Equally important is the search for specific pesticides that may be useful in improving the quality and quantity of fish harvests. The need for this research does not imply that the widespread use of pesticide formulations automatically constitutes a serious threat to marine life. Rather, it emphasizes the fact that we have too little knowledge of this environment to predict the effects of natural or man-made changes, or to write meaningful regulations to protect the marine habitat.

There are many separate but related problems to be solved in order to realize the objective. Some of the problems dealing with pesticides, such as levels of acute toxicity, are clearly defined; others are more obscure and appear only as the research progresses. A significant part of the research is still concerned with development of reliable investigative techniques, for the toxicity of specific compounds may vary considerably depending on the assay methods used or the field conditions

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*In calendar year 1963, twelve biologists and technicians worked full time on pesticide projects: P. A. Butler, D. L. Coppage, R. A. Croker, W. R. Gould, V. T. Gammell, R. P. Hannah, H. T. Holland, L. D. Lively, J. I. Lowe, R. J. Reed, A. J. Rick, and A. J. Wilson. Other members of the staff of the Gulf Breeze Biological Laboratory have contributed in some measure their time and efforts.

under which the pesticide is employed. The development of uniform testing methods is an essential phase of the Bureau's program. Routine screening of new chemicals and formulations for their toxicity to marine fauna is a part of the research and development program required for the registration of a new product.

This progress report defines the major fields of investigation, and summarizes under each heading the current status of the Bureau's pesticide research.

ACUTE AND CHRONIC TOXICITY STUDIES

Are pesticides intrinsically toxic to commercial fishery resources? The answer to this question is an unqualified yes. The question suggests, however, that we are considering levels of pollution expected to occur in nature and at such concentrations as may be relatively less toxic. Even the most harmless of chemicals, including water, is toxic when present in sufficient quantity. More than 15 years ago, Bureau scientists observed that under certain conditions DDT could be beneficial in the collection of oyster set and advocated its use. Recent research has clearly demonstrated that, depending on the concentration, not only does DDT decrease oyster growth rates but it can also accumulate in their tissues in high concentrations.

The degree of toxicity depends on environmental factors too, and there is need for evaluating the relative toxicity of the many pesticides under standard test conditions in which water quality, temperature, and similar external factors are known, as well as the concentration of the test chemical.

The screening projects conducted during the past 4 years have demonstrated that different groups of marine forms may react quite differently to the same chemical. Even closely related species of fish, for example, may show differential levels of susceptibility to a pesticide. As a result, although four representative groups of marine forms have been selected for the routine evaluation of acute toxicity of each important pesticide, it is recognized that this is a minimum standard for testing. If a pesticide is proposed for direct application to a marine area, a far more thorough evaluation of its toxicity would be mandatory.

Tabulated below are selected data demonstrating the different levels of sensitivity of the four bioassay groups to specific pesticides (+ to ++++ shows increasing sensitivity; 0 indicates no effect).

Pesticide	Phytoplankton metabolism	Oyster shell growth	Shrimp survival	Fish survival
Dyrene	++	+++	+	++++
Methyl parathion	+	0	++++	0
Phosdrin	0	0	++	+
Phosphamidon	0	0	++	0

Chemicals are tested at winter and summer water temperatures, and since naturally flowing sea water is used in the test aquariums it may require a year to complete the minimum number of assay tests on a specified pesticide. Test conditions and acute toxicity criteria are described for each group used to screen pesticides during the past year; summaries of the pertinent data are presented in the indicated tables.

Phytoplankton

The microscopic plants of the sea are the essential link in the conversion of solar energy into food for more complex marine animals. Chemicals interfering with phytoplankton growth and reproduction could seriously interfere with the production of commercial fish harvests. One measure of the well-being of phytoplankton is the rate at which a sample incorporates inorganic carbon molecules into its cellular matrix. This may be measured precisely with carbon C^{14} under constant light conditions. By mixing known amounts of C^{14} with two samples of phytoplankton, one of which contains a know concentration of pesticide, it is possible to measure how much the pesticide interferes with growth in a given period of time. Using the decrease in growth, or decrease in carbon fixation, as a standard, the toxicities of a series of pesticides may be compared (table A-1).

Crustacea

Much of our concern about pesticide pollution of the marine environment continues to stem from the fact that shrimp, the most valuable fishery, are crustacean members of the arthropod phylum. Since most pesticides have been specially selected to kill terrestrial members of this group, and since juvenile shrimp spend their first growing season in estuaries near the source of land drainage, the effects of pesticides on them are examined with particular interest. Three species are seasonally available for testing and there is no evidence that the adults of one type are more sensitive to pesticides than the others.

Some pesticides paralyze shrimp and other crustaceans with which they come in contact rather than kill, so that the criterion for toxicity with this group is the effective concentration causing paralysis or death to half of the sample within a stated period, usually 24 or 48 hours. As in our other bioassay tests, pesticides dissolved in acetone are metered into flowing sea water aquariums to maintain the desired concentration. Summaries of the screening tests are presented in table A-2.

Mollusks

Clams and oysters are of particular interest because of their known ability to store in their tissues high concentrations of chemicals that exist in only trace amounts in the surrounding sea water. Since most of them normally flourish in estuarine waters, they may be particularly sensitive to chemicals washed in from the adjacent river basin. Mollusks, however, are able to close their valves and protect themselves from toxic substances in the environment. Tests to evaluate their sensitivity to pesticides must be conducted at sublethal concentrations, and we find that, as with phytoplankton, growth is an objective index to measure.

Juvenile oysters grow at quite uniform rates under similar conditions and it is possible to compare shell deposition (growth) in a control group of oysters with shell deposition in oysters exposed to various concentrations of a test chemical. The relative toxicity of a series of pesticides to oysters can then be expressed as EC_{50}, the effective concentration of a pesticide causing a 50 percent decrease in growth in experimental as compared with control oysters during a definite period, usually 96 hours. Surviving oysters are then transferred to unpolluted water and observations are made on the time required for growth rates to return to normal. Such data are presented in table A-3.

Fish

The sensitivity of estuarine fish to pesticides varies unpredictably with the species, but in general the younger specimens are most quickly affected. Consequently, juveniles are used in the reported tests. Since not all species are available throughout the year, few comparative data are available at this time. Tests are conducted in flowing sea water; stock solutions of pesticides are made up in acetone, and fish are exposed to four or more dilutions of the pesticide. EC_{50} values are determined by graphical interpolation. In some cases, the solubility of the chemical limited the maximum concentrations that could be used, and EC_{50} values were not determined. Summaries of the data are presented in table A-4.

Chronic exposure of fish to pesticides

Estuarine fish have been found sensitive to low concentrations of a wide variety of pesticides. There is often evidence of a threshold of sensitivity, and slightly below a near-lethal concentration, fish may show no ill effects. Observations were conducted for a 5-month period to determine whether juvenile spot, Leiostomus xanthurus, would be affected by continuous exposure to a sublethal concentration of toxaphene, an insecticide used extensively on agricultural crops.

The fish were established in suitable aquariums in a continuous-flow sea-water system and exposed to two concentrations, 0.1 and 0.01 parts per billion (ppb). Toxaphene at 0.5 ppb will cause a 50 percent mortality within 6 days under these conditions. During the test, there were no significant differences between experimental and control fish in mortality, growth, or behavior. Post-mortem examination revealed a thickening of gill lamellae in the experimental but not in the control fish. Acute toxicity tests conducted at the end of the 5 months' exposure showed that surviving fish had acquired no resistance to toxaphene.

Bioassay techniques

Most pesticides have been developed for their specific toxicity to terrestrial arthropods and there is reason to expect them to be equally harmful to aquatic members of this group. Many marine arthropods (crustaceans), including shrimp, lobsters, and crabs, are of obvious economic importance to man. Perhaps even more important than the arthropods listed are the related minute crustaceans which make up a significant part of the zooplankton of the sea, the basic food for a majority of our commercial fishery species.

Mass mortalities could occur among copepods and other zooplankton crustaceans and not be noticed because of their small size. Their absence, however, could mean the loss of the entire crop of fish dependent on them for food. With these facts in mind, exploratory tests have been undertaken using copepods as bioassay animals.

Tests were conducted in battery jars of standing water at an average salinity of 22 parts per thousand (ppt) and temperature of 17°C. Pesticides were added in acetone solution, and the amount was determined which caused a mortality of half of the exposed population within 2 days. In general, chlorinated hydrocarbons exhibit the same degree of toxicity to copepods as to shrimp or crabs. The phosphorus compounds are much less toxic, presumably because they are hydrolyzed in the test containers of standing water.

The collection and maintenance of copepods pose technical problems which limit their usefulness as bioassay animals. Eggs of the salt lake brine shrimp, Artemia salina, are readily obtained and easily cultured in artificial sea water. Exploratory tests were conducted to determine the usefulness of brine shrimp as bioassay animals to replace marine forms.

Brine shrimp larvae, of selected ages, were exposed to a series of pesticide solutions from 0.001 to 10.0 parts per million (ppm) over periods ranging from 4 to 72 hours. Surprisingly, the shrimp were relatively insensitive under these test conditions and exhibited great variability in response in duplicate tests.

The small size of both copepods and brine shrimp prevents their use in flowing sea water systems where we have obtained the most reliable toxicity data. Consequently, further tests on these animals have been abandoned.

RESIDUE STUDIES

Uptake and retention of pesticides by shellfish

Most pesticides and particularly the chlorinated hydrocarbons have a toxic effect on marine shellfish. Oysters exposed to minute concentrations of agricultural chemicals show abnormal pumping activity, decreased shell growth and, at summer water temperatures, significant mortalities. Animals that are affected but not killed, when returned to clean water, soon recover from all outward aspects of damage.

Earlier experiments showed that oysters exposed to DDT at levels of 1 to 1,000 ppb (µg/liter) show a progressive decrease in shell deposition as compared with controls, from approximately 20 percent at 1 ppb to 100 percent at 1,000 ppb. When such oysters are returned to unpolluted water, growth rates return to normal within 4 weeks.

The objectives in the present study were to determine the amounts of selected pesticides stored by shellfish, where they were stored, and how long they persisted.

Pacific and eastern oysters (C. gigas and C. virginica) and the hard clam (M. mercenaria) were maintained in flowing sea water aquariums. Acetone solutions of DDT were added continuously to maintain concentrations of 0.1 to 10 ppb. At appropriate intervals groups of oysters and clams were sampled. In addition, eastern oysters were exposed to 1.0 ppb of dieldrin, 1.0 and 5.0 ppb of lindane, and 1.0 ppb of heptachlor. Chemical analyses were made by paper chromatography. DDT was stored in

the tissues at all exposure levels. Of the other pesticides tested, only dieldrin was detected as a residue; tissues contained 3.5 ppm after 60 days of exposure. Within 20 days this amount was flushed from the tissues of oysters held in clean water. Tabulated below are the results of analyses made at the termination of the indicated exposure periods.

	Amount of DDT in environment ppb	Exposure days	Amount of DDT in tissues ppm
Eastern oyster	10.0	7	151.0
	1.0	40	30.0
	0.1	40	7.0
Pacific oyster	1.0	7	20.0
Hard clam	1.0	7	3.0 - 9.0

Samples of the eastern oysters were dissected and tissues of the different regions were analyzed separately; clam analyses were not made.

Tissue	Parts per million of DDT	Percent
Intestinal tract and gonad	18	67
Mantle and gills	7	26
Muscle	1+	4
Tissue fluids	1-	3

In later experiments, separate analyses showed that gonad tissue stored relatively twice as much DDT and its metabolites as did the liver and intestinal tract. There is the possibility that the gonad pesticide residues are actually in the eggs. Further research is required to determine whether these residues can affect larval development.

The remaining oysters and clams were maintained in clean sea water, and whole-body analyses for DDT were made at intervals. These data are summarized as follows:

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Clams		Oysters			
Days in clean water	Residual DDT ppm	Days in clean water	Residual DDT ppm after exposure to: 0.1 ppb	1.0 ppb	10.0 ppb
0	3.5	0	7.0	30.0	151.0
10	0.88	10	2.0	11.3	128.0
20	0.161	30	0.0	4.5	119.0
		50		1.7	44.0
		70		0.0	24.0
		90			6.0

Accumulation of DDT in other marine animals

The fact that oysters in our experiments accumulated DDT residues 70 thousand times greater than the amount present in their environment in a little more than a month may be a peculiarity of oyster physiology. Exploratory experiments were conducted to determine the ability of other forms to store DDT and its possible effect on them or their predators.

Shrimp.--Commercial shrimp, Penaeus setiferus, are routinely used in 48-hour bioassay tests. DDT levels of 0.001 ppm and above are extremely toxic to them, and for these tests, to check accumulation, a concentration of 0.0005 ppm was selected. After 72 hours, considerable mortality occurred, and surviving shrimp were analyzed. Paper chromatography analysis revealed 0.14 ppm of DDT in whole body samples.

Scaled sardine.--Juvenile sardines, Harengula pensacolae, were selected for accumulation studies. This is a clupeid fish similar to the commercially important menhaden, and it spends much of its life in estuarine waters. Fish were exposed for 7 days to a sublethal concentration of 0.0001 ppm. Residue analysis showed a biological magnification of more than 1,000X; whole body samples contained DDT residues of 0.11 ppm.

Sea squirts.--The ascidian, Styela plicata, is a solitary sea squirt, member of a group well known for its ability to extract and accumulate inorganic metals from sea water. Under our test conditions, these animals tolerated relatively high levels of DDT. Two groups were exposed for 10 days at levels of 0.1 and 0.01 ppm of DDT. Water intake was reduced in the group at the high concentration but no mortalities occurred. Half of each group was analyzed on the 10th day, the remainder were placed in clean flowing sea water for a period of flushing. Control animals contained no detectable amount of DDT. Analyses of whole body residues indicated an accumulation of about 10.0 ppm in the low-concentration group (0.01 ppm) and about 20.0 ppm in the high-concentration group

(0.1 ppm). Although the high-concentration group was exposed to 10 times the level of DDT used on the low-concentration group, the amount accumulated was only twice that of the low-concentration group. The high-concentration group had an accumulation rate of 200 times the treatment level, while the low-concentration group had a rate of 1,000 times the treatment level.

Sea hare.--The sea hare (gastropod), Bursatella leachii, is an omnivorous benthic detritus feeder often associated with oyster reefs in the South. Observations were made on its possible role in the fixation of pesticides present in estuaries and the extent to which the accumulation rate might be affected by its association with oysters.

A concentration of 0.01 ppm of DDT was maintained in a series of small aquariums with continuously flowing sea water for a period of 10 days. Various combinations of sea hares, oysters, or both were held in the experimental and control aquariums. Residue analyses for DDT were made on suitable samples of the animals, substrates, and containers to determine the path of the DDT in the systems. The following table summarizes the data; figures in parentheses indicate percent recovered of total amount of DDT delivered:

Animals	Total mg. DDT in oysters	Total mg. DDT in sea hares	Total mg. DDT in feces	Total mg. DDT on oyster shells	Total mg. DDT on aquariums	Total
Oysters and sea hares	15.836 (4.36)	4.482 (1.23)	10.340 (2.85)	0.110 (0.03)	0.334 (0.09)	31.102 (8.56)
Sea hares	-- --	1.78 (0.49)	0.522 (0.14)	-- --	0.336 (0.09)	2.639 (0.73)
Oysters	13.517 (3.72)	-- --	8.360 (2.30	0.110 (0.03)	0.358 (0.10)	22.345 (6.15)

Oysters alone, for example, removed or fixed approximately 6 percent of the DDT flowing through the system. Sea hares alone removed less than 1 percent, but in the aquariums where the sea hares had opportunity to feed on oyster feces contaminated with DDT more than 8 percent of the pesticide was removed from the water and fixed in animal tissues or on the bottom detritus.

This experiment suggests some of the pathways by which pesticides suspended and dissolved in estuarine waters may accumulate, both in the fauna and in the physical substrates. It is reasonable to assume that in estuaries having large animal communities and a moderate flushing rate, a major portion of the pesticide burden of the inflowing waters may be rapidly accumulated and made available for transport in the food web.

Effects of DDT in a tidal marsh

Field studies were initiated to verify laboratory results and to investigate the effects and kinetics of pesticides under natural conditions. In the tidal marsh habitat several species are dependent on vegetation and detritus, and provide opportunity to trace pesticide residues in a short food chain.

A tidal marsh creek was treated with 0.2 pound of DDT to the acre in March, 1963, and observed for 4 months. The purpose of the experiment was to determine (1) the effect of a recommended mosquito-control application of DDT on some animals of a typical tidal marsh habitat, (2) the distribution and concentration of DDT in the system, and (3) the persistence of toxicity. Bioassay animals included seven fish species and fiddler crabs. Quantitative data for mortality, species composition of survivors, and whole-body residues of DDT for dead and living animals were obtained for fish and fiddler crabs held at several sites. Residue analyses for DDT and metabolites were also performed on samples of water, vegetation, bottom sediments, and snails.

Ninety-eight percent of the total mortality of animals occurred within 3 weeks after treatment, under conditions of low rainfall, semi-isolation from tidal flushing, and increasing temperatures. The distribution of DDT was affected by wind and water movement. Fish populations were restored by subsequent reproduction of surviving and introduced fish.

DDT was not detectable in bottom and surface water samples after 1 and 14 days, respectively, while DDT residues in vegetation and sediments reached a maximum between 3 and 6 weeks' post-treatment. Maximum DDT residues from all samples ranged from 0.05 ppm (water) to 90 ppm (fish). With 0.05 ppm of DDT equal to 1, maximum residues for other samples were: 66, sediments; 99, crabs; 144, snails; 1,480, vegetation; and 1,784, fish.

The data indicate a significant localization of DDT residues in the tidal marsh habitat; further work is needed to determine the effect of these residues in omnivorous marsh animals.

Similar field experiments were completed with phosphamidon at 1.0 pound/acre, and malathion at 0.1 and 0.2 pound/acre. Fish, when exposed to phosphamidon for 10 days, suffered no mortality, while malathion at both dosages resulted in mortality only to the sheepshead minnow (Cyprinodon variegatus) in isolated areas after 2 days' exposure. Strong tidal currents were present during both bioassays.

FOREST INSECT CONTROL PROGRAMS

Hemlock looper

Bureau personnel from Seattle and Gulf Breeze laboratories participated in a multi-agency study of the effects of spraying 60,000 acres of forest land in southeastern Washington to control the hemlock looper, Lambdina fasciolaria lugubrosa, a forest defoliating insect. Both Sevin and DDT were used. There was concern whether either or both of these pesticides might be carried into Willapa Bay, where a valuable oyster fishery is located.

Field bioassay sites were chosen in the Nemah, Naselle, and Willapa (control) estuaries, for the placement of live-boxes containing Dungeness crabs, little neck clams, and Pacific oysters.

Spraying with Sevin began on July 5 and was completed on July 29. DDT was used only during the last 3 days on 11,000 acres of looper-infested timber. No direct evidence of animal mortality due to pesticides was found, nor were any fish or other animal kills noted in Willapa Bay through late August. Nineteen samples of oysters and clams (a total of 172 animals) collected just after spray through late August, yielded no detectable residues of Sevin or DDT.

Spruce budworm

Spraying with DDT has been shown to be an effective control for various forest insects. In some cases the effects on migrating salmon and resident fish in streams in treated areas have been drastic, in others, negligible. The Bureau of Commercial Fisheries Biological Laboratory at Auke Bay, Alaska, has completed the third year of a 4-year cooperative study to analyze the effects of a controlled spraying program in which two watersheds were sprayed with 1/4 pound of DDT per acre and two similar watersheds were studied as controls.

Appropriate samples were collected to determine the prespray condition of the environment; the water, resident fish, insects, clams, and plankton were analyzed for DDT residues. Seasonal variations in the diet of resident fish and the numbers of aquatic insects were determined during the 2 years prior to spraying. In 1963, following the June 21 spray date, samples were collected at regular intervals until late summer.

The most obvious result of the spraying was the change in abundance of aquatic insects. In the two sprayed streams, 40 marked stones were examined and found devoid of aquatic insects. Large numbers of dead insects both aquatic and terrestrial were found drifting. The following tabulation shows the changes in the numbers of drifting insects per sample before spraying (June 16 and 18) and on day of spraying (June 21) in four Alaska streams, 1963:

	Number of insects per sample in --			
	Test creeks		Control creeks	
	Cabin	Virginia	Old Tom	Saltery
No. of Samples*	3 - 6	7 - 6	3 - 6	7 - 6
Aquatic insects:				
Diptera	1.1 - 21.5	4.9 - 8.0	2.3 - 1.8	0.3 - 0.8
Ephemeroptera	7.7** - 59.5	4.7** - 40.0	10.0** - 4.3**	6.9** - 3.8**
Trichoptera	0.0 - 4.3	0.0 - 0.8		
Plecoptera	0.0 - 9.2	0.3 - 5.8		
Other	0.0 - 0.8	0.0 - 6.7	0.0 - 0.3	
Terrestrial insects:				
Diptera	0.0 - 1.5	0.7 - 11.3		
Coleoptera	0.0 - 2.0	0.0 - 6.7		
Hymenoptera	0.0	0.0 - 0.8		
Hemiptera	0.0 - 1.0			
Other	0.0 - 3.3	0.0 - 2.3		

* First number = before spraying; second number = day of spraying; pre-spray samples at Virginia and Saltery Creeks taken on June 16 and 18, pre-spray samples taken at Cabin and Old Tom Creeks on June 16 only.

** Nymphal skins

There was no mortality after spraying among fish held in live cars, nor were any dead fish observed in the streams. A high percentage of fish from the test streams had empty stomachs as compared with fish from the control streams where the insect food supply persisted.

It is significant that analyses for residues of DDT plus its metabolite DDE showed only trace quantities (less than 5 ppb) in essentially all samples with the exception of fish.

Following the spray treatment, residues of DDT plus its metabolite DDE increased markedly in fish samples from test streams while only traces were found in control fish. The data are tabulated below in parts per million on a wet weight basis.

	Test Creeks		Control Creeks	
Date	Cabin	Virginia	Old Tom	Saltery
1962	0.82	0.62	0.11	0.11
1963 pre-spray	0.11	trace	trace	0.07
1963 post-spray				
June	trace	0.05	trace	trace
July	3.2	4.5	trace	trace
August	6.9	4.2	trace	trace
September	4.3	9.5	trace	trace

Apparently these residues resulted from eating contaminated insects. There is no explanation for the residues observed in 1962 before the spray treatment was initiated.

In summary, the major effects of the spraying were the eradication of aquatic insects with the resulting change in the diet of resident fish and the persistence of DDT in fish tissues. Sampling in the following year will show whether these changes had any permanent effect on the fish populations.

BENEFICIAL USE OF PESTICIDES

The obvious economic benefits and saving in manpower resulting from the use of pesticides in agriculture have prompted a search for chemical controls of predators and parasites which interfere, for example, with fish populations in the Great Lakes and oyster production in our coastal waters.

The Bureau's Milford Biological Laboratory has pioneered in the screening of formulations suitable for the control of oyster drills. These marine snails cause great loss to the industry and prevent oyster culture in many areas.

Some of the formulations that have been developed for drill control (gastropodicides) include combinations of chemicals having considerable toxicity to drills. They are being evaluated in several geographic areas to determine their effectiveness and specificity under various environmental conditions as well as toxicity to other marine forms.

It has been thought that since these formulations were relatively insoluble in water they would probably offer no particular threat to nontarget animals. These compounds are:

Polystream (a mixture of polychlorinated benzenes).
Drillex (Sevin 2%, polychlorinated benzenes 98%).
Sevin 10G (Sevin 10%, florex 90%).
Sevin 3CB (Sevin 3%, polychlorinated benzenes 93%, dimethylformamide 4%).

Initial experiments were conducted to determine the solubility of the active or toxic elements in the formulations. Copepods, marine crustaceans, were selected as the bioassay animals because of their close relationship to other economically important marine animals.

Beakers filled with filtered sea water were treated with the equivalents of 100 pounds per acre of Sevin 10G, 3 cubic yards per acre of Sevin-3CB-treated sand, 3 cubic yards per acre of Drillex-treated sand, and 3 cubic yards per acre of Polystream-treated sand. After 2 hours' steeping time, the effluent was siphoned off and diluted to various concentrations. Ten milliliters of each concentration were placed in Syracuse glasses and copepods were added. Mortalities in percent, calculated after 4 hours, were:

Concentration of effluent	Compound				
	Sevin 10G	Sevin 3CB	Drillex	Polystream	Control
100.0	100.0	100.0	100.0	22.9	3.3
60.0	95.0	100.0	100.0	18.8	
40.0	50.0	100.0	100.0	15.2	
20.0	15.9	100.0	100.0	15.9	

Obviously, all the formulations were soluble, and the use of Drillex, for example, on a large oyster reef in a restricted body of water could have a serious effect on the zooplankton, on other economically important crustaceans, and on fish species dependent on plankton for food. Polystream, the least toxic of the chemicals tested, appears to be the most suitable pesticide now available for treatment of drills on oyster reefs.

Three of the formulations were tested on commercial shrimp and drills simultaneously. Animals were placed in small plastic aquariums with flowing sea water and clean sand bottoms. Representative data are as follows:

Pesticide	Rate tested	Shrimp mortality % in 24 hours	Drills affected % in 24 hours
Drillex	*3 yds/acre	100	100
Sevin 10G	100 lbs/acre	100	20
Sevin 3CB	*3 yds/acre	100	60

*Dry sand treated at rate of 10 gal. of formulation per cubic yard of sand.

At all concentrations, there was a complete kill of shrimp within 24 hours, and in some instances as few as 20% of the drills were affected. Under these test conditions, the minimum amounts causing a 100% loss of shrimp were without visible effect on drills at the end of 24 hours.

Further tests were conducted in small pools and under field conditions to determine the effects of the several drill-control formulations on commercial shrimp and the southern oyster drill. In general, pool tests indicated greater toxicity than under field conditions, perhaps because of crowding. In field tests, all drills were immobilized by Polystream, but even at double the recommended rates the mortality of shrimp was negligible. Shrimp were irritated, however, and the presence of Polystream prevented their normal burrowing during daylight hours. While this might make shrimp more available to predation in treated areas, it also indicates that shrimp would stay away from such areas if possible. Pool and field tests showed both Sevin 3CB and Sevin 10G to be much more toxic to shrimp with as much as 100% mortality after 6 days' exposure. Their effect on drills was, in general, unsatisfactory for control purposes.

The initial tests to assay the toxicity of these drill control compounds to copepods showed that the active ingredients were soluble and could be detected in the effluent from experimental aquariums.

Additional tests were conducted to determine the relative toxicity to drills and shrimp following various periods of flushing the pools or treated field sites. The following selected data demonstrate that after 2 to 7 days' flushing the treated areas remained highly toxic to fresh specimens of shrimp while the toxicity to drills decreased markedly.

Pesticide rate	Test site	Days flushed	Shrimp		Drills	
			Exposure hours	Mortality percent	Exposure hours	Mortality percent
Drillex						
5 yds/acre	Pool	5	24	100	120	60
5 yds/acre	Field	7	24	60	72	15
Sevin 3CB						
5 yds/acre	Pool	5	24	100	144	18
Sevin 10G						
200 lbs/acre	Field	7	48	40	192	0

Further evidence of the solubility of the active ingredient in these formulations is shown by the fact that 45 percent of the sample of test shrimp was killed within 48 hours when they were placed 15 to 30 feet away from the test site under field conditions. The effluent from aquariums containing moderate applications of Sevin 10G caused the death of 80 percent of test animals even after 4 days of flushing the aquarium. These tests are indicative of the inherent danger to nontarget animals when broad-spectrum pesticides are used in areas conducive to their dispersal.

FUTURE CONSIDERATIONS

The pesticide research program has made significant progress this past year in the standardization of techniques, the initiation of field observations of large scale control programs, and preliminary investigations of the movement of pesticide residues in food webs.

The complexities and size of these problems make much greater effort necessary in the near future in order to gain the necessary knowledge soon enough to be useful. We anticipate a much more concerted effort on the part of biologists, pesticide manufacturers, and government agencies to assess the pesticide problem and to adopt the measures necessary for its solution.

In addition to the planned expansion of the present program, estuarine monitoring stations must be established at strategic locations in coastal areas that support commercial fishery harvests. We need to know the amounts and kinds of pesticides being drained into the estuarine areas, where they lodge, how long they persist, and whether they may contaminate our food supplies or endanger individual links in the food chains.

