THE EFFECTS OF PESTICIDES ON FISH AND WILDLIFE

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FISH AND WILDLIFE SERVICE

EFFECTS OF PESTICIDES ON FISH AND WILDLIFE

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INTRODUCTION

This is the fifth report on expanded studies of pesticides since the enactment of Public Law 85-582 (August 1, 1958), which authorized and directed the Secretary of the Interior to undertake comprehensive continuing studies on the effects of pesticides upon fish and wildlife resources. The preceding reports in this series are Fish and Wildlife Circulars 84, 143, 167, and 199.

The report, as the ones before it, summarizes pesticide research by the Bureau of Commercial Fisheries and the Bureau of Sport Fisheries and Wildlife. Most of the studies were carried out at the Biological Research Laboratory of the Bureau of Commercial Fisheries, located at Gulf Breeze, Florida; the Fish-Pesticide Research Laboratory and Denver Wildlife Research Center, both of which are located at the Federal Center in Denver, Colorado; and the Patuxent Wildlife Research Center at Laurel, Maryland. Fish phases of the work were under Dr. Philip A. Butler at Gulf Breeze and Dr. Oliver B. Cope at Denver. Bird and mammal investigations were carried out under Mr. Cecil S. Williams at Denver and Dr. Eugene H. Dustman at Laurel, Maryland.

Much of the earlier work was concerned with the field evaluation of pest control operations, improvements in analytical techniques, and the measurement of residue levels in fish and wildlife species collected from or near sprayed areas. The present report reflects an increasing effort to determine the physiological effects of pesticides, particularly those related to reproduction and survival under conditions encountered in the natural environment. The findings present additional evidence of the almost universal presence of chlorinated hydrocarbon insecticide residues in ecosystems. Biological magnification of these chemicals in the food chain and effects upon fish and wildlife are topics of high research priority.

Research workers who wish to use or quote statements or data should first communicate with the responsible investigators listed for the studies.

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.

WILDLIFE STUDIES
PATUXENT WILDLIFE RESEARCH CENTER

by

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The question of whether or not pesticides are instrumental in the population decline of bald eagles, ospreys, peregrines, pelicans, and other species is presently one of the more critical conservation problems. Research in 1964 therefore included both monitoring pesticide residues in important species and experiments to determine the effects of these residues on reproductive success and mortality. Field residues were sampled in bald and golden eagles, black duck eggs, osprey eggs, and Canada geese. Procedures were developed for experimentally evaluating both short-term and long-term effects of pesticides. Black ducks, mallards, pheasant, bobwhite, Coturnix quail, and cowbirds were placed on experimental diets. The sparrow hawk was selected as a test species to represent the predatory birds, and a colony was established. A protocol for making short-term tests to compare the toxicities of different chemicals was developed, tested, and prepared for recommendation to the chemical industry.

The problem of endangered species, however, is only part of a more general problem: the distribution of pesticide residues has become universal, and we know little of the effects of these residues. Research on particular species is one important approach. Research on whole ecosystems or selected parts of ecosystems, such as food chains, is a second. Such research requires both sampling (monitoring and surveillance) and ecological studies. In 1964, sampling included exploration of the use of waterfowl wings as a monitoring technique, sampling of residues in Antarctic animals, surveys of residues in animals found dead after pesticide treatments, and others. Ecological studies included the pesticide ecology of a portion of Lake Michigan, uptake of DDT by ducks and their foods in an Ohio marsh, the ecology of robins in Dutch elm treatment areas, and a survey of the effects of malathion used in cereal leaf beetle control. Experiments were undertaken on the effects of certain types of environmental stress on response to pesticides.

Special emphasis was given to tests of chemical methodology, and transition from colorimetric methods to gas and thin-layer chromatography was nearly complete by the end of the year.

Discussions included here are compiled from research in progress, therefore final tabulations may differ.

EFFECTS OF PESTICIDES ON BIRDS OF PREY

The peregrine falcon no longer breeds in the eastern United States. Bald eagles are scarce in the East and their reproductive sucess is exceedingly low. They produced young in only 3 of 16 nests studied in 1964 in the mid-Atlantic States. Nationwide, the percentage of young birds has declined gradually each year since 1961 (Sprunt and Ligas 1963). Ospreys are rare north of Chesapeake Bay and some of their colonies are reproducing poorly and dwindling rapidly (Ames and Mirsereau 1964). In Great Britain, numbers of predatory birds

have declined drastically, particularly the kestrel, sparrow hawk, and peregrine (Cramp et al. 1961). Reproductive success of the Scottish golden eagle has declined greatly in recent years (Lockie and Ratcliffe 1964).

Apparently a widespread decline in populations of birds at the top of the ecological food chain is underway. These birds live in environments that are subject to many changes, including increasing pollution of all kinds. Pesticides are the most recent addition to the roster. They now are universally distributed in living animals. Certain characteristics of these pesticides, such as DDT and dieldrin, have caused concern. Residues accumulate in animal tissues even at low dosages (Laug and Fitzhugh 1946). Such accumulations can become hazardous when food supply is reduced. They are transported across the placenta (Finnegan et al. 1949) and are incorporated into milk (Woodard et al. 1945). Minute quantities cause liver damage (Laug et al. 1950). Certain pesticides have reduced reproductive success in experimental studies of various birds and mammals. Field effects on reproduction have been demonstrated for lake trout in New York (Burdick et al. 1964) and pheasants in California (unpublished reports). Circumstantial evidence suggests reproductive effects of DDD on grebes (Hunt and Bischoff 1960) and of seed-dressing chemicals on hawks in Great Britain (Cramp et al. 1961).

It is essential to determine whether pesticides are in fact a strongly contributing force in population declines. Sufficient evidence on this is not obtained, however, simply by showing that there are pesticide residues in eggs or animals of species that are declining. One of our approaches is to measure residues in wild birds and their eggs, and to determine experimentally the effects of such residues on life and reproduction. Experimental studies are not possible for all species; hence, we try to work with closely related or ecologically similar forms.

Progress is reported here for our principal current studies, which concern eagles, ospreys, and sparrow hawks.

Ospreys in Connecticut and Maryland (L. F. Stickel, F. C. Schmid, W. L. Reichel, and Peter L. Ames, Cooperator)

Relationships between DDT residues in eggs and reproductive success of ospreys in the field were studied on the Connecticut and Potomac Rivers during the summers of 1963 and 1964. The Connecticut colony was chosen because it was known to be declining and to have very poor reproductive success. The Potomac colony was chosen for contrast, for it was known to be flourishing.

The plan was to take for analysis one egg from each of a number of nests in both colonies, and then study nest success in both areas to see if it was correlated with residues in eggs. The eggs were analysed for DDT residues at Patuxent.

Reproductive success in the two areas differed sharply, as shown below.

The percentage of egg-bearing nests that produced young was conspicuously lower in Connecticut although there was essentially the same number of eggs per nest. In both areas, however, once young were hatched, they ordinarily fledged successfully. Most losses occurred in the egg stage. Many eggs disappeared in both areas, but in Connecticut 9 eggs passed the incubation period without hatching, whereas

Area	Egg Production		Hatching Success		Fledging Success		Percentage
	Nests	Eggs	Nests	Hatchlings	Nests	Fledglings	Success
Potomac	26	78(51)*	16	27	15	24	58
Connecticut	15	44(29)*	2	4	2	4	13

* Numbers in parentheses show eggs remaining after sample eggs were removed.

only 3 remained unhatched in Potomac nests, although more nests were observed in the Potomac area.

DDT residues in the Connecticut eggs averaged higher than those in the Potomac eggs, although there was considerable overlap in the ranges. Comparisons below show parts per million wet weight (adjusted).

Parts per million wet weight of DDT +DDE+DDD	DDT Residues in Osprey Eggs	
	Connecticut Number of eggs	Maryland Number of eggs
1	0	2
2	0	15
3	2	8
4	4	6
5	0	3
6	5	5
7	4	1
8	1	1
9	2	0
10	2	0
11	1	0
Average	6.5	3.4

Adjusted values were used for these eggs because misinterpretations arise almost automatically when the customary wet-weight basis of expressing residues is used. This problem can be illustrated by comparing residues in Connecticut osprey eggs collected early in the season with residues in eggs collected after they failed to hatch (see table p. 6). The eggs that failed to hatch always contained higher parts per million of pesticide. Often the difference was great. It is natural to assume that the higher figures represented a greater pesticide content and accounted for the failure of the eggs to hatch. Some workers have drawn this very conclusion from such data. The trouble is that old eggs have lost much of their weight, especially their moisture, through drying and decay. Residues expressed as parts per million, usually mean one part of pesticide per million parts of the wet weight. If wet weight is down to half the original weight in an old egg, then the parts per million will be twice that of the fresh egg even though the amount of pesticide is unchanged. As an example, if there were 50 micrograms of pesticide in a 50-gram egg, this would be 1 part per million. If the egg dried to 25 grams, the same 50 micrograms would represent 2 parts per million. As eggs can lose most of their weight, this error can be enormous.

To correct for this, we adjusted egg weights to be as close as possible to the fresh wet weights. Contents of fresh eggs weighed an average of 0.8 gram per cubic centimeter. The volume of each egg had been recorded in cubic centimeters. Therefore the volume of each egg was multiplied by 0.8 to obtain a good estimate of the original wet weight of the egg's contents. This was the adjusted weight. The weight of the shell did not matter, for only contents are used in calculating pesticide content. The adjusted weight was used to calculate the pesticide content of old eggs. The table on p. 6 shows how much bias actually was produced by failure to allow for drying of over-age eggs.

The bias still appeared when residues were expressed as parts per million of fatty material (lipids) in the egg. Apparently some of the lipids also are lost in a decomposing egg. The bias would still appear if a dry weight basis were used, for lipids are part of the dry weight, and presumably lipids are not the only materials that could be lost through decomposition and escape of vapors. The use of a fat-free dry weight was not explored, for dry weights were not determined for these eggs.

This point is critical in all egg residue studies, and could cause erroneous conclusions.

The contrast in amount of residues in eggs from Connecticut and Maryland is clear. Obviously there is a difference in DDT contamination. However, massively greater residues (J. A. Keith, this report) were present in gull eggs from a colony where reproduction continued (although low) and in the eggs of pheasants (Genelly and Rudd 1956).

It is theoretically possible that the osprey is extremely sensitive to DDT residues, and could be affected by the amounts found. It is certain, however, that other causes--possibly other pesticides (only DDT was determined here) or other pollutants--should be considered, as should unrelated environmental factors.

Multiple chemical analyses will be made for eggs collected in 1964; the first three

Nest No.	Collected Early or Late (Past Hatching)	DDT Residues (DDE + DDT + DDD) Unadjusted ppm(wet weight)	Adjusted ppm(wet weight)
14	E	6.4	6.6
14	L	9.9	6.9
16	E	9.2	8.8
16	L	28.5	7.0
61	E	10.3	5.9
61	L	11.4	4.1
61	L	44.0	9.3
107	E	9.6	9.9
107	L	10.8	3.9
201	E	9.8	9.5
201	L	15.2	7.7
201	L	43.5	5.5

completed (from the Potomac area) all contained dieldrin residues.

Bald Eagles (L.F. Stickel, V.A. Adomaitis, G.E. Bagley, N.J. Chura, L.N. Locke, C.M. Menzie, R.M. Prouty, W.L. Reichel, and P.A. Stewart)

The dangerous situation of the bald eagle is the cause of much concern to both biologists and the public. The Bureau has undertaken the continuing responsibility of monitoring the pesticidal contamination of these birds. It has also undertaken experimental studies to assess the significance of specific quantities of pesticide in tissues and organs.

Field-Collected Eagles. Monitoring done thus far shows the amounts of residues present in various tissues of many eagles that were found dead. The residues, determined colorimetrically, consist primarily of DDE, a breakdown product of DDT. The nationwide distribution of the sample and the quantities of residue in muscle tissue are shown on p. 7 for all bald and golden eagles examined to date. It is apparent that some eagles were subjected to considerably more DDT exposure than others, but the differences were not associated with the collection localities. Measurements of storage in fat were made for the most recent specimens. Residues in fat were 13, 14, 19, 34, 81, 238, 260, and 363 ppm in eight bald eagles, and 23 ppm in one golden eagle.

It has become increasingly apparent that eagles are exposed to other chemicals than DDT. Identifying these compounds and the different DDT breakdown products is important to the interpretation of pesticide hazard. Hence eagles received during the past 6 months have been held while new methods that would provide identification of several compounds from a single sample were being placed in effect. Dieldrin residues were found in five of six specimens rerun for chemicals other than DDT; three also contained traces of heptachlor epoxide.

Sources of Field-Collected Eagles Analyzed for DDT

Area	Bald Eagles (No.)	Golden Eagles (No.)	Area	Bald Eagles (No.)	Golden Eagles (No.)
Alaska	8		Mont.	1	
Ark.	4		N.B.	1	
Calif.	1	1	N.J.	1	
Conn.	1		N.D.	1	
Fla.	4		Ohio	3	
Ill.	1	1	Ont.	1	
Iowa	7	1	S.D.	1	1
Maine	3		Tenn.	1	
Mich.	8	1	Vt.	1	
Minn.	7	1	Va.	1	
Mo.	0	1	W. Va.	1	
			Wis.	4	

DDT* Residues in Muscle Tissue of Field-Collected Eagles

Parts per Million wet weight	Bald Eagles (number)	Golden Eagles (number)
0	1	
trace	2	3
1-4	26	3
5-9	15	1
10-14	4	
15-19	4	
20-24	3	
25-29	1	
30-34	1	
35-39	1	
40-44	1	
68	1	
118	1	

* colorimetrically determined, primarily DDE

Experimental Studies. Analyses of eagles that died of DDT poisoning in experimental studies in Alaska were completed. The most significant result was that the brains of eagles that died of DDT poisoning contained very similar quantities of DDT despite differences in dosage levels and how long it took to die. Thus readings of brain levels are shown to be an important clue to DDT poisoning.

The table on the following page shows residues in brain, liver, and muscle of the five birds on the highest dosages.

DDT and Spermatogenesis. In an effort to determine whether DDT might interfere with reproduction of eagles by affecting the testes, a histological study was made of testes from all male eagles that died or were killed during experimental DDT treatments. Testes were histologically normal in three bald eagles fed for 60 days on a diet containing 10 ppm DDT; in two fed 10 ppm DDT for 120 days; in three fed 10 ppm for 60 days, then clean food for 60 days; and in one fed 160 ppm for 71 days. This latter bird died. Sperm were present in six of seven eagles that were killed or died in May, the peak of the eagle nesting season in Alaska.

Marked histological changes were found in the testis of one bald eagle fed a diet containing 4000 ppm DDT (wet weight), a level well above the minimum lethal level.

Reproductive failure can result from lack of motility of sperm or volume of sperm as well as from structural abnormality, and sections of preserved material would be unable to show these facts. Reproductive failure also can occur in many ways, of course, but the examinations reported here indicate that spermatogenesis itself probably is not affected by DDT ingested in sublethal amounts.

Sparrow hawks. (W. H. Stickel and F. C. Schmid)

Ospreys and eagles can hardly be used in extensive experiments with reproduction. Their relative, the sparrow hawk, is far more suitable in many ways. A colony of 42 pairs of these hawks has been established at Patuxent. If the first breeding season is successful, these birds will be used to test the hypothesis that then seems most pertinent to the problem of the eagle and osprey decline.

DDT Residues in Bald Eagles that Died of DDT Poisoning in Experimental Studies

DDT in diet (ppm)*	Days on dosage before death	DDT Residues**		
		Brain ppm	Muscle ppm	Liver ppm
800	62	63	73	280
800	59	80	291	--
4000	23	58	112	715
4000	15	85	149	--
4000	18	86	169	391

* Dry weight basis, on assumption of 70 percent moisture content in diet.
** Residues, primarily DDT and DDD, colorimetrically determined.

EFFECTS OF PESTICIDES ON WATERFOWL

The high recreational and esthetic position of waterfowl and the importance of waterfowl in the ecology of wetland areas make the welfare of these birds a primary concern. Wetlands have been subject to pesticide applications for many years, particularly in control of mosquitoes and other pest insects. Pesticides applied to forests and farms are washed into small ponds and potholes used as nesting and brooding areas, as well as into other inland and coastal waters. Many different chemicals reach the waterfowl environment. The mallard duck, therefore, was selected as one of the principal species in the chemical rating program, which is discussed later. Field and experimental studies to determine effects of pesticides on waterfowl and their foods have been initiated only recently and on a small scale. Some are summarized and others are listed to provide a record of work in progress.

Monitoring Pesticide Residues in Waterfowl (R. G. Heath, L. F. Stickel)

The dangers that residues in the body may offer to birds require experimental studies for interpretation. Also needed, however, is continuing knowledge of the quantities of pesticidal residues in wild waterfowl. We are now exploring the use of waterfowl wings (which are collected from hunters in great numbers for other purposes) to measure general contamination levels of the continental population. First, several samples of wings were analyzed to see if pesticides appeared in them in measurable quantities. All but one of twenty unselected wing samples of 14 waterfowl species collected in the fall of 1963 contained DDT residues, as shown on following page.

The next steps are to explore the practical operational problems, and to determine the degree of variation in levels and kinds of chemical contamination. This is being undertaken on a small scale. Samples of mallard and black duck wings of young and adult birds shot in Pennsylvania and New York will provide the data. Wings have been assembled on a random basis from the samples of the 1964 fall hunting.

Occurrence and Significance of Pesticide Residues in Black Duck Eggs (L. F. Stickel, W. L. Reichel, and C. E. Addy, Cooperator)

Declining reproductive success of black duck populations in the Atlantic Coastal region and the failure of the population to respond to restrictive hunting regulations resulted in initiation of an exploratory survey of residues in field-collected eggs. Eggs were obtained from cooperators in eight states from Maine to Maryland in the spring of 1963. DDT and its breakdown products were present in the eggs of all but one of 37 clutches.

Developments of chemical methodology, including procedures to permit determinations of

Pesticide Residues in Waterfowl (Sampled from wings submitted by hunters from various States)

Species	Number in Pool	DDT+DDE+DDD ppm wet weight	Analyses
			Muscle fragments only, gas chromatography
Mallard	2	0.14	"
Black duck	2	0.03	"
Widgeon	2	--	"
Green-winged teal	2	0.17	"
Shoveler	1	0.11	"
Pintail	2	tr.	"
Wood duck	2	2.74	"
Wood duck	2	0.17	"
Common goldeneye	1	0.08	"
Barrow's goldeneye	1	0.42	"
Bufflehead	1	2.28	"
Ruddy duck	2	0.59	"
			Muscle, bone and tissues of wing base, thinlayer chromatography
Pintail	4	3.90	"
Blue goose	1	0.20	"
Blue goose	1	0.80	"
Mallard	4	1.00	"
Black duck	3	2.20	"
Black brant	2	0.24	"
Black brant	3	0.15	"
Wood duck	4	0.30	"

dieldrin and other chemicals, as well as DDT, made it desirable to collect again in 1964. Eggs were submitted from more than 60 localities in 11 states and 2 Canadian provinces. Twenty-seven cooperators participated in the sampling. Results are not yet available.

Experimental studies to compare reproductive success with associated quantities of DDT in the eggs were initiated in December 1964. Adult black ducks obtained during the summer were started on diets containing $2\frac{1}{2}$, 10, and 40 parts per million of DDT in oil. Eighteen hens and six drakes were started in the experiment, with equal numbers on each dosage. These birds will be kept on pesticide diet through at least two laying seasons and additional birds soon are to be placed on dosage. Similar, but more extensive studies, are underway with the closely related mallard.

Pesticide Residues in Canada Geese of the Mississippi Flyway. (L. F. Stickel, R. G. Heath, J. L. Sincock)

Differences in reproductive success in Canada goose populations in the three segments of the Mississippi Flyway Population prompted a determination of residue levels among the groups to see if pesticides could logically be considered a part of the problem.

Samples were taken in late winter of 1964, after the full season's exposure to the foods and environment of the wintering grounds. Fifty-five adult hens were collected from the Mississippi Valley flock at Horseshoe Lake, Union County, and Crab Orchard National Wildlife Refuges in Illinois; sixty-four hens were taken from the TVA flock at the Wheeler National Wildlife Refuge in Alabama; and sixty-four from the Eastern Prairie flock at Swan Lake National Wildlife Refuge in Missouri. These samples were obtained through the cooperation of G. Fooks, R. G. Personius, T. Z. Atkeson, and R. H. Timmerman.

Samples of muscle, fat, liver, and ovaries are being analyzed for pesticidal residues.

DDT Accumulation by Waterfowl in an Ohio Marsh (T. J. Peterle and D. L. Dindal, Ohio State University, Cooperators)

DDT labeled with radioisotopic chlorine was distributed over a 4-acre marsh area on Sandusky Bay in the summer of 1964, with the purpose of following transport and accumulation of the compound in plants and animals. Dosage rate was 0.2 pound per acre, a rate commonly used in mosquito control. Scaup and mallards are being released on the marsh at stated intervals and for specified periods in an effort to establish a pattern of uptake and loss in relation to time. The labeled compound has been detected in water, aquatic vegetation, invertebrates, and the waterfowl.

Effects of Eurasian Watermilfoil Control Procedures on Waterfowl and other Organisms in Aquatic Environments (J. H. Steenis and personnel of Chesapeake Biological Laboratory of the University of Maryland Natural Resources Institute, and the Virginia Institute of Marine Science, Cooperators)

Control of Eurasian watermilfoil by 2,4-D and Diquat in tidal waters results in replacement by native aquatic plants, including the more desirable duck food plants. On the Susquehanna flats, where waters are nearly fresh, production of najas and wildcelery have proved a principal attraction for waterfowl. Similarly, parts of the Sassafras River and other areas have been improved for waterfowl.

The effects of 2,4-D treatments on shellfish, crabs, fish and associated marine life have been studied on certain experimentally treated areas without evidence of harm. However, analysis of data from these studies has not been completed.

The problem of the possible presence of 2,4-D residues in oysters and other aquatic organisms has been explored, but lack of good methods for residue determinations in animal tissues has hampered progress. Preliminary readings indicated 3-4 ppm of 2,4-D residues in oysters 3 days after a field treatment and less than 1 ppm in crabs, showing that the chemical is picked up from the water.

The rates and quantities of absorption under different conditions, and the rates of loss of residues in clean water, have not been determined and cannot be until methodology is developed.

PESTICIDES IN THE ENVIRONMENT

The universal distribution and exchange of pesticides among living animals, their foods, and the air, water, and soil are becoming increasingly apparent. It has long been difficult for experimentalists to locate uncontaminated material; chemical residue laboratories are seeking museum material from the 1930's or earlier in an attempt to obtain chemical blanks for comparisons. By 1963, fish in remote streams and those that spent their lives on the high seas proved to contain residues.

The widespread distribution of pesticides is well shown by the occurrence of residues in animals collected at large, without reference to particular pesticide treatments or programs. It is shown further by the fact that animals found dead following pesticide treatments with

one chemical, often prove to contain other chemicals as well. Effects of the multiple contamination have not been assessed.