REPORTS

The large number of agencies conducting pesticide research and the time required for publication of manuscripts make difficult the timely dissemination of research data. In order to make results available as early as possible, the Biological Laboratory at Gulf Breeze summarizes and prepares in mimeographed form its pesticide research data at quarterly intervals during the year. Although these reports may contain preliminary data, it is our purpose to inform other workers as quickly as possible. The reports are mailed to approximately 40 agencies concerned with pesticide research.

The following research reports have been approved for publication:

Croker, Robert A., and Alfred J. Wilson, Jr.
Kinetics and effects of DDT in a tidal marsh ditch, Santa Rosa Island, Florida.

Lowe, Jack I.
Chronic exposure of spot, Leiostomus xanthurus, to sublethal concentrations of toxaphene in sea water.

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

Pesticide	Percent decrease
Defoliants	
DEF	75.3
Fungicides	
Chemagro 4497	86.1
Gastropodicides	
Polystream (mixed poly-chlorinated benzenes)	31.8
Herbicides	
2,4-D butoxy ethanol ester	16.3
2,4-D, 2 ethyl-hexyl ester	48.7
2,4-D propylene glycol butyl ether ester	44.2
Dacthal	37.3
Dalapon	0.0
Diquat	45.1
Kurosal (SL 60% silvex)	0.0
MCP Amine Weed Killer	0.0
N-Serve	15.0
Paraquat	53.2
Shell SD 7961	0.0
Sodium TCA	0.0
Tordon 22K	8.4
Tordon 101	0.0
Venon 245	0.0
Zytron	58.8
Insecticides	
Chlorinated hydrocarbon	
BHC (45% Gamma Isomer)	16.0
Strobane	87.7
Telodrin	76.8
Organophosphorus	
Bayer 38156	54.8
Bayer 37289	84.0
Bayer 41831	14.9
Bidrin	0.0
Ciodrin	0.0
CO-RAL	27.4
Dimethoate (Cygon)	0.0
Methyl parathion	5.1
Phosdrin	0.0
Phosphamidon	0.0
Shell 4072	13.1
Shell SD 7438	44.0
Shell SD 8447	7.2
Shell SD 8448	10.0
Phorate (Thimet)	41.5
Parathion (Thiophos)	9.9
DDVP (Vapona)	0.0
Nematocides	
Nellite	0.0

Table A-2. Concentration of pesticides in sea water causing mortality or loss of equilibrium in 50 percent of adult shrimp tested.

Pesticide	Test Animal	Average salinity per mil	Average temp. °C	24-hour EC_{50} ppm (mg/liter)	48-hour * EC_{50} ppm (mg/liter)
Acaricides					
Sulphenone	[1] B	31	18	30% at 1.0	40% at 1.0
Tedion	B			0.25	0.25
Fungicides					
Bayer 47531	B	28	27	30% at 1.0	1.0
Chemagro 2635	B	25	29	0.55	0.44
Chemagro 4497	B	23	29	0.074	0.055
Dyrene	B	24	29	10% at 0.1	10% at 0.1
Gastropodicides					
Polystream (mixed poly-chlorinated benzenes)	B	31	24	0.55	0.55
Herbicides					
2,4-D dimethyl-amine salt	B	24	30	[2] ne at 2.0	10% at 2.0
2,4-D butoxy-ethanol ester	P	24	30	ne at 1.0	ne at 1.0
2,4-D propylene glycol butyl ester	P	27	28	ne at 1.0	ne at 1.0
Dacthal	B	29	15	ne at 1.0	ne at 1.0
Eptam	W	29	15	20% at 1.0	0.63
Monuron	W	30	24	ne at 1.0	ne at 1.0
Neburon	W	30	24	[3] ir at 1.0	0.55
Shell 7961	W	28	14	ne at 1.0	ne at 1.0
Tillam	W	29	15	ne at 1.0	ne at 1.0
Insecticides					
Carbamate					
Bayer 37344	P	27	27	0.060	0.055
Bayer 39007	P	26	28	0.085	0.066
Bayer 44646	B	27	29	0.55	0.55

(continued)

Table A-2. Concentration of pesticides in sea water causing mortality or loss of equilibrium in 50 percent of adult shrimp tested (continued).

Pesticide	Test Animal	Average salinity per mil	Average temp. °C	24-hour EC_{50} ppm (mg/liter)	48-hour * EC_{50} ppm (mg/liter)
Insecticides					
Chlorinated hydrocarbon					
BHC	B	24	30	0.0050	0.0036
Strobane	B	25	28	0.036	0.0085
Telodrin	B	30	17	0.00033	0.00007
Organophosphorus					
Amer. Cyanamid 43,913	B	33	19	10% at 1.0	0.63
Amer. Cyanamid 52,160	B	31	18	0.0043	0.0028
Aspon (ASP-51)	B	26	29	0.0082	0.0048
Bayer 25141	B	25	29	0.044	0.01
Bayer 37289	P	29	29	0.0006	0.0005
Bayer 38156	P	27	27	0.0036	0.0006
Bayer 41831	B	25	29	0.0050	0.0025
Bidrin	B	30	21	0.50	0.25
Ciodrin	B	31	20	0.36	0.055
CO-RAL	P	29	28	0.0044	0.0036
DDVP (Vapona)	P	25	26	0.055	0.044
Dimethoate (Cygon)	B	30	22	ne at 1.0	20% at 1.0
Di-syston	B	28	25	0.050	0.025
Dylox	P	27	27	0.44	0.36
Ethion	P	29	30	0.055	0.036
Imidan	B	27	29	0.049	0.0045
Methyl parathion	B	29	25	0.0055	0.0055
Methyl trithion	B	28	27	0.0006	0.0005
Parathion (Thiophos)	B	28	24	0.0055	0.001
Phorate (Thimet)	B	33	18	0.0044	0.0007
Phosdrin	B	32	24	0.50	0.25
Phosphamidon	P	25	25	0.60	0.44
Shell 4072	B	30	16	0.62	0.25
Systox	P	27	26	0.40	0.063

* Solubility of the pesticide limited the maximum concentration that could be tested in some cases.

1/ B, P, W = brown shrimp, Penaeus aztecus; pink shrimp, Penaeus duorarum, and white shrimp, Penaeus setiferus, used as test animals.

Table A-3. Concentration of pesticides in sea water causing a 50% decrease in oyster shell growth, EC_{50}.

Pesticide	Average salinity per mil	Average temp. °C	96-hour * EC_{50} ppm (mg/liter)	Recovery period weeks
Acaricides				
Tedion	27	27	0.53	3
Tedion	25	13	0.39	2
Defoliants				
DEF	27	27	0.38	1
DEF	27	10	0.1	3
Fungicides				
Bayer 47531	25	29	0.059	5
Chemagro 2635	29	29	0.01	8+
Chemagro 4497	23	28	0.24	2
Dyrene	22	30	0.046	7+
Dyrene	24	10	0.064	8+
Gastropodicides				
Polystream (mixed polychlorinated benzenes)	29	24	0.57	2
Herbicides				
2,4-D, 2 ethyl hexyl ester	27	15	38% at 5.0	1
Dacthal	29	15	0.25	1
Diuron	25	22	1.8	4
Fenuron	26	22	1/ ne at 2.0	0
Kurosal SL (60% silvex)	28	25	ne at 1.0	0
Monuron	25	22	12% at 2.0	0
Neburon	27	25	0.41	2
N-Serve	29	10	0.28	1
2,4,5-T polyglycol butyl ether ester	25	13	0.14	1
Tillam	25	28	20% at 1.0	0
Insecticides Carbamate				
Zectran	26	9	ne at 1.0	0

(continued)

Table A-3. Concentration of pesticides in sea water causing a 50% decrease in oyster shell growth, EC_{50} (continued).

Pesticide	Average salinity per mil	Average temp. °C	96-hour * EC_{50} ppm (mg/liter)	Recovery period weeks
Insecticides				
Chlorinated hydrocarbon				
Aldrin	19	11	0.055	8
Aldrin	27	30	0.025	3
Dieldrin	20	11	0.44	5
Dieldrin	25	22	0.034	0
Endrin	21	12	0.40	2
Endrin	22	24	0.033	0
Strobane	29	25	0.059	2
Telodrin	33	18	0.055	5
Thiodan	21	19	0.38	7
Thiodan	22	28	0.065	2
Organophosphorus				
Bayer 37289	28	28	0.07	4
Bayer 38156	29	23	0.084	9
Bayer 41831	29	27	0.69	2
Baytex	23	15	0.58	1
Bidrin	29	14	21% at 1.0	0
Ciodrin	28	10	1.0	1
CO-RAL	23	30	0.95	1
CO-RAL	21	9	0.51	1
Dimethoate (Cygon)	31	20	10% at 1.0	0
Dylox	22	30	ne at 1.0	0
Dylox	28	12	12% at 1.0	0
Ethion	23	30	0.059	2+
Ethion	29	10	0.07	1
Methyl parathion	29	24	ne at 1.0	0
Parathion (Thiophos)	31	24	22% at 1.0	1
Phosdrin	30	22	ne at 1.0	0
Phosphamidon	25	25	ne at 1.0	0

* Solubility of the pesticide limited the maximum concentration that could be tested under the described conditions and in some cases prevented a determination of EC_{50} values.

1/ ne = no effect.

Table A-4. Concentration of pesticides in sea water causing 50% mortality, 24- and 48-hour EC_{50}, to juvenile fish.

Pesticide	Kind of fish	Average salinity per mil	Average temp. °C	24-hour EC_{50} ppm (mg/liter)	48-hour * EC_{50} ppm (mg/liter)
Acaricides					
Tedion	[1] C	28	11	[2] ne at 1.0	ne at 1.0
Fungicides					
Dyrene	S	23	29	0.0	0.0085
Dyrene	M	21	29	20% at 0.1	20% at 0.1
Gastropodicides					
Polystream (mixed poly-chlorinated benzenes)	C	29	24	ne at 1.0	ne at 1.0
Herbicides					
Dacthal	C	29	15	ne at 1.0	ne at 1.0
Insecticides					
Carbamate					
Bayer 39007	C	28	25	ne at 1.0	ne at 1.0
Bayer 44646	C	28	25	ne at 1.0	10% at 1.0
Chlorinated Hydrocarbon					
Aldrin	S	28	24	[3] 0.0082	0.0055
DDT	S	20	12	[3] 0.005	0.002
DDT	C	25	9	ir at 0.1	0.005
Dieldrin	S	25	12	0.0055	0.0055
Endrin	S	24	12	0.0044	0.0006
Heptachlor	S	20	12	0.055	0.025
Kepone	S	26	22	0.3	0.17
Lindane	S	23	15	0.03	0.03
Methoxychlor	S	26	22	0.03	0.03
Mirex	S	27	22	ne at 2.0	ne at 2.0
Strobane	C	29	25	0.055	0.0085
Telodrin	C	30	17	0.0055	0.0036
Thiodan	S	26	22	0.0009	0.0006
Toxaphene	S	26	28	0.0022	0.001

(continued)

Table A-4. Concentration of pesticides in sea water causing 50% mortality, 24- and 48-hour EC_{50}, to juvenile fish (continued).

Pesticide	Kind of Fish	Average salinity per mil	Average temp. °C	24-hour EC_{50} ppm (mg/liter)	48-hour * EC_{50} ppm (mg/liter)
Insecticides					
Organo-phosphorus					
Aspon (ASP-51)	C	27	26	ne at 1.0	ne at 1.0
Bayer 41831	C	25	9	ir at 1.0	ir at 1.0
Bayer 38156	M	30	27	0.010	0.0067
Baytex	S	23	19	1.72	1.22
Bidrin	K	32	20	ne at 1.0	ne at 1.0
Ciodrin	C	30	16	ne at 1.0	ne at 1.0
DDVP (Vapona)	S	25	28	0.55	0.55
Dibrom	S	20	20	0.50	0.44
Dimethoate (Cygon)	K	32	20	ne at 1.0	ne at 1.0
Di-syston	C	28	25	0.74	--
Dylox	C	23	13	ne at 1.0	ne at 1.0
Ethion	C	25	12	0.42	0.069
Guthion	S	21	21	0.055	0.050
Malathion	S	24	19	0.55	0.55
Methyl para-thion	C	28	24	ir at 1.0	ir at 1.0
Parathion (Thiophos)	C	31	24	0.065	0.060
Phorate (Thimet)	K	32	18	0.0032	0.0004
Phosdrin	C	31	24	0.83	0.83
Phosphamidon	S	29	23	ne at 1.0	ne at 1.0
Phosphamidon	M	24	30	ne at 1.0	ne at 1.0
Systox	S	27	26	0.55	0.55

* Solubility of the pesticide limited the maximum concentration that could be tested in some cases.

1/ C - Cyprinodon variegatus, sheepshead minnow
S - Leiostomus xanthurus, spot
M - Mugil cephalus, striped mullet
K - Fundulus similis, longnose killifish

2/ ne = no effect

3/ ir = irritated

SPORT FISHERY INVESTIGATIONS

by

Oliver B. Cope
Division of Fishery Research
Bureau of Sport Fisheries and Wildlife

Investigations on pesticides and fish went ahead in 1963 at the Fish-Pesticide Research Laboratory in Denver and at field facilities at Marion, Ala.; Tishomingo, Okla.; La Crosse, Wis.; Jackson, Wyo.; and Laurel, Md. There was little change in physical features this year, and the staffs were able to devote full effort to working with fish and economic poisons. Advances were made in long-term studies at outlying stations and in acute toxicity bioassay at Denver and Laurel.

The summations that follow describe the chief results of the experiments in 1963. For each type of work, principal researchers are named in parentheses.

LABORATORY STUDIES AND TOXICOLOGY

Fish toxicity tests at Denver (W. R. Bridges, A. K. Andrews, John Gerdes, and Bruce Dart)

Toxicity tests at Denver included time-temperature studies, preliminary bioassay tests on new pesticides or different species of fish, and comparisons of toxicities of pyrethrum and rotenone.

1. Toxicity of DDT and toxaphene to bluegills was determined at various temperatures and times of exposure (table B-1). In general, the toxicity of DDT increased with decrease in temperature, with the effect of temperature tending to level off at 45° and 85°F. The 24-hour LC_{50} at 45° appears somewhat high; in this test, fish were completely immobilized at concentrations one-third to one-fourth of the LC_{50} values, but they were not dead. The toxicity of toxaphene increases moderately with increase in temperature. The inverse relation seen with DDT agrees with toxicity-temperature relations seen in insects.

2. The toxicity of similar emulsifiable formulations of rotenone and pyrethrum to rainbow trout, channel catfish, and bluegills is presented in table B-2. It is clear that the rotenone formulation is more toxic to these species than is the pyrethrum formulation.

3. Results of various bioassay tests made during the year with miscellaneous insecticides and herbicides are reported in table B-3.

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Fish toxicity tests at Laurel (Anthony Inglis, E. L. Davis)

Bioassay was carried on at the Patuxent Wildlife Research Center at Laurel after the new laboratory was put into operation in 1963. The work at Laurel consisted primarily in testing of herbicides against fish, with emphasis on the effects of water hardness on toxicity.

Table B-4 shows some toxicity measurements with silvex and bluegills and pygmy sunfish. Trends toward higher toxicity in harder waters are seen, but the differences in toxicity are not statistically significant.

Insect toxicity tests at Denver (W. R. Bridges, H. O. Sanders

Bioassay tests were conducted on wild aquatic insects collected in the field, held in the laboratory, and tested under standardized conditions. Table B-5 summarizes the results of tests involving six species of insects and nine insecticides, and shows that relative toxicities to these insects do not correspond to toxicities to fish.

C^{14} DDT in a microenvironment at Denver (W. R. Bridges, B. J. Kallman, A. K. Andrews)

Six hundred micrograms of C^{14}-labelled DDT were placed in 30 liters of water in each of 4 aquariums, together with soil and aquatic vegetation. Small bluegills were added to 2 of the aquariums after 28 days and snails of the genus Ampullaria after 6 weeks. The objectives of the study were to measure the breakdown of DDT in parts of the system and to learn if DDT would return to the water from high-residue components of the environment after the toxicant had reached near-zero levels in the water.

Samples of water, soil, and vegetation were taken from all aquariums for analysis by radioisotope detection. The vegetation was consumed by the snails in the two aquariums where they were present. Vegetation samples taken after that time were from the other two aquariums and the snails subsisted on lettuce fed every 2 days.

Fourteen days after the addition of the DDT, the level in the water was down to 0.42 ppb, was 6.0 ppb in the soil, and had reached 15,600 ppb in the vegetation. At 4 weeks when the fish were added, the water contained 0.30 ppb. The fish accumulated residues to more than 1,000 ppb in a week and a half, while the amounts in the mud were decreasing and those in the vegetation were still increasing. When the snails were added at 6 weeks, the water had 0.19 ppb, the soil had 1.1 ppb, the vegetation had 23,400 ppb, and the fish about 1,000 ppb. Two weeks later, the water was reduced to 0.08 ppb, the vegetation reduced to 20,700 ppb, and the snails contained 160 ppb. At 15 weeks, all parts of the environment still contained some DDT, but declines were apparent. Had the snails eaten vegetation containing DDT from the 8th to 15th week, the decline in DDT levels in the snails might not have occurred. Table B-6 summarizes the residue measurements made in the study.

Malathion and cutthroat trout at Jackson, Wyo. (Don Allison, B. J. Kallman, C. C. Van Valin, Joseph McCraren)

A long-term experiment dealing with chronic effects of malathion on cutthroat trout began at Jackson, Wyo. Measurements were made on brain cholinesterase activity, growth, mortality, and histopathological changes in the first phase of the work, in which lots of fish were given malathion in bath form every 35 days; other lots were given malathion in their feed, also at 35-day intervals; the control lot was given no malathion. The second phase, which will also consider reproductive success, will begin in January 1964.

Cumulative mortality in both lots of bathed fish exceeded that of the control lot throughout most of the year. Mortality in the fed lots approximated that of the control lot. The number of mortalities exhibiting traumatic injury or disease symptoms was about the same in all lots. This is in contrast to what happened in the DDT experiment, where disease symptoms occurred more often in dead fish in low-dosage lots than in high-dosage lots.

Hematocrit values decreased in all lots from January through September. There was no consistent pattern of hematocrit values from lot to lot; hematocrit values did not seem to be related to amount of exposure to malathion.

Consistent relations appeared among lots of trout with respect to inhibition and recovery of brain cholinesterase levels. Highest activity was always seen in control fish, and lowest activity in the high-treated bathed fish. Patterns of recovery between treatments were fairly uniform. Figure B-1 shows brain cholinesterase measurements, by colorimetry, for lot I, (the control lot), lot II, (the lot bathed at 1.0 ppm), and lot IV (the lot fed 8.0 mg/k. in the feed).

Sodium arsenite and bluegills at La Crosse, Wis. (Philip Gilderhus)

In 1963, considerable work was done in the laboratory to analyze samples of water, bluegills, and soils exposed in outdoor plastic pools in the 1962 experiment. In addition, a new chronic-effects experiment was carried on in 1963, with many measurements made and samples preserved for chemical analysis during the winter.

The amount of arsenic in water samples collected in 1962 was proportional to the amount of sodium arsenite applied to the pools, according to colorimetric analysis. The more frequent the application the greater the amount of chemical in the samples. The arsenic content of fish collected in 1962 was generally proportional to the strength of the exposures. In soil samples taken at 8 weeks there was more arsenic from pools treated once than from pools treated with more total arsenic at weekly intervals. Table B-7 shows these results.

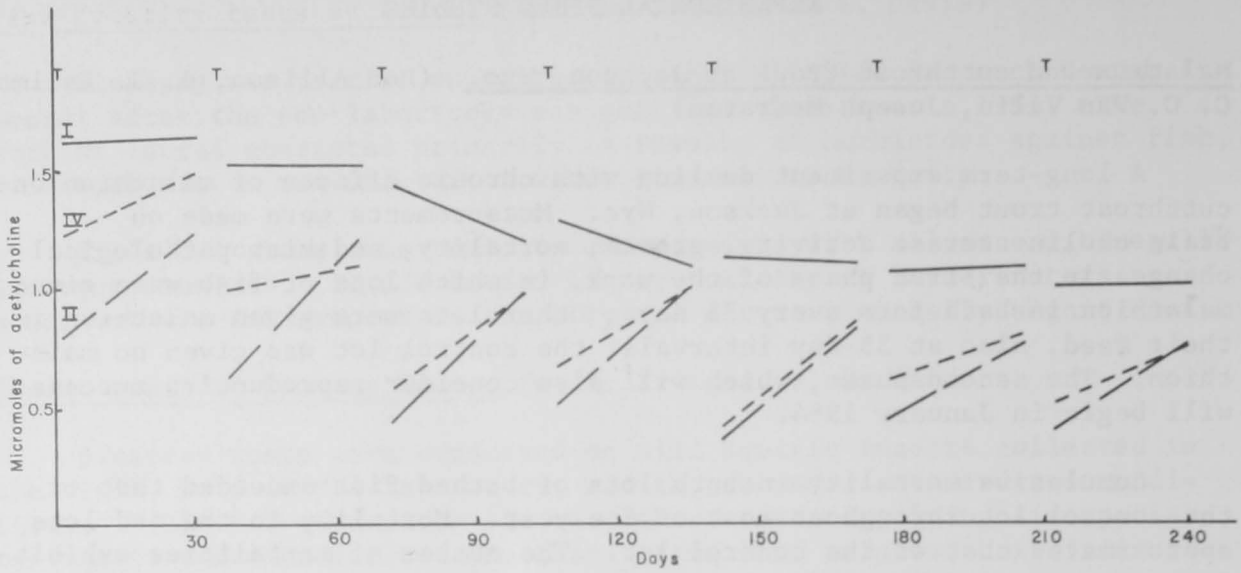

Figure B-1. Effects on brain cholinesterase of periodic exposure of three lots of cutthroat trout to malathion. Enzyme activity expressed as micromoles of acetylcholine hydrolyzed per 2 mg. of brain tissue in 30 minutes at 25°C. Treatments given at times shown by "T". Lot I was the control group. Lot II was bathed in malathion solution. Lot IV was given malathion in feed.

Results available from the 1963 exposures show that microhematocrit measurements were not correlated with concentrations of sodium arsenite in the pools. The ratio of gonad weight to body weight in bluegills collected after 8 and 16 weeks showed no difference directly attributable to amount of exposure to sodium arsenite. Survival of young-of-the-year fish after 16 weeks seemed to be influenced by degree of exposure to the chemical; survival was highest in the control and lowest in groups receiving 1.2 ppm at weekly and monthly intervals. The survival of adults, however, was not proportional to strength of treatment. Table B-8 shows survival measurements for immatures and adults.

Growth in length and weight of young-of-the-year bluegills tended to be inversely proportional to concentration of sodium arsenite in the pools, as seen in table B-9.

Adult bluegills in general sustained losses in weight, probably due to a failure of the natural food supply. The greatest losses in weight occurred in pools with the highest concentrations of arsenite in each treatment series.

Pathological examination of exposed fish from the ponds in 1963 revealed that few changes took place in the first few weeks. After that, some startling changes were apparent. No nematodes were found in the pyloric caecae, in contrast to the earlier condition. Kidney and liver damage appeared in the later samples. There is also a possibility of degenerative lesions in the ovaries of some fish.

Herbicides and sunfish in Okla. (Ronald Elkin)

Pond tests at Durant, Okla., were run with Kuron (propylene glycol butyl ether esters of 2,4,5-TP) to measure chronic effects on redear sunfish. Four ponds were used, one being an untreated control and others receiving 1, 3, and 10 ppm. Applications of Kuron were made on March 21, April 20, and May 22. The experiment was terminated on June 14.

Residues of Kuron in the ponds, as measured by gas chromatography, increased with time, degradation of the herbicide proceeding at slower rates than the addition of new chemical under the schedule. The result was the development of residues in the water to levels greatly exceeding what would be used in aquatic weed control. Some apparent effects on the fish were measured. The average sizes of fish taken in trap samples had greater lengths and weights in the ponds that were treated than in the control pond, and the stronger the treatment, the larger and heavier were the fish. Microhematocrit measurements were higher in treated fish than in untreated fish, but the heavily treated fish did not have higher hematocrits than those exposed to lighter treatments. Definite pathology was found associated with exposure to Kuron. Well-defined liver degenerative lesions were found from 2 weeks on in the 3- and 10-ppm treated fish, but not at any time in the control or the group treated at 1 ppm. From the 5th week, testicular degenerative lesions were seen in the 3- and 10-ppm groups, resulting in apparent exhaustion atrophy of the spermatic tubules and production of immature, atypical, and abnormal spermatozoa. No comparable changes were seen in ovaries.

Experiments in ponds at Tishomingo, Okla., were begun in September to measure chronic effects of Kurosal SL (potassium salt of 2,4,5-TP) on bluegills. Applications were made to ponds at 0, 10, 25, and 75 ppm. There was no immediate effect on the fish, but filamentous algae and Potamogeton began to die at the outset. Nais was eliminated after 3 weeks, but Chara was not affected. Fish died in limited numbers in the 75-ppm ponds after a few weeks, and about 35 percent of the fish in these ponds had died after 2-1/2 months. Mortality in the 25-ppm ponds was at lower rates, but none was seen in the 10-ppm ponds or in the control ponds.

Kurosal in the pond water has not been as persistent as was the Kuron, according to bioassay with cucumber seeds. The Kurosal broke down at faster rates than the repeated additions to the ponds once a month.

Heptachlor and bluegills at Marion, Ala. (A. K. Andrews, Bruce Stebbings)

Two outdoor experiments were carried on at Marion, Ala., to measure chronic effects of heptachlor on bluegills. In one study, heptachlor was added at various levels to the diet of bluegills in plastic wading pools; in the other study, heptachlor was added to the water in 1/10-acre earthen ponds holding bluegills.

The pond study, with exposure at 0.05, 0.0375, 0.025, 0.0125, and 0 ppm of heptachlor, showed rapid development of residues of heptachlor, heptachlor epoxide, and related compounds in pond water, bottom sediments, and fish. Measurement of residues was done by paper chromatography. Buildup of residues in fish and water was immediate, but no residues were found in bottom sediments until after the 14-day sample. Heptachlor measured in water never approached the amounts of toxicant added. Residues in soils were found only in the two highest levels of treatment, never exceeded by much the amounts added to the ponds, and were not found after the 56-day sample, in June. Table B-10 summarizes the residue data for fish.

Pathological examination of 99 fish collected through 28 days in these tests revealed no tissue or cell changes associated with exposure to heptachlor.

Growth of fish in the ponds, as indicated by average sizes of fish taken in samples throughout the experiment, was different from pond to pond. In general, the average size of fish taken from heavily treated ponds was greater than in fish from the control and lightly treated ponds.

No heptachlor was found in bluegills after the 56-day sample. Unexplained was the appearance of DDT and its degradation products in fish at about the time of the disappearance of the heptachlor. DDT was not found in water, mud, or vegetation, and the source of the DDT in the fish is not known.

Numbers of invertebrates sampled from the ponds by traps and with dredges indicated that the control pond had greater numbers of organisms, exclusive of the gastropods, than did the treated ponds during most of the experiment. Greatest numbers of invertebrates were generally trapped at the 24-inch depth.

The plastic pool study, with feeding of heptachlor at 25, 10, 5, and 0 mg/kg, showed development of residues of heptachlor, heptachlor epoxide, and related compounds in the bluegill whole bodies. Residues did not form as fast or to as great an extent as in the pond studies described above. Also, as in the pond studies, DDT and its products appeared in many fish; in this experiment the appearance was in the 112-day sample in October.

Growth in bluegills in these tests appears to have been influenced by exposure to heptachlor, with greater growth in groups given no treatment or low treatments. Table B-11 summarizes data on size of fish.

These results do not agree with those derived from the pond study described above, but differences in sampling methods and treatment rates may explain the discrepancy.

Residues in fish from hatcheries (C. C. Van Valin, John O'Donnell, Ernest Giedd, Henry deHoll)

Rainbow trout fed for 26 months on various diets in a large-scale hepatoma induction experiment were submitted to this laboratory from nine National Fish Hatcheries in various sections of the United States. Chemical analyses by paper chromatography were made of whole bodies of the fish, and the only pesticide found in any fish was the DDT complex. DDT and its metabolites were found in every fish examined, and there was variation from sample to sample, from station to station, and from diet to diet. We conclude that the greatest cause of variation in DDT residues was differences in diets. Table B-12 summarizes the findings.

EFFECTS ON FIELD POPULATIONS

Grasshopper control (W. R. Bridges, C. C. Van Valin)

A grasshopper control program was carried on by the U. S. Department of Agriculture on the Boise National Forest in July. Malathion was sprayed from airplanes at the rate of 3/4 pound per acre on 65,000 acres. Our staff studied short-term effects in the water at strategic sites. Observations on resident fish in the streams and on hatchery trout placed in live-cars indicated no mortality or other adverse effects on fish. Brain cholinesterase activity in rainbow trout in live-cars, as measured colorimetrically, was slightly reduced in some cases, but had returned to normal levels within 15 days. Numbers of aquatic insects were killed or immobilized by the spray in at least two small tributaries of the Boise River. However, many apparently healthy insects remained in the stream after the dissipation of the malathion.

EQUIPMENT, METHODS, AND TECHNIQUES

Chemistry methods (C. C. Van Valin, John O'Donnell)

Methods for the determination of Kuron and Mirex in fish and water have been partially developed.

The procedure for Kuron in fish consists in extraction with 10% ethyl ether in petroleum ether, followed by partitioning of the extract residue between hexane and acetonitrile, elution through a column of acid-washed chromatographic adsorption alumina, and final estimation by paper chromatography using silver nitrate spray to develop the spots of Kuron. Early data indicate a recovery of 54%. The paper chromatograph development will afford detection and estimation of as little as 0.1 µg on the paper.

Mirex determination in fish consists of extraction with 10% ethyl ether in petroleum ether, passage of the extract residue through an MgO

celite column, treatment with fuming sulfuric acid, and paper chromatography-silver nitrate development. Early data indicate 83% recovery with sensitivity to 0.1 μg on the paper.

Kuron and Mirex can be recovered from water by adsorption on activated charcoal. The recoveries are 49 and 84%, respectively.

Tests have been conducted to compare the efficiencies of different activated charcoals used in the analysis of DDT in water samples. Those tested were Nuchar C-190 (+30 mesh), coconut charcoal (6-14 mesh), and coconut charcoal (powdered). The granular and powdered coconut charcoals are roughly equivalent in their ability to absorb DDT from water. They are inferior in this respect to the Nuchar C-190, but they contribute less contaminating material which interferes in the chromatographic development. The recovery factor for Nuchar C-190 is 70% in the range of 10-20 μg DDT per gallon of water. The factor for granular coconut charcoal is 40%.

Table B-1. Influence of time and temperature on toxicity of two insecticides to bluegills averaging 1.2 grams in weight.

Insecticide and Temperature	LC_{50} in µg./1 at: 24 hrs.	48 hrs.	96 hrs.
DDT and bluegills			
45	21.0	2.4	1.6
55	5.0	2.6	2.0
65	7.4	5.4	4.5
75	9.2	7.0	6.6
85	9.8	6.4	5.6
Toxaphene and bluegills			
45	35.0	9.6	4.3
55	9.6	5.5	3.4
65	7.2	4.9	2.6
75	6.6 (7.0)[1/]	3.8 (6.0)[1/]	2.5 (5.0)[1/]

1/ Figures in parentheses are LC_{50} values for DDT for this lot of fish.

Table B-2. Toxicity of rotenone and pyrethrum formulations[1/] to three species of fish

Exposure period	LC_{50} in µg. of total material/1 Pyrethrum	Rotenone
Rainbow trout (0.3 gram) Tested at 55°F. for --		
24 hours	56	32(3.0)[2/]
48 hours	54	28(2.0)
96 hours	54	26(1.5)
Channel catfish (0.5 gram) Tested at 75°F. for --		
24 hours	96	32(4.9)
48 hours	82	29(4.1)
96 hours	78	26(3.3)
Bluegills (0.6 gram) Tested at 75°F. for --		
24 hours	78	24(8.4)
48 hours	70	22(6.0)
96 hours	70	22(4.7)

1/ Rotenone 0.7% active ingredient; pyrethrum 1.0% active ingredient. Same materials used to prepare both formulations, except that the rotenone preparation contained 6% more xylene than did the pyrethrum.

2/ Figures in parentheses are LC_{50} values for DDT in µg/1. for the lot of fish tested.

Table B-3. Toxicity measurements of various pesticides versus fish.

Toxicant	Species	Wt. or Length	Temp. °F.	Estimated LC_{50}, µ/l at: 24 hrs.	48 hrs.	96 hrs.
Aldrin, tech.	Black bullhead	1.5g	75	22	19	19
Apholate, tech.[1/]	Rainbow	1.5g	55	Not affected at 40,000 µ/l. for 96 hours.		
DDT, p,p'	Black bullhead	0.9g	75	65	47	27
DDT, p,p'	Channel catfish	1.4g	75	4.2	3.3	2.9
Dieldrin, tech.	Black bullhead	1.5g	75	11	10	10
Dimethoate, tech.	Rainbow	1.5g	55	20,000	12,000	8,500
Endrin, tech.	Black bullhead	1.5g	75	1.3	1.1	1.1
	Channel catfish	1.4g	75	0.45	0.34	0.29
Ethyl Guthion, tech.	Bluegill	0.8g	75	3.8	1.4	-
Heptachlor, tech.	Black bullhead	0.9g	75	76	50	34
Lindane, tech.	Bluegill	0.8g	75	61	26	-
	Rainbow	5.4g	55	49	-	-
Toxaphene, tech.	Black bullhead	0.9g	75	7.7	6.4	5.8
PGBE esters of:						
2,4-D, E.C. 70.5%	Rainbow	1.5g	55	1,200	1,100	1,100
2,4-D, Tech. 100%	Rainbow	1.5g	55	1,200	1,100	1,100
2,4,5-TP,E C.65.5%	Rainbow	1.5g	55	750	650	600
2,4,5-TP,Tech.100%	Rainbow	1.5g	55	1,500	1,400	1,300

1/ Insect sterilant

Table B-4. Toxicity of butoxyethanol ester of 2,4,5-TP to two species of fish, in waters of various conductivities and pH values.

Species and conductivity in micromhos	pH	LC_{50} in mg/l at: 24 hrs.	48 hrs.	96 hrs.
Bluegills, 0.75 g				
101.6	7.4	.460	.415	.405
171.2	8.1	.575	.520	.500
176.4	7.4	.440	.395	.390
404.3	8.1	.545	.530	.520
439.6	7.4	.450	.432	.418
650.5	7.8	.565	.550	.535
654.0	6.1	.400	.360	--
Pygmy sunfish				
82.2	5.3	.763	.758	.720
155.0	5.5	.738	.699	.633
408.4	4.8	.603	.532	.489
646.5	6.3	.635	.590	.580

Table B-5. Toxicities of various insecticides to some immature aquatic insects, tested at 60°F.

Genus and Toxicant	LC_{50} in µg./l at: 24 hours	48 hours	96 hours
Pteronarcys (stonefly)			
Aldrin	30.0	8.0	1.3
DDT	45.0	16.0	9.0
Dibrom	27.0	16.0	8.0
Dieldrin	6.0	1.1	0.70
Endrin	4.0	0.96	0.25
Heptachlor	8.0	5.6	1.1
Lindane	1.2	1.0	--
Malathion	40.0	20.0	10.0
Phosphamidon	1400.0	460.0	130.0
Toxaphene	18.0	7.0	2.3
Pteronarcella (stonefly)			
DDT	--	5.0	3.0
Malathion	12.0	6.0	--
Heptagenia (mayfly)			
DDT	0.6	0.3	0.1
Malathion	0.4	0.2	--
Lestes (damselfly)			
DDT	30.0	10.0	--
Hydropsyche (caddisfly)			
DDT	7.4	3.4	1.7
Malathion	26.0	13.0	5.0
Toxaphene	33.0	27.0	4.2
Atherix (snipefly)			
DDT	84.0	25.0	17.0

Table B-6. Total amounts of DDT and its metabolites measured in components of the micro-environment. 20 μ/l of C^{14}-labelled DDT placed in the system in one application.

Time elapsed	Total residues, μ/l				
	water	soil	vegetation	fish	snails
10 days	1.1	--	--		
2 weeks	0.42	6.0	15,600		
3 weeks	0.07	3.5	19,800		
4 weeks	0.30	--	--	1/	
6 weeks	0.19	1.1	23,400	Approx. 1000	2/
8 weeks	0.08	--	20,700	--	160
11 weeks	0.06	--	9,500	--	120
15 weeks	0.03	--	4,100	--	140
21 weeks	0.03	--	1,960	--	28

1/ Bluegills were added to the system at 4 weeks. 3-day samples averaged 270 μ/l.
2/ Snails were added to the system at 6 weeks. 4-day sample measured 140 μ/l.

Table B-7. Arsenic residues in water, bottom soils, and fish from pools treated for an 8-week period in 1962.

Pool No.	Herbicide application rate (ppm As^{o})	Residues at end of 8 weeks (ppm As^{o})		
		Water	Fish Flesh	Soil
1	2.31 yearly	1.01	0.38	92.1
2	0.69 yearly	0.056	0.35	37.3
3	0.23 yearly	0.024	1.02 1/	10.7
4	0.69 monthly	0.43	0.17	38.1
5	0.23 monthly	0.12	0.20	22.5
6	control	ND 2/	ND	ND
7	0.69 weekly	4.81	3.88 1/	44.9
8	0.23 weekly	0.98	0.78 1/	36.7
9	0.023 weekly	0.12	0.09	6.5

1/ Small fish
2/ ND denotes no detectable amount

Table B-8. Survival of immature and adult bluegills after a 16-week exposure to various concentrations of sodium arsenite in plastic pools in 1963.

Pool No.	Herbicide application rate (ppm As^{o})	Young-of-year		Adult		
		Number stocked	Number surviving	Number after sampling	Number surviving	Percent surviving
1	4.0 yearly	200	103	154	95	62
2	1.2 yearly	200	108	154	89	58
3	0.4 yearly	200	159	154	80	52
4	1.2 monthly	200	90	134	81	60
5	0.4 monthly	200	163	151	78	52
6	control	200	179	133	80	60
7	1.2 weekly	200	35	143	44	31
8	0.4 weekly	200	145	163	82	50
9	0.04 weekly	200	147	163	89	55

Table B-9. Growth of young-of-the-year bluegills during a 16-week exposure to various concentrations of sodium arsenite in plastic pools in 1963.

Pool No.	Herbicide application rate (ppm As^{o})	Average length (in.)		Average weight (gm.)		Weight ratio final/orig.
		Orig.	After 16 weeks exposure	Orig.	After 16 weeks exposure	
1	4.0 yearly	1.37	1.98	0.54	1.92	3.5
2	1.2 yearly	1.37	2.42	0.54	3.93	7.3
3	0.4 yearly	1.37	2.57	0.54	4.48	8.3
4	1.2 monthly	1.37	2.06	0.54	1.76	3.3
5	0.4 monthly	1.37	2.53	0.54	4.62	8.6
6	control	1.37	2.40	0.54	3.32	6.1
7	1.2 weekly	1.37	1.93	0.54	1.53	2.8
8	0.4 weekly	1.37	2.27	0.54	2.88	5.3
9	0.04 weekly	1.37	2.48	0.54	4.21	7.8

Table B-10. Total residues, in ppm, of heptachlor, heptachlor epoxide, and related compounds in whole bodies of bluegills exposed to one application in ponds at Marion, Alabama, beginning in April 1963.

Time after treatment	Pond 1 Treated at 0.05 ppm	Pond 2 Treated at 0.0375 ppm	Pond 3 Control	Pond 4 Treated at 0.025 ppm	Pond 5 Treated at 0.0125 ppm
16 hours	9.2	5.7	--	--	--
1 day	28.7	27.2	ND[1/]	--	11.1
3 days	27.7	45.1	ND	4.0	3.8
7 days	52.6	29.5	ND	9.4	0.9
15 days	56.8	19.8	ND	11.1	3.2
28 days	15.7	8.1	ND	8.0	0.5
56 days	0.15	0.18	ND	0.15	--
84 days	ND	ND	ND	ND	ND
125 days	ND	ND	ND	ND	ND
140 days	ND	ND	ND	ND	ND

1/ ND denotes no detectable amount

Table B-11. Average weights of bluegills on August 30 after receiving heptachlor in feed for three months at Marion, Alabama.

Heptachlor concentration in feed, mg/kg.	Number of fish sampled	Total weight of samples, g.	Average weight per fish, g.
25	15	93	6.20
10	37	316	8.54
5	69	681	9.87
Control	48	573	11.94

Table B-12. Total amounts of DDT and its products measured in rainbow trout from nine hatcheries and fed various diets for 26 months. DDT content expressed as ppm., each number representing one sample.

Diet	McNary	Norfolk	Leetown	Hagerman	Lamar	Quilcene	Ennis	Manchester	Spearf
Clark	0.66	1.05 0.56	0.58 0.88	0.69	0.72	1.22 0.75	1.30 0.85	0.65 0.80	1.18 1.21
Rangen	0.62	0.86 0.92	0.97 0.34	0.97	0.59 0.82	0.71 0.50	0.59 0.56	0.47 0.45	0.37 0.50
Glencoe	--	0.34 0.34	--	--	--	--	--	--	0.27 0.12
Strike	--	0.71 0.58	--	--	0.62 0.44	--	--	--	--
Purina	0.18	--	0.16 (20%)	0.25	--	--	--	0.37 0.37	--
Hill	--	--	--	0.33	--	--	--	--	--
Murray	0.13	--	--	0.13	--	0.10 0.23	0.19 0.17	--	--
Stockton	--	--	--	0.12	--	--	--	--	--
Small's Dina Fish	--	--	--	0.88	--	0.36 0.77	0.82 1.08	--	--
Cortland #6	--	--	--	--	0.80 0.69	--	--	--	--
Oregon Moist Pellet	--	--	--	0.60	--	--	--	--	--
100% Meat	--	--	0.07 0.10	--	--	--	--	0.11 0.09	0.25 (liver) 0.18

WILDLIFE STUDIES, DENVER WILDLIFE RESEARCH CENTER

by
D. Glen Crabtree
Division of Wildlife Research
Bureau of Sport Fisheries and Wildlife

The broad aim of all pesticide-wildlife studies at the Denver Wildlife Research Center is to develop a firmer understanding of the relation between wide-spread applications of various pesticides and the welfare of the wildlife species in the western United States. For the most part, pesticide-wildlife studies are of a continuing nature; therefore, the data and any conclusions drawn before the studies are completed can only be regarded as tentative.

The pesticide-wildlife studies of the Denver Center during 1963 are discussed under 5 categories:

1. Improvement of analytical methods for identifying and measuring pesticide and residues in organic and inorganic materials.

2. Experiments to judge the effects of pesticides on confined wildlife.

3. Studies of experimental field plots to evaluate gross effects of pesticides on wildlife.

4. Surveillance of pesticide programs.

5. Miscellaneous field investigations relating to pesticide-wildlife problems.

With one exception, all pesticide residues listed in this report were obtained by a paper chromatographic analytical method. This procedure has a maximum sensitivity of 5×10^{-7} grams for the detection of the chlorinated hydrocarbon compounds involved. The material from Tule Lake as outlined in table C-9 was analyzed with a microcoulometric chromatograph which has a maximum sensitivity for the detection of many of the organo-phosphate insecticides. The maximum sensitivity of the colorimetric field test kit for measuring cholinesterase inhibition is approximately a 0.20 pH unit change in the substrate.

IMPROVEMENT OF ANALYTICAL METHODS FOR IDENTIFYING AND MEASURING PESTICIDES AND RESIDUES IN ORGANIC AND INORGANIC MATERIALS

Comparison of gas chromatographs (William H. Robison)

There is a need for improving existing methods or developing new ones for the identification and measurement of pesticide materials as they occur in plants and animals, soils, and waters. A number of studies of the Denver Center have been directed toward this goal.

One such study compared the use of two different kinds of gas chromatograph, one equipped with a thermal conductivity and hydrogen flame detector and the other having an electron capture cell detector. The results show that the gas chromatograph equipped with an electron capture cell detector is superior in the determination of traces of chlorinated hydrocarbons in animal tissues. By this method, small amounts may be easily detected and measured with a relatively high degree of accuracy.

Blood cholinesterase activity as an indication of organo-phosphate exposure (James K. Peterson and Charles W. Hall)

A second study sought better methods of detecting and measuring small amounts of organo-phosphate insecticides. Organophosphates alter the blood cholinesterase activity; this phenomenon can be measured by several methods. Two methods that appeared to have the highest potentiality were compared: (1) the colorimetric method of Hestrin (1949) in which unreacted acetylcholine is measured chemically, and (2) a modification of the electrometric measurement technique of Michel (1949) in which the cholinesterase is allowed to act on acetylcholine in a standard buffer solution. The comparison showed that the electrometric method seems preferable because of its greater sensitivity as well as its better reproducibility of results. Tables C-1 and C-2 summarize the findings as they relate to various test animals. A major disadvantage of the colorimetric method is the necessity of filtering each of the samples containing red blood cells.

EXPERIMENTS TO JUDGE THE EFFECTS OF PESTICIDES ON CONFINED WILDLIFE

Metabolic fate of DDT in the rat (James E. Peterson and William H. Robison)

Earlier studies which demonstrated that partial *in vivo* conversion of DDE to TDE can take place, were extended in early 1963 to include additional research into the metabolic fate of DDT in the rat. The results now more completely describe the steps through which DDT passes during the course of detoxication and elimination. In addition to the well-known DDE and DDA, TDE (DDD, Rhothane) was proved to be a DDT metabolite. Four previously unreported compounds were isolated and identified. These are 1-chloro-2, 2-bis(p-chlorophenyl)ethylene ("DDMU"), 1-chloro-2,2-bis(p-chlorophenyl) ethane ("DDMS"), unsymmetrical bis(p-chlorophenyl)ethylene ("DDNU"), 2,2-bis(p-chlorophenyl)ethanol ("DDOH").

Prairie grouse: toxicity and tissue residues of Sevin and dieldrin
(Lowell C. McEwen, James E. Peterson, Milton H. Mohn and George H. Ise)

Acute oral toxicities of Sevin and dieldrin were determined in adult male sharp-tailed grouse and greater prairie chickens that were live-trapped in North Dakota and Nebraska and studied in Denver. Tissues of the birds were analyzed for Sevin or dieldrin residues after administration of measured dosages in gelatin capsules.

Acute toxicity of Sevin and tissue residues for the few birds studied are summarized in table C-3. The limited results indicate a relatively low acute toxicity to prairie grouse since two sharptails and three prairie chickens survived single oral doses of Sevin ranging from 1,020 to 1,860 mg/kg. Toxicity of this chemical was also low for ringneck pheasants, and for bobwhite quail in other studies (U. S. Bureau of Sport Fisheries and Wildlife, 1963). Sharptails that died from Sevin did so within 24 hours. This was found to be true also of pheasants in similar tests at the Denver Center. However, the two prairie chickens survived 2-3 days before death. Droppings were collected for analysis from the two sharptails surviving large doses of Sevin. The curve in figure 1 illustrates the rapid rate of elimination of Sevin in the feces of these birds.

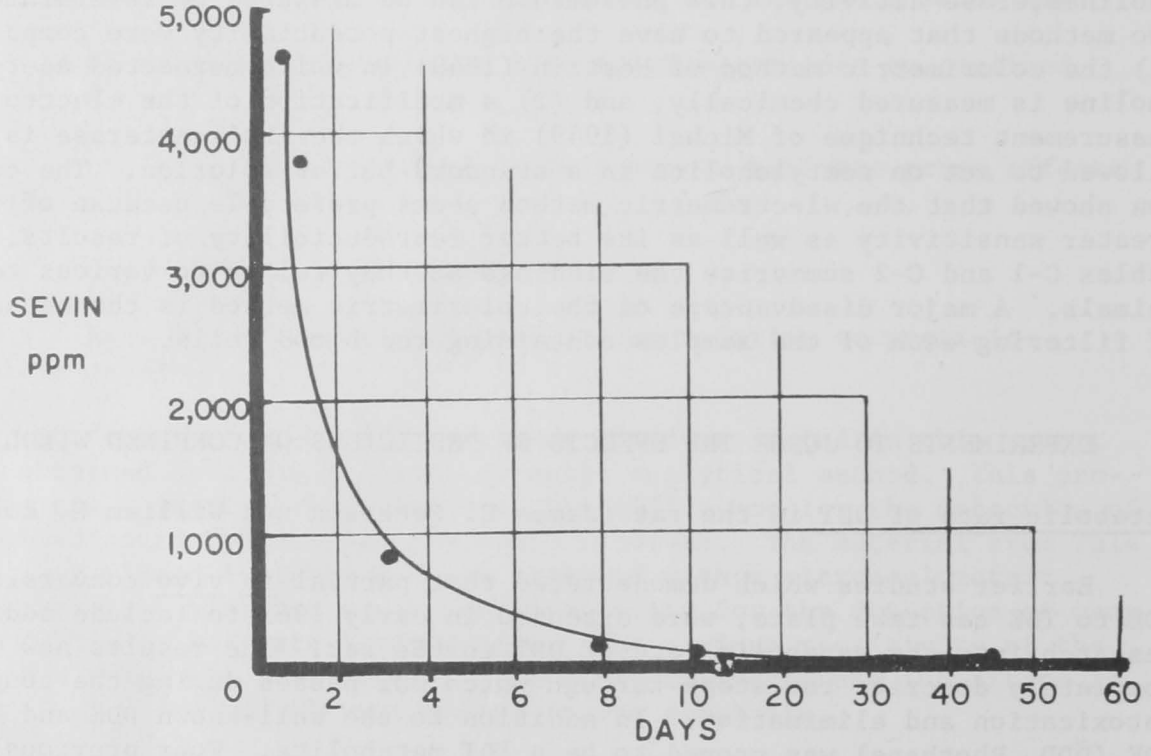

Figure C-1. Sevin concentration in droppings of two sharptails dosed at 1,021 and 1,500 mg/kg respectively.

Tissue residues of Sevin, which ranged from 0.1 to 21 ppm in tissues, were relatively low in five birds that died after large dosages (table C-3). No Sevin was recovered in the brain, kidneys, or liver of one surviving bird that was sacrificed in good condition 169 days after pesticide administration.

Dieldrin was found to be highly toxic to both sharptails and prairie chickens (table C-4). Adult male sharptails were killed by all oral doses of dieldrin down to 2.5 mg/kg. Survival occurred only at the level of 1.5 mg/kg. The prairie chickens showed slightly greater resistance to dieldrin than did the sharptails. Three control birds given lactose in capsules showed no ill effects.

Of interest was the fact that one prairie chicken survived a 17 mg/kg dieldrin dose. When this bird was sacrificed 189 days later, the brain, kidneys and liver were found to contain slightly less than 1 ppm dieldrin. Subcutaneous and visceral fat deposits in this specimen contained 15 ppm dieldrin.

Reaction of Canada geese to a diet containing 2,4-D (Mitchell G. Sheldon, Paul Johnson, James E. Peterson and William H. Robison)

In an experiment to more fully understand the effect of 2,4-D on Canada geese, 10 specimens were fed an ad-libitum diet containing 1000 ppm of Carbon-14 tagged 2,4-D. A control group of 10 geese was also maintained. Geese were sacrificed after the following periods of exposure to the herbicides: 22, 50, 62, 73, 113, 134, 197 and 230 days. When an experimental bird died or was sacrificed, a control goose was also sacrificed and both were examined for gross and microscopic changes in vital organs. Various tissues were reserved for future residue analysis. In general, findings were as follows: Enlargement of kidneys and a general jaundiced appearance of other organs occurred in birds subjected to the 2,4-D diet. However, no gross external changes were apparent when treated birds were compared with untreated birds. The average weight gain for the treated birds, including two that died after 197 and 230 days, was 333.3 gms. The untreated control birds gained an average of 481.0 grams.

Histological examination of various organs indicated a general progressive "disorganization" of cellular structure. Hepatic cell destruction, round cell invasion and fatty degeneration were observed in the liver. Kidney damage included evidence of capillary degeneration and thrombosis, dissolution of Malphigian tubules, fatty degeneration, invasion of connective tissue, arteriole wall enlargement, and increase in size of undissolved glomeruli.

Two geese were removed from treatment after 192 days and were placed upon a herbicide-free control diet. Histological examination of one goose sacrificed 4 months after return to a "clean" diet showed moderate improvement. Micro-examination of tissues of the goose killed 7 months after removal from a 2,4-D diet indicated that presumed damage had been largely corrected and the visceral organs had returned to approximately normal appearance and structure.

DDT fed to mule deer (Richard E. Pillmore and Richard E. Wilson)

In order to study the rate of accumulation and elimination of DDT or its metabolites in subcutaneous fat, a 2-year old, male, mule deer was given p,p'DDT at the rate of 10 mg/kg/day. The pesticide was administered in the feed for 10 consecutive days, starting September 27. Subcutaneous fat over the rump was sampled surgically.

During the experiment which continued 31 days, no abnormal behavior or other symptoms associated with DDT poisoning were noted. At the end of this period the deer was sacrificed and autopsy samples taken. Analyses of both biopsy and autopsy samples are given in table C-5. The build-up of DDT in the fat during the period of administration progressed as expected.

In another experiment an acute dose of 500 mg/kg of p,p'DDT was administered to a pregnant Rocky Mountain mule deer on April 11, 1963. The DDT was given in 220 ml of olive oil by intrarumen injection. On April 12 the deer exhibited recurrent tremors, loss of muscular coordination, and a nasal discharge which became thick, stringy and yellow-green later in the day. The deer died the following morning.

Post-mortem examination revealed no visible fat deposits in the deer. Results of chemical analyses of several tissues for DDT and derived residues were as follows:

Tissue	DDT	TDE*
Brain	16.0 ppm	3.6 ppm
Liver	1.0 "	38.0 "
Kidney	7.0 "	4.0 "
Adrenals	11.0 "	65.0 "
Placental membranes	2.0 "	0.8 "
Fetus	7.0 "	13.0 "

* Only metabolite of DDT found

The 500 mg/kg dose of p,p'DDT was lethal to this deer; however, the poor general condition of the animal may have influenced this susceptibility. A rapid transfer of ingested DDT across the placental membranes into the fetus was also demonstrated.

Mule deer: systemic control of nose bot (Richard E. Pillmore)

The control of nose bot larvae (Cephenomyia) in penned deer would be of value. Famophos, an organic phosphate, was administered by intramuscular injection in accordance with the manufacturer's recommendation for sheep, 60 mg/kg. This dosage killed a mule deer fawn and caused intoxication of two domestic goats. A second fawn and 7 adult deer died after receiving 40 mg/kg; one deer showed intoxication but survived after receiving 30 mg/kg. The experiments will be continued.

Relative toxicity of five organo-phosphate insecticides to mallard ducks (James O. Keith, in cooperation with Mir S. Mulla, University of California at Riverside and Arthur F. Geib, Kern Mosquite Abatement District)

Evaluation of long-term effects of organo-phosphate insecticides on waterfowl, the direct effects of which have been reported previously (U. S. Bureau of Sport Fisheries and Wildlife, 1963, p. 52), were completed in 1963. Briefly, five mallards of both sexes, hand reared after artificial incubation, were held in each of 10 ponds subjected to 6 biweekly treatments of Baytex, Guthion, parathion, SD-7438 and Sumithion at the rate of 0.4 pounds per acre. Three birds were present in each of two control ponds. Observations were made for 6 months after completion of treatments in November 1962. The ducks showed no apparent effects from the treatment during this period.

The chronic feeding trials reported here were begun in 1963 to determine effects on mallards of diets contaminated at 25 parts per million with Guthion, Baytex, parathion, SD-7438, Sumithion and with pure corn oil, which was used as a carrier for the insecticides. Eight ducks of mixed sexes were held in each of six pens, and each group was continuously offered food contaminated with one of the materials. No supplemental food was given. Birds were kept on Baytex diets for 36 days and on other diets for 42 days. The following weight data were collected:

Insecticide	Ave. food consumed in gms/bird	Ave.grams gained	Ave.grams lost by: Surviving birds	Dying birds
Baytex	38		137	347
Guthion	77	152		
Parathion	50		219	376
SD-7438	78	113		
Sumithion	72	57		
Control	74	163		

Ducks on diets containing Guthion, SD-7438, and Sumithion ate amounts of food comparable to those consumed by control birds, gained weight, and survived.