Ecological studies of specific areas have shown residues in all parts of the environment, including mud, invertebrates, plants, fish, and birds. Recent data from both survey and ecological studies are summarized below.

Pesticides in the Lake Michigan ecosystem (J. J. Hickey and J. A. Keith, University of Wisconsin, Cooperators)

The exploration of residues in the Green Bay area of NW Lake Michigan, begun in 1963, was completed in 1964. The preliminary sampling of shallow-water sediments reported last year was followed up by analyses of nine deep-water (33-96 ft.) samples in 1964 on both the bay and lake sides of the Door County peninsula. These muds averaged 0.014 ± 0.005 ppm of DDT, DDE, and DDD on a wet-weight basis. Wet-weight levels of the same compounds in the crustacean (Pontoporeia affinis) ran 0.41 at a depth of 90-100 ft. in Ellison Bay, 0.44 in old-squaw ducks, and 0.54 in whitefish taken 5 miles off Bailey's Harbor in the lake.

Similar levels averaged 3.35 ppm in 13 alewife meals taken by (and away from) herring gulls, 4.2 in alewife fertilizer oil, 4.52 in 10 chubs analyzed as whole fish, and 5.60 in the muscle tissue of 5 whitefish. No correlation of these levels with age of the fish was evident.

Wet-weight residues of the three compounds in the brain, breast muscle, and body fat of three juvenile old-squaw ducks collected in midwinter averaged 0.74 ± 0.02, 2.03 ± 0.04, and 72.9 ± 8.8 ppm. For two adults these values were 1.67 ± 0.12, 6.33 ± 1.48, and 138.0 ± 11.6, respectively. Residue levels in two juvenile ring-billed gulls collected during the nesting season were quite similar to those of the young ducks: 0.72 ± 0.14, 3.6 ± 1.3, and 77.9 ± 24.7 ppm; but those in two adult ring-billed gulls were considerably higher: 7.1 ± 0.6, 28.0 ± 4.8, and 976 ± 179, respectively.

Twelve seemingly healthy adult herring gulls collected on their nesting islands had 20.8 ± 2.1 ppm of the three compounds in their brains, 98.8 ± 9.2 in their breast muscle, and 2,441 ± 334 in their body fat. Residues in six banded herring gulls ranging in age from 1.25 to 4.08 years were not stratified according to age. In this area, young herring gulls appear to attain the same general residue levels of the adults at least by the time the birds become yearlings.

This preliminary study leads to the conclusion that the biological concentration of pesticides previously reported for aquatic ecosystems of the size of Clear Lake (Hunt and Bischoff, 1960), Big Bear Lake (Hunt and Keith, 1962), and Tule Lake (Keith, Wilson, and Ise, 1963) is occurring in a similar fashion in the Green Bay area of Lake Michigan. In Pontoporeia, the concentration factor is about 50 times that of the residue level in mud, the increase being greatest in DDE. In fish, this factor is roughly 10 times that of the level of Pontoporeia. The birds sampled in this study spend at least part of their lives away from the Great Lakes, and their residue levels cannot at this time be solely ascribed to the dietary levels we found for them in the winter or summer parts of their annual cycle.

Door County, from which these residues are reported, has been using about 70,000 lbs. of DDT a year. While our results involve small samples that limit extrapolation to other parts of Lake Michigan, insecticide usage on lands bordering the NE side of the lake is probably lower than in Door County and as heavy or heavier on the E and SE sides of the lake where a large orchard industry is located.

Reproductive success in a DDT-contaminated population of herring gulls (J. A. Keith, University of Wisconsin, Cooperator)

In the 12 adult nesting herring gulls reported on by Hickey and Keith above, mean wet-weight residues in body fat averaged 390 ± 46 ppm of DDT, 1,925 ± 274 of DDE, and 126.4 ± 16.9 of DDD; 11 of these birds were collected on nesting islands in Green Bay in 1963; the 12th on the Lake Michigan side of the Door County peninsula in 1964.

Nine eggs taken from nine different nests on Sister's Island, Green Bay, early in the incubation period of 1964 averaged (wet weight) 19 ± 3 ppm of DDT, 202 ± 34 of DDE, and 6.0 ± 0.9 of DDD (total 226.8 ± 38.2. Nests at

this one colony were systematically checked from May 25 to July 17, 1964.

Three chicks found dead at the age of 1 week had (wet weight) residues of 295, 353, and 428 ppm of DDT, DDE, and DDD in their entire carcasses. Similar means for 5 healthy chicks at 35-42 days of age averaged 1.92 ± 0.74 in brain tissue, 7.0 ± 2.1 in breast muscle, and 180 ± 22 in body fat.

The reproductive success in these 114 nests was 0.41 young fledged per breeding pair--the lowest thus far found for this species. Similar statistics have been reported elsewhere (or recalculated here) as 0.54 in Denmark (Paludan, 1951), 0.7 in Germany (Drost et al., 1961), 0.91 in Canada (Paynter, 1949), and 0.9 and 1.1 in the Irish Sea (Darling, 1938).

Of the eggs calculated to have been laid, 29 percent disappeared and 38 percent failed to hatch. The disappearance rates of eggs that would and would not hatch were assumed to be the same. The 44 percent of eggs laid that hatched is well below 90 percent reported for Denmark (Paludan, 1951), 86 and 96 percent for the Irish Sea (Darling, 1938), and 68 percent recalculated for New Brunswick (Paynter, 1949). The hatching failure of 38 percent contrasts with published figures of 9.2 and 6.2 percent recalculated (Paynter, 1949; Paludan, 1951). The mortality in these eggs was spread rather evenly throughout the 27-day incubation period, and the high residues of DDT and its metabolites in these eggs appear to represent the causative factor for the low reproductive success encountered in 1964 in this area.

Kinetics of pesticide poisoning in Dutch elm disease control (L. B. Hunt, University of Wisconsin, Cooperator)

Based upon pesticide residues (DDT, DDE, DDD, and methoxychlor) in 133 samples of soil, earthworms, and birds from Wisconsin communities with known spray histories, the following relationships were noted: (1) Pesticides were concentrated in the top inch of 6-inch soil cores, but total residues in earthworms from the same soil were eight times greater than those in this surface layer. Soil residues from different sprayed areas showed poor correlation with elm size, elm density or reported spray history, but these residues averaged nearly 50 times those found in control area soils. (2) The three dominant earthworm groups sampled--adult Lumbricus terrestris, immature Lumbricus sp., and adult Allolobophora caliginosa--contained comparable total pesticides. The first species, however, contained a significantly lower percentage of DDT and a significantly higher DDE content, and the percentage of DDT declined from May to June following April spraying. (3) Residues in adult robins found on the sprayed Madison campus were far above those in two control areas, and slightly above those in experimentally poisoned birds. Residue differences in dead versus tremoring robins were not significant. (4) No methoxychlor was detected in any bird tissue tested. Adult robins exposed to both DDT and methoxychlor had significantly higher DDE and total residues in the brain and significantly higher DDE in the breast than those exposed only to DDT. In robins, the principal metabolite of methoxychlor may be DDE. Nesting robins on 61.2 acres of Madison campus jumped from three pairs to 29 pairs following the change to methoxychlor. (5) Female robins had significantly higher brain fat, brain residues, breast fat, and breast residues than males, and adult robins collected in May had significantly higher body weight, brain residues and breast residues than those found in April. Peak robin mortality occurred during late April, and a small sample of normal robins, weighed and banded, showed April weight losses following late March arrival. High fat content may allow greater residue levels to accumulate before mortality results, and it is suggested that April robins may be physiologically more susceptible to lower pesticide doses than those dying in May. (6) Breast residues in young robins were approximately one-third those in adults, and death could not be definitely attributed to pesticide poisoning. Nesting success was comparable to that found in pre-spray studies. (7) Adult golden-crowned kinglets contained the highest breast residues of all birds tested, and one group died a year after elms were last sprayed. (8) In the DDT-sprayed elm environment, total pesticide residues (dry weight) accumulated from 9.9 ppm in soil

to 140.6 ppm in earthworms to 443.9 ppm in adult robin brains, but the proportion of DDT in these totals declined from 74.7 to 70.9 to 6.5 percent as DDT was converted to its metabolites.

Bird mortality following DDT spray for Dutch elm disease (C. F. Wurster, Jr., D. H. Wurster, and W. N. Strickland, Dartmouth College and Dartmouth Medical School, Hanover, New Hampshire, Cooperators*)

Population studies of robins and other birds were made in 1963 in Hanover, New Hampshire, where 2,300 elms occurring on 670 acres were sprayed with DDT for Dutch elm disease control. Concurrently, dead and dying birds were collected for residue analysis. Similar studies were conducted in nearby Norwich, Vermont, where no spraying was done. By June 1, the Hanover robin population had fallen 70 percent below the original May 1 population, but the population in Norwich remained unchanged. Chickadees, nuthatches, creepers, and woodpeckers declined similarly. During 1963, 151 specimens of 34 species were found dead or tremoring in Hanover. Residue analyses of tissues and organs indicated that concentration in brain is a useful criterion in judging cause of death, in line with the conclusions of Bernard (1963). When methoxychlor replaced DDT in 1964, field studies showed that mortality was considerably reduced.

*Residue analyses provided by Bureau.

DDT in loons (L. N. Locke and G. E. Bagley)

Approximately 3,000 loons died on the beaches of Lake Michigan and other Great Lakes

DDT residues in loons found dead

Specimen number	DDT residues in parts per million wet weight*		
	Brain	Liver	Muscle
18888	trace	0.3	2.2
18889	not analyzed	0.3	2.3
18890	not analyzed	2.7	2.5
18891	not analyzed	2.7	2.8
18892	not analyzed	0.9	0.7
18909	3.3	2.2	1.5
18910	4.9	2.4	2.5
18911	6.7	2.3	3.0
18912	3.5	7.6	6.4
18913	2.8	4.2	4.5
18914	trace	3.8	2.8

*Determinations by thin-layer chromatography. These residues are DDE, a breakdown product of DDT. Traces of DDD also were detected in six of the loons. Samples were heated at 85° C for 4 hours to destroy botulinus toxin before analysis. Tests on other tissues indicated that similar determinations of residues were obtained whether material was cooked or uncooked.

in 1963 and possibly an even greater number in 1964. Examination of specimens for botulinus toxin was made by the Bureau and by the Michigan Conservation Department. Type E botulism was confirmed in nearly all specimens examined. Experimental verifications are not possible with loons, since their care in captivity is not understood. Attempts at verification have been largely unsuccessful with gulls, also involved in the die-off. Various experimental complications, however, make it unsuitable to draw conclusions; botulism must still be considered suspect and further studies made.

Loon tissues were analyzed chemically to determine whether pesticide effects also should be considered. The residue levels shown in the table are far below the amounts that would be considered fatal on the basis of studies with other species of birds. Nothing is known about the effects of pesticides in combination with other pollutants or with disease, but it seems very unlikely that pesticides contributed to the death of the loons.

DDT in the Antarctic (C. M. Menzie, W. L. Reichel, and W. J. L. Sladen, Cooperator)

Six Adelie penguins and a crabeater seal collected in the Antarctic in February 1964 contained DDT and its metabolites in amounts from 1.3 to 152 parts per billion (wet weight) in fat and liver. Both species spend their entire lives in the Antarctic and feed on nonmigrating crustaceans and fish. Quantities determined by electron capture gas chromatography are shown below. Identification was confirmed by thin-layer chromatography with reconfirmation by gas from material removed from the thin-layer plates. Identifications also

Specimen Number	Species	Tissue	DDT residues (parts per billion, wet weight)
6151	Penguin	Liver	36
6160	Penguin	Liver	16
6160	Penguin	Fat	45
6161	Penguin	Liver	25
6161	Penguin	Fat	24
6162	Penguin	Liver	24
6162	Penguin	Fat	87
6163	Penguin	Liver	115
6163	Penguin	Fat	152
6169	Penguin	Liver	20
6164	Seal	Blubber	39
6164	Seal	Liver	13

were confirmed in portions of the same samples sent to the Wisconsin Alumni Research Foundation.

Dieldrin, DDT, and heptachlor epoxide in birds found dead in an area treated with dieldrin

The extent of multiple contamination is shown in a series of birds found dead in an urban area treated with dieldrin. Homeowners and others use various chemicals in their yards and gardens, so the number of chemicals shown in the residue analyses is not surprising. The question of interpretation is an open one, however, for experimental studies have been devoted largely to single chemicals.

Pesticide residues in birds found dead in Virginia following dieldrin treatments at 2 pounds per acre for white-fringed beetle control

Parts per million, whole bodies, wet weight (gas chromatography)

Species	Dieldrin	DDT and metabolites	Heptachlor epoxide
Cardinal (pool of 3 birds)	1.8	1.2	nd
Dove, mourning	1.4	0.6	nd
Egret, common	3.3	10.0	nd
Grackles (pool of 5)	0.9	0.8	0.6
Grackles (pool of 6)	7.8	4.3	nd
Grackles (pool of 6)	3.0	3.2	0.4
Grackles (pool of 6)	7.8	8.7	0.6
Grackle	1.8	1.6	0.1
Grackle	0.9	3.6	0.4
Grackle	2.9	2.7	0.4
Grackle	1.2	1.5	0.2
Heron, great blue	2.8	8.9	nd
Mockingbird (pool of 2)	2.5	0.7	nd
Mockingbird	0.5	0.6	nd
Quail, bobwhite	0.1	0.4	0.1
Quail, bobwhite	2.6	nd	0.3
Robins (pool of 6)	5.6	6.0	nd
Robins (pool of 6)	6.9	6.6	0.3
Robins (pool of 6)	10.2	11.9	nd
Robin	6.9	2.0	0.3
Robin	4.2	3.9	0.2
Skimmer, black	7.5	2.0	nd
Sparrow, house (pool of 2)	1.3	0.2	nd
Starling (pool of 6)	11.0	2.6	0.2
Starling (pool of 6)	6.0	2.5	0.6
Starling (pool of 6)	5.4	2.6	0.3
Starling (pool of 5)	1.4	0.7	0.7
Starling	4.1	2.3	0.4
Thrasher, brown (pool of 3)	4.3	3.8	0.9
Thrasher, brown	1.0	1.6	nd
Towhee (pool of 2)	0.5	1.0	nd
Waxwing, cedar (pool of 5)	1.6	3.2	nd

nd indicates none detected.

DDT in an industrially contaminated area

Wildlife habitats sometimes encompass rivers or streams that receive waste effluents from pesticide manufacturing plants. Land animals collected from one such area in the Southeast were analyzed for DDT residues, with results shown below.

Field survey of large-scale malathion application (C. T. Black and G. L. Zorb, Michigan Department of Conservation, Cooperators)

Extensive field work in 1963 revealed no harm to vertebrates from aerial application of 1 pound per acre of malathion in aqueous spray. In 1964, applications consisted of undiluted technical-grade malathion at rates of 0.64 and 0.43 pounds per acre. Some areas received two applications. One to 3 days after spraying, searches for dead animals were made along 47 miles of good wildlife edge habitat and along 43.5 miles of similar unsprayed habitat. No dead animals were found.

Birds were counted along the same search lines. Counts remained as high in treated areas as in untreated areas.

DDT Residues in Wild Animals Living in an Industrially Contaminated Environment

Species	DDT residues[1] parts per million, wet weight	
	Muscle	Fat
Crow	21.7	873
Crow*	51.4	770
Crow*	52.9	1,603
Crow*	119.3	774
Crow	24.5	616
Crow*	6.9	510
Swamp rabbit	0.5	3
Swamp rabbit	0.7	not analyzed
Swamp rabbit	0.6	14
Cottontail rabbit	1.6	50
Cottontail rabbit	0.5	16
Cottontail rabbit	trace	7
Gray fox	27.4	50

1/Residues in specimens marked with an asterisk (*) were primarily DDT. Residues in all others were primarily DDE. Colorimetric methods.

Young pheasants survived in an area treated twice in the same season with malathion, but the sample was small. It was not determined whether other young pheasants were affected by the spray directly or by reduction of food supply, but there was no concrete reason to believe that such effects occurred.

Bluebird nests were studied on treated and untreated areas. In untreated areas, 26 nests succeeded and 8 failed. In treated areas, 11 succeeded and 2 failed. Thus, bluebird reproduction was as successful on treated as untreated land.

MEANING OF PESTICIDE RESIDUES IN ANIMALS

Delayed mortality of DDT-dosed cowbirds in relation to disturbance (W. H. Stickel)

A long-term study of DDT kinetics in cowbirds included one group of approximately 50 birds that received diets containing 40 parts per million DDT in oil for eight weeks, from December 19, 1963, to February 13, 1964. During this time two birds died (January 14 and January 16). On February 13, to obtain a random sample needed for chemical analyses, two persons entered the cage and caught all birds in mist nets; birds were placed in individual compartment cages and were released after about 40 minutes. That night a bird was observed in tremors and was dead the next day. The remaining birds were fed only clean food thereafter. They were caught again on February 20: one was dead on February 21 and another February 24. Next disturbance was February 27: three birds were dead next day. Next disturbance was March 12, and a bird died in tremors that afternoon. Examination showed all in a condition typical of DDT deaths in which fat is essentially gone from all obvious storage sites, yet there is no muscular emaciation; gall bladders are full.

Thus, mortality occurred 1, 8, 11, 15 (3 birds), and 28 days after removal of toxic food and six of the seven deaths followed unusual disturbances.

Augmentation of a pesticide effect by disturbance is reasonable in the light of other evidence concerning DDT response. In acute oral dosage studies, response of rats has been delayed or diminished by protecting the animals from environmental disturbance and effects have been increased by such disturbance. (Deichmann et al. 1950).

Death related to DDT Residues in the Brain (L. F. Stickel and W. H. Stickel)

Probabilities that deaths of birds in the field are due to pesticides are difficult to establish because neither time of exposure nor dosage is known. To be diagnostic of death, residue levels must be similar regardless of time or dosage level. These criteria have been met by brain residues found in experimental studies of sparrows (Bernard 1963), of rats (Dale et al. 1962, 1963) and of bald eagles (discussed elsewhere in this report). Correlative evidence has been obtained from robins collected tremoring or dead after DDT treatments for Dutch elm disease.

Residue levels were determined in the brains and certain other tissues of 11 cowbirds that died during a period of 12 days on a diet of 500 parts per million DDT, in 3 that were removed from toxic diet after 8 days but died after 2, 9, and 40 days on clean diet, and in 3 that survived 8 days of toxic diet plus 112 days of clean diet and were killed. Only brain residues will be discussed here.

DDT and DDD in the brains of dead birds showed no time-related trends, being similar in birds that died on dosage for the various lengths of time and in those that died after long periods on clean food. DDT plus DDD averaged 66±6 ppm wet weight, with a range of 35–99 ppm. DDE, however, appeared not to be critical in this series, for more was present in survivors than in those that died.

Quantities of DDT residues were remarkably similar to those reported for the other species listed above, when expressed comparably and with allowances for differences in chemical methodology. Thus it appears that similar brain levels of DDT+DDD are diagnostic of DDT-

induced mortality over a wide range of species, mammals as well as birds.

Effects of DDT on Coturnix Quail under Stress (N. J. Chura and L. F. Stickel)

Wildlife populations are subject to many forms of stress that may affect their vulnerability to pesticides, including stress due to disturbance, disease, and extremes of weather. Birds migrating to northern breeding grounds may encounter both severe weather and reduced food supply. When they arrive in the north and begin to establish breeding territories, they are further subject to both social and physiological stresses. Egg laying represents a heavy drain on a bird's supply of fat and protein.

An experimental study is under way to measure the effects of reproductive and nutritional stress on pesticide-induced mortality. Coturnix quail of a single age are being reared to adult size under two lighting regimes, one of which permits normal development (day lengths similar to summer) and the other (day lengths similar to winter) which prevents reproduction. When the birds are of adult size and suitable age, part of each group will receive reduced rations to produce weight loss and others will continue on normal diets. Dosages of DDT will then be incorporated in the diets. Mortality in the different groups will be measured to see if there are differences. Thus the effects of reproduction and reduced food supply on pesticide mortality will be tested both separately and in combination.

Extensive preliminary trials were necessary to properly establish the lighting cycle needed to inhibit reproduction, to gauge food consumption to weight loss, and to solve practical problems. Lighting trials with 24 pairs of birds showed that with 14 hours' daylight initiated at 50 days' age, most hens laid eggs at 66-68 days. With 8 hours of daylight, there was little gonadal development and only one hen produced eggs. Reduction of diet to about 25 percent of normal produced weight losses of about 20 percent in 5 days. Dosage trials resulted in survival of some birds at levels as high as 1600 parts per million for 10 days.

TOXICOLOGICAL TESTING

Pen Studies of Avian Toxicity at Patuxent (Robert G. Heath, James W. Spann, Clyde Vance)

The most perfectly designed laboratory tests of toxicity can be expected to yield only a partial evaluation of the total effect of pesticides on wildlife populations. However, laboratory tests can show whether or not a chemical is detrimental to penned animals when administered at levels expected in the field. They can also produce measurements of the relative toxicity of one chemical to another.

The question of "best experimental design" is invariably complex and at times debatable. Often the research worker must choose between several approaches, none perfect, and hopes one will furnish the most complete answers in a practicable manner. Under these conditions, then, we have developed the following protocol for testing the acute and relative toxicities of pesticides and their effects on reproduction of penned birds.

Protocol for Testing the Acute and Relative Toxicity of Pesticides to Penned Birds (R. G. Heath and L. F. Stickel)

The primary purpose in conducting acute toxicity tests is to provide, for a particular species of animal, a quantitative rating of the toxicity of pesticidal chemicals in relationship to each other. The tests also provide a measurement of the amount of chemical needed to produce a prescribed level of mortality under the conditions of the experiment. Tests are conducted on birds of several species, since there may be great differences in susceptibility from one species to another. Our tests employ the mallard duck, bobwhite quail, and ring-necked pheasant. (Coturnix is presently being incorporated in the testing schedule.)

Chemical is introduced as a component of the diet, because ingestion is believed to be the principal source of exposure in the wild. Moreover, injection could yield atypical results, and force-feeding of encapsulated pesticide has been unsatisfactory. Dermal or inhalation toxicity may be important in certain instances but will need attention as specific instances arise.

The basic plan utilizes the probit methods of Finney (1952. Probit Analysis. Cambridge University Press) that have been generally adopted for toxicological tests. These procedures provide statistical confidence limits that show the reliability of the determinations and permit comparisons of toxicity of one chemical with another. We have introduced the use of DDT as a tentative standard, in addition to untreated "controls," in order that results obtained in different times or at different laboratories can be compared with each other "within experiments" as defined by Finney (1952).

Toxicant is administered as a dietary component at six dosage levels, geometrically spaced over the range that is judged likely to produce mortality from 20 to 80 percent. However, levels producing 0 and 100 percent mortality may be useable. Some range-finding tests ordinarily are necessary to gauge the dosage span.