Ducks that consumed Baytex and parathion ate much less food than control birds and lost weight. They showed chronic poisoning after 3 weeks on test; symptoms were decreased activity, bills and feet cold to the touch, and movement by a slow, accentuated "goose step". Three birds on Baytex diets died at the expiration of 28, 32, and 35 days on test, and three on parathion diets after 28, 39 and 41 days. Five birds survived on each of these diets; surviving birds lost less weight than birds that died.

Ducks that survived exposure to Baytex and parathion were returned to untreated diets and gained an average of 145 and 123 grams, respectively, during the first week. They lost all recognizable symptoms of poisoning within 2 days, but were weaker and in poorer condition than the other experimental birds after 2 weeks.

These tests indicate that mallard ducks continuously exposed to the relatively light contamination of 25 ppm of Baytex and parathion in their food would suffer serious intoxication and death.

Acute toxicity tests also were undertaken to further evaluate the relative hazards of these insecticides to mallards. Seven weeks after termination of feeding trials, the experimental ducks were given corn oil solutions of insecticides encapsulated in gelatin. Approximate acute oral LD_{50} of these organophosphates for mallards were:

Compound	No. of birds used	Approx. LD_{50} in mg/kg
Baytex	13	1.0
Guthion	7	150.0
Parathion	13	1.0
SD-7438	6	400.0
Sumithion	6	125.0

Because chronic poisoning of these specimens may have affected the results, the tests were repeated in January 1964 with birds not previously exposed to the insecticides. Results of the second test were quite similar to those reported above, and verified the high toxicity of Baytex and parathion.

Toxicity of DDT and toxaphene to young white pelicans (James O Keith)

During 1960, 1961, 1962 and 1963 an unusual mortality of fish-eating birds of several species occurred at the Tule Lake National

Wildlife Refuge. Over 1,100 birds were found dead at the refuge during this period. Fish and birds collected from refuge ponds contained residues of DDT and toxaphene which apparently were transported into marsh habitats in waste agricultural water. Experimental work was attempted to determine the significance of these findings.

Acute toxicity trials were conducted in June with young white pelicans at the Refuge to determine the approximately lethal doses of toxaphene and DDT (table C-6). The data indicate that toxaphene is much more toxic to these birds than DDT, but there is apparently no potentiation due to the presence of DDT. In the trials involving both insecticides, all birds except one receiving 400 mg/kg reacted in a manner that could have been expected on the basis of amounts of toxaphene ingested.

Experiments are in progress to ascertain the effects of chronic assimilation by pelicans of diets containing 10 ppm toxaphene, 50 ppm DDT, and 50 ppm DDT plus 10 ppm toxaphene.

STUDIES OF EXPERIMENTAL FIELD PLOTS TO EVALUATE GROSS EFFECTS OF PESTICIDES ON WILDLIFE

Experimental application of dimethoate (Richard E. Pillmore)

A pilot test of dimethoate for the control of spruce budworm was conducted by the U. S. Forest Service with the assistance of the American Cyanamid Company on the Carson National Forest of New Mexico. Biologists from the Denver Center set up enclosures in the test area for domestic rabbits, and observed the treatment for any visible effect upon wildlife. No wildlife casualties were found on a 52-acre plot treated with 8 ounces of this insecticide per acre and no effect on bird activity was observed.

Before spraying, blood samples were withdrawn from the ear vein of each of three domestic rabbits in each of four 10 x 20 foot enclosures. Blood samples were again collected after the spraying, and paired samples were tested for indications of decreased cholinesterase activity resulting from exposure to the dimethoate. The method used was basically that of Limperos and Ranta (1953).

The blood cholinesterase activity did not decrease in the rabbits in two enclosures sprayed at a rate of 8 ounces per acre. The rabbits in the two enclosures sprayed at an estimated rate of 32 and 48 ounces per acre, respectively, showed a definite reduction in blood cholinesterase activity. The test rabbits were transported to Denver for further observation but no other effects of the dimethoate exposure were noted in the ensuing months.

Hemlock looper control (Richard E. Pillmore and James E. Peterson)

Pilot tests with phosphamidon, conducted in southwestern Washington by the U. S. Forest Service, were studied for the effect of this pesticide on birds and mammals. One Washington varying hare and two domestic rabbits were placed in each of four enclosures which were constructed by the Weyerhaeuser Company on lands owned by the Crown-Zellerbach Company. Blood samples were taken from the ears of the rabbits before and after treatment. Filter papers sampled the amounts of phosphamidon reaching the pens. The enclosures were sprayed from helicopter at the rate of 1.5 pounds of phosphamidon per acre (instead of the intended 1 pound per acre) on July 6 and 7. Because of wind drift, it was necessary to respray three of the pens; for two of these (II and III) the applications were made from a lower elevation than previously.

Results were as follows:

Enclosure	Site	Lbs./acre of phosphamidon received	Decrease in cholinesterase activity
I	under canopy	1.25**	slight to moderate
II	in open	5.57**	moderate to large
III	in open	1.73*	slight to moderate
IV	under canopy	0.00**	slight to moderate

*estimated ** as measured by filter paper

None of the rabbits exhibited any gross symptoms of organo-phosphate poisoning. The lowered cholinesterase activity suggested that all the rabbits ingested an organo-phosphate compound. The varying hares were affected to a greater extent than the domestic rabbit.

Malathion applications at Yosemite (James O. Keith and Merle L. Killpack)

The program to control infestations of lodgepole pine needle miner in sites receiving high recreation use was continued in Yosemite National Park in 1963. Although studies in 1961 and 1962 indicated that the application of one pound per acre of malathion was not detrimental to wildlife populations, additional evaluations were made this year because of continued concern for wildlife expressed by conservation groups.

Censuses of wild birds and mammals were made before and after pesticide treatment and various materials in the environment were collected for malathion residue analysis at intervals after treatments. Three species of songbirds and 5 species of rodents were exposed to spray applications and later held on foods similarly exposed. Captive animals were also held on experimental diets contaminated with between 100 and 5,000 parts per million of malathion. Tissues of both captive and wild animals were collected for residue analysis at intervals after treatments.

Data are not presented herewith because they have not been completely checked and analyzed; a more comprehensive report will be prepared later. Present summarization of the data permit only tentative conclusions. The treatments made in 1963 appeared to have little immediate impact on wildlife. Animals exposed to applications of malathion showed no apparent effects from this exposure and mammals on diets contaminated with up to 5,000 ppm of malathion survived and gained weight.

SURVEILLANCE OF PESTICIDE PROGRAMS

Crop insecticides in the Klamath Basin (James O. Keith, Milton H. Mohn and George Ise)

In 1962 studies were begun at Tule Lake and Lower Klamath National Wildlife Refuges in California to determine the origin of pesticide contamination, the mode of pesticide transportation in water systems and the accumulation and transfer of pesticide in aquatic food chains. During 1962 and 1963 analyses were completed of samples of water and a few other materials.

Tule Lake Refuge serves as a sump for a large agricultural irrigation system and is being contaminated continually with pesticides carried in the return-flow irrigation water. Mortality of fish-eating birds during the last 4 years as discussed on page 50 of this report, was due mainly to toxaphene, the residues of which concentrated in and became available through aquatic food chains. Toxaphene was used for several years to control insects on adjacent lands, but its use was discontinued in 1960 and its residues are no longer being found in refuge water. Chemicals most consistently found on both refuges are related to DDT, which was widely used in agriculture until 1953 and is still being used to a more limited extent.

Samples of water in the two refuges and entering Tule Lake Refuge were filtered and residue determinations were made for pesticides in both the filtrate and the suspected material. Residues given for total samples were calculated from those found in the two sub-samples. Sample size was 1 gallon.

Collection dates and results of the analyses of samples of waste irrigation water entering Tule Lake Refuge are shown in table C-7. The samples were composited weekly from 11 stations and bi-monthly from 4 stations. All samples contained residues of DDE, present in 83 percent of the samples; DDD, in 11 percent; or "DDMU" in 33 percent. Residues in the total water samples, ranging from 0.2 to 6 ppb, indicate a relatively low contamination. Birds found dead at the refuge contained DDT and its products in amounts ranging from 3 to 264 ppm. These residues probably could have been acquired from concentrations of insecticides accumulated by food chain organisms from small amounts entering the Refuge in return-flow agricultural water. However, since the birds affected are migratory, pesticides could have been obtained from other areas.

During 1962 water was collected at 10 stations in the marsh of the two refuges in order to compare pesticide residues there with those entering the area through the irrigation system. Collection dates and analyses of these samples are given in table C-8. At marsh stations DDE was again the most common chemical found; DDD and dieldrin were found in one sample each. The residues were comparable to the lower amounts found in water from pump stations. Water in Lower Klamath Refuge was contaminated to about the same degree as water in the Tule Lake Refuge, into which irrigation waters were received; this indicates the persistent nature of these chlorinated hydrocarbons.

In 1963 the Tule Lake Irrigation District and the Tule Lake National Wildlife Refuge made possible the continuation of these studies by providing funds for water analyses. Collection dates for the 1-gallon water samples are recorded in table C-9. Water is received by the Irrigation District through "J" canal and "J" drains. Samples from the private drains lease drains and plant "D" were taken to determine quality of the water as it progressed through the irrigation drainage system. Water is pumped out of the Irrigation District at plant "D". Individual water samples were obtained at "J" canal and plant "D", but the other stations are represented by composite samples of water from several points.

The samples were analyzed for chlorinated hydrocarbons and thiophosphates by Stoner Laboratories, Campbell, California. Results shown in table C-9 are based on residues in the total sample. They show that pesticides of the DDT group and heptachlor epoxide were present in water entering the Tule Lake Irrigation District in August and November. Residues of BHC, Lindane and the thiophosphates evidently originated within the district. It is apparent that much more intensive sampling would have been necessary to determine the source of residues and variations in their dispersal during the year.

Aldrin contamination of lakes at Rocky Mountain Arsenal (Mitchell G. Sheldon Milton H. Mohn and George H. Ise)

Recent investigations of pesticide contamination in industrial lakes at the Rocky Mountain Arsenal consisted only of periodic surveillance. On September 6, 1963, agreement was reached by the Department of the Interi Department of Defense, Shell Chemical Company, and the Colorado Game, Fish and Parks Department that aldrin and/or dieldrin contamination existed and corrective measures were outlined.

A sampling plan to determine the extent and degree of aldrin-dieldrin contamination in three industrial lake beds has been drawn up for use by the Department of Defense. This includes procedures of sampling and method for chemical analysis of the soil samples.

Specimens were collected to ascertain the extent of pesticide contamination in plants and animals at the Arsenal. Samples and the results of their analysis are listed in table C-10. Ducks contained the greatest amounts of residues and pheasants, which feed on seeds and insects in the dry lake beds and adjoining uplands, had the next greatest amounts. Mule deer inhabiting the more remote parts of the Arsenal contained only a

Eight marked, wing-clipped mallards were released on Ladora Lake in the Arsenal in an attempt to determine the rate of pesticide accumulation. They will be collected for analysis at intervals over a period of 3 months.

Pesticide residues in waterfowl collected in the field (Mitchell G. Sheldon Milton H. Mohn, George A. Ise and Richard A. Wilson)

Collections during 1963 for a continuing program consisted of waterfowl taken during the breeding season, waterfowl eggs from nesting grounds in the United States and Canada, and waterfowl from migration and wintering areas. Also, samples of aquatic vegetation and snails from far-north waterfowl breeding grounds were collected to investigate pesticide contamination of that habitat. Air in the United States and Canada was sampled for chlorinated hydrocarbon residues to learn possible sources of these persistent pesticides in remote, untreated areas.

Table C-11 lists the species of plants collected from waterfowl breeding areas in Canada and residues found therein. The collection areas have not been known to receive pesticide application. DDT metabolites, ranging from undetectable amounts to 0.7 ppm, are higher than those measured in 1962 collections. The source of these chemicals and their effect upon duck reproduction is unknown.

Air samples were taken with a 2-inch glass tube loosely packed with glass wool coated with mineral oil. The tube was attached to the outside of an aircraft and air was collected at altitudes from 100 to 2500 feet during May, June, July and August. The unit of measure was actual flying time taken by the plane's tachometer.

Pesticide residues from the air samples are recorded in table C-12 as weight recovered because air volume was not measured. The New Mexico sample, made immediately after an application of DDT, indicates that it occurs high above the treated area. All other exposures sampled residual pesticides in the atmosphere above untreated areas; it is significant that such areas contained measurable amounts of DDT or its products.

As in past years, approximately 60 percent of samples of waterfowl and waterfowl eggs collected in 1963 contained pesticide residues. The eggs consisted of composites of entire clutches from active and abandoned nests, and individual eggs from active or terminated nests.

Unhatched eggs and carcasses of bald and golden eagles, submitted to the Bureau by cooperators in Arizona, Colorado and Alaska, were analyzed and pesticide residues detected are listed in table C-13.

Effects of pesticides on the reproductive success of wild mallards (Mitchell G. Sheldon, Milton H. Mohn and Richard A. Wilson)

Pesticide residues have been found in wild duck eggs and are known to have a marked effect on reproduction of some species. To further investigate this, 10 clutches of duck eggs, totaling 62 eggs, were collected at Monte Vista Wildlife Refuge, Colorado, and artificially incubated at the Denver Center. Hens associated with five clutches were collected for determination of pesticide residues in their tissues. In addition, 10 wild duck nests were sampled by collecting one egg from each clutch for pesticide analysis.

Table C-14 records the data from the analysis of duck hens and unhatched eggs of clutches removed to the laboratory. Visceral tissues of each hen were compounded for analysis but the unhatched eggs were analyzed individually. Residues detected in eggs from wild mallard nests are recorded in table C-15.

A hatching of 83 percent (49 ducklings from 59 fertile eggs) of the incubated eggs indicated relatively good success from the incubation procedure. Embryos in unhatched eggs ceased to develop between the 21st day of incubation and pipping. Several factors may have killed these embryos, but the responsible one could not be identified. Duckling survival during 6 weeks of observation following hatching was good; the deaths that did occur were mostly caused by accidents.

Residues in forest birds in New Mexico (Richard E. Pillmore, James E. Peterson, Richard A. Wilson, Milton A. Mohn, George H. Ise and Charles W. Hall)

Forest birds were collected by the New Mexico Game Department before a forest spray program on June 15, 1962, and again 15 and 30 days after this application of 1 lb/acre of DDT. The specimens were analyzed at Denver Center. Pesticide residues found are recorded in table C-16, and are based on analysis of an aliquot of the undried whole body expressed as parts per million.

DDE found in the pre-spray collections indicate a previous unknown exposure to DDT or DDE.

As expected, the residues show a marked increase after spraying, with various degradation products of DDT appearing after the lapse of 15 or more days following the application.

Phosphamidon application in Montana (Robert B. Finley, Jr., and Merle Richmond)

The U. S. Forest Service in 1963 tested several insecticides for use against forest insects in hopes of finding a substitute for DDT. One of these, phosphamidon, was aerially sprayed on 5000 acres of private forest

land at the rate of 1 pound per acre in 1 gallon of water. The test plot was on rough, forested terrain near Missoula, Montana.

To investigate possible adverse effects of the operation, pre-spray bird populations were counted for 1 week on both test and unsprayed check areas. Censuses and systematic searches for casualties were conducted for 1 week after spraying and for a similar period 5 weeks after treatment. Censuses were moving station counts taken for 2 hours beginning at sunrise. Personnel of the U. S. Forest Service and the Montana Department of Fish and Game assisted with the post-spray operation.

Census data are recorded in table C-17. Bird abundance and activity on the spray plot were low for montane coniferous forests, but were more nearly normal on the unsprayed plot. Blue grouse, however, were moderately numerous on the test plot but scarse on the check plot. Bird activity dropped abruptly to an extremely low level on the spray plot while it increased somewhat on the non-spray plot. In the second post-spray period populations on both plots had increased -- on the sprayed plot to more than the pre-spray count. In the late post spray period, birds in both plots were moving more widely in flocks than earlier, hence they were seen more easily and counts on successive days were more erratic.

Six carcasses were found in 44 man-hours of searching on the spray-plot and one carcass in 10 man-hours on the unsprayed areas. One additional carcass was found in the course of bird census work. Each casualty and its associated circumstance was examined for evidence of cause. Four birds were deemed to have been killed by phosphamidon and one possibly so. Because of the dense ground cover over most of the study area, the few casualties found indicate that a larger number may have occurred.

An evening grosbeak and two blue grouse, cock and hen, were found sick. The evening grosbeak tumbled out of a tree and the grouse showed poor alertness, a stumbling walk, and poor control in flight and landing. The hen grouse died in 2 weeks (after the grouse were captured) but the cock recovered normal alertness and coordination.

The two grouse were transported to the Denver Center where cholinesterase determinations, made with whole blood, were compared with cholinesterase activities of six grouse believed to have had no history of exposure to any organo-phosphate pesticide. Cholinesterase activity of the two sick grouse, 11 days after spraying, was 54 and 58 percent lower than that of the 6 presumably normal birds. Six weeks after exposure to phosphamidon, the blood of the cock had returned to normal activity.

Grasshopper control: Sevin - North Dakota (Lowell C. McEwen, James O. Ellis, James E. Peterson, Milton H. Mohn and George H. Ise)

The effects of Sevin on wildlife are being studied at Lostwood Refuge, North Dakota, in a long-term study begun in 1961. In 1962 Sevin was sprayed for grasshopper control at the rate of 1 pound active ingredient

in 1 gallon of water per acre, on one of two 2000-acre study areas. The study areas are located in typical prairie pot-hole country characterized by rolling terrain and numerous small pot-holes and aspen groves.

Intensive census has been conducted periodically on birds, small mammals, insects, and aquatic invertebrates on both study areas before and after the spray application. Game species such as sharp-tailed grouse and ducks received special attention for possible effects from the insecticide.

Total insect kill on the sprayed uplands was estimated at 50 to 60 percent, by weight. No emigration of birds due to reduction of food supply was observed, and bird counts on permanent upland census lines did not differ between the sprayed and unsprayed areas (table C-18). Sharp-tailed grouse showed no effects of the spray application based on flush records, dancing ground counts and brood observations. Small mammals declined slightly on the sprayed area one year post-spray (table C-19).

Aquatic invertebrates in the pot-holes were sampled but variation was too great to closely estimate the kill on the sprayed area. Bird counts in the pot-hole habitat indicated lower numbers on the sprayed area in 1963 (table C-18). The decline was chiefly in waterfowl brood use. Small mammals also were fewer around the sprayed pot-holes (table C-19).

A few animal and environmental samples were collected for chemical analysis one year post-spray. Traces of Sevin ranging from <0.1 to 0.8 ppm were found in 5 of 16 samples.

Grasshopper control: aldrin - Wyoming (Lowell C. McEwen, Milton H. Mohn and George H. Ise)

A cooperative Federal-State-private operation to control grasshoppers was observed to learn its effects on wildlife. Aldrin was aerially sprayed on 93,000 acres of range near Guernsey, Wyoming, at the rate of 2 ounces in 1 gallon of diesel oil per acre. Pre-spray and post-spray conditions for 90 days were observed during four periods. The sprayed area was typical short grass plains interspersed with "breaks" dominated by woody plants; about 6 miles of permanent stream draining into the North Platte River was also sprayed. Game species, song birds and small mammals were present on the area.

Spray cards placed in the small-mammal trapping plots were ruined by rain before retrieval. There was little evidence, however, that the areas were hit by spray. Dead grasshoppers were nearly impossible to locate and two soil and litter samples were negative for dieldrin residues. Post-spray populations of small mammals remained high.

Sixteen dead vertebrates were located, mainly incidental to other work. Of these, six trout and three birds appeared to have been direct casualties of the spray. Dieldrin was recovered from two of the birds in the amounts of 3.0 and 2.6 ppm, wet weight (whole body basis). Dieldrin residues ranged from less than 0.1 to 0.3 ppm in four other vertebrate samples collected from the sprayed area.

Two soil and litter samples from an area where dead grasshoppers were easily found contained 0.02 and 0.03 ppm dieldrin and similar levels of DDE. Three samples of dying and dead grasshoppers yielded from 0.6 to 0.8 ppm dieldrin and 0.2 to 0.3 ppm DDE. Dieldrin in the animal results from metabolism of aldrin. Of interest is the recovery of measurable amounts of DDE up to 4 ppm from all but 6 of 20 samples of all kinds. The origin of this material in the environment and local fauna is unknown.

MISCELLANEOUS FIELD INVESTIGATIONS RELATING TO PESTICIDE-WILDLIFE PROBLEMS

Investigation of goose mortality resulting from the use of zinc phosphide

(James O. Keith and Vernon A. Perry)

Between October 23 and 25, 1963, 453 geese were found dead in a small local area on the Tule Lake National Wildlife Refuge. Most evidence suggested that the mortality was due to zinc phosphide poisoning, although one in ten of the carcasses examined showed indications of disease.

During the summer of 1963 about 1,000 acres of farm lands, adjacent to the locality where the dead geese were found, were treated with 6 to 8 pounds of a 1 percent zinc phosphide bait to control meadow mice. The bait consisted of semi-crushed oat groats to which a green dye and zinc phosphide were applied. Because barley stubble on one 90-acre field was burned, waste grain from harvesting and the poison oat groats applied on July 30 were exposed and became parched and scorched. It was observed that large numbers of geese fed in the field shortly after it was burned.

Examination of gizzards from dead geese showed that most birds had eaten some green-colored oat groats and larger amounts of barley and wild oats. Much of the grain in gizzards was parched or scorched. These findings implicated zinc phosphide as a possible cause of death and the burned field as the source of poison bait.

Because zinc phosphide was not believed to persist on bait under field conditions, samples of the poison oat groats were collected from the burned field for chemical analysis and for feeding to captive geese. Two samples of the baits analyzed by the Denver Wildlife Research Center and the California State Department of Agriculture contained residues of 0.35 and 0.31 percent, respectively, of zinc phosphide. Evidently about one-third of the original amounts of zinc phosphide remained on the bait after 3 months exposure to field conditions.

Four geese given 100 kernels of freshly prepared 1-percent bait survived. Four of 6 geese given 200 kernels died and all 9 geese given either 300 or 400 kernels of fresh bait died. These findings suggest that the LD_{50} of fresh zinc phosphide baits to snow geese is between 200 and 300 kernels.

About 900 kernels of bait, collected from the burned field by 2 men who worked a full day to gather this supply, were tested on captive geese. Individual snow geese given 300 and 400 kernels died.

Movements of white pelicans (James O. Keith)

In 1962 the white pelican was selected as an indicator species among migratory water birds for monitoring the level of pesticides in the environment. The movements and migrations of the white pelican, therefore, are important from the standpoint of how and when migratory water birds come into contact with pesticides and the effect of repeated contacts. Further, the study will help define the source of pesticides which have caused bird mortality in the Klamath Basin of California.

Studies of movements of pelicans continued in 1963 with the use of better dyes. Young, flightless birds were banded and color-marked in July at nesting areas as noted below:

Colony	Number banded	Number color-marked	Color of dye
Anaho Island Refuge, Nev.	405	115	green
Lower Klamath Refuge, Calif.	134	89	yellow
Clear Lake Refuge, Calif.	293	47	brown

Banding is a more permanent label than dye, and permits long-term studies of movement and the calculation of relative mortality among birds in different nesting colonies.

In 1963, 431 colored pelicans were reported from throughout western United States; colors and locations for most of them are shown in figure C-2 Results have established principal areas used by pelicans from the various colonies between July and November. Areas of heaviest use will be sampled in 1964 to determine their contamination with pesticides.

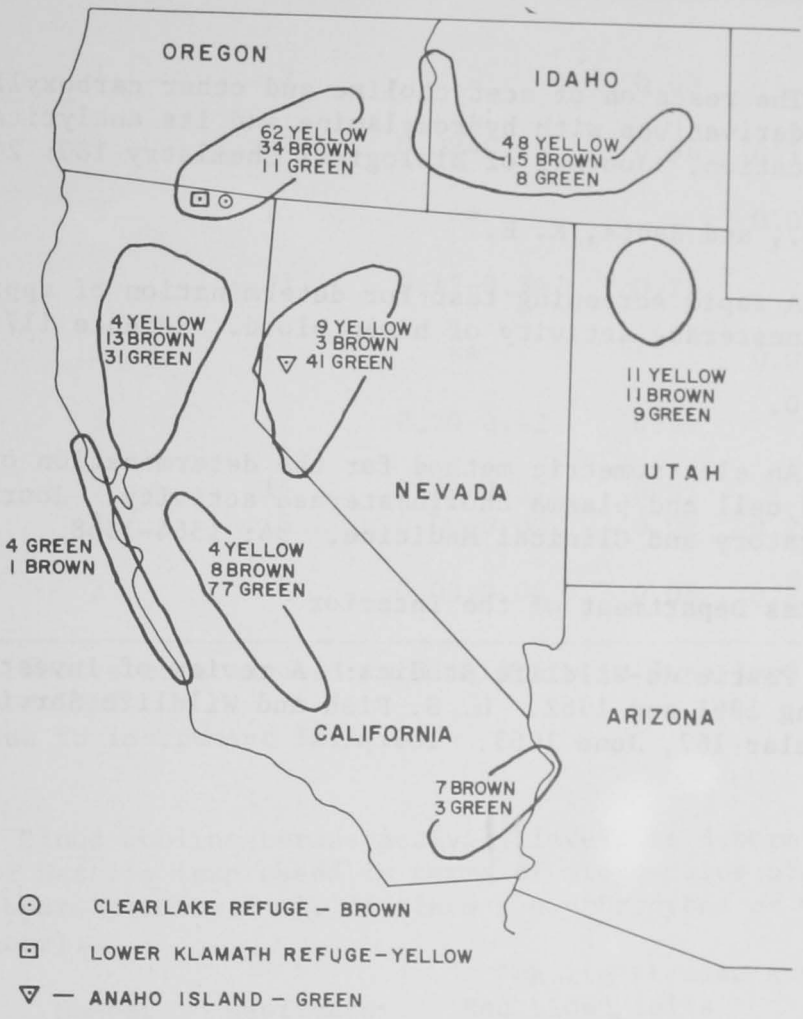

Figure C-2. Number of color-marked pelicans observed between August and December 1963, within eight geographic areas of the western United States.

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Table C-1. Blood cholinesterase activity levels as determined by Michel's method expressed in terms of the change in pH per hour.

Species	Number Used	Replicates	Cholinesterase activity level (pH change/hr.) Red blood cells Range	Red blood cells Average	Plasma Range	Plasma Average
Cottontail rabbit	1	2	-	0.08	-	0.14
Domestic rabbit*	4	2	0.03-0.15	0.96	0.15-0.28	0.21
Domestic goat	5	10	**		0.03-0.08	0.05
Domestic goat	5	33	0.17-0.34	0.25	**	
Confined mule deer	3	7	**		0.05-0.12	0.06
Confined mule deer	8	37	0.20-0.42	0.31	**	
Wild mule deer	7	14	0.29-0.37	0.32	**	
Pheasant	3	5	0.00-0.02	0.01	0.22-0.28	0.25
Mallard duck	2	4	0.03-0.08	0.05	0.23-0.33	0.21

* Domestic rabbits were exposed to dimethoate in June 1963. Tests made September 1963.

**Not run due to instrument failure.

Table C-2. Blood cholinesterase activity levels as determined by the method of Hestrin (expressed in terms of micro-moles of acetycholine bromide hydrolyzed by 1 ml. of plasma, erythrocytes or whole blood, in one hour).

Species	Number Used	Replicate blood samples	Cholinesterase Activity Red blood cells Range	Red blood cells Average	Plasma Range	Plasma Average
Canada goose	1	3	No activity			0.92
Mallard duck	2		" "		0.76-1.0	0.88
Sharptailed grouse	3	3	" "		3.12-3.84	3.41
Prairie chicken	2	3	" "		3.80-3.84	3.82
Pheasant	1	3	" "		-	1.2
Domestic goat	3	3	1.28 - 1.88	1.63	Negligible activity	
Mule deer	2	3	2.00-2.12	2.06	"	"

Table C-3. Acute oral toxicity of Sevin and resulting tissue residues in adult male sharptails and prairie chickens.

Species and Number	Sevin dose[1] mg/kg	Result	Sevin residues in tissues[2] ppm ("wet basis")
Sharptail # 6	2,000	Death	9.0
" #10	1,750	"	17.0
" #79	1,650	"	0.1
" #38	1,500	Survival	Not analyzed
" #18	1,020	"	" "
" #15	0	"	" "
Prairie chicken #59	2,750	Death	0.2
" #65	2,000	"	21.0
" #57	1,860	Survival[3]	0.0
" #61	1,730	Survival	Not analyzed
" #58	1,390	"	" "
" #34	0	"	" "

1/ Acute oral administration via gelatin capsule.
2/ Composite of brain, heart, kidney, liver and muscle.
3/ Sacrificed 169 days after dosage.

Table C-4. Acute oral toxicity of dieldrin to adult male sharptails and prairie chickens.

Bird and Number	Dieldrin dose mg/kg	Result	Bird and Number	Dieldrin dose mg/kg	Result
Sharptails			Prairie Chickens		
74	27	Death	32	107	Death
98	20	"	33	53	"
100	12 in oil	"	25	35	"
69	11 in oil	"	48	22	"
10	5 in oil	"	63[1]	17	Survival
71	5 in oil	"	64	16 in oil	Death
86	2.5 in oil	"	78	15 in oil	"
99	2.5 in oil	"	14	10 in oil	Survival
12B	1.5 in oil	Survival	77	5 in oil	"
11B	0	0	19,34	0 in oil	"

1/ Sacrificed 189 days after dosing.

Table C-5. Results of the chemical analysis of deer tissue samples for DDT and derived metabolites, 1963.

Date	Tissue sample	Residues in parts per million of: DDE	DDT	TDE	DDMU*
Sept.24	Subcutaneous fat from rump (Biopsy)	0.5	(total residues)		
Oct. 3	" "		17	0.5	
Oct. 8	" "		27	1.6	
Oct. 23	" "		58	7.3	
Nov. 8	Subcutaneous fat from rump (Autopsy)		9.6	2.4	
Nov. 8	Subcutaneous fat from brisket		12	2.4	
Nov. 8	Visceral fat from omentum		12	1.6	
Nov. 8	Visceral fat from kidney area		6.8	1.9	
Nov. 8	Visceral fat from heart		9.6	1.8	
Nov. 8	Adrenal glands	TR	TR	TR	0.84
Nov. 8	Thyroid and parathyroid glands	TR	1.6	TR	0.5
Nov. 8	Brain	TR	TR	0.43	0.12
Nov. 8	Liver	TR	0.12	TR	0.16
Nov. 8	Testes	TR	TR	TR	

* DDMU 1-chloro-2, 2-bis(p-chlorophenyl)ethylene.

Table C-6. Results of acute, oral, toxicity trials with young white pelicans.[1/]

Chemicals and dose rates in mg/kg	Weight of birds gms	Amounts of solutions administered in ml[2/] DDT	Toxaphene	Results Status	Time
Toxaphene					
400	6113	-	8.558	Death	In 20 hours
200	5760	-	4.032	Death	In 20 hours
100	5198	-	1.819	Death	In 20 hours
100	5115	-	1.790	Death	In 20 hours
100	6680	-	2.338	Survival	Intoxication
50	7480	-	1.309	Death	In 20 hours
50	4970	-	0.870	Death	In 20 hours
50	7200	-	1.243	Survival	Intoxication
DDT					
800	4185	67.000	-	Survival	No intoxicatio
400	6190	49.520	-	Survival	No intoxicatio
200	5580	22.320	-	Survival	No intoxicatio
100	4085	8.170	-	Survival	No intoxicatio
50	7160	7.160	-	Survival	No intoxicatio
Toxaphene and DDT					
400	6460	25.840	4.522	Survival	Intoxication
200	5447	10.894	1.906	Survival	No intoxicatio
100	4302	4.302	0.753	Death	In 20 hours
50	5855	2.928	0.512	Survival	No intoxicatio

1/ Sex of birds not known.

2/ Corn oil solutions of 5.0% DDT and 28.6% Toxaphene.

Table C-7. Residues of chlorinated hydrocarbons in composite samples of waste irrigation water entering the Tule Lake National Wildlife Refuge, 1962

Date Collected	Vol. of Sample	Wt. of Suspended Material	DDT Metabolite Residues		
			Filtrate in ppb	Suspended Material in ppm	Entire Sample in ppb
1962	ml	gms air-dried			
May 25	3435	0.25	T[1/]	15.0	1.0
June 1	3600	0.14	T	7.0	0.4
June 8	3925	0.30	N.D.[2/]	78.0	6.0
June 15	3720	0.19	T	1.9	0.2
June 20	3470	0.11	T	3.3	0.2
June 29	3520	0.16	0.1	3.5	0.3
July 6	3500	0.20	T	69.0	4.1
July 11	3500	0.20	T	49.0	2.9
July 20	3600	0.25	T	60.0	4.0
July 30	----------	----------	Sample Lost	----------	----------
August 3	3500	0.18	T	6.5	0.4
August 10	3600	0.05	N.D.	10.0	0.2
August 17	3650	0.03	0.1	42.0	0.3
August 23	3790	0.08	0.2	4.6	0.3
August 31	3630	0.04	T	4.2	0.2
Sept. 6	3650	0.10	0.2	3.7	0.3
Sept. 14	3550	0.10	0.1	3.0	0.3
Sept. 21	3390	0.15	T	1.8	0.2
Oct. 24	3510	0.12	T	3.0	0.2

1/ Trace amounts - less than 0.1 part per billion

2/ None detected

Table C-8. Residues of chlorinated hydrocarbons in water collected at marsh stations in the Tule Lake and Lower Klamath National Wildlife Refuges during 1962.

Location and date	Vol.of Sample	Wt. of Suspended Material	DDT Metabolite Residues Suspended Material in ppm	Filtrate in ppb	Entire Sample in ppb
1962	ml	gms air-dried			
Tule Lake Refuge					
April 25	3640	0.19	5.8	0.3	0.6
June 29	3556	0.06	5.2	0.1	0.2
August 23	3660	0.09	6.1	0.2	0.4
October 22	3630	0.18	7.0	0.3	0.6
Lower Klamath Refuge					
April 26	3575	0.08	5.9	0.3	0.4
June 30	3730	0.13	3.0	0.2[1/]	0.3
August 23	3650	0.05	22.0	0.2	0.5
October 24	2700	0.07	5.2	0.1	0.2
Unit 2					
April 26	3730	0.02	4.8	0.2	0.2
August 22	3645	0.06	3.0	0.1	0.1

1/ Also trace of dieldrin in filtrate

Table C-9. Pesticide residues in water collected at several locations in the Tule Lake Irrigation District during 1963. Residues are expressed in parts per billion.

Location and Date	Chlorinated Hydrocarbons			Thiophosphates
	DDT[1]	Heptachlor Epoxide	Lindane and Others	Unidentified
"J" Canal				
July 10	-[2]	-	-	-
August 13	0.20	0.06	-	-
November 3	0.06	-	-	-
"J" Drains				
July 10	-	-	-	-
August 13	0.20	0.06	-	-
November 3	0.25	0.05	-	-
Private Drains				
July 10	-	-	T[3]	0.10
August 13	0.14	0.06	0.07	1.70
November 3	-	-	-	-
Lease Drains				
July 10	-	-	-	-
August 13	0.06	-	0.06	-
November 3	0.13	0.06	0.06	-
Plant "D"				
July 10	-	-	-	0.13
August 13	0.06	-	0.06	1.10
November 3	0.15	-	0.10[4]	-

1/ Includes DDD and DDE

2/ Dash indicates no residues detected

3/ Trace of BHC - less than 0.1 ppb

4/ Unidentified

Table C-10 Pesticide residues in samples collected at the Rocky Mountain Arsenal in 1963. Parts per million are based on wet weight of specimens except for vegetation which was air-dried.

Sample	Residues in ppm Aldrin	Dieldrin	DDT metabolites
Pheasant visceral tissues	NA*	1.8	0.4
Pheasant visceral tissues	NA	6.0	0.5
Pheasant visceral tissues	NA	14.4	1.4
Deer visceral fat	NA	<0.1	<0.1
Deer visceral fat	NA	<0.1	<0.1
Deer liver	NA	<0.1	<0.1
Aquatic snail tissue	3.7	20.0	<0.1
Aquatic snail tissue	0.0	11.0	<0.1
Leech	<0.1	14.0	0.7
Mallard tissue	NA	NA	NA
Mallard visceral fat	NA	1400.0	NA
Mallard visceral fat	NA	2400.0	NA
Pintail visceral fat	NA	720.0	NA
Gadwall visceral fat	NA	545.0	NA
Scaup tissue	NA	24.0	NA
Terrestrial vegetation from dry bed of Upper Derby Lake	NA	1.2	1.0
Aquatic vegetation - Ladora Lake	26.4	17.6	<0.1
Aquatic vegetation - Ladora Lake	NA	14.0	<0.1
Aquatic vegetation - Ladora Lake	NA	4.5	<0.1

* None apparent by paper chromatography

Table C-11. Pesticide residues in aquatic plants and snails collected in North West Territory Canada, 1962-63. Residues from vegetation are based on air-dried weight.

Sample	Area	Residues in ppm DDT	"DDMU"*	DDE
1962				
P. richardsonii	Yellowknife Bay	-	-	-
S. latifolia	25 mi. pond	-	-	-
P. richardsonii	30 mi. pond	-	-	-
L. trisculca	30 mi. pond	-	-	<0.1
P. zosteriformia	30 mi. pond	-	<0.1	<0.1
S. multipedunculatum	35 mi. pond	-	-	<0.1
U. vulgaris	35 mi. pond	-	-	-
Snail tissue (Lymna)**	Stagg Lake	-	-	<0.1
1963				
P. richardsonii	25 mi. pond	-	-	-
P. richardsonii	30 mi. pond	-	-	-
Potomogeton mixed sp.	30 mi. pond	-	-	<0.1
P. richardsonii	Stagg River	-	-	0.7
P. richardsonii	McKenzie River	-	-	0.4

* DDMU = 1-chloro-2,2-bis(p-chlorophenyl)ethylene

** Residue based on wet weight

Table C-12. Chlorinated hydrocarbon residues from air samples collected in the United States and Canada, 1963.

Origin	Exposure Time	Micrograms of DDT recovered
North and South Dakota	2.5 hrs.	0.7
New Mexico	0.5 hrs.	45.0
N.W.T. Canada	12.8 hrs.	Filter material lost in flight
N.W.T. to Central Canada	25.0 hrs.	Trace
Central Canada to Denver, Colorado		Trace
Unopened control sample tube	-	None detected

Table C-13. Chlorinated hydrocarbon residues found in golden and bald eagles or their eggs, collected in 1963.

Species sample	Area	Residues in ppm (wet weight) DDT	DDE	Other chlorinated hydrocarbons
Golden eagle egg	Colorado	-	<0.1	-
Bald eagle viscera	Arizona	-	-	-
Bald eagle viscera	Alaska	-	1.0	-
Bald eagle egg	Alaska	-	<0.1	-
Bald eagle egg	Alaska	<0.1	0.4	-
Bald eagle egg	Alaska	-	0.8	Trace*
Bald eagle egg	Alaska	-	2.4	Trace*
Bald eagle egg	Alaska	-	0.5	Trace*

* Trace of what appeared to be dieldrin was also present

Table C-14. Pesticide residues found in wild duck eggs collected at Monte Vista National Wildlife Refuge, Colorado, and incubated at Denver Wildlife Research Center, 1963.

Species	Number of eggs in clutch	Hen collected with clutch	Number of eggs failing to hatch	DDT residues in ppm (wet weight) Hen	Unhatched eggs
Mallard	8	No	2	-	1.5 and 1.0
Mallard	5	No	0	-	*
Mallard	8	No	2	-	*
Mallard	3	No	1	-	*
Mallard	5	No	2	-	<0.1 and<0.1
Mallard	8	Yes	0	6.0	*
Mallard	6	Yes	1	0.7	1.2
Mallard	5	Yes	1	7.9	*
Mallard	8	Yes	1	0.1	<0.1
Shoveller	6	Yes	3 infertile	13.3	1.0, 0.7 and *

* Awaiting analysis

Table C-15 Egg clutches of wild mallards sampled at Monte Vista Wildlife Refuge Colorado, for pesticide residues in 1963.

Number of eggs in clutch	Number of eggs collected per nest	DDT residues in ppm (wet weight)
3	1	0.2
3	1	0.3
7	1	0.3
7	1	0.2
5	1	0.3
3	1	0.4
5	1	0.3
5	1	0.0
5	1	0.9
5	1	0.7

Table C-16. Residues found in songbirds collected before and after 1962 spruce budworm control program in New Mexico. Unless indicated otherwise the specimens were collected in the Pot Creek area, Taos County.

Species	Residues found in ppm			
	DDE	DDT	TDE	DDMU*
PRE-SPRAY COLLECTION				
Western robin (Coralles Canyon)	2.4			
Western robin (2)	0.5			
Williamson's sapsucker	0.4			
Hermit thrush	0.5			
Green-tailed towhee	0.3			
Western Wood pewee	0.6			
Mountain chickadee	0.4			
Lincoln sparrow	0.7			
Virginia warbler	0.9			
15 DAY POST-SPRAY COLLECTION June 28, 1962				
Western robin	0.5	9.6		
Williamson's sapsucker	(<0.5 total residues)			
Violet green swallows (4)	2.4	8.0		
Vireo (unidentified)	2.0	2.5		
Fly catchers (4) Empidonax and Western Wood pewees	2.9	2.9		
30 DAY POST SPRAY COLLECTION July 16, 1962				
Olive-sided flycatcher	7.5	15.0	<3.0	
Western Wood pewee	3.0	3.7	<0.5	
Mac Gillivray's warbler	5.1	2.6	<0.5	
Chipping sparrow	3.7	2.6	<0.5	
Evening grosbeak (2)	1.9	1.2	<0.5	
Wren (unidentified)	(<0.5 total residues)			
BLUE GROUSE COLLECTION (Sebadilla Creek) July 29, 1962				
Adult female	2.7	<0.1	<0.1	1.3
Chick - half grown	0.3	<0.1		<0.1
Chick - half grown	-	<0.1		<0.1
Chick - half grown	-	<0.1	<0.1	<0.1

* 1-chloro-2,2-bis(p-chlorophenyl)ethylene

Table C-17. Bird activity on the phosphamidon test plot and on an unsprayed check area in Missoula County, Montana, 1963

Period and Date	Birds seen per 2-hr. count	
	Sprayed area	Check area
Pre-Spray		
June 20	27	45
June 22	20	49
June 23	38	47
June 25	33	46
Average	30	47
Early post-spray		
June 30	4	49
July 1	8	49
July 2	9	65
July 3	2	53
July 4	10	62
July 5	8	77
Average	7	59
Late post-spray		
August 4	141	61
August 5	42	67
August 6	36	85
August 7	44	95
August 10	16	122
August 11	33	117
Average	52	91

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Table C-18. Bird counts on Sevin-sprayed and unsprayed test plots at Lostwood Refuge, North Dakota

Period	Number of birds per hour			
	Upland lines		Pot-hole lines	
	Sprayed	Unsprayed	Sprayed	Unsprayed
Pre-spray				
July 1i61	100	110	92	130
October 1961	5	9	98	123
June-July 1962	87	83	139	116
Post-spray				
July-August 1962	83	82	127	110
September 1962	37	35	119	134
July-August 1963	84	84	132	259

Table C-19. Small mammal censuses on Sevin-sprayed and unsprayed test plots at Lostwood Refuge, North Dakota

Period	Average initial captures per 100 trap nights			
	Upland lines		Pot-hole lines	
	Sprayed	Unsprayed	Sprayed	Unsprayed
Pre-spray				
July 1961	5	12	9	6
October 1961	3	8	1	3
June-July 1962	5	7	2	3
Post-spray				
July-August 1962	8	11	7	5
September 1962	24	23	2	11
July 1963	24	32	10	30
September-October 1963	19	32	12	14

WILDLIFE STUDIES, PATUXENT WILDLIFE RESEARCH CENTER

by

Lucille Stickel
Division of Wildlife Research
Bureau of Sport Fisheries and Wildlife

Pesticide research at Patuxent in 1963 combined field and laboratory studies to measure direct and indirect effects of pesticides on wildlife, to measure and interpret the occurrence and importance of pesticide residues in wild animals, and to develop more efficient methods of pesticide evaluation.

Eagle studies were continued through investigations in Alaska and by residue surveys in field specimens.

A long-term study of the field effects of heptachlor on quail in Georgia was completed, and the immediate effects of low-dosage heptachlor treatments were tested in Arkansas. Field results were tested further by continuation of enclosure studies of bobwhite on heptachlor-treated land. Studies of the effects of malathion were initiated in Michigan in cooperation with the Michigan Department of Conservation.

Problems of pesticide residues in wildlife were attacked by field surveys of ospreys and black ducks and by studies of gain and loss of pesticide residues in captive cowbirds. Studies of pesticides in the Lake Michigan area were begun in cooperation with the University of Wisconsin.

Effects of nonchemical methods of mosquito control were studied in cooperation with the Florida Department of Game and Fish. Some residue readings were made of birds collected in an area where silvex was applied for alligatorweed control.

Methods development continued through testing of field techniques; development of new laboratory criteria was undertaken in cooperation with Michigan State University.

Toxicological testing of new or widely used pesticidal chemicals was continued, with pheasants, quail, and mallards as the principal subjects. Less extensive testing was done on certain other species.

Discussions included here are compiled from research in progress, and hence final tabulations may differ. Other research workers wishing to use or quote statements or data should first communicate with the responsible investigators, who are listed for each study.

EVALUATION OF CHEMICALS

Toxicity tests on birds (James B. DeWitt, Calvin M. Menzie, James W. Spann, Clyde Vance)

Data on toxicities of compounds and formulations are required in applications for registration of pesticides, and serve as bases for regulations designed to minimize hazards associated with the use of these materials. However, there are marked differences between species in susceptibility to various toxicants, and tests on the usual laboratory animals may not furnish adequate measures of pesticide hazards to wildlife. Toxicological studies at Patuxent are made on captive birds of several wild species. Toxicant is incorporated in the diet, on the hypothesis that ingestion constitutes the major route by which birds acquire pesticides from treated environments.

Principal test subjects are young and adult bobwhite, ringnecked pheasants, and mallard ducks obtained from pen-reared stock. In 1963, some tests were also made on cowbirds, grackles, red-winged blackbirds, and herring gulls, obtained by trapping wild birds. An important phase of the 1963 studies was the development of a colony of coturnix quail, and the inclusion of these birds in the test program. Coturnix mature early so that 4 or 5 generations may be reared in a year. Females may remain in breeding condition 30 or more weeks, producing 3 to 6 eggs per week.

Birds are treated in groups and are allowed free access to food and water. Tests on young birds begin when the birds are 1 or 2 days old; in tests of adult birds, approximately equal numbers of males and females are included in each test group. Controls are fed regular game-bird diets. The other groups receive similar diets modified by the addition of test compounds. Records of average food consumption are kept as an indication of the quantities of toxicant ingested, and are useful also in suggesting any marked repellency.

Results of tests made in 1963 are shown in table D-1. The form of presentation differs from that used previously. In earlier reports the effort was to provide an overall summary, where general conclusions could be obtained by scanning, but where details were omitted. Although the general reader may not find the new plan as useful to him, the change was adopted to make all facts quickly available to research

workers and others concerned with the more technical details of chemical testing. Some of the chemicals listed were tested in other years also; only the 1963 data are presented for these compounds.

EFFECTS OF DDT ON BALD EAGLES

Studies of the effects of pesticides on eagles were continued in 1963, both by experiment and by determination of DDT residues in eagles found dead in various places in North America.

Alaskan experimental studies (John L. Buckley, Nicholas J. Chura, and Louis N. Locke)

The 1961-62 feeding experiments demonstrated that eagles can be killed by DDT and suggested that a median lethal level is on the order of 160 ppm in the diet within 100 days. Chemical analyses of those birds showed that they were quite variable in their accumulation of residues. Residues were determined by colorimetric methods, as described in a later section.

In the 1962-63 studies, 16 eagles were captured in November and December along the Chilkat River near Haines, Alaska, and were housed at the Experimental Fur Station, Petersburg, Alaska. Feeding experiments were planned to learn the rates of gain and loss of residues that might be expected in wild birds. The results showed that the birds gained residues when fed on contaminated food and eliminated them when fed on clean food. At the level fed, and if no significant organ damage occurred, preliminary analysis of the data suggests that an equilibrium might have been reached in about a year. The predicted level is of the same magnitude as many of the residue levels in field specimens. This estimate must be considered tentative until results are available from additional residue analyses that are under way. Previous reports that 10 ppm of DDT (dry weight) is the maximum likely to be found in fish in the wild was based on examination of data available to us in the fall of 1961. More recent data suggest contamination of wild fish may be 3 to 12 times this amount; thus the 10 ppm feeding level may have been unrealistically low. A more detailed report is listed under Publications (Buckley and DeWitt, 1963).

Residues in field collected eagles (James B. DeWitt, Vyto A. Adomaitis, George E. Bagley, Calvin M. Menzie, Richard M. Prouty, and William L. Reichel)

We have received 58 specimens of bald eagles found dead or incapacitated in 20 States and 2 Canadian Provinces. Autopsy showed that at least 16 (possibly 24) of the birds had been shot; 3 were sick; the others were dead of unknown causes. Residue analyses have been made of at least some organs of 56 of the eagles (table D-3). All but one, a bird from Alaska, contained detectable DDT residues. In addition, 5 bald eagle

eggs have been analyzed. An egg from a nest in New Jersey in 1962 contained 24.3 ppm; 2 eggs from another 1962 New Jersey nest contained 11.4 and 36.9 ppm. Two eggs from a 1963 Missouri nest contained 1.1 and 5.6 ppm.

These findings show that eagles in the wild have access to and in fact do ingest substantial quantities of DDT. They strongly suggest that a high percentage of bald eagles carry DDT and its metabolites in their tissues. The existence of residues, however, does not tell what effects, if any, these residues may have, either on adults or on eggs.

EFFECTS OF HEPTACHLOR ON WILDLIFE

Quail studies in Decatur County, Georgia, and in Alabama (Walter Rosene,Jr

Final summarization of results of these studies was completed in 1963 The abstract follows:

A study of the effects of field applications of heptachlor on bobwhite quail (Colinus virginianus) and other animals was conducted on three like areas, two in Decatur County, Ga., and one in Escambia County, Ala., from February 1958 to March 1962. Heptachlor in granules was applied by air on the Georgia areas for eradication of the imported fire ant (Solenopsis saevissima richteri). Applications were directed by personnel of U.S. Department of Agriculture, Plant Pest Control Division. The Alabama area remained untreated. Transects where whistling cocks were counted were superimposed on areas where coveys were counted. Size of areas varied from 14,000 to 20,000 acres. Each area had six transects, totaling 11,000 acres. Whistling cock quail and conveys averaged 28 and 20 per 1,000 acres respectively the year before treatment on a Georgia area, and cocks and coveys averaged 25 per 1,000 acres for the duration of the study on the untreated Alabama area. Where portions of an area were treated at 2 pounds heptachlor per acre, whistling cocks and coveys were reduced significantly, with a greater reduction where a greater proportion of land was treated on the area or its transects. A decline of cocks and coveys also followed ½-pound applications (approaching statistical significance for coveys). Three years after treatment, cock and covey numbers were below those recorded before treatment. Whistling cocks and coveys also declined on adjoining land which remained untreated (significant for cocks, approaching significance for coveys). This decline was attributed to movement of quail from untreated land to treated land. There is evidence that some loss occurre in quail after they made this movement. The decrease in quail numbers in each instance could be ascribed to the application of heptachlor. Song birds were listed on the two Georgia areas. The first summer, eight more species and 458 more individual permanent resident birds were listed on the untreated than the treated area. After half of the untreated area was treated with heptachlor at 2 pounds per acre the following winter, the number of resident birds declined 37 percent. Some species of summer resident birds could not be found on treated land after heptachlor was applied. A small plot of 4 acres was

intensively searched for dead and dying animals, and observations were made on living animals. Forty-seven days after treatment, no live animals were seen or heard on the plot and a total of 38 dead animals had been found. Soils were sampled twice after treatment, and residues had declined in the second lot of samples. Animals were secured for analysis at two periods, and residues declined in the second group of animals. Numbers of birds observed on the area increased in the same period.

Enclosure tests of bobwhite (James B. DeWitt, Richard M. Prouty, and James W. Spann)

These studies were undertaken as a step in bridging the gap between field and laboratory studies. Land was treated with granular heptachlor at rates that have been recommended for use in the field, either currently or in the past. Adult birds from pen-reared stock were placed in 20-by 50-foot wire-covered pens, one pair of birds in each pen. If one of the pair died, the other was killed and a new pair was introduced. Birds used in these tests were in breeding condition, but had not been mated before being placed in the pens.

The 1963 tests involved two sets of pens. One set of 32 pens had been used in experiments in 1962. Of this set, 8 pens had been left untreated as controls and the others had been treated with granular heptachlor on May 2, 1962, as follows; 6 pens at 0.25 pound per acre, 6 pens at 0.5 pound per acre (treated at 0.25 pound per acre May 2, 1962, and retreated at the same rate August 2, 1962), 6 pens at 1.25 pounds per acre, and 6 pens at 2.0 pounds per acre. The second set, a group of 36 pens, was used for the first time in 1963. One set of 9 pens was kept untreated; the others were treated May 8, 1963, at the rates of 0.125, 0.25, and 1.25 pounds per acre (9 pens at each rate).

Data presented in table D-2 show results for 1963, and supplement the earlier information summarized in Circular 167 (U. S. Bureau of Sport Fisheries and Wildlife, 1963).

Effects of quarter-pound heptachlor application in Arkansas (Douglas James, University of Arkansas, Cooperator)

A field investigation was made in the summer of 1962 to determine whether significant wildlife mortality could be detected following heptachlor application at the rate of ¼ pound per acre. Four study areas in Union Company, Ark., were selected for good bird populations and for habitat similarity between treated and untreated tracts. Two areas were on forested land and two were on clear-cut land with shrubby

regrowth; one area from each vegetative type was scheduled for treatment to control the imported fire ant. Treatment was applied June 21 and 27. More than half the shrubby tract was treated both days and thus received ½ pound of heptachlor per acre, double the intended amount. Searches were made every few days from June 1 until August 29 on the shrubby land and from May 31 until July 20 on the forest land.

Hours spent searching for carcasses of animals were as follows:

	Forest land		Shrubby land	
	Treated	Untreated	Treated	Untreated
Before treatment	18	7	16	7
After treatment*	12	18	41	18
Days of search	14	11	28	19

*Hours of searching were distributed both before and after the second treatment on the forested area.

No carcasses were found at any time on any area during the searches (totaling 134 ¼ hours) except for two toads, one lizard, and one fledgling bird, all of which were killed by a bulldozer. These findings provide strong evidence that: (1) Immediate mortality from ¼ pound heptachlor applications is too low to be measured by practicable field techniques. (This study, of course, does not give evidence concerning possible delayed effects through the food chain), and (2) unless there is unusually high mortality, dead animals are not readily found in the field.

EFFECTS ON WILDLIFE OF CHEMICALS USED IN CONTROL OF CEREAL LEAF BEETLE

Studies in Michigan (Gordon L. Zorb and C. T. Black, Michigan Department of Conservation, Cooperators)

The Department of Agriculture's 1963 program for suppression of cereal leaf beetle infestation recommended treatment of grain fields with malathion at the rate of 1 pound per acre in an aqueous spray. Wildlife studies were planned to include search for dead animals on treated and untreated areas, counts of songbirds, and experimental pen studies with ringnecked pheasants.

Searches for dead animals were made by teams of biologists from the Conservation Department. Before spraying, 31 miles of search routes were established in good or excellent wildlife edge cover around grain

fields on 93 farms in 8 townships of Berrien County, Mich. These edge habitats were chiefly fencerows, woods edges, and ditchbanks; other habitats were swale, roadside and windbreak. The 31 miles of routes were searched in late April before treatment. Beginning May 11, 1 pound of malathion per acre was applied to 33,768 acres in Berrien and Cass Counties, Mich. Part of the area was treated twice. During days 1-7 after spraying, 26 miles of routes were studied; during days 5-11 after spraying, 21 miles of the same routes were searched again. Ornithologists censused birds along 19 miles of the routes before treatment. On two censuses after treatment, they covered 16 and 12 miles.