The pesticide is dissolved in corn oil (or, as necessary, propylene glycol), then mixed thoroughly with the feed. The amount of solvent added to the feed is the same for all chemicals and dosage levels; an equal amount of clean solvent is added to the diet of the controls. We have found that 20 grams of oil per kilogram of feed is sufficient to permit dissolving the maximum necessary amounts of all chlorinated hydrocarbons tested to date. Other solvents (e. g., propylene glycol) will replace corn oil only when the latter is unsuitable.

Test birds should be obtained from stock that has been reared and maintained on a standard game bird ration. Food and water should be available continuously. Thermostatically controlled heating units are needed in cages used for young birds.

Ideally, a minimum group of 10 birds is tested at each dosage level (although we have used as few as 6 birds in an emergency). Birds are placed on toxic diet between the 5th and 7th day of life to avoid excessive interference of chemical intake by yolk sac absorption. Birds are statistically randomized into the treatment groups, then kept with their associates and provided with clean food for at least 2 days before being put on toxic diet.

The test period consists of 5 days' feeding on toxic diet plus 3 days' feeding on clean diet. We wanted time on toxic diet to be as short as feasible and still produce the necessary percentage kills. The object was to approach the single dose as nearly as possible, within the limits imposed by dietary introduction of toxicant, and thus to mitigate problems produced by differences in storage and excretion rates of different chemicals. The 3-day observation period following the dosage period was used to help avoid bias due to overestimating the required lethal dosage by measuring mortality before a dosage had full time to act. (This phenomenon is sometimes referred to as "overkill.") Longer periods of posttreatment observation were tried but found unnecessary. The short test period also reduces the likelihood of mortality among the controls, a factor tending to weaken the precision of results.

Results are expressed as LC_{50}, here defined as the concentration of pesticide, measured in parts per million in the diet, computed to produce 50 percent mortality at the end of the test period. The percentage mortality at each dosage level is based on the number of birds dead at the end of the 8-day period. These percentages are used to compute the LC_{50} by the methods of Finney, cited above.

So defined, the LC_{50} is designed to "hit a target," and it is essential that the length of the dosage and observation period be constant. The LC_{50}, determined by standardized methods, permits quantitative comparisons of relative toxicities of different toxicants under the prescribed test conditions.

The LC_{50} for each chemical is accompanied by an LC_{50} for the "standard" (tentatively

DDT) obtained concurrently, and by a determination (when necessary) of percentage mortality among untreated controls, for which data also are obtained concurrently. If mortality occurs among the control groups, mortality levels among the treatment groups must be adjusted accordingly (see Finney, 1952). The LC_{50} for DDT is obtained in the same manner as for the other chemicals except that it may be desirable to use more dosage levels. The importance of the DDT standard in interpretation of the other results has led us to use eight dosage levels rather than six as extra insurance that its LC_{50} can be computed with adequate precision. Failure to obtain an estimate for any one chemical in a set is much less serious, for only a single repetition is needed in a subsequent test, whereas poor data from the DDT standard may reduce the usefulness of the results of the entire set.

In comparing the toxicities derived in different tests, the use of some predetermined toxicant as a "standard" becomes virtually imperative. By using a standard with each set of toxicants tested, effects of differences in experimental conditions among tests conducted at different times and/or locations can be largely accounted for and results adjusted accordingly. Otherwise such differences go undetected and can result in biased conclusions. (DDT is currently proposed as the standard because it has been so widely used and studied, although current findings suggest that for the species under test another chemical such as dieldrin might give less variable results.)

The untreated controls include at least three, and preferably six, sets of 10 birds each. Thus one set of DDT standards and one set of controls serve for comparison with several chemicals, provided that all tests are run at the same time and location, that birds are distributed at random among all groups, and that treatments are randomly assigned to the pens.

It is not considered essential that an LC_{50} always be obtained, for some chemicals will not be ingested in amounts large enough to kill. In these cases the important fact is that birds do not die at doses well above those to which they reasonably can be expected to be exposed. We have set a ceiling at 5000 ppm for the chemicals currently under test at Patuxent, but other chemicals may require modification.

A rough measure of ingestion of toxicant can be obtained by weighing amounts of toxic food supplied, amounts remaining, and estimating spillage. Birds can be weighed as a cage group at the time of randomization, and intake of toxicant expressed roughly as mg/kg/day (allowing for deaths of birds during the dosage period). However, we believe an LC_{50} expressed in terms of ppm toxicant in the diet provides the more useful measurement of toxicity (see "rationale" below). Estimates of intake of food and of toxicant may be useful in interpreting anomalous test results. For example, some chemicals may be repellent and tend to reduce food intake at higher dosages, and others perhaps could stimulate food intake.

Rationale of Acute Toxicity Testing

The LC_{50}, derived as prescribed, pinpoints within statistical limits both the defined lethal dosage of a chemical and its relative toxicity in comparison with other chemicals under the test conditions. Such comparisons could at best be only loosely approximated if a less rigorous protocol were employed, and very possibly statistical confidence limits of such estimates could not be computed.

In comparing the toxicities of pesticides, the necessity of using some predetermined toxicant as a standard has been discussed. The standard allows differences in experimental conditions among tests conducted at different times and/or locations to be largely accounted for, so that comparisons can be adjusted accordingly. Otherwise such differences go undetected, and biased comparisons result.

The decision to express lethality in terms of parts per million (ppm) of toxicant in the diet instead of milligrams of toxicant consumed per kilogram of body weight (mg/kg) was derived only after considerable thought. Neither expression is perfect. Were the toxicant administered instantaneously (i.e., by injection or encapsulation), the expression mg/kg would be appropriate; but injection could yield atypical results, and instantaneous dosage by force-feeding of encapsulated pesticide has proved

unsatisfactory. Moreover, oral intake is undoubtedly the most common in the wild. Thus it seems more plausible to deal not with an instantaneous dosage but with one administered through the diet over a restricted period of time.

We have considered the danger in expressing toxicity in terms of ppm in the diet should some toxicants, especially at higher dosages, act as repellents and reduce the intake of food. Or some might for a time stimulate food consumption. In such cases pesticide intake would not be directly proportional to ppm in the diet. Even so, if a toxicant has repellent or attractant properties, we would expect birds in the field to react accordingly; and we believe that this factor should be considered in measuring the toxicity of a chemical at a given dietary level, especially if we wish to associate laboratory results with field conditions.

Expressing toxicity in terms of mg/kg when the dosage is continuous instead of instantaneous can be quite misleading. Birds on a high dietary level of toxicant may die rapidly and in so doing consume fewer total milligrams of chemical than birds surviving for some time on a lower level. The implication, if length of time on toxicant is not considered, would be that fewer mg/kg will kill a higher portion of the birds; i.e., that lower dosages are more toxic than higher dosages.

If length of time on toxicant is considered, an expression of "mg/kg/day" (average milligrams of toxicant consumed per kilogram of body weight per day of feeding) can be derived. This could be a useful approximation in short-term tests, but is soon confounded by "excretion" of toxicant (which proceeds continuously, and not at the same rate for all chemicals) and may be misleading if the dosage period is very long. Furthermore, an accurate measurement of mg/kg/day is not easily obtained. It requires that (1) every bird be banded; (2) individual bird weights be recorded before, during, and at the completion of the test, as well as at death, so that individual daily weights can be estimated; and (3) total food consumption be measured accurately, which requires an estimate of food spillage. Thus the estimated mg/kg/day is costly and time consuming to compute and is of unknown accuracy due to the need to estimate food spillage. Further, it requires considerable handling of the birds, the stress of which could confound the mortality pattern among levels and chemicals.

Any estimate of the hazard of environmental levels of pesticide is better expressed in terms of ppm of toxicant in, say, the natural diet than in some abstract expression of mg/kg/day. Therefore, toxicity measurements based on ppm in the diet would seem more directly applicable in environmental studies.

In acute short-term tests (e.g., 5 days), a measurement of the weight of food consumed per bird-day on toxicant can be used to detect any marked differences in food consumption due to repellency or impaired appetite resulting from toxicity.

Results of 1964 Tests

Tables on pages 23 and 24 present the findings of the acute toxicity studies conducted at Patuxent during 1964. Pesticides are listed in the tables in high to low order of toxicity. Poor pheasant reproduction in 1964 greatly reduced the numbers of chicks available for acute studies with this species.

Pen Studies of Pesticidal Effects on Avian Reproduction

Knowledge of the highest nonlethal level of a pesticide that an animal can sustain in the diet is only a part of the information needed to evaluate the safety of a chemical. Even more important but less easily attainable (and presently unattainable for some species) is knowledge of the effect of otherwise "safe" levels of a chemical on the reproductive processes of a species. There is strong evidence to indicate that a chemical can be innocuous in terms of direct mortality and yet reduce reproductive success.

Pen studies of avian reproduction, although decidedly more efficient than field studies, are costly in terms of pen space, time, and manpower. This is especially true, as at Patuxent, in working with species having annual reproductive cycles. Our tests have therefore

been designed to first screen chemicals for reproductive effects, and then to study intensely those chemicals showing positive effects.

The screening procedure is as follows: A chemical known to be directly lethal as generally applied in the field is tested at two levels; the higher level is our best estimate (based on previous studies) of the highest nonlethal dietary level the animal can sustain for at least a year, while the lower level is about 60 percent below the higher one. The latter not only affords information about that level but acts as a "safety level" should results prove the higher level to be lethal prior to the reproductive period. If, however, a chemical is expected to produce no appreciable mortality at the highest recommended field levels, it is screened at only one dosage level, estimated to be slightly above the highest recommended field level.

We are presently conducting screening tests on three species of game birds: bobwhite quail, ring-necked pheasants, and mallards. Coturnix will be incorporated in the program as soon as feasible. We are also testing three levels of DDT in a special study of black duck reproduction. The tests employ a completely randomized design wherein the pen is the sampling unit. Several pens must be used for each level of a chemical to distinguish between natural variation in reproductive success and any real changes produced by a chemical. One set of pens of untreated birds serves as the "control" for several chemicals under simultaneous test. All birds are from "pesticide-free" lineage unless otherwise specified, and are assigned to pens by means of random numbers. Treatments are also assigned to pens randomly. Eggs are gathered daily, stored under controlled conditions, and incubated biweekly.

Estimates will be made of the following parameters: eggs laid per hen-day of laying, eggs fertile per egg set in incubator, eggs hatched per fertile egg, proportion of crippled chicks, and chick survival on clean diet to 14 days of age.

The above testing protocol was initiated during the summer of 1964, so that no tests have yet been completed. However, the experiments outlined in table, page 24 are underway at this time.

Progress in Testing the Toxicity of TFM

An attempt in June 1964 to estimate the acute LC_{50} of the lampricide TFM (3-trifluormethyl-4-nitrophenol) for mallard ducklings resulted in no mortality at levels ranging from 500 to 5,000 ppm in the diet. The 60 test birds were continued on the same levels of TFM until January 14, 1965, when all birds were placed on 2,000 ppm to simplify the experiment. This level is very much higher than would conceivably occur in the wild. The birds will be maintained at the 2,000 ppm level through the reproductive season to test the effects of TFM on reproduction and duckling survival.

To date, food consumption, growth, and survival of the test birds have been completely normal.

On January 14, seven of the drakes, surplus to the reproduction study, were sacrificed and autopsied at Patuxent by Dr. Lou Locke. He found all birds to be grossly and histopathologically normal.

Acute and Relative Toxicities of Pesticides to Bobwhite Quail Chicks

(Patuxent Wildlife Research Center, 1964)

Chemical	No. Pens of Birds	Birds/ Pen	LC_{50}* (ppm toxicant in diet)	95% Conf. Limits of LC_{50}	Relative Toxicity** (95% Confidence Limits in Parentheses) endrin	aldrin	dieldrin	chlordane	toxaphene	ddt
ENDRIN	6	10	15	10--22	1	2.5 (1.6--3.7)	2.6 (1.7--4.0)	19.0 (12.1--29.6)	48.2 (26.5--87.7)	52.4 (32.7--84.0)
ALDRIN	12	8 to 10	39	36--42	.40 (.27--.63)	1	1.1 (.8--1.4)	7.6 (6.2--9.1)	19.4 (12.3--30.6)	21.1 (17.9--24.9)
DIELDRIN	12	6 to 10	40	36--45	.38 (.25--.59)	.91 (.70--1.3)	1	7.4 (4.7--11.2)	18.4 (12.4--29.4)	23.6 (15.9--36.3)
CHLORDANE	12	6 to 8	320	276--371	.05 (.03--.08)	.13 (.11--.16)	.14 (.09--.21)	1	2.5 (1.7--3.9)	3.0 (2.2--4.2)
TOXAPHENE	6	6	834	625-1110	.02 (.01--.04)	.05 (.03--.08)	.05 (.03--.08)	.40 (.26--.59)	1	1.3 (.9--1.8)
DDT	14	6 to 8	881	796--975	.02 (.01--.03)	.05 (.04--.06)	.04 (.03--.06)	.33 (.24--.45)	.77 (.56--1.11)	1

*LC_{50} defined as ppm of toxicant in an ad libitum diet expected to produce 50 percent mortality at the completion of an 8-day period, 5 days of toxic diet plus 3 days of clean diet.

** Relative toxicity in a given square is that of the chemical in the left-hand column (capital letters) to the associated chemical in the upper line (small letters). Example: DIELDRIN is .38 times as toxic as endrin (confidence limits .25--.59) and 18.4 times as toxic as toxaphene, etc.

Acute and Relative Toxicities of Pesticides to Mallard Ducklings

(Patuxent Wildlife Research Center, 1964)

Chemical	No. Pens of Birds	Birds/ Pen	LC_{50}* (ppm toxicant in diet)	95% Conf. Limits of LC_{50}	Relative Toxicity** (95% Confidence Limits in Parentheses) endrin	aldrin	dieldrin	toxaphene	ddt
ENDRIN	6	10	21	16--29	1	7.3 (4.6--11.4)	9.4 (6.0--14.1)	24.9 (16.1--38.6)	40.9 (30.3--52.2)
ALDRIN	12	8 and 10	164	143-188	.14 (.09--.22)	1	1.3 (.8--2.0)	3.4 (2.8--4.2)	5.6 (3.5--8.4)
DIELDRIN	6	10	200	151--265	.11 (.07--.17)	.77 (.5--1.23)	1	2.7 (1.7--4.2)	4.4 (2.5--5.7)
TOXAPHENE	6	8	564	495--641	.04 (.03--.06)	.29 (.24--.36)	.37 (.24--.59)	1	1.6 (1.1--2.6)
DDT	8	10	875	650--1140	.02 (.02--.03)	.18 (.12--.29)	.23 (.18--.40)	.63 (.39--.91)	1

*LC_{50} defined as in table on Bobwhite Quail.

**Relative toxicity read as in table on Bobwhite Quail.

Acute and Relative Toxicities of Pesticides to Pheasant Chicks

(Patuxent Wildlife Research Center, 1964)

Chemical	No. Pens of Birds	Birds/ Pen	LC_{50}* (ppm toxicant in diet)	95% Conf. Limits of LC_{50}	Relative Toxicity** (95% Confidence Limits in Parentheses) endrin	ddt
ENDRIN	6	10	11.0	9.5--12.5	1	74 (61--90)
DDT	4	10	804	686--942	.014 (.01--.02)	1

*LC_{50} defined as ppm of toxicant in an ad libitum diet expected to produce 50 percent mortality at the completion of an 8-day period, 5 days of toxic diet plus 3 days of clean diet.

**Relative toxicity read as in table on Bobwhite Quail.

A Listing of the 1965 Penned Reproduction Studies (in Progress)

	Bobwhite Quail (3 females, 2 males per pen)		Pheasants (5 females, 1 male per pen)		Mallards (6 females, 2 males per pen)	
Chemical	ppm in diet	pens/level	ppm in diet	pens/level	ppm in diet	pens/level
Endrin	0.4;1	8;8	0.4;1	3;3	-	-
DDT	10;25	8;8	10;25	3;3	2.5;10;40	5;5;5
Dieldrin	1.5;4	8;8	1.5;4	3;3	4;10	4;4
Heptachlor	4;10	8;8	8;20	3;3	-	-
Lindane	75	8	75	3	75	4
TFM	-	-	-	-	2,000	1
Control	0	8	0	5	0	10

Effects of malathion on penned pheasants (G. L. Zorb and C. T. Black, Michigan Department of Conservation, Cooperators)

An experiment with the effects of malathion on pheasants was conducted in the fall of 1963. Malathion was applied at rates of 1, 5, and 10 pounds per acre. Each dosage was applied to 9 pens, each of which held 1 cock and 5 hens. Birds, ground, and food for a month were sprayed. There were also 9 control pens. No deaths attributable to the spray occurred. Autopsy revealed no internal effects. There was, however, an off-taste in birds from the highest treatment group.

All birds that were not autopsied were held over winter and allowed to breed in the spring of 1964. No further treatment was applied, but treatment groups of 1963 were kept separate and their reproductive success was studied. Their eggs were collected and incubated in the period of April 27 to May 7. All young were reared to the age of three weeks so that any crippling loss could be detected.

Reproduction of treated birds proved to be fully as successful as that of controls whether judged by numbers of eggs, fertility, or survival of young.

These results indicate that when malathion is applied at the usual rates of one pound per acre or less, there is a wide safety factor for birds that are at all similar to pheasants in their reaction to malathion. This conclusion is in agreement with the results of laboratory toxicity studies made at Patuxent and elsewhere, all suggesting that small doses of malathion are well tolerated by warm-blooded vertebrates and that these animals are able to metabolize malathion rather rapidly.

MEASUREMENT OF PESTICIDE RESIDUES

Analytical methodology for determination of pesticide residues is undergoing extensive and rapid change as new techniques are developed and details of old methodology are examined. Dependability of identification and measurement of residues is essential because monitoring, surveillance, and experimental studies all require the ability to reliably detect differences in quantities and kinds of pesticides. Analytical procedures suitable for plant tissues may not be satisfactory for animal tissues. For this reason, even thoroughly satisfactory published methods may require adaptation; they certainly require testing with the specific materials of concern. The variation that is inherent in measurements of any kind occurs at many stages in an analytical determination. Since this variation can be reduced but not removed, it must be subject to planned measurement to know the limits within which the readings are reliable. The analytical process requires several steps, and at each step problems may arise. The tissue must first be ground and dried; the fatty material (carrying the chlorinated hydrocarbon pesticides) must be extracted; the various organic impurities that would interfere with final readings must be removed, often in a series of steps; finally, the readings must be made and interpreted.

Problems of identification of unknown peaks on gas chromatograms and unknown spots on thinlayer plates challenge chemical ingenuity and require a knowledge of pesticide breakdown products. Various practical problems of technique require no less effort for solution.

Results of certain comparative studies are summarized below. Other questions under investigation include the following:

(1) Are pesticides extracted in greater percentages from rotting than from fresh material so that readings of content are distorted?
(2) Which extraction solvents are most effective in removing fatty material (and pesticides) from particular tissues?
(3) Do residues measured in tissues preserved by freezing differ from those in tissues preserved in formaldehyde or other materials? Does storage time influence the readings?

Comparison of recovery of DDT and metabolites from eggs using acid-wash cleanup vs. acetonitrile partitioning (W. L. Reichel, C. M. Menzie, V. A. Adomaitis, G. E. Bagley, and R. Prouty)

Egg samples collected to survey residue levels in black ducks first were cleaned by the modified acid wash procedure used by Schechter, Pogorelskin, and Haller (1947) for DDT and by Murphy and Barthel (1960) for heptachlor. When this method of cleanup proved inadequate for dieldrin, for which acetonitrile partitioning is required, tests were made to see whether the acetonitrile procedure gave DDT recoveries that were as satisfactory as those obtained with the acid wash.

To accomplish this, 34 fresh chicken eggs were homogenized in a blender and subdivided into equal portions. Ten portions were fortified with each of the quantities of DDT, DDE, and DDD outlined in the table which follows. Four samples were left unfortified. The samples were ground with anhydrous sodium sulfate and extracted with petroleum ether in Soxhlet apparatus.

Extracts from half of the samples were transferred to separatory funnels, made up

to 300 ml with petroleum ether, and 50 ml of a 2:1 mixture of concentrated and fuming (15%) sulfuric acid was added without shaking. The acid layer was drawn off, a second 50 ml portion of the mixed acids was added, and the funnel shaken 3 times. Emulsions were broken by the addition of 5 ml water. The acid layer was removed and the petroleum ether was washed with 50 ml portions of water until washings were neutral to litmus. The petroleum ether was dried by filtration through Dri-Rite, concentrated, and placed on a 2x15 cm. column of activated florisil; and the pesticides were eluted with 200 ml of a mixture of 3:1 hexane-benzene.

Extracts from the remaining samples were concentrated, taken up in 50 ml hexane and partitioned three times with 50 ml portions of acetonitrile saturated with hexane, essentially the method of Jones and Riddick (1952). The acetonitrile was evaporated to dryness at room temperature and chromatographed on florisil as previously described.

The samples were analyzed by thin-layer chromatography using 200 x 200 mm glass plates, coated with aluminum oxide G (0.25 mm thick) and activated in a circulating air oven at 130°C for 1/2 hour. The plates were developed with 200 ml hexane, dried and sprayed with Mitchell's (1958) silver nitrate reagent and exposed to ultraviolet light until spots appeared. A semiquantitative determination was made by comparing the sample run at several dilutions with standards of DDT, DDE, and DDD. The sensitivity of this system, adopted from Baumler and Rippstein (1961), was 0.1 microgram.

Average percentage recoveries are shown in the table below. Data were subjected to an analysis of variance, from which the following conclusions may be drawn:

(1) Acetonitrile partitioning and acid wash cleanup yielded percentage recoveries that were not significantly different from each other.

(2) Percentage recovery was significantly poorer in eggs fortified with larger amounts of chemical.

Method of Cleanup	Micrograms of Each Pesticide Added per Egg	Percentage Recoveries		
		DDE	DDT	DDD
Acid Wash	100	92	73	69
Acetonitrile	100	99	92	93
Acid Wash	200	54	46	42
Acetonitrile	200	63	67	74
Acid Wash	400	40	36	36
Acetonitrile	400	36	40	43
Acid Wash	none	t	0	0
Acetonitrile	none	0	0	0

Dieldrin recovery from animal tissue by gas chromatographic methods using different cleanup procedures (C. M. Menzie, V. A. Adomaitis, W. L. Reichel, G. E. Bagley, and R. Prouty)

A series of field-collected specimens from a dieldrin-treated area was used in a comparison of cleanup procedures for dieldrin.

Samples were prepared by removing keratinous material and skinning. The remainder was then homogenized. After drying under slight vacuum at less than 40° C, the samples were exhaustively extracted with petroleum ether in Soxhlet apparatus. After removal of solvent, the lipoidal residue was divided into two equal portions. One portion was cleaned by an acid wash procedure used for heptachlor (Murphy and Barthel, 1960); and the other by acetonitrile partitioning (Jones and Riddick, 1952). For each, cleanup was completed by chromatography on florisil (Gannon, 1958).