In pre-spray searches, the only natural remains found were one rabbit skull and three piles of pheasant feathers -- all clearly old. Five cats and two chickens were found at one spot, evidently where someone dropped them. Thus, on 31 miles of good wildlife edge, no recently dead wildlife was found. After spraying, the only dead wildlife found was one raccoon killed by a dog. Numerous frogs, tadpoles, mosquitoes, and other insects remained alive. Young in 12 bird nests were developing normally. About 500 snails of the genus Limnaea were dead in one ditch, but many other snails remained alive there; the spray probably did not cause this mortality. The number of birds counted along routes dropped one-third between the second and third post-spray censuses. This decline probably resulted from bad weather, but the presence of fewer insects in the fields also may have had an effect.

Studies of the effects of malathion spray on pheasants were conducted in a set of 20 pens (12 by 150 feet each). One male and five females occupied each pen. Pens were sprayed with water formulations at rates of 1, 5, and 10 pounds of malathion per acre. The vegetation in the pens, a month's supply of ear corn, and the pheasants themselves all received the spray. The pheasants seemed to show no ill effects, and no birds died until 3 months after spray, when several birds flew into the side of the cage. Gross autopsy of 12 hens (1 from each treatment and 1 from the controls, 6, 21, and 29 days after the spray) showed no differences between control and experimental birds. The remaining birds are being retained for observation.

Since pheasants survived 10 pounds of malathion per acre in aqueous solution, it is evident that direct effects would not be expected to follow a 1-pound per acre treatment of malathion, as used in 1963.

THE MEANING OF PESTICIDE RESIDUES IN ANIMALS

One of the most common and important ways of studying the effects of pesticides on animals is by analysis of residues. Unless mortality

is evident, the study of residue levels is the chief current line of attack in understanding persistent chemicals such as DDT and other chlorinated hydrocarbons. The questions of what a given residue level indicates about the level of contamination in the animal's food are largely unanswered. Basic information on rates of accumulation and loss of residues under controlled feeding are necessary for a reasonable interpretation of hazards in the field.

Some information of this kind was obtained with captive eagles in Alaska, as described above, but studies with eagles have practical limits. Therefore, fuller understanding is sought with species more easily kept; understanding of similarities and differences among species also is necessary, for animals of different kinds may react differently, or in different degrees.

Rates of build-up and loss of DDT in cowbirds (William H. Stickel; Don W. Hayne; and Merrill Jackson, North Carolina State College, Cooperators)

Two pilot trials were run to establish suitable sublethal and lethal dosage levels for the principal experiment. The first pilot trial was made with powdered DDT in a dry, mealy diet. Wholebody analyses (gastrointestinal tract not included) were made at the Pesticide Residue Laboratory, North Carolina State College, by electron capture gas chromatography. Results are expressed as parts per million on a wet weight basis. Average residue content of groups of birds was obtained by pooling several birds in an analytical sample. Separate chemical cleanup was done for three samples of the extract from each pool (these are the aliquots in the table below).

Fed 5 ppm DDT for 2 weeks	DDE ppm	DDT ppm	DDT / DDE
Replicate 1 (pool of 4 birds)			
Aliquot 1	0.78	3.02	3.80
2	1.44	3.59	5.03
3	1.32	3.00	4.32
Average			4.38
Replicate 2 (pool of 4 birds)			
Aliquot 1	1.35	3.30	4.65
2	1.04	2.81	3.85
3	1.07	3.03	4.10
Average			4.20

	DDE ppm	DDT ppm	DDT / DDE
Fed 100 ppm DDT for 1 week (pool of 4 birds)			
Aliquot 1	1.19	24.2	25.39
2	2.62	25.7	28.33
3	3.25	20.6	23.85
Average			25.86
Fed 100 ppm DDT for 1 week, then clean food for 1 week (pool of 4 birds)			
Aliquot 1	2.99	20.9	23.89
2	2.19	19.7	21.89
3	1.75	18.5	20.25
Average			22.00
Fed 100 ppm DDT for 2 weeks (pool of 3 birds)			
Aliquot 1	4.18	58.3	62.48
2	2.65	55.1	57.75
3	2.45	63.2	65.65
Average			61.96

These data are of interest in showing the amount of variation that may be expected in repeated chemical analyses of single batches. They also show the magnitudes of residues to be expected in short-term feeding at two levels, and they suggest that a small but perceptible loss of residues occurs within 1 week.

The second pilot trial was made similarly, but with crystalline DDT (p, p'isomer) dissolved in oil and with a crumbles diet. Results of this test are incomplete, but indicate a considerably greater proportional absorption of DDT from the feed than when the chemical was mixed with the food as a dry powder.

On the basis of these trials, the time schedules for the main experiment were established on multiples of a 2-week interval and the dosage rates as 2.5, 10, and 40 ppm. Dosage began in late December. Experiments in keeping the birds were conducted concurrently with the trial runs.

Pesticide residues in eggs of black ducks (Lucille F. Stickel, William Reichel, and C. Edward Addy, Cooperator)

Black duck populations in the Atlantic Coastal Region reached a peak during the 1954-55 fall-winter period, according to the Winter Survey. Populations then declined to a low in January 1958, and have increased only slightly since then, despite more restrictive hunting regulations and fewer duck hunters. Further, wing surveys of the last 3 years have shown declining age ratios which even at the initial 2:1 ratio in 1960 can be considered barely adequate to build the population if it is

hunted significantly. There is no reason to believe that the breeding ground habitat has altered drastically during this period or that climatic changes have been sufficient to explain the reduced breeding success.

The possible effect of pesticides on black duck populations was considered worthy of exploration. Since black ducks have a diverse diet of both animals and plants, they may have more opportunity for exposure to pesticides than species that feed only on plant material. During the winter, black ducks often feed heavily on worms, small clams, mussels, snails, small crabs, and other crustaceans, and various minnows and other small fish.

An exploratory survey of residues in black duck eggs was undertaken as a first step. Requests were made to widely distributed cooperators in the Atlantic Flyway for collection of three eggs from a nest and three to five nest samples for each general area. Sample clutches were received from Nova Scotia, New Brunswick, Maine, Vermont, Massachusetts, Connecticut, New York, New Jersey, Delaware, Maryland, and Michigan. Eggs were opened and examined for freshness and stage of embryonic development, then prepared for chemical analysis of residues. The three eggs from a single nest were pooled for a sample unless they differed in developmental stage or freshness.

Analysis of this series of duck eggs is being made by the thin-layer chromatographic technique, as described elsewhere in this report. Exploration and standardization of the method and its adaptation to this material were completed in 1963, and analyses of the black duck egg specimens was begun.

Reproductive success of ospreys in relation to pesticidal residues in their eggs and in their environment (William H. Stickel, Frederick C. Schmid, Lucille F. Stickel, William Reichel, and Peter L. Ames, Cooperator)

Osprey populations have been declining in Atlantic coastal areas for some years. The question has been raised whether pesticides are involved in this decline. Ospreys subsist on fish that they obtain in shallow water and thus the birds could be exposed consistently to toxicant through the food chain.

The presence of pesticides in osprey eggs was shown by studies made in 1962 at Old Lyme, Conn., by Peter Ames of the Yale Peabody Museum. Chemical analyses showed DDT or its metabolites in all samples tested, including 6 eggs, 1 nestling, and 1 embryo. Three fish samples taken from osprey nests at Old Lyme also contained residues.

Investigation of the effects of pesticides on osprey populations and reproductive success is therefore important, and parallel studies were initiated in two areas: Old Lyme, Conn., where Ames' investigations of the past few years indicated poor reproductive success and a declining population; and the Lower Potomac area in Maryland, where a colony apparently was thriving.

Single eggs were taken from a series of nests in each locality, and the success of the remaining eggs in the same nests was followed by periodic observation. Embryo development and freshness were determined in the laboratory. Chemical analysis of the eggs has not yet been done. Nest food samples and a series of fecal samples were collected for analysis if egg residues indicate this is desirable.

A comparison of histories of nests from which eggs were taken showed Lower Potomac nestings to be more successful, as was anticipated. In the Potomac area, 17 of 26 nests with eggs hatched 27 nestlings, fledged 24 young. Eleven eggs disappeared from nests; 3 passed incubation without hatching and were collected. In contrast, 2 of 15 Connecticut nests with eggs hatched 4 nestlings (3 in 1 nest, 1 in another) and all fledged; 16 eggs disappeared; 9 passed incubation and were collected.

Pesticides in Lake Michigan (Joseph J. Hickey and J. Anthony Keith, University of Wisconsin, Cooperators)

An environmental study of pesticides in the Green Bay area of Lake Michigan was initiated in cooperation with the University of Wisconsin. Green Bay lies just off Door County, which is Wisconsin's main fruit-growing area using about 15 percent of all the agricultural pesticides applied annually in the State. The 1963 exploration of levels and amounts of pesticides in wildlife and its environment included: Samples of gulls (36), bottom mud (20), food fish (10) and eggs (4). Residue determinations have been made for part of the series by the Wisconsin Alumni Research Foundation using gas chromatographic techniques. Residues were determined in five fish (all alewives) regurgitated by young gulls or taken from gull nests. Total combined residues of DDT, DDE, and DDD in these fish were 1.5, 2.1, 3.3, and 5.2 ppm, wet weight basis (average 3.0 ppm).

Total residues of DDT, DDE, and DDD (ppm wet weight) in tissues of herring gulls of different ages were as follows (individual values are in parentheses):

	Brain	Breast muscle	Fat
Juveniles (pre-flight)	Av. 2.4 ppm (1.1, 1.3, 4.9)	Av. 9.5 ppm (7.4, 6.2, 14.9)	Av. 206 ppm (173, 189, 257)
Subadults (collected at a dump)	Av. 19.9 ppm (13.5, 20.6, 21.4, 24.2)	Av. 86.7 ppm (53.7, 74.4, 110.7, 108.0)	Av. 2035 ppm (1563, 2066, 2543, 1969)
Adults (collected on nesting territory)	Av. 19.8 ppm (16.0,18.5,19.8, 20.0, 20.6,23.8)	Av. 107.3 ppm (145.8,122.0, 122.6,89.2, 105.6, 58.6)	Av. 2753 ppm (2705,1949,3450, 2540,4273,1600)

Full presentation of data awaits completion of residue analyses and some additional field sampling.

EFFECTS OF PESTICIDES APPLIED TO AQUATIC AREAS

Effects of alligatorweed control on wildlife (Frank B. McGilvrey, Jr., and John H. Steenis)

A few waterfowl were taken for chemical analysis as a step in learning whether they accumulate herbicide residues from waters treated for alligatorweed control. Fourteen waterfowl were collected on Lake Marion, S. C., in February 1962 from an area that had been treated with granular silvex at 20 pounds acid equivalent per acre in mid-July 1961. How long these birds had been feeding in the area was not known. Residue determinations were made for the center by the Wisconsin Alumni Research Foundation. The waterfowl were skinned and the lower legs, wings, and heads were removed. The Marquardt and Luce method, with modifications in extraction methods, was used to determine 2,4,5-T (silvex). Results indicated that uptake of silvex will occur under field conditions. Four ringnecked ducks contained 0.06 ppm, 0.15 ppm, 0.16 ppm, and 0.20 ppm of silvex (wet weight basis); one coot contained 0.06 ppm; five other coots, one shoveler, one lesser scaup, one green-winged teal, and one gadwall contained no detectable residues.

Florida impoundments for mosquito control -- a non-chemical method (Charles H. Trost, University of Florida, Cooperator)

Mosquito control districts along sections of the Atlantic coast of Florida are convinced of the efficiency of controlling saltmarsh mosquitoes by means of permanent impoundment of marshes. They report far less need for application of insecticides. Under contract to Patuxent Wildlife Research Center, the Florida State Board of Health has supervised an evaluation of the effect of these impoundments on wildlife. Graduate student Charles H. Trost, under supervision of Dr. Maurice W. Provost, has conducted monthly inventories of birds

using impounded and unimpounded saltmarsh study areas in Volusia, Brevard, Indian River, St. Lucie, and Martin Counties. These inventories have consistently shown about 10 times more bird use of the impounded than of the unimpounded marshes. The impoundments provide a greater amount of accessible open water and increased quantities of submerged aquatic plants and fish that serve as a food supply for water birds.

DEVELOPMENT AND REFINEMENT OF METHODS FOR EVALUATING EFFECTS OF PESTICIDES

Measurement of a known mortality rate in a small bird population

(Chandler S. Robbins and others)

A detailed comparison was made of several ways of detecting population loss among small birds. The study was made on a 100-acre area of moist deciduous forest at the Patuxent Wildlife Research Center in the summer of 1962. Initial censuses were made by counts of territorial males (Williams method) and by mist netting. Mortality up to 50% was then imposed on netted birds of common species and counts again were made by both methods.

Data are summarized in the table below. The species listed ("removal" species) are the ones upon which mortality was imposed; data for all other species are grouped as "nonremoval" species. Column 1 (Banding) shows the size of the breeding population before birds were removed, as estimated from recaptures of banded birds; column 2 (Census A/B) shows estimates by the territorial male counts. In the next two columns are independent estimates of the population as determined by observers A and B, one using east-west census lines for his field trips, the other using north-south lines through the same area. The next two columns show the estimated number of birds removed (through intensive netting, June 6-8, 1962) as determined independently by A and B in 8 census trips each after removal of 170 birds (88 males). The final column shows the number of birds of each species that were removed.

Species	Est. Breeding Males/100 Acres				Est. Males Removed		Actual Males Removed
	Banding*	Census A/B	Census A	Census B	A	B	
R.-e. Vireo	82	60	56	56	14	15	25
Wood thrush	32	25	18	27	7	9	21
Ac. Flycatcher	18	22½	23	21	7	3	8
Ovenbird	8	16	11	15	5	10½	8
Ky. Warbler	12	14½	15	13½	5	5	10
Hd. Warbler	6	12½	13	10	8	3½	7
Redstart	25**	22	16	15	0	4	2
Sc. Tanager	48**	17½	15½	15	½	5	5
Cardinal	17**	10	9	9	1½	1½	2
Total	193	200	176½	181½	48 (27%)	56½ (31%)	88 (44%)
Total, nonremoval species	85	76		82	12½ (16%)	16 (20%)	0

* Figures in this column are corrected for territory size; the total, 193, is a weighted figure.

** Sample too small; only 1 or 2 banded birds were recaptured during sampling period as compared with 7 to 23 for the other species.

The observed decreases in nonremoval species are a measure of the decrease in singing as the season progresses. This decrease takes place in the removal species as well as in nonremoval species. When each observer's estimate of the decrease in nonremoval species is subtracted from his estimate of the decrease in removal species, the remainder (11 percent for each observer) is the estimated loss resulting from removal. Since 88 birds out of an estimated population of 200 (44 percent) were removed, each observer detected only one-fourth of the loss that occurred.

The banding data showed that an influx of new birds took place during and immediately after the 3-day removal period and it was this increase that made it impossible to measure accurately the number of birds removed.

Although the decrease in population resulting from the removal of birds could not be measured adequately, both the loss of marked birds from the population and the change in ratio of marked to unmarked birds were quite apparent. Recapture records of 198 birds of the nine removal species were compared with recapture records of the same species on essentially the same dates the preceding year, when no birds were removed. In the first sampling period, before the removal date, the 1962 recaptures were 8 percent below the 1961 recaptures. In the second sampling period, after the removal date, the 1962 recaptures were 44 percent below the 1961 recaptures. In the third sampling period, the 1962 recaptures again were 44 percent below the 1961 recaptures.

Carcass search technique (William H. Stickel and Nicholas J. Chura)

Tests of the value of standardized searches in measuring mortality of birds in the field were extended in 1963 by several experiments and by practical use in connection with actual pesticidal treatments in Georgia, Alabama, Louisiana, Arkansas, and Michigan. The field trials were encouraging in demonstrating that dead animals are very seldom found in normal areas, and hence that numbers of dead animals are strong evidence for the occurrence of unusual mortality.

Experimental tests compared the effectiveness of different searchers in locating carcasses and the proportions of carcasses found in different habitats.

Since searchers do not find all carcasses present, a method for estimating numbers of carcasses was explored in 1963. The method shows promise. The plan was to distribute known numbers of marked objects along the search routes where carcasses also were present and to see whether carcass numbers could be estimated from the proportion of marked objects found, according to the Lincoln Index formula (possibly adjusted by introduction of a factor to compensate for differences in findability of carcasses and marked objects).

The objects used were woodcock wings (available from woodcock wing survey), which seemed likely to be more similar to carcasses than most objects, and which still were durable enough for possible use in actual field studies. Results of one set of searches are shown in the table below. Search was conducted along four 2,650-foot routes in a woods-field edge habitat at the Patuxent Center. Each of the four areas was searched on separate days by two men working independently. Wings and carcasses were distributed in an area 12 feet wide, with placement determined from random number tables.

Area	Items	Number Present	Number Found		Estimated Carcasses Present1/	
			Observer 1	Observer 2	Observer 1	Observer 2
A	Carcasses	4	3	3	6.0	5.2
	Wings	12	6	7		
B	Carcasses	12	4	7	6.9	12.0
	Wings	12	7	7		
C	Carcasses	12	8	8	16.0	16.0
	Wings	12	6	6		
D	Carcasses	4	3	2	4.5	2.0
	Wings	12	8	12		
All	Carcasses	32	18(56%)	20(63%)	32.0	30.0
	Wings	48	27(56%)	32(67%)		

1/ Using Lincoln Index formula, assuming that wings and carcasses are equally likely to be found: Wings found:Wings present::Carcasses found:Carcasses present.

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Carcass disappearance rates (William H. Stickel)

The rate at which dead animals disappear in the field is important in determining the numbers that can be found and hence the extent of mortality that has occurred.

Tests made in 1963 followed the earlier results in showing rapid disappearance of a substantial portion of the carcasses and also indicated considerable variability in rates.

Results of these experiments lead to two conclusions of importance in pesticide field work: (1) Remains of small animals seldom persist for long periods as evidence of what has happened; (2) a large proportion of the remains do persist long enough to be found if searches are made at intervals of 2 or 3 days after treatment.

The table below summarizes the percentages of carcasses remaining visible in all tests after different lengths of time. Thirty birds were used in each test except that of the bobwhite in Maryland, in which there were 21.

Locality	Species	Day 1	Day 2	Day 3	Day 4	Day 8	Week 2	Week 5
Alabama*	Bobwhite	87%	80%	67%	53%	--	--	--
Texas*	Bobwhite	93%	90%	87%	87%	--	--	--
Maryland	Bobwhite	76%	57%	33%	10%	--	--	--
Maryland	Grackle	90%	77%	73%	70%	67%	60%	57%
Maryland	Grackle	70%	53%	37%	33%	30%	23%	23%
Maryland	Red-winged Blackbird	67%	47%	37%	--	27%	27%	--

*/ From Rosene and Lay (1963). Journal of Wildlife Mgt. 27(1): 134-142.

Development of new laboratory criteria (Ralph A. Ernst, and Robert K. Ringer, Michigan State University, Cooperators)

Studies were initiated, using coturnix quail as the test animal, to develop assay methods for ascertaining the effects of pesticides. After establishment of the colony, exploratory studies of blood cell relations were made of birds on diets containing different levels of DDT. There is some evidence of effects on differential counts of blood cells. Additional exploration of this type is being undertaken with Zectran, Zytron, and Tordon. Work on injecting embryonating eggs with pesticides was begun. Current studies are aimed at developing techniques of injection and determining what stage of incubation will give consistent results.

MEASUREMENT OF PESTICIDAL RESIDUES

(James B. DeWitt, Vyto A. Adomaitis, George E. Bagley, Calvin M. Menzie, Richard M. Prouty, and William L. Reichel)

Chemical analyses of pesticide residues are made as part of field and laboratory research programs, and are essential for delineation and resolution of certain problems. With certain compounds, they offer conclusive proof of exposure and may constitute strong presumptive evidence of the severity of exposure. However, they are not directly applicable to materials which do not leave identifiable residues (e.g. some organophosphate insecticides) or those whose metabolic products are unknown.

In 1963, techniques of gas and thin-layer chromatography were under development as a supplement to the colorimetric and infrared spectrophotometric methods currently employed.

Paper and thin-layer chromatographic techniques, as described by Mills (1959) and others (Baumler, and Rippstein, 1961; Onley and Mills, 1962) have been investigated and applied as rapid sensitive methods for estimation of several chlorinated pesticides from a single sample. Techniques have been developed or adapted for the resolution and estimation of DDT, DDE, DDD, DDA, lindane, heptachlor, heptachlor epoxide, dieldrin, methoxychlor, thiodan, kepone, perthane, and 2,4-D derivatives. The sensitivity achieved was approximately 0.5 mmg.

Colorimetric analyses were made of approximately 1,000 samples of animal tissues and soils, using analytical procedures described below.

Preparation of samples

Animal tissues.--Birds and mammals were skinned, and keratinized tissues (feathers, fur, beak, feet) were discarded. For experimentally dosed animals the contents of gastrointestinal tracts were also discarded. Remaining tissues were (a) dried to constant weight under slightly reduced pressures or in a gentle current of air at temperatures of not more than 40°C., macerated, and extracted with acid-cleaned petroleum ether in a Soxhlet type extractor;[1/] or (b) ground with anhydrous Na_2SO_4 (2 or 3 g Na_2SO_4 per g of tissue) to form a fine, freeflowing powder, and extracted as above.

1/ Many field specimens were partially dehydrated or decomposed at time of receipt. All analyses of such specimens were made on dehydrated tissues.

Soils.--Moisture content of the samples was adjusted to approximately 5 percent by air drying at room temperature and/or the addition of sufficient quantities of distilled water. Samples were extracted by end-over-end tumbling with a 2:1 mixture of hexane-isopropanol.

Clean-up procedures, animal tissues

Heptachlor, and heptachlor epoxide.--Extracts were transferred to a separatory funnel, made up to 300 ml with petroleum ether, and 50 ml of a 2:1 mixture of concentrated and fuming (15 percent) H_2SO_4 added without shaking. The acid layer was drawn off, a second 50 ml portion of the mixed acids was added, and the funnel shaken gently 3 times. Emulsions were broken by the addition of 5 ml H_2O. The acid layer was removed, and the petroleum ether layer was washed with 50 ml portions of H_2O until washings were neutral to litmus. (Emulsions which formed at this stage were broken by the addition of small amounts of anhydrous Na_2SO_4.) The petroleum ether layer was dried by filtration through Drierite, concentrated at room temperature under a gentle stream of air, and placed on a 2 x 15 cm. column of activated florisil. Solvents used to develop columns and elute pesticides were: Aldrin - 200 ml hexane; heptachlor - 250 ml hexane; heptachlor epoxide - 200 ml of a 3:1 mixture of hexane-benzene.

DDT.--Volume of extract was adjusted to approximately 150 ml, and mixed with 40 ml of a 1:1 mixture of concentrated and fuming (15%) H_2SO_4. After 30 minutes, the petroleum ether layer was decanted onto a Davidow column containing a slurry of 30 g celite 545 and 18 ml of the acid mixture. Flask was washed 3 times with 50 ml petroleum ether, and each wash added to the column.

Clean-up procedures, soils

Heptachlor and heptachlor epoxide.--Extracts were dried by filtration through Drierite, concentrated, and chromatographed on a 2 x 15 cm column of activated florisil. Heptachlor was eluted with 25 ml of hexane, and heptachlor epoxide with 200 ml of a 3:1 mixture of hexane-benzene.

Determinations

DDT.--The purified extracts were concentrated and analyzed according to the Schechter-Haller procedure (Schechter, et al, 1945).

Heptachlor.--The purified extracts were concentrated, treated with 1 ml of the chlordane reagent of Ordas, (Ordas et al, 1956), and heated for 15 minutes. Optical density (absorbency) of the reaction product was determined at 410 mmu.

Heptachlor epoxide.--The purified extracts were concentrated, treated with 1 ml of the Polen-Silverman reagent, (Polen and Silverman, 1952), and heated for 15 minutes. Optical density (absorbency) of the reaction product was determined at 567 mmu.

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Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963

ount of toxicant in the diet is expressed as parts per million (ppm); toxicant consumed, expressed as milligrams of toxicant per kilogram of bird (mg/kg), is an average index figure, showing magnitude of intake of toxicant; birds designated as young were started on test at 1 or 2 days of age

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	to End of Test
Tests on bobwhite quail: (1) Young birds												
Bayer 37344	3-3a	1,000	25	12	88	--	--	92	3	410	1,230	4,920
	3-3a	500	25	40	48	84	--	96	12	354	4,248	14,160
Bayer 39007	5-5a	1,000	21	14	86	--	--	100	8	483	3,864	6,762
	5-5a	500	21	111	48	48	52	52	56	76	4,256	8,436
	3-3a	200	25	135	24	32	64	64	85	35	2,975	4,995
	3-3a	100	25	40	56	60	--	60	5	18	90	720
2,4-D acetamide	3-3a	2,500	25	12	72	--	--	76	3	2,258	6,774	27,096
2,4-D butoxyethanol ester	3-3a	5,000	25	135	28	44	56	60	50	1,212	60,600	155,790
2,4-D dimethylamine salt	3-3a	2,500	24	138	12	29	42	42	--	513	--	70,794
Dacthal	4-4c	2,500	25	14	68	--	--	80	9	817	7,353	11,438
	6-6a	2,500	25	12	88	--	--	100	8	5,328	42,624	63,936
	6-6a	1,000	25	22	40	--	--	100	12	1,049	12,588	23,078
	4-4c	1,000	25	15	52	--	--	84	10	127	1,270	1,905
Diuron	5-5a	5,000	25	5	100	--	--	100	3	4,250	12,750	21,250
	5-5a	2,500	25	14	88	--	--	100	3	2,792	8,376	39,088
Endothal	4-4c	5,000	25	14	80	--	--	84	3	3,583	10,749	50,162
	4-4c	2,500	25	125	32	64	68	68	14	512	7,168	64,000
Fenuron	5-5a	5,000	25	111	24	32	44	44	--	775	--	86,025
	5-5a	2,500	23	111	9	26	30	30	--	500	--	55,500
Maleic hydrazide	4-4c	5,000	25	125	40	64	64	64	20	1,386	27,720	173,250
	4-4c	2,500	25	125	8	16	20	20	--	330	--	41,250
	6-6a	2,500	25	24	52	--	--	56	4	2,333	9,332	55,992
	4-4c	1,000	25	14	76	--	--	96	9	317	2,853	4,438
	6-6a	1,000	25	97	28	48	52	52	46	208	9,568	20,176
	6-6a	500	25	97	20	32	32	32	--	56	--	5,432
Monuron	5-5a	5,000	21	14	86	--	--	100	4	3,065	12,260	42,910
	5-5a	2,500	21	14	57	--	--	100	5	2,218	11,090	31,052
2,4,5-TP ester	6-6a	2,500	25	24	60	--	--	64	6	2,273	13,638	54,552
	6-6a	1,000	24	41	17	38	--	100	34	500	17,000	20,500
Control	3		25	138	0	16	32	32				
	3a		26	138	0	12	23	27				

Table D-1 . Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	to End of Test
Tests on bobwhite quail: (1) Young birds -- continued												
Control	4		25	125	0	4	16	16				
	4a		25	125	8	20	20	20				
	4b		24	125	0	12	17	21				
	4c		26	125	12	15	15	19				
Control	5		27	111	18	18	37	37				
	5a		25	111	20	36	36	36				
Control	6		25	97	20	36	--	40				
	6a		25	97	24	44	--	48				
Tests on bobwhite quail: (2) Adult birds												
Bayer 38920	1-1g	25	13	111	0	0	8	8	--	3	--	289
	1-1g	25	14	111	7	7	7	7	--	3	--	333
2,4-D acetamide	*	2,500	13	50	0	0	--	0	--	127	--	6,350
	*	2,500	37	50	3	3	--	3	--	206	--	10,300
	1-1g	1,000	21	111	0	0	0	0	--	93	--	10,323
	1-1g	1,000	17	111	0	0	0	0	--	92	--	10,212
DDT	*	200	13	50	0	0	--	0	--	9	--	430
	*	200	15	50	0	0	--	0	--	25	--	1,250
Kelthane	1-1g	100	21	111	10	10	14	14	--	10	--	1,088
	1-1g	100	17	111	0	0	0	6	--	10	--	1,077
Mirex	1-1g	300	17	111	0	0	12	12	--	28	--	3,108
	1-1g	300	16	111	0	0	25	31	--	35	--	3,885
Rhothane (DDD, TDE)	1-1g	250	19	111	10	10	16	21	--	27	--	2,997
	1-1g	250	20	111	5	5	5	5	--	25	--	2,775
Control	1		20	112	0	0	0	0				
	1a		20	112	0	0	0	0				
	1b		20	112	0	0	0	0				
	1c		20	112	0	0	5	5				
	1d		19	112	0	0	0	0				
	1e		22	112	0	0	0	0				
	1f		20	112	0	0	5	5				
	1g		20	112	0	0	0	0				

* No control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets. These tests were conducted to get maximum utilization of birds that would have been destroyed.