Eluates were concentrated and were then analyzed by electron capture gas chromatography (Jarrell-Ash): column - 150°C; injector 180°C; 30# N_2; 10^{-9} amps; 5% SE-30 on 80/90 mesh Anakrom ABS in 44" glass column.

Results of these analyses are shown in the accompanying table. In each instance, recovery was far lower after acid wash than after acetonitrile partitioning.

	Acetonitrile	Acid-Wash
Identification	Dieldrin ppm (dry weight)	Dieldrin ppm (dry weight)
Rabbit Liver	55.6	0.6
Bobwhite	6.9	0.3
Bobwhite	7.7	0.1
Bobwhite	6.9	0.2
Bobwhite	7.8	0.2
Bobwhite	12.6	0.2
Mourning Dove	13.4	0.7
Mourning Dove	17.8	0.2
Cardinal	5.0	0.3
Junco	13.2	0.3
House Sparrow	7.1	0.1
Common Grackle	11.3	1.4

Identification	Acetonitrile Dieldrin ppm (dry weight)	Acid-Wash Dieldrin ppm (dry weight)
E. Meadowlark	20.7	2.0
P. maniculatus	16.5	0.1
House Rat	1.3	0.06
2 Robins	18.3	7.7
4 Robins	13.0	2.9
Catbird	23.9	3.7
Brown Thrasher	27.5	4.7
Brown Thrasher	22.3	1.5

PRINCIPAL RESEARCH NEEDS

To learn the effects of pesticides in combination with each other and with other environmental pollutants, such as metals; and to determine which, if any, pollutants may be responsible for the decline of such birds as the eagle and osprey.

To determine the levels of pesticide residues in the brain or other tissues that are diagnostic of death from the common residual chemicals.

To learn the quantities of pesticides in eggs that affect the hatchability of the eggs and survival of the chicks, and to learn whether residue levels in adults can be used to predict levels in eggs.

To learn, at least approximately, the rates at which residues of the common residual chemicals are stored in the body and the rates at which they are lost.

To find valid, feasible ways of testing effects on birds of organophosphorus insecticides that vary widely in toxicity and persistence.

To learn how various environmental and physiological stresses such as disease, reduced food supply, and social pressures affect reactions to pesticides.

To learn how pesticides may affect behavior, in particular those behavioral changes that may cause parental failure in birds.

To find a quick and practical method that will permit at least preliminary testing of a much wider range of pesticides.

Perhaps most needed are analytical studies of critical factors acting on whole environments. This means locating all major ecosystems and food chains that are seriously contaminated with persistent pesticides. It also means sampling animals from as many populations as possible that are suspected of declining because of pesticide pollution.

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WILDLIFE STUDIES
DENVER WILDLIFE RESEARCH CENTER

by

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During 1964, emphasis on pesticide-wildlife studies at Denver Wildlife Research Center was changed from general surveillance and limited monitoring to intensive investigation of new pesticides. This was done to find suitable substitutes for the chlorinated organic insecticides, which are applied to vast areas of the West for the control of forest and range pests.

As the first step, the Denver Center signed a cooperative agreement with the Chemical Evaluation Laboratory, U.S. Forest Service, Berkeley, California, to evaluate the effects on captive wildlife species of nonpersistent insecticides found to be effective against forest insects by the entomological screening program of the Berkeley Laboratory.

When a compound is found that effectively controls an insect pest and does not have undue acute or chronic toxic effects, it will be field tested by Denver Center biologists on an experimental area set aside in the Bitter Root National Forest near Hamilton, Montana.

The immediate objective of these cooperative studies is to replace DDT, widely used for forest insect control, with a less persistent insecticide having a minimum effect on wildlife. Although much of the latter half of 1964 was spent in "tooling up" for the study of new insecticides, limited surveillance and monitoring studies were conducted at the request of other government agencies.

Summaries of the 1964 Denver Pesticide-Wildlife studies fall into six categories:

1. Improved or new methodology to identify and measure pesticide residues in organic and inorganic materials.
2. Toxicity of pesticides to captive wildlife.
3. Toxicity of insecticides to wild grouse in natural habitat.
4. Pesticide residues in endangered wildlife species.
5. Pesticide surveillance and monitoring activities.
6. Miscellaneous pesticide-wildlife studies and observations.

All pesticide residues listed were obtained by one of these analytical procedures:

1. Paper chromatography--maximum sensitivity 5×10^{-7} grams.
2. Gas chromatography using an electron capture detector--maximum sensitivity 1×10^{-12} grams.
3. Gas chromatography using a microcoulometric detector--maximum sensitivity 1×10^{-8} grams.

IMPROVED OR NEW METHODOLOGY TO IDENTIFY AND MEASURE PESTICIDE RESIDUES IN ORGANIC AND INORGANIC MATERIALS

Analytical Methods for the New Avicide DRC-1339 (James E. Peterson)

Three methods were developed to permit analysis of concentrates of DRC-1339, prepared baits, and residues in tissues of birds

and mammals. One is a volumetric method that involves the neutralization of an acidic salt of an aromatic amine (DRC-1339) with a dilute solution of a strong base. This method is rapid, simple, and easily reproduced with a high degree of accuracy. With care, reproducibility of results with ± 0.02% is possible. The second method estimates the toxicant gravimetrically as the N-acetyl derivative--a procedure best suited to the analysis of nominal 1% baits prepared for control of certain birds. An accuracy of approximately ±0.1% is possible by gravimetric means. The third method involves a paper chromatographic technique that permits identification and estimation of microgram quantities of the new pesticide. Adaptations of this method allow the estimation of two metabolites of DRC-1339; the N-acetyl derivative and an N-acetylated carboxy acid. Like other paper chromatographic estimations, accuracy is limited to about ±10% for levels as low as 0.1 to 0.2 ppm in the original sample.

Metabolism of DRC-1339 by warm-blooded animals (James E. Peterson)

While analytical methods were being developed for the recovery and estimation of DRC-1339 from animal tissue, some facts about the metabolic fate of this new avicide were learned. Being an acid salt of an aromatic amine, it was assumed that low recovery of the DRC-1339, per se, from a "spiked" liver homogenate was due to partial acetylation of the amine (a normal liver function). This would alter solubility characteristics in such a way as to make the acetylated form non-extractable with the solvents employed for the extraction of the free amine. The validity of this assumption was borne out by administration of DRC-1339 to rats. The acetylated derivative was recovered and identified by physical characteristics such as melting point determination and infrared spectroscopy. It was learned almost simultaneously that an additional conversion product was produced from the acetylated compound. This second metabolite was recovered from the urine of rats and was identified as a carboxy acid derivative of the acetylated amine. Again, identification was made by comparing the infrared spectogram and physical characteristics of the metabolite with those of the known laboratory synthesized compound.

Identification of Baytex in Bird Tissue Samples (Charles W. Hall)

Studies were made of the recovery of Baytex, an organo-phosphate, from animal tissue, and paper chromatographic and colorimetric methods were successfully developed for detecting trace quantities. The colorimetri determination is preferred because of a greate specificity for the detection of Baytex in bir tissues.

The colorimetric procedure measures or product of hydrolysis of Baytex, 4-methylthic m-cresol, which is condensed with the col producing 4-aminoantipyrene in the presence sodium metaperiodate. The absorbance of tl resulting colored solution is read at 458 mμ or spectrophotometer and the concentration Baytex determined from a standard curve.

Blood Cholinesterase Activity--Organc phosphate Treatment Studies of Various Dc mestic and Wild Animal Species (Charles W Hall)

Many blood determinations were made t ascertain: (1) the blood cholinesterase activit of various species of animals that had not beer treated with pesticides, and (2) the comparative degree of blood acetylcholinesterase inhibition produced by treatment with several organo-phosphate insecticides. Domestic goats, deer, coyotes, and rabbits were used in these studies. Avian species included pheasants, grouse, turkeys, chickens, ducks, geese, eagles, hawks, prairie chickens, Hungarian partridges, and terns. The pesticides involved included malathion, phosphamidon, Baytex, Dibrom, DRC-714, parathion, and dimethoate. Treatment with any of these pesticides produced marked depression of blood cholinesterase activity. Normal blood cholinesterase activity varied widely between species, and the activity of no two species responded identically to treatment with a single organo-phosphate compound. It was learned that mammals generally have the blood cholinesterase activity

concentrated in the red blood cells, while in the case of avian species, the activity is found in the plasma. Gophers, rabbits, and coyotes were interesting exceptions to this rule of thumb because most of the cholinesterase activity in these species is found in the blood plasma. The electrometric method of Michel (1957) was employed in these determinations.

Rapid "Clean-up" Procedures for Residue Analysis (D. Glen Crabtree, William H. Robison, James E. Peterson, and Richard A. Wilson)

Two new "clean-up" procedures have been added to the laboratory methodology. In one method, environmental samples such as water, soils and muds, are passed through a Florisil column which adsorbs both the elemental sulfur and the pesticides. The sulfur is then eluted with a nonpolar solvent. The pesticides are in turn eluted from the column using proper polar solvents and are identified by conventional procedures. Elemental sulfur, if not removed, seriously interferes with chromatographic methods of pesticide analysis.

The second new "clean-up" technique may be applied to extracts from plant and animal tissues. It provides a means of rapid "survey" to identify and measure chlorinated organic pesticides present in amounts of one part per million or more. This method takes advantage of the preferential adsorption of biological extractives on Florisil that was added to the extract solution. The pesticides remain in the solution and may be directly injected into a gas chromatograph equipped with an electron capture detector for qualitative and quantitative estimation.

TOXICITY OF PESTICIDES TO CAPTIVE WILDLIFE

Mammalian Metabolism of DDE (James E. Peterson)

Following a single administration of DDT by stomach tube to a deer, a substantial amount of an unknown material (Compound X) was detected in the blood of the test animal by means of a gas chromatograph equipped with an electron capture detector. The unknown component was not found in normal deer blood. By repeating the experiment on rats, enough of compound "X" was recovered to allow identification by infrared spectrum analysis, and by melting point and mixed melting point determinations employing the purified laboratory-synthesized compound as a reference. Further feeding studies with rats established that this newly identified metabolite was produced from DDT and DDE[1] (a major metabolite of DDT) and not from either DDMU[2] or DDNU[3] (homologs of DDE) (Peterson and Robison, 1964). Thus, compound "X" appears to be a degraded form of DDE. One or two additional abnormal materials that were also detected in the blood of DDT-treated deer and rats may be degradation products of the now identified compound "X".

Toxicity of Dimethoate to Mule Deer (Richard E. Pillmore and Charles W. Hall)

Technical grade dimethoate was dissolved in ethyl alcohol and injected into the rumen of two adult male mule deer. No gross symptoms were observed in one deer following a single dose of 100 mg/kg; however, the cholinesterase activity in the red blood cells was reduced 66% in 12 days. After 3 months, blood cholinesterase activity had increased, but not to the pretreatment levels.

Salivation was apparent within 1 hour after a single administration of 200 mg/kg to the second deer. In 7 hours, contracted eye pupils, skeletal muscle tremors, continued salivation, and some muscular weakness or loss of coordination were apparent--all symptoms of organo-phosphate intoxication. Blood cholinesterase activity of this deer declined 77% within 24 hours, but approached the pretreatment blood level 3 months later.

Both deer survived and presented a normal outward appearance within one week following the dimethoate administration.