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	of Test
Tests on coturnix:												
(1) Young birds:												
Aldrin	30-30a	100	27	5	100	--	--	100	3	8	23	38
	30-30a	50	27	7	78	--	--	78	4	9	35	62
	30-30a	25	27	14	52	--	--	74	10	7	71	99
	27-27a	25	23	22	4	--	--	74	19	3	63	73
	28	5	25	3	100	--	--	100	3	1	2	2
BHC	47-47a	1000	25	7	72	--	--	72	4	150	600	1050
	47-47a	500	25	7	52	--	--	52	7	151	1057	1057
Baytex	30-30a	200	28	7	82	--	--	82	5	10	50	70
	30-30a	100	36	7	81	--	--	81	6	3	20	23
	46-46a	50	17	179	0	12	35	88	170	15	2550	2685
Chlordane	28	1000	25	12	96	--	--	100	3	477	1431	5724
	28	500	25	13	60	--	--	88	8	351	2808	4563
	29-29a	250	30	7	55	--	--	55	6	50	300	350
	47-47a	200	25	21	36	--	--	84	17	37	629	777
	47-47a	100	25	173	36	40	56	64	73	35	2555	6055
2,4-D acetamide	34-34a	2500	25	21	44	--	--	56	12	1191	14292	25011
2,4-D amine salt	35	2500	25	62	12	12	--	12	--	800	--	49600
2,4-D ester	31-31a	5000	24	60	38	38	--	38	--	645	--	38700
	31-31a	2500	25	60	8	28	--	28	--	595	--	35700
DDT	26-26a	2000	21	6	100	--	--	100	3	367	1101	2202
	26-26a	1000	19	9	79	--	--	79	5	181	905	1629
	26-26a	500	16	16	44	--	--	56	11	101	1111	1616
	28	500	25	17	60	--	--	100	10	175	1750	2975
	29-29a	250	25	18	16	--	--	100	17	78	1326	1404
	34-34a	250	25	21	36	--	--	56	14	93	1302	1953
	33-33a	200	25	24	32	--	--	100	14	60	840	1440
	49	100	25	12	48	--	--	80	10	20	200	240
	49	50	25	12	32	--	--	60	12	8	98	98
	*	10	25	104	12	20	28	28	--	4	--	416
Dieldrin	30-30a	200	37	7	86	--	--	86	3	39	117	273
	30-30a	100	36	7	78	--	--	78	5	9	46	64
	27-27a	50	22	15	27	--	--	59	12	7	86	108
	27-27a	25	23	29	0	--	--	9	--	5	--	136
	28	10	25	139	40	40	48	72	101	11	1111	1529

* Control data abnormal for this group

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	to End of Test
Tests on coturnix:												
(1) Young birds -- continued												
Dimethoate	29-29a	200	25	62	24	48	--	52	61	18	1,098	1,116
	29-29a	100	25	14	64	--	--	68	8	21	168	294
Endrin	27-27a	25	27	8	74	--	--	74	6	3	19	25
	27-27a	10	27	29	7	--	--	52	21	2	34	46
	47-47a	5	24	152	38	42	54	100	66	2	110	254
Heptachlor	30-30a	500	38	5	100	--	--	100	3	18	54	90
	31-31a	200	25	6	80	--	--	80	5	63	315	378
	27-27a	100	25	22	28	--	--	72	17	14	206	308
	27-27a	50	28	29	14	--	--	32	--	8	--	226
	28	50	25	25	52	--	--	60	10	25	250	625
	29-29a	25	36	28	42	--	--	53	19	5	93	137
Parathion	47-47a	200	25	4	100	--	--	100	2	26	52	104
	47-47a	100	25	6	100	--	--	100	4	27	108	162
	47-47a	50	25	7	72	--	--	72	5	8	39	54
Rhothane (DDD, TDE)	29-29a	2500	25	7	56	--	--	56	7	583	4081	4081
Thiodan	28	1000	29	25	66	--	--	72	7	923	6461	23075
	28	500	32	25	50	--	--	56	9	182	1638	4550
Zectran	29-29a	1000	25	5	100	--	--	100	3	114	342	570
	29-29a	500	25	7	60	--	--	60	5	25	125	175
	48	500	25	5	100	--	--	100	3	250	750	1250
Control	26		20	15	0	--	--	0				
	26a		20	15	10	--	--	10				
Control	27		23	29	0	--	--	0				
	27a		25	29	0	--	--	4				
Control	28		25	139	4	4	16	44				
Control	29		25	63	40	40	--	40				
	29a		25	63	12	12	--	12				
Control	30		27	20	15	--	--	22				
	30a		27	20	26	--	--	26				
Control	31		25	153	28	48	52	56	54			
	31a		25	153	16	44	48	48				
Control	33		25	34	12	16	--	16				
	33a		25	34	32	40	--	40				

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed to 50% Mortality: Daily	to 50% Mortality	to End of Test (mg/kg)
ests on coturnix:												
1) Young birds -- continued												
Control	34		25	102	4	4	4	4				
	34a		25	102	8	8	12	12				
Control	35		25	62	16	24	--	28				
Control	46		22	185	9	9	14	27				
	46a		22	185	0	9	14	18				
Control	47		25	179	12	20	24	36				
	47a		25	179	8	12	28	28				
Control	48		25	146	24	24	24	24				
Control	49		17	12	18	--	--	29				
ests on coturnix:												
2) Adult birds												
Aldrin	25-25i	100	10	4	50	--	--	50	4	14	54	54
	25-25i	50	10	18	10	--	--	100	13	4	55	76
Apholate	37 **	(1000	10	16	10	--	--	30	--	68	--	1088
		(2000	7	17	29	--	--	86	15	182	2730	3094
	37	500	8	16	0	--	--	0	--	63	--	1008
	37	250	9	16	0	--	--	0	--	43	--	688
2,4-D acetamide	*	5000	24	97	0	4	--	21	--	661	--	64,117
	*	5000	16	85	0	0	--	50	77	375	28,875	31,875
	38	5000	15	100	20	33	47	47	--	991	--	99,100
	*	2500	16	85	0	19	--	19	--	570	--	48,450
	38	2500	15	100	0	0	0	0	--	607	--	60,700
2,4-D amine salt	38	5000	15	100	7	27	33	33	--	935	--	93,500
	38	2500	16	98	0	0	--	100	94	604	56,776	59,192
2,4-D ester	*	5000	16	85	6	6	--	44	--	1140	--	96,900
	*	2500	16	85	0	6	--	6	--	641	--	54,485
DDT	*	500	24	97	12	29	--	88	48	83	3984	8,051
	24-24i	500	10	16	60	--	--	90	7	175	1225	2,800
	25-25i	500	10	8	60	--	--	60	8	76	608	608
	25-25i	250	10	105	0	40	70	70	57	83	4731	8,715
	24-24i	200	10	112	10	40	80	80	49	28	1372	3,136

o control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets.
hese tests were conducted to get maximum utilization of birds that would have been destroyed.
irds were fed 1000 ppm for 16 days, then level was increased to 2000 ppm.

Table D-1. Toxicity test of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	to End of Test
B. Tests on coturnix: (2) Adult birds --continued												
Dibrom	24-24i	500	10	100	10	20	20	20	--	50	--	5000
	25-25i	250	11	101	0	0	18	18	--	76	--	7676
Dieldrin	25-25i	100	10	8	50	--	--	50	8	17	136	136
	25-25i	50	10	105	0	30	70	70	66	14	924	1470
	24-24i	20	10	112	0	10	80	80	67	4	235	392
	24-24i	10	10	100	0	0	0	0	--	1	--	133
Heptachlor	25-25i	100	10	35	30	90	--	100	12	25	300	875
	25-25i	50	11	70	0	27	--	100	66	11	726	770
	24-24i	50	10	53	20	60	--	90	16	7	110	366
	24-24i	25	10	110	20	30	60	70	51	4	179	385
Control	24		8	112	0	0	0	0				
	24a		9	112	11	11	11	22				
	24b		9	112	0	0	0	11				
	24c		8	112	0	0	0	0				
	24d		13	112	15	15	15	15				
	24e		10	112	0	0	10	10				
	24f		9	112	0	11	11	11				
	24g		10	112	0	0	0	0				
	24h		9	112	0	0	0	0				
	24i		7	112	0	14	14	29				
Control	25		8	105	0	12	25	25				
	25a		8	105	0	12	38	38				
	25b		9	105	11	11	11	11				
	25c		8	105	0	0	0	0				
	25d		11	105	0	0	0	0				
	25e		9	105	0	0	11	22				
	25f		8	105	0	0	12	12				
	25g		10	105	0	0	0	0				
	25h		9	105	0	0	22	22				
	25i		7	105	14	14	14	14				
Control	37		10	16	0	--	--	0				
Control	38		15	51	0	7	--	27				
C. Tests on mallards: (1) Young birds												
Chlordane	17	5,000	25	5	--	--	--	100	4	652	2,608	3,260
	17	2,500	25	5	--	--	--	100	3	91	273	455
	19-19b	1,000	25	7	--	--	--	100	5	283	1,415	2,264
	20	500	25	12	76	--	--	76	6	71	426	852
	21-21a	250	25	31	76	80	--	80	5	44	220	1,364

Table D-1. Toxicity test of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	Mortality	to End of Test
C. Tests on mallards: (1) Young birds -- continued												
Dacthal	17	5,000	25	3	--	--	--	100	2	471	942	1,413
	18-18a	5,000	25	16	80	--	--	92	6	1,351	8,106	21,616
Dimethoate	17	5,000	25	4	--	--	--	100	2	235	470	940
	17	2,500	25	3	--	--	--	100	2	288	576	864
	18-18a	200	25	20	60	--	--	88	8	108	864	2,160
Endrin	18-18a	50	25	6	--	--	--	100	4	10	39	59
	18-18a	25	25	16	80	--	--	88	5	13	65	208
	18-18a	10	25	26	52	--	--	72	9	5	46	133
Heptachlor	17	1,000	26	6	--	--	--	100	4	103	412	618
	17	500	26	6	--	--	--	96	5	27	135	162
	18-18a	500	25	5	--	--	--	100	3	59	177	295
	17	250	26	6	--	--	--	100	4	19	76	114
	18-18a	250	25	6	--	--	--	100	4	18	72	108
Kelthane	18-18a	5,000	25	6	--	--	--	100	4	517	2,068	3,102
	17	5,000	25	7	--	--	--	100	4	323	1,292	2,261
		5,000	25	9	--	--	--	100	6	367	2,202	3,303
	18-18a	2,500	25	6	--	--	--	100	5	274	1,370	1,644
	17	2,500	25	9	--	--	--	100	6	367	2,202	3,303
Kepone	16	100	25	175	48	48	60	64	30	18	540	3,150
	18-18a	100	25	14	76	--	--	76	4	67	268	938
	18-18a	100	31	161	10	35	35	35	--	12	--	1,932
MCPA	20	2,500	22	27	45	--	--	77	10	67	670	1,809
Mirex		500	27	182	78	81	81	81	5	106	530	19,292
		500	26	182	73	73	73	77	5	89	445	16,198
	16	500	26	175	0	15	15	15	--	66	--	11,550
Parathion	17	1,000	25	4	--	--	--	100	1	168	168	672
	17	500	25	6	--	--	--	100	4	36	144	216
	17	250	25	7	--	--	--	100	3	36	108	252
Perthane	21-21a	5,000	25	59	8	12	--	16	--	957	--	56,463
	20	5,000	25	100	24	28	32	32	--	836	--	83,600
	21-21a	2,500	25	100	40	44	44	44	--	493	--	49,300
Sodium arsenite	19-19b	1,000	25	13	92	--	--	92	6	293	1,758	3,809
	19-19b	500	25	154	32	44	60	60	32	86	2,752	13,244
	19-19b	250	25	154	8	8	12	12	--	34	--	5,236
Thiodan	17	500	26	6	--	--	--	92	4	58	232	348
	17	250	16	13	94	--	--	94	5	103	515	1,339

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	to End of Test
C. Tests on mallards: (1) Young birds -- continued												
Zectran	18-18a	5,000	25	6	--	--	--	100	4	163	652	978
	18-18a	2,500	25	6	--	--	--	100	4	169	676	1,014
	18-18a	1,000	25	13	68	--	--	100	7	33	231	429
Control	16		25	175	48	48	48	48				
Control	17		29	92	0	0	--	0				
Control	18		30	86	20	20	--	20				
	18a		25	161	12	20	--	20				
Control	19		25	78	0	0	--	0				
	19a		25	153	8	8	8	8				
	19b		35	153	29	31	31	31				
Control	20		25	148	52	52	56	56	6			
Control	21		26	101	4	4	4	4				
	21a		26	101	8	12	19	19				
C. Tests on mallards: (2) Adult birds												
Aldrin	14-14e	100	16	126	0	6	62	81	84	9	756	1,134
	14-14e	50	16	127	0	0	6	12	--	5	--	648
	15-15e	50	14	82	7	21	--	86	51	5	235	377
	15-15e	25	16	83	0	0	--	31	--	2	--	191
	15-15e	25	16	82	19	19	--	31	--	3	--	238
	14-14e	25	22	127	0	0	4	4	--	2	--	267
	14-14e	25	16	127	0	0	0	0	--	2	--	279
American Cyanamid #43913	23-23d	1,000	10	88	0	20	--	50	69	60	4,140	5,280
	23-23d	500	10	63	0	10	--	30	--	31	--	1,953
Bayer 38920	*	5,000	10	34	0	90	--	100	22	41	902	1,394
	*	2,500	10	27	20	--	--	100	17	44	748	1,188
	*	1,000	10	37	0	20	--	100	27	33	891	1,221
	*	500	10	89	0	10	--	100	50	31	1,550	2,759
	*	250	9	67	0	11	--	100	50	16	800	1,072
	*	100	10	98	0	10	--	80	74	7	518	686
Chlordan	*	5,000	10	37	0	90	--	100	20	81	1,620	2,997
	*	2,500	10	48	0	50	--	100	27	66	1,782	3,168

* No control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets. These tests were conducted to get maximum utilization of birds that would have been destroyed.

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	tp End of Test
C. Tests on mallards: (2) Adult birds												
2,4-D butoxyethanol ester	15-15e	200	16	82	0	0	--	0	--	21	--	1,722
	14-14e	200	16	126	0	0	0	0	--	16	--	2,016
DDT	15-15e	200	9	12	89	--	--	100	6	6	36	72
	15-15e	200	13	16	92	--	--	100	8	4	30	61
	14-14e	200	16	126	0	0	0	19	--	17	--	2,142
	14-14e	200	11	126	0	0	18	18	--	15	--	1,890
	14-14e	100	16	126	0	0	0	0	--	8	--	983
	15-15e	100	16	84	6	--	--	12	--	10	--	840
Dieldrin	*	1,000	8	30	0	100	--	100	28	20	560	600
	*	1,000	11	34	0	64	--	82	30	19	570	646
	*	500	10	33	0	80	--	100	24	10	238	327
Dimethoate	*	5,000	10	33	20	80	--	100	21	16	336	528
Diuron	23-23d	5,000	11	47	0	45	--	100	33	59	1,947	2,773
Heptachlor	*	1,000	10	35	0	90	--	100	23	31	713	1,085
	*	500	10	27	10	--	--	80	17	19	323	513
	*	500	10	22	10	--	--	20	--	37	--	814
	*	500	13	21	8	--	--	69	--	20	--	420
	*	500	16	21	6	--	--	81	19	18	342	378
	*	250	10	27	0	--	--	70	23	14	322	378
Kelthane	*	5,000	11	34	0	91	--	100	24	92	2,208	3,128
	*	5,000	16	34	0	69	--	94	28	94	2,632	3,196
Kepone	*	5,000	11	20	0	100	--	100	15	44	660	880
	*	5,000	6	15	50	--	--	50	10	11	110	165
	15-15e	100	13	83	0	0	--	0	--	13	--	1,079
	15-15e	100	10	85	0	0	--	10	--	11	--	935
	15-15e	100	10	90	10	10	--	10	--	11	--	990
	14-14e	100	13	127	0	0	0	0	--	9	--	1,105
Lindane	*	5,000	10	27	10	--	--	100	16	33	528	891
	*	2,500	10	27	0	--	--	70	20	10	200	270
Mirex	15-15e	1,000	11	81	9	36	--	45	--	97	--	7,857
	14-14e	1,000	13	128				31	--	69	--	8,832
	14-14e	500	13	126	0	0	0	0	--	38	--	4,788
	15-15e	500	15	81	0	7	--	13	--	57	--	4,617
	15-15e	200	18	81	11	11	--	11	--	22	--	1,782
	14-14e	200	19	126				5	--	13	--	1,638

* No control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets. These tests were conducted to get maximum utilization of birds that would have been destroyed.

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	to End of Test
C. Tests on mallards: (2) Adult birds -- continued												
Rhothane (DDD, TDE)	15-15e	1,000	11	43	27	91	--	91	14	54	756	2,322
	14-14e	1,000	14	128	0	0	14	36	--	73	--	9,344
	15-15e	500	9	83	0	0	--	0	--	55	--	4,565
	15-15e	500	10	84	0	0	--	20	--	49	--	4,116
	14-14e	500	12	126	8	8	17	25	--	40	--	5,040
	14-14e	500	16	126	0	0	0	0	--	41	--	5,166
	14-14e	250	16	126	0	0	0	0	--	19	--	2,394
	15-15e	250	16	83	0	0	--	0	--	27	--	2,241
Sodium arsenite	14-14e	100	26	128	0	0	0	0	--	8	--	973
Thiodan	*	1,000	8	27	0	--	--	100	22	14	308	476
	*	1,000	10	31	0	90	--	100	25	14	350	434
	*	500	11	21	0	--	--	9	--	8	--	168
	*	500	8	21	12	--	--	12	--	6	--	128
Zectran	*	5,000	10	27	10	--	--	90	16	13	208	351
	*	2,500	10	27	0	--	--	70	25	9	220	238
	*	1,000	10	27	0	--	--	60	27	7	176	176
Control	14		16	128	0	0	6	6				
	14a		16	128	0	0	0	0				
	14b		16	128	0	0	0	0				
	14c		16	128	0	0	0	0				
	14d		16	128	0	0	0	0				
	14e		16	128	0	0	0	0				
Control	15		11	81	0	0	--	0				
	15a		11	81	0	0	--	9				
	15b		11	81	0	0	--	0				
	15c		11	81	0	0	--	0				
	15d		11	81	0	0	--	0				
	15e		11	81	0	9	--	9				
Control	23		15	133	0	0	0	0				
	23a		14	133	0	0	0	0				
	23b		14	133	0	0	0	0				
	23c		14	133	0	0	0	0				
	23d		15	133	0	0	0	0				

* No control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets. These tests were conducted to get maximum utilization of birds that would have been destroyed.

Table D-1. Toxicity of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	to End of Test
D. Tests on pheasants:												
(1) Young birds												
American Cyanamid #43913	12-12a	2,000	25	7	--	--	--	100	4	847	3,388	5,929
	12-12a	1,000	25	13	88	--	--	96	6	445	2,670	5,785
Bayer 37344	12-12a	1,000	25	127	12	64	64	64	22	205	4,510	26,035
	12-12a	500	25	35	4	76	--	80	13	225	2,925	7,875
	12-12a	250	25	127	16	20	32	32	--	22	--	2,794
Baytex	10-10b	200	25	7	--	--	--	100	4	100	400	700
	10-10b	100	25	41	44	60	--	80	15	8	122	332
2,4-D amide	10-10b	5,000	25	26	4	--	--	52	26	1,618	42,068	42,068
2,4-D amine salt	10-10b	5,000	25	41	8	84	--	84	19	842	15,998	34,522
	10-10b	2,500	25	155	0	28	36	36	--	338	--	52,390
2,4-D butoxy ester	10-10b	5,000	25	26	12	--	--	76	15	2,571	38,565	66,846
Dalapon	9-9a	5,000	25	169	12	16	24	24	--	431	--	72,839
DDT	9-9a	2,000	25	7	--	--	--	100	5	2,667	13,335	18,669
Dieldrin	10-10b	500	24	6	--	--	--	100	4	133	532	798
	10-10b	200	25	7	--	--	--	100	4	143	572	1,001
Endrin	10-10b	50	24	7	--	--	--	100	4	11	44	77
Kelthane	9-9a	1,000	25	14	72	--	--	100	4	511	2,044	7,154
Kepone	9-9a	100	25	169	44	56	80	80	20	11	220	1,859
	9-9a	100	25	169	12	28	76	76	41	11	431	1,775
	11-11a	100	25	141	28	44	64	64	48	12	576	1,692
MCPA	9-9a	5,000	31	14	48	--	--	100	12	1,472	17,664	20,608
Parathion	9-9a	500	25	6	--	--	--	100	5	187	935	1,122
	10-10b	200	25	13	60	--	--	64	8	47	376	611
	10-10b	100	25	155	36	40	56	56	41	19	779	2,945
Perthane	9-9a	5,000	25	27	52	--	--	52	5	1,091	5,455	29,457
	9-9a	2,500	25	27	52	--	--	52	8	574	4,592	15,498
Rhothane (DDD, TDE)	9-9a	200	25	27	32	--	--	68	14	36	504	972
	9-9a	100	25	169	4	16	40	44	--	9	--	1,521
Thiodan	10-10b	200	25	155	4	16	44	44	--	20	--	3,100
	10-10b	100	25	155	8	8	16	20	--	9	--	1,426

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	to End of Test
D. Tests on pheasants: (1) Young birds -- continued												
Control	9		25	169	16	16	20	20				
	9a		25	169	16	20	24	28				
Control	10		25	155	12	16	20	20				
	10a		25	155	0	0	0	0				
	10b		25	155	4	12	16	20				
Control	11		25	141	0	4	40	40				
	11a		25	141	0	8	48	48				
Control	12		25	127	4	12	44	48				
	12a		25	127	8	48	52	52	70			
D. Tests on pheasants: (2) Adult birds												
Bayer 38920	*	1,000	23	42	17	74	--	100	23	14	322	588
	*	500	18	49	11	44	--	100	31	13	403	637
	7-7k	50	12	111	8	8	8	8	--	2	--	266
	8-8s	50	6	111	0	17	33	33	--	3	--	333
	8-8s	50	6	111	0	17	33	33	--	3	--	366
	8-8s	50	5	113	0	0	0	0	--	2	--	249
	7-7k	25	13	109	0	0	0	0	--	1	--	120
	8-8s	25	5	111	0	0	20	20	--	1	--	144
	8-8s	25	7	111	14	14	14	43	--	2	--	167
Chlordane	*	2,500	19	49	32	84	--	90	23	60	1,380	2,940
	7-7k	100	10	111	0	20	40	40	--	4	--	444
	8-8s	100	7	110	0	28	43	43	--	4	--	484
	7-7k	50	11	111	0	0	0	18	--	3	--	311
	8-8s	50	8	111	0	12	12	25	--	3	--	366
Dalapon	13-13k	5,000	10	24	10	90	--	90	18	142	2,556	3,408
DDT	*	500	18	56	6	28	--	78	42	14	588	784
	7-7k	300	17	67	35	41	--	100	31	10	310	670
	*	250	18	56	0	11	--	39	--	10	--	538
	7-7k	100	20	168	0	5	30	75	160	4	688	722
	7-7k	10	16	118	6	6	31	38	--	1	--	59
Diuron	13-13k	5,000	10	38	50	90	--	100	6	592	3,552	22,496

* No control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets. These tests were conducted to get maximum utilization of birds that would have been destroyed.

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) to 50% Mortality: Daily	to 50% Mortality	to End of Test
D. Tests on pheasants: (2) Adult birds -- continued												
Kepone	7-7k	100	12	109	8	8	25	33	--	5	--	556
	8-8s	100	7	111	0	0	14	14	--	6		611
	8-8s	50	6	111	0	0	0	0	--	3	--	333
	8-8s	25	6	111	0	0	17	17	--	2	--	189
Mirex	*	5,000	13	57	23	38	--	85	43	74	3,182	4,218
	*	5,000	9	49	67	67	--	89	4	93	372	4,557
	8-8s	500	5	113	0	0	0	0	--	3		339
	8-8s	500	4	113	0	0	0	0	--	21		2,373
	7-7k	500	16	109	19	38	50	56	85	25	2,125	2,725
	8-8s	200	6	113	0	0	17	17	--	10		1,107
	8-8s	200	6	113	0	17	33	33	--	18		2,034
	7-7k	200	11	111	9	18	18	18	--	8	--	910
	8-8s	200	5	113	0	20	20	20	--	7		746
Monuron	*	5,000	10	87	0	50	--	80	24	348	8,352	30,276
Rhothane (DDD, TDE)	7-7k	500	11	74	9	36	--	100	39	40	1,560	2,960
	7-7k	200	11	109	0	0	36	36	--	11	--	1,199
	8-8s	200	5	109	0	0	0	0	--	12	--	1,308
	8-8s	200	6	107	0	0	67	67	--	17	--	1,819
	7-7k	100	11	111	9	9	9	9		8		833
	8-8s	100	6	107	0	17	17	17	--	9	--	1,006
	8-8s	100	5	107	0	0	0	0	--	9	--	974
Thiodan		5,000	16	49	12	31	--	100	31	67	2,077	3,283
	7-7k	200	11	111	0	0	0	0	--	7	--	766
	8-8s	200	5	110	0	0	0	0	--	13	--	1,430
	8-8s	200	5	110	0	20	20	20	--	25	--	2,750
	7-7k	100	12	111	0	0	17	17	--	6	--	699
	8-8s	100	5	110	0	0	20	20	--	6	--	616
	8-8s	100	4	110	0	0	0	0	--	9	--	1,012
Zectran		5,000	19	42	16	79	--	100	20	87	1,740	3,654
	7-7k	500	10	109	10	30	40	40	--	29	--	3,161
	8-8s	500	6	111	0	17	33	33	--	40	--	4,440
	7-7k	100	9	109	0	0	11	11	--	4	--	469
	8-8s	100	6	111	0	0	17	17	--	5	--	511

* No control groups. Experience has shown that little or no mortality occurs among birds of this age group when fed normal diets. These tests were conducted to get maximum utilization of birds that would have been destroyed.

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	to End of Test
D. Tests on pheasants: (2) Adult birds -- continued												
Control	7		11	111	0	0	0	0				
	7a		11	111	0	0	0	0				
	7b		11	111	0	0	0	0				
	7c		11	111	0	0	18	18				
	7d		11	111	0	0	0	0				
	7e		11	111	0	9	9	9				
	7f		11	111	0	0	0	0				
	7g		12	111	0	8	17	17				
	7h		11	111	0	0	9	9				
	7i		11	111	0	0	0	0				
	7j		11	111	0	0	9	9				
	7k		11	111	0	0	0	0				
Control	8		6	112	0	0	0	0				
	8a		6	112	0	0	0	0				
	8b		6	112	0	0	0	0				
	8c		6	112	0	0	0	0				
	8d		6	112	0	0	0	0				
	8e		6	112	0	0	0	0				
	8f		6	112	0	0	0	0				
	8g		5	112	0	0	0	0				
	8h		5	112	0	0	0	20				
	8i		5	112	0	0	0	0				
	8j		5	112	0	0	0	0				
	8k		5	112	0	0	0	0				
	8L		5	112	0	0	0	0				
	8m		5	112	0	0	0	0				
	8n		6	112	0	0	0	0				
	8o		7	112	0	0	14	14				
	8p		6	113	0	0	0	0				
	8q		6	113	0	0	0	0				
	8r		6	113	0	0	0	0				
	8s		6	113	0	0	17	33				
Control	13		11	111	0	0	18	18				
	13a		11	111	0	0	9	9				
	13b		11	111	0	0	0	0				
	13c		11	111	9	9	9	18				
	13d		11	111	0	0	0	0				
	13e		11	111	0	0	18	18				
	13f		12	111	0	0	0	0				
	13g		11	111	0	0	9	9				
	13h		11	111	0	9	9	9				
	13i		11	111	0	0	18	18				
	13j		11	111	0	0	0	0				
	13k		11	111	0	0	0	0				

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	to End of Test
E. Tests on adult cowbirds:												
Dimethoate	39	100	12	30	75	92	--	92	5	18	90	540
Mirex	39	1,000	12	30	0	83	--	83	25	238	5,950	7,140
	39	500	12	30	0	8	--	42	--	132	--	3,960
Rhothane (DDD, TDE)	39	2,500	12	14	67	--	--	100	8	338	2,704	4,732
	39	1,000	11	19	36	--	--	100	14	195	2,730	3,705
	40	500	12	24	50	--	--	100	10	74	444	1,776
Thiodan	40	500	10	6	100	--	--	100	5	3	14	17
	40	200	10	12	90	--	--	100	6	10	60	120
Zectran	40	1,000	12	7	100	--	--	100	6	46	276	322
Control	39		11	30	0	0	--	0				
Control	40		12	24	0	--	--	0				
F. Tests on adult grackles:												
American Cyanamid #43913	41	100	12	30	0	42	--	42	--	22	--	660
	41	50	12	30	0	67	--	67	23	13	299	390
	41	25	12	30	0	42	--	42	--	5	--	124
	41	10	12	30	8	42	--	42	--	1	--	42
Control	41		12	30	0	17	--	17				
G. Tests on adult red-winged blackbirds												
American Cyanamid #43913	42	100	12	31	8	75	--	83	22	28	616	616
	42	50	12	31	42	42	--	42	--	12	--	372
	42	25	12	31	0	17	--	42	--	6	--	195
	42	10	12	31	25	33	--	33	--	2	--	74
Bayer 39007	43	100	12	30	0	33	--	33	--	20	--	600
	43	50	12	30	0	25	--	25	--	11	--	318
	43	25	12	30	0	25	--	25	--	6	--	171
	43	10	12	30	0	42	--	42	--	2	--	72
Control	42		12	31	8	25	--	25				
Control	43		12	30	0	8	--	8				

Table D-1. Toxicity tests of selected chemicals at Patuxent, 1963 -- continued

Chemical	Control Number	Amount in Diet (ppm)	Birds in Pen (number)	Length of Test Period (days)	Percentage Mortality at End of: 10 Days	30 Days	100 Days	End of Test	Time to 50% Mortality (days)	Toxicant Consumed (mg/kg) Daily	to 50% Mortality	to End of Test
H. Tests on adult herring gulls:												
DDT	44	500	10	22	20	--	--	100	13	23	299	506
	44	250	10	50	20	70	--	100	20	14	280	700
	45	160	4	39	25	75	--	100	14	7	101	281
	45	160	4	32	25	75	--	100	23	10	228	317
	45	10	4	99	25	25	--	50	90	1	55	60
	45	10	4	99	50	75	--	75	10	1	4	35
Control	44		10	50	10	20	--	20				
Control	45		4	99	0	0	--	75	62			

Table D-2. Mortality and reproduction of bobwhite confined on land treated with granular heptachlor at Patuxent.[1/]

Treatment (lb/acre)	No. pens	No. pairs	Percentage Cumulative Mortality at End of 10 days	20 days	30 days	50 days	100 days	No. hens producing chicks	Total chicks produced
Birds placed in pens within 1 week after treatment[2/]									
0	9	9	0	0	11	22	22	5	38
0.125	9	9	0	0	11	33	44	7	100
0.25	9	9	22	22	33	33	33	4	27
1.25	9	9	11	67	78	78	78	2	21
Birds placed in pens 2 or 4 weeks after treatment[2/]									
0.25	2	2	0	50	100	100	100	0	0
1.25	6	6	16	50	50	50	50	1	7
Birds placed in pens 6 or more weeks after treatment[2/]									
0	2	2	0	0	50	50	50	1	3
0.125	1	1	0	0	0	0	100	1	17
0.25	3	3	33	33	33	33	67	2	20
1.25	4	4	0	0	25	75	75	0	0
Birds placed in pens 9 to 12 months after treatment[3/]									
0	8	11	0	18	36	36	64	2	14
0.25	6	9	0	0	11	11	44	3	20
0.50	6	8	0	0	0	12	38	1	7
1.25	6	8	0	12	12	25	25	5	36
2.0	6	8	0	12	12	12	38	4	43

1/ Birds were kept in 20x50-foot pens, with one pair per pen. If one of the pair died, the other bird was killed and a new pair introduced. All birds introduced more than 1 week after treatment were replacements.

2/ Pens treated May 8, 1963.

3/ Pens treated May 2, 1962; pens listed as 0.50 lb/acre received 0.25 lb/acre May 2, 1962 and 0.25 lb/acre August 2, 1962.

Table D-3. Pesticide residues in field specimens analyzed at Patuxent during 1962 and 1963. Unless otherwise specified, the entire carcass, exclusive of skin, feathers, fur, feet, and gastrointestinal tract was analyzed. Minus indicates no detectable residues by methods employed. Averages and ranges are based on analyses showing residues. The letter (w) designates wet weight basis; all other analyses are on a dry weight basis. The letters (HE) indicate heptachlor epoxide.

Species	Toxicant	Minus	Plus	Average (ppm)	Range (ppm)
Birds					
Chickadee, Carolina	HE	0	1	17.3	-
Cuckoo, yellow-billed	HE	0	1	2.8	-
Hawk	HE	0	1	0.6(w)	-
Mockingbird	HE	0	1	5.9	-
Osprey (brain)	HE	1	0	0	-
Robin	HE	0	1	8.7	-
Snipe	HE	0	1	2.5	-
Sparrow, Bachman's	HE	0	1	5.5	-
Warbler, black & white	HE	0	1	Trace	-
Woodcock					
From the north	HE	1	92	3.9	Trace - 31.1
" " "	DDT	6	100	2.3	Trace - 17.9
" " south	HE	0	54	2.3	0.4 - 6.0
" " "	DDT	7	47	1.1	Trace - 7.7
Woodcock livers	HE	0	6	11.5(w)	8.6 - 15.4(w)
" "	DDT	1	5	6.7(w)	3.5 - 10.6(w)
" eggs	HE	12	2	0.4(w)	Trace - 0.8 (w)
" "	DDT	0	14	1.6(w)	0.4 - 4.9 (w)
" chicks	HE	0	1	Trace (w)	-
" "	DDT	0	1	1.5(w)	-
Wood pewee	HE	0	1	3.5	-
Vireo, red-eyed	HE	0	1	9.9	-
" white-eyed	HE	0	1	6.0	-
Eagles, golden					
Liver	DDT	0	1	2.5(w)	-
Muscle	DDT	0	1	2.8(w)	-

Table D-3. Pesticide residues in field specimens analyzed at Patuxent during 1962 and 1963 -- continued

Species	Toxicant	Minus	Plus	Average (ppm)	Range (ppm)
Eagles, bald 1/					
Liver	DDT	1	53	7.9(w)	Trace - 82.1(w)
Muscle	DDT	1	51	9.4(w)	0.2 - 68.1(w)
Pheasant					
Liver	DDT	0	6	6.7	2.0 - 19.5
Muscle	DDT	1	5	1.1	Trace - 3.5
Black duck					
Liver	DDT	0	1	21.5	-
Muscle	DDT	0	2	5.1	2.4 - 7.7
Red breasted merganser					
Liver	DDT	0	7	11.6(w)	3.9 - 17.2(w)
Heart	DDT	0	4	7.4(w)	4.0 - 11.7(w)
Swan	DDT	1	3	0.6(w)	0.3 - 0.8(w)
Fish					
Catfish	HE	0	1	2.3(w)	-
Killifish	DDT	0	1	1.9(w)	-
Gizzard shad	DDT	0	1	1.5(w)	
Shad	HE	0	2	4.8(w)	1.6 - 7.9(w)
Misc. fish	HE	0	1	0.7(w)	0.5 - 0.8(w)
Mammals					
Black bear					
Fat	DDT	0	1	1.4	
Peromyscus leucopus	DDT	1	16	3.7	0.9 - 11.9
Peromyscus maniculatus	DDT	1	29	2.8	0.6 - 9.3

1/ Combined results of analyses made in all years, not restricted to 1962-63.

WILDLIFE STUDIES BY THE COOPERATIVE WILDLIFE RESEARCH UNITS

by

Lee E. Yeager
Division of Wildlife Research
Bureau of Sport Fisheries and Wildlife

Cooperative Wildlife Research Units have been established at 18 Land Grant universities. Each Unit is supported by the participating university, the State game and fish department, the Wildlife Management Institute, and the Bureau of Sport Fisheries and Wildlife. The Units are centers for training as well as research, and nearly all of the studies serve as thesis projects for graduate students working under the direction of the Unit Leader or cooperating faculty members at the universities. Four Units conducted pesticide research in 1963: Alabama, Louisiana, Massachusetts, and Ohio.

AUBURN UNIVERSITY, ALABAMA

Field-pen tests of Mirex with bobwhite quail (Maurice F. Baker)

Mirex, a polychloro polycyclic hydrocarbon, is highly effective as a stomach-poison insecticide but has relatively low mammalian toxicity. It has been used as a bait for the imported fire ant and was commercially available in a formulation used in these tests: corncob grits, 85.000 percent; soybean oil, 14.025 percent; and Mirex, 0.075 percent. Previous field work indicated that this bait had no observable effects on wildlife, but further studies were needed to determine possible effects at higher rates of application.

In this study, 3 rates of bait application were used on different paired plots: 10 pounds per acre, the regular rate of application used in field control; 100 pounds per acre; and 1,000 pounds per acre. Two untreated plots served as experimental controls. Three pens, each containing 2 male and 2 female bobwhites, were placed on each plot. Water and shelter were provided and feed was placed on the ground as needed. All pens were moved weekly to a new site on the plot.

The first part of the test continued for 8 weeks, during which no effects attributable to Mirex were observed in the birds. One bird died of coccidiosis.

In a second phase of the study, the birds were wintered on the treated plots and eggs were collected and incubated during the breeding season. Fertility and hatchability of the eggs are given in table E-1. Some suggestion of adverse effects on reproduction was noted among the birds that were wintered on plots treated at the 100- and 1000-pound rates of application; however, differences are not statistically significant at the 5 percent level. The necessity of wintering the birds in pens no doubt contributed to the variability of data and low hatchability of the eggs.

Conclusions are that the regular field rate of use of Mirex bait will not affect quail; nor did the higher rates of application affect the caged birds. Significant evidence was not obtained to make any definite conclusion about the effects of higher rates on reproduction.

LOUISIANA STATE UNIVERSITY

The effects of aldrin-treated seed rice on wildlife (John D. Newsom)

The objectives of this study are to determine: (1) the effects of sub-lethal dosages of aldrin on egg production and fertility and on duckling survival in the fulvous tree duck, blue-winged teal, and mottled duck; (2) the occurrence of aldrin residues in wild ducks and rails and in their eggs; (3) the effect of aldrin seed treatment on crayfish production; and (4) the residual build-up of aldrin in the soil.

The project has not progressed to the point that data are reportable.

OHIO STATE UNIVERSITY

The effects of endrin on meadow vole reproduction (Donald B. Snyder, Tony J. Peterle, E. E. Good)

A study of the effects of endrin on vole reproduction utilized two 5-acre areas of bluegrass meadow in 1961 and 4 acres in 1962. Censusing of voles was performed in April of each year and population samples were retained for analyses of reproduction. Endrin was applied in May with a single application from a low-pressure tractor sprayer. Areas received water and 0.6 lb./acre of endrin in 1961, and water and 0.9, 1.3, and 2.0 lb./acre of endrin in 1962. Censusing was again performed 2 months after spraying, near the end of the breeding season. Voles were collected during each of the latter censusing periods and examined for reproductive capabilities and endrin residues.

Reproduction was investigated with reference to difference in susceptibility of sexes, difference in susceptibility of immatures and adults, body weight at time of breeding, number of females in different breeding conditions, ovulation rates, and prenatal losses. A bioassay technique involving guppies (Lebistes) was developed to estimate the quantity of residual endrin in the voles. Residues were obtained by steam distillation and a cleanup procedure preceded bioassay. Tissue fortified with 1.6 ppm yielded 10 percent recovery, and 21 voles which consumed from 5.4 to 126.0 mg/kg in the laboratory contained an estimated total of from 0.16 to 1.92 ppm in their tissues.

Endrin applied at 0.6 pound/acre caused no reduction in a meadow-vole population, while applications of 0.9 to 2.0 pounds/acre caused reductions of 71 to 95 percent. Reduction of numbers was significantly greater in the female than in the male population at 2.0 pound/acre, where there was an intermediate lowering in the overall population. Application of both 0.6 and 2.0 pound/acre caused a reduction in the number of litters produced 2 months after treatment. Other investigated aspects of reproduction were not significantly affected.

Bioassay indicated an absence of endrin in animals from the area sprayed with 0.6 pound/acre, while from zero to 0.73 ppm was detected in those from the area sprayed with 2.0 pound/acre. Endrin was detected in too few specimens to enable comparison of parts per million in tissue with reproductive capabilities.

The effect of endrin on the helminth parasites of the meadow vole
(Dorothy Adalis, John Crites)

This study was made on the specimens obtained for the project, "The Effects of Endrin on Vole (Microtus pennsylvanicus) Reproduction in Bluegrass Meadows." Study areas and methods are described under that study.

In bluegrass habitats, the meadow vole harbors certain helminth parasites of the Classes Nematoda, Cestoda, and Trematoda. All of these helminth with the exception of Syphacia obvelata, have an arthropod or mulluscan intermediate host. Some of the concentrations of endrin used in the study were high enough to control effectively the intermediate hosts. The accompanying table reveals that fewer parasites were found in voles of the endrin-treated plots than in voles of the control plots.

On the basis of the statistical analyses made, we conclude that endrin had an effect on the degree of parasitism in the voles taken from the treated plots. Reduction in the degree of parasitism was due to: (1) the lethal effect of endrin on the intermediate hosts of the parasites, which resulted in the prevention of new vole infections; and (2) the lethal effect of endrin in a 1.8 pounds/acre concentration, on the parasites in the alimentary canal of the voles.

The reduction in the number of parasites was not due to their seasonal variation because analysis indicates no interaction in the control plots due to seasons.

	Total Numbers	Total No. In Control Plots	Total No. In Endrin Plots
Q. quinqueserialis	120	120	0
E. thompsoni	5	3	2
S. obvelata	95	87	8
M. muris	73	68	5
A. macrocephala	20	17	3
P. troeschi	18	18	0

New tracer techniques for evaluating the effects of malathion on the ecology of a forest fauna (Robert H. Giles, Jr., Tony J. Peterle)

The distribution of malathion in a forested area of east-central Ohio was studied during the summers of 1962 and 1963. This broad-spectrum insecticide was selected for study because of its increased use in the control of many important forest insect pests. The use of a radioisotope to label the insecticide would provide a means of tracing the movements of the insecticide in the eco-system, of determining the effect of the insecticides on the fauna, and of learning details of the faunal ecology of the forest. The development of techniques for detecting the movement and location of a tagged insecticide would be required.

A sulphur isotope, S^{35}, was selected as the label because of its low beta energy (0.167 MeV) and its adequate half-life (87.1 d). Preliminary studies on one tenth-acre plot in the summer of 1961 provided potential application rates in terms of total radiation, and also allowed development of a sample-preparation technique. A faunal survey of two 20-acre watersheds was conducted during the summer of 1961. In May of 1962, one of the watersheds was treated with an application of 2 pounds of technical-grade malathion per acre in a formulation of xylene, triton X-155 emulsifier, and water. The malathion was synthesized with S^{35} by the Radiochemical Centre, Amersham, England. One c (curie) of activity was aerially applied to 1 of the 20-acre forested areas on 15 May and 25 May 1962. The specific activity of the synthesized malathion was 1 .5 mc/mM.

The distribution of components of the aerial spray within the forest was measured. Electrically-operated air samplers provided estimates of drift off the area; helium filled balloons bearing frosted-glass discs measured above-canopy application; bark samples and glass discs suspended vertically measured quantities settling out at different layers in the canopy; glass discs and spotting-enamel paper not only allowed a measure

of horizontal distribution but a check of a detection device for standard spray distribution. Soil samples and monitoring of marked stakes allowed sub-surface distribution studies.

Samples of insects, mammals, reptiles, birds, and of water from intermittent streams indicated initial and subsequent distribution of the insecticide and its metabolites in the ecosystem. Population studies of the faunal system continued throughout the summers of 1961-62 and a limited amount of survey data was collected in the summer of 1963. Preliminary results indicate that the insect populations returned to normal in about 3 weeks and that a detectable effect on the densities of the mammals on the sprayed area occurred.

Residue studies in the summer of 1963 indicated that the isotope S^{35}, originally applied as malathion S^{35}, had become equally distributed over both treated and untreated watersheds 1 year following application. There was no significant difference between the two watersheds in radioassay tests of insect, bird, and mammal tissues. The lack of difference in residual values suggests that drift from the aerial applications, or from translocation of the S-35 label by the fauna of the 2 areas, nullified any differences caused by the original application.

Predaceous insect families contained higher levels of radioactivity than phytophagous groups, but differences between the two were not significant. Families containing the highest residue values seemed to be those with the larval stages occurring in aquatic habitats. Nine of the 12 families which contained no evidence of S^{35} residue were largely phytophagous in their food habits.

Although bird specimens from the treated watershed had a higher level of activity than those from the untreated area, the difference was not significant. The level of activity in bird tissue tended to decline as the season progressed. The Baltimore oriole (I. galbula), a canopy feeder, had the highest level of radioactivity. Residue levels of other canopy feeding species taken in both 1962 and 1963 showed that the 1963 level of activity was approximately 16 percent of that recorded in postspray measurements in 1962.

Among the 60 mammal specimens assayed, females contained significant levels of activity more frequently than males. No difference was apparent in residue levels between specimens from the 2 watersheds. The shrew (B. brevicauda) had the highest level of activity among the mammals tested.

Measurable levels of radioactivity were found also in earthworms, crayfish, toads, and frogs.

The cycling of Cl^{36} labeled DDT in a marsh ecosystem (Robert L. Meeks, Tony J. Peterle)

There exists at present a great need for knowing the fate of the vast quantities of insecticides released into the natural environment. DDT has been used extensively as an insecticide for mosquito control on marshes in the past and will probably continue to be used in the future. Although much is not understood about its behavior in the environment, we do know that it is accumulative in action, retains its chemical identity in soils for years, is stored in large amounts by some organisms with no apparent ill effects, can affect vertebrate physiology, and may cycle in food chains with lethal results.

Five acres of a natural marsh will be sprayed with chlorine^{36}-labeled DDT at a rate currently used for mosquito control. This labeled DDT will be traced throughout the ecosystem for 2 growing seasons. The amounts accumulated at each trophic level, movement between levels, and rate of return to the abiotic portion of the environment will be determined.

UNIVERSITY OF MASSACHUSETTS

Measurement of the body burden of DDT in rufous-sided towhees from sprayed and unsprayed areas (Frederic W. Davis, William G. Sheldon, Frederick Greeley, Lawrence W. Bartlett)

In controlled tests over three seasons, aerial spraying of DDT over 500-acre experimental plots produced on-ground deposition rates which varied 15 to 1 between years and even more within years. These variations, resulting from errors in flight patterns, variation in droplet size, minor thermal air currents and electrostatic effects, were not correlated with density of the canopy. Spray not apparent on ground adjacent to the treated areas drifted at least to several miles. The disappearance of DDT residue from foliage was not correlated with rainfall.

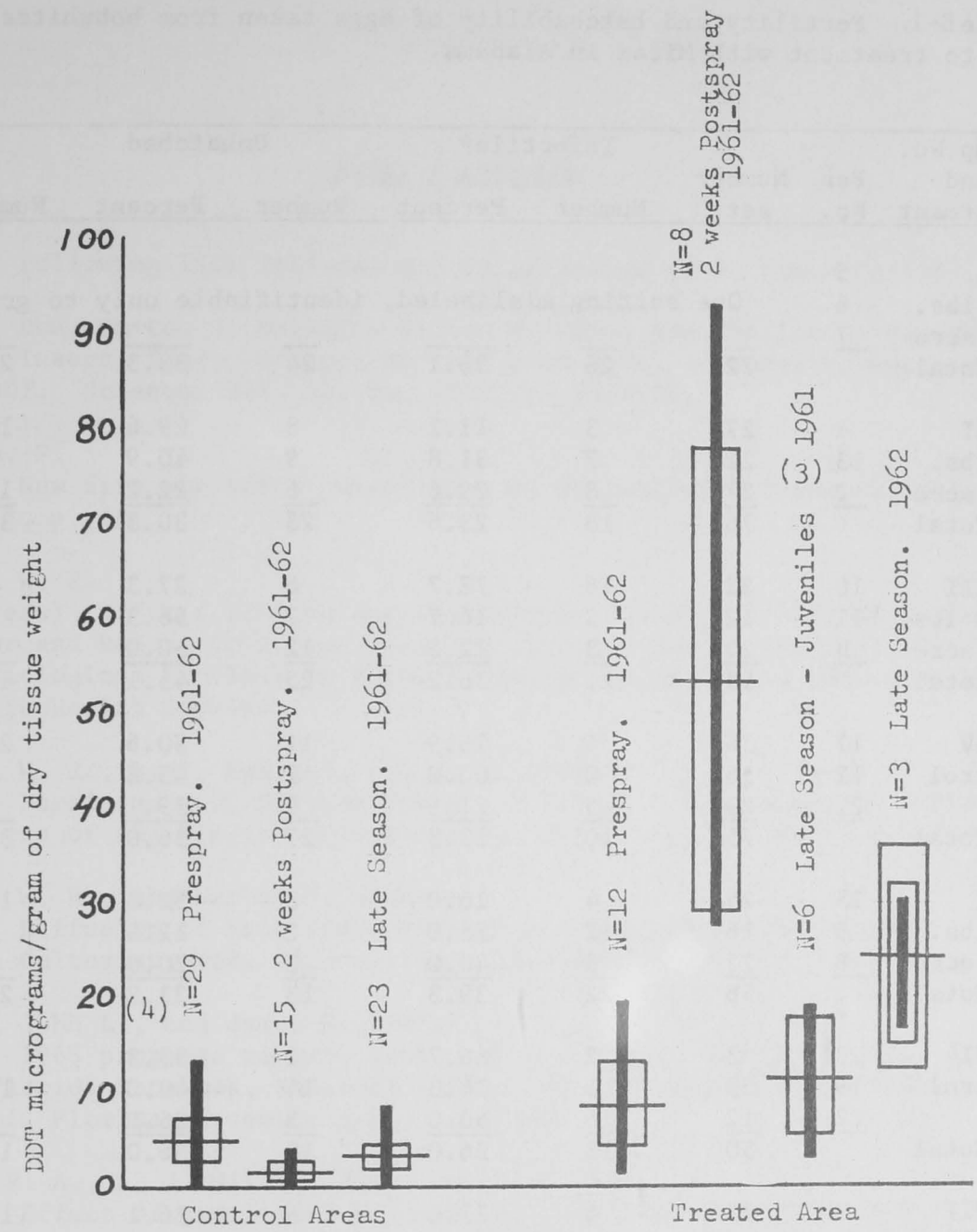
Insect larvae incapacitated or killed by DDT in 1962 contained an average of 28.4 mcg/g dry weight of DDT. They probably constituted the source of appreciable amounts of DDT accumulated by adult, nestling, and juvenile towhees, since pre-spray levels of DDT in towhees did not differ between control and experimental plots (Figure E-1).

Significant reduction of towhee survival of fledging size occurred in 1961, the year of heaviest on-ground DDT deposition. Foraging for food shifted from the tree canopy to the ground immediately after DDT application in all years.

There were no significant effects on sperm production, clutch size, hatching, survival to fledging in 1960 and 1962, adult mortality, emigration or immigration, population density, bird weights, or size of territories.

Sevin treatments reduced the number of towhees that foraged in trees immediately after treatment, and also shortened the period of arboreal foraging. This presumably is because the Sevin reduced the number of insects available to the birds for feeding. No other detectable effects were noted. Sevin treatment resulted in lower body tissue residues than did DDT under similar conditions.

Laboratory studies of captive towhees have been undertaken to compare residue levels in birds administered DDT at different dosages, then held for different lengths of time after dosage. Seasonal differences in susceptibility of DDT poisoning were suggested by the following facts: (1) No birds died from a group of 24 dosed November 25, 1962 at 500 mg/kg of technical DDT (in corn oil); (2) 10 of 16 birds died from a group dosed at 1000 mg/kg on March 29, 1963; (3) 4 of 16 birds died (and 4 others were observed in tremors) from a group dosed at 250 mg/kg on March 30, 1963; and (4) no birds died from DDT poisoning among three groups of 10 birds each, tested at levels of 250, 500, and 1000 mg/kg on November 26, 1963. Residue analyses of birds from these groups are partially completed.



(1) DDT combined.
(2) Birds were skinned, debeaked, delegged and eviscerated prior to analysis.
(3) All birds were adult or subadult except where noted.
(4) N=number of birds.

Legend: Vertical bar indicates range.
Horizontal line indicates mean.
Open box equals $\pm$ 3 SE.

Figure E-1. Residue levels(1) in carcasses(2) of towhees collected from the field.

Table E-1. Fertility and hatchability of eggs taken from bobwhites subjected to treatment with Mirex in Alabama.

Group No. and treatment	Pen No.	Number set	Infertile*		Unhatched		Hatched	
			Number	Percent	Number	Percent	Number	Percent
I	5							
100 lbs.	6	One setting mislabeled, identifiable only to group.						
per acre	7							
Total		72	26	36.1	24	33.3	22	30.5
II	4	27	3	11.1	8	29.6	16	59.2
10 lbs.	13	22	7	31.8	9	40.9	6	27.3
per acre	2	27	8	29.6	6	22.2	13	48.2
Total		76	18	23.6	23	30.3	35	46.0
III	10	22	16	72.7	6	27.3	0	00.0
1000 lbs.	11	12	2	16.7	7	58.3	3	25.0
per acre	B	24	3	12.5	12	50.0	9	37.5
Total		58	21	36.2	25	43.1	12	20.7
IV	17	36	5	13.9	11	30.6	20	55.5
Control	12	16	0	00.0	7	43.8	9	56.2
	21	23	5	21.9	9	39.1	9	39.1
Total		75	10	13.3	27	36.0	38	50.7
V	15	25	4	16.0	8	32.0	13	52.0
10 lbs.	9	16	12	75.0	2	12.5	2	12.5
per acre	B	15	6	40.0	3	20.0	6	40.0
Total		56	22	39.3	13	23.2	21	37.5
VI	23	3	2	66.7	1	33.3	0	00.0
Control	19	35	5	14.3	14	40.0	16	45.7
	22	12	6	50.0	3	25.0	3	25.0
Total		50	13	26.0	18	36.0	19	38.0
VII	3	23	4	17.4	6	26.1	13	56.5
100 lbs.	24	26	10	38.5	12	46.2	4	15.3
per acre	20	22	14	63.7	5	22.7	3	13.6
Total		71	28	39.4	23	32.4	20	28.2
VIII	14	23	6	26.1	6	26.1	11	47.8
1000 lbs.	1A	8	4	50.0	3	37.5	1	12.5
per acre	16	14	8	57.1	5	35.7	1	7.2
Total		45	18	40.0	14	31.1	13	28.9

* Eggs were candled after 5 days incubation to determine fertility.

PUBLICATIONS

The following list includes papers published or in press in 1963.

Allison, Don, Burton J. Kallman, Oliver B. Cope, and Charles C. Van Valin
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