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[1] DDE - 1, 1-dichloro-2,2,-bis(p-chlorophenyl)ethylene

[2] DDMU - 1-chloro-2,2-bis(p-chlorophenyl)ethylene

[3] DDNU - 2,2,-bis(p-chlorophenyl)ethylene

Acute Toxicity of Dieldrin and Malathion to Sharp-tailed Grouse (Lowell C. McEwen and Donald B. Knapp)

Dieldrin and malathion are two insecticides likely to be sprayed on sharp-tailed grouse and prairie chicken ranges. The acute oral toxicity of these two chemicals was determined with captive, adult, male sharptails. For dieldrin an LD_{50} dose proved to be 6.4 mg/kg (3.3 to 12.5, $p = .05$).

Malathion was much less toxic; the critical dosage level was approximately 220 mg/kg. All 9 grouse dosed below 220 mg/kg (60-219 mg/kg) survived, and 8 receiving more than this amount (220-599 mg/kg) died. Due to a shortage of grouse, the LD_{50} of malathion was not determined.

Effects of DDT and Toxaphene in Diets of Young White Pelicans (Edward L. Flickinger and James O. Keith)

Chronic exposure of white pelicans to DDT and toxaphene in diet was undertaken under pen conditions to help define the role of these insecticides in the death of fish-eating birds at the Tule Lake National Wildlife Refuge during 1960-1962. For 3 months birds were fed fish injected with corn-oil solutions of insecticides. The pelicans received amounts of insecticides in the diet somewhat comparable to those present in fish eaten by wild birds. Twenty-five birds in groups of five were maintained on fish contaminated at each of the following rates: 10 ppm toxaphene, 50 ppm toxaphene, 50 ppm DDT, and 10 ppm toxaphene in addition to 50 ppm DDT. A group was also maintained on noncontaminated fish to serve as controls in the trial.

The pelicans did not respond well to conditions of captivity, but the trial did permit an assessment of the gross effects of the insecticides on birds. Results are summarized in Table C-1. As in acute toxicity trials reported earlier (United States Department of the Interior, 1964:50), pelicans were much more susceptible to toxaphene than DDT. Definite

Table C-1. Results of 3-month chronic toxicity trials with four groups of young white pelicans

Insecticides	PPM In Diet	Effects Attributable to Diets
Toxaphene	50	Elimination of parasites[1/] Tremors, convulsions and death in 4-6 weeks
Toxaphene	10	Reduction in parasites
DDT	50	Elimination of parasites
DDT and Toxaphene	50 and 10	Elimination of parasites
None (Control)	0	None

1/ Both endo and ectoparasites of pelicans on all experimental diets were affected.

symptoms of intoxication occurred only in birds receiving 50 ppm toxaphene in the diet. Sublethal doses of toxaphene and/or DDT caused elimination of endoparasites.

While the interactions of insecticides with the physiology of wild birds found dead at Tule Lake Refuge were undoubtedly quite different from those of captive birds, the trial did help in assessing the relative toxicity of DDT and toxaphene, and in defining their general effects on birds. Analyses will be made of tissues from birds exposed to insecticides in this trial, and this information will also help in interpreting the significance of residues found in dead birds at Tule Lake Refuge.

TOXICITY OF INSECTICIDES TO WILD GROUSE IN NATURAL HABITAT

Acute Toxicity of Insecticides to Wild Grouse in Natural Habitat (Lowell C. McEwen, Robert L. Brown [Montana Fish and Game Department] and Milton H. Mohn)

It is questionable whether insecticide toxicity to wildlife is similar under field and laboratory conditions. The extent of similarity was investigated in a pilot study conducted cooperatively with the Montana Fish and Game Department.

Male sharp-tailed grouse were trapped on their breeding grounds, treated, and released. Treatment consisted of acute, oral dosages of either dieldrin or malathion, or control dosages of sugar in capsules. Reactions of the 52 test birds were checked by observing them on or near the breeding grounds. Most of the grouse were marked with neck tags and leg bands. Twelve were instrumented with radio transmitters (of the type developed by Marshall in Minnesota) and tracked for more intensive data.

Acute toxicity results were similar to those obtained with captive grouse. The lethal malathion level appeared to vary between 200 and 250 mg/kg with the wild grouse, a somewhat more variable range than recorded for captive birds. The LD_{50} for dieldrin with wild grouse was 6.9 mg/kg (4.4 - 10.9, $p = .05$). This was close to the LD_{50} of 6.4 mg/kg (3.3 - 12.5, $p = .05$) obtained with the penned birds.

Radiotracking and telemetry provided information on movements, symptoms, and reactions of the wild grouse that had been given various doses of malathion and dieldrin. It also made possible the recovery of dead birds and the collection of survivors for examination and residue analysis. Residues found in the visceral organs of dieldrin-treated grouse agreed well with the severity of the symptoms observed. Observations on behavior of the test birds and susceptibility to predators were of particular interest, but they were too limited to be conclusive.

PESTICIDE RESIDUES IN ENDANGERED WILDLIFE SPECIES

Bald Eagle Studies (William H. Robison, Charles W. Hall, George H. Ise and Mitchell G. Sheldon)

Four bald eagle carcasses were examined for pesticide content. Two of the eagles died from unknown causes in Colorado and two in Oklahoma. The Colorado specimens contained 2.08 ppm and 3.50 ppm of DDT and its metabolites in body aliquot samples. Body fat was present in these specimens in insufficient amounts to permit a separate analysis of this tissue.

One of the two Oklahoma bald eagles contained 2.51 ppm of DDT and metabolites in a body aliquot sample, and 68.40 ppm in its body fat. The second contained 158 ppm of DDT and metabolites in its fat, and 44 ppm of dieldrin. All analyses were conducted by paper chromatographic methods.

Whooping Crane Studies (William H. Robison, Mitchell G. Sheldon and Richard A. Wilson)

Two infertile whooping crane eggs, and two basic maintenance diets from the New Orleans Audubon Park Zoo, as well as tissues from the remains of an immature whooping crane found on the Aransas Refuge, were analyzed for pesticide residues. Both paper and gas chromatographic analytical techniques were used.

Special efforts in the "clean-up" procedures were made in order to detect the lowest possible limits consistent with sample size and procedures used. An electron capture detector was employed in the gas chromatograph which permitted the quantitation and detection of nanogram quantities of the chlorinated pesticides. The residues found are listed in Table C-2.

The quantitation between the methods varies at extremely low levels, but the qualitation does not vary when the detectability limits are taken into consideration.

Table C-2. Pesticide residues in whooping crane eggs, diet and carcasses

	Pesticide Residues in ppm[1]					
	Total p,p',DDT & metabolites	Endrin	Dieldrin	Aldrin	Heptachlor	Heptachlor Epoxide
Paper Chromatograph Method						
Egg No. 6	2.60	0.43	T[2]	T	0	0
Egg No. 7	2.86	0.65	T	0	0	0
Diet No. 1	0.85	0	0.15	0	0	0
Diet No. 2	0.16	0	T	0	0	0
Feathers[4]	T	0	0	0	0	0
Bone[4]	T	0	T	0	0	0
Connective Tissue[4]	0.32	0	0	0	0	0
Gas Chromatograph Method						
Egg No. 6	2.4830	0.509	0.148	0.021	T[3]	0
Egg No. 7	1.8020	0.611	0.165	0	T	T
Diet No. 1	0.6550	0.283	T	T	T	T
Diet No. 2	0.0330	0.087	T	T	T	T
Feathers[4]	0.1010	0	0	0	0	0
Bone[4]	0.0199	0	T	0	0	0
Connective Tissue[4]	0.0315	0	T	0	0	0

1/ ppm was based on the sample weight as received
2/ T = Trace (less than 0.1 ppm) for paper chromatography
3/ T = Trace (less than 0.01 ppm) for gas chromatography
4/ The only tissue available from the carcasses

PESTICIDE SURVEILLANCE AND MONITORING ACTIVITIES

Effects on Wildlife of Rangeland Spraying of Malathion for Grasshopper Control (Lowell C. McEwen, Charles W. Hall, Paul Johnson, and Nebraska Game, Forestation and Parks Commission)

Malathion was aerially applied on short-grass plain and ponderosa pine vegetative types at the rate of 8 ounces per acre (no carrier) for grasshopper control. The application was made in early June 1964 on 10,000 acres near Harrison, Nebraska.

Bird census lines, totaling approximately 10 miles on the treated area and 4 miles on the untreated area were censused 17 times prespray and 19 times postspray. The average number of birds per 2-hour-early-morning census decreased 16 percent on the sprayed area and increased 57 percent on the unsprayed area. The increase on the unsprayed area was attributed to both young birds emerging from the nests, and movement of individuals from the sprayed area.

Small mammals, taken in a total of 882 operational trap nights, showed no apparent effects from the treatment, but the data were limited.

Four, young, male Merriam's turkeys collected from the sprayed area showed possible decreases in blood plasma cholinesterase, but no visible poisoning symptoms. Histological examination of various tissues indicated slight degeneration in kidneys and testes. Whether these changes were related to malathion exposure could not be determined. All wild turkey broods observed were 4 weeks of age or older. No information was obtained on possible effects of the pesticide on newly hatched or young turkeys up to 4 weeks old.

Ten, young, female, domestic turkeys were caged on the treated area for close observation. Cholinesterase activity of blood plasama before spraying averaged 0.60, measured as pH change per hour (six birds sampled). Postspray cholinesterase levels in these birds dropped to an average of 0.43. Blood samples were checked periodically until 68 days postspray when cholinesterase had returned to prespray levels. No external symptoms of poisoning were seen in any of the turkeys.

Malathion Application in Montana (Richard E. Pillmore, and Philip South, U.S. Forest Service)

During 1964, malathion was applied by the U.S. Forest Service at a rate of 0.75 pound per acre in a gallon of oil for spruce budworm control on approximately 140,000 acres of the Lolo National Forest, Montana. A study area was selected within the spray boundaries to investigate the effect of the malathion application on bird numbers. Two-hour morning trend counts of songbirds were conducted along two routes following descend ridge tops. They were made on four mornings before the spraying and on three consecutive mornings following spraying. Only slight differences were evident between the average number of birds seen per hour on the two routes. The combined average for the two routes was 19.5 before and 22.0 after the spraying, while individual counts ranged from 11 to 30 before, and from 16 to 27 after spraying.

The total number of species tallied was 27 before and 22 after spraying, but no importance is associated with this reduction. Prespray counts included blue grouse, duck hawk, Clark's nutcracker, Steller's Jay, Townsend's Solitaire and a kinglet. While the kinglet was not observed after the spray application, mountain bluebirds, a species not encountered in the prespray, were seen during the final postspray period. Kinglets were heard during the postspray counts but were not identified visually. Some Coturnix quail shipped from Denver were exposed to the spray in an enclosure and returned to the Denver Research Center for observation. No mortality or other visible effects attributable to the spray occurred in these birds.

Pesticide Contaminations in Wildlife Refuges (James O. Keith, Milton H. Mohn and George Ise)

A survey of pesticide contaminations at the Tule Lake and Lower Klamath National Wildlife Refgues was initiated in 1962 to determine how dead fish-eating birds accumulated the insecticides found in their tissues. Results

showed that most components of the refuge environments that were sampled contained chlorinated hydrocarbon residues, but certain deficiencies were found in the simple hypothesis that birds were largely exposed through an accumulation of the insecticides via an aquatic food chain. Certain basic elements of the food chain, such as organics and other materials suspended in water, may also contribute directly as they were found to contain greater relative residues than the fish eaten by the birds.

Birds affected at Tule Lake were all migratory, and they could have been exposed to high residues in fish in other regions used during their annual movements. Therefore, the survey was expanded in 1963 to include two additional refuges. The objectives were to find if pesticide combinations existed in other areas and, if so, what the relative residue levels were in various components from these areas. Another purpose of the 1963 work was to determine if any single component of the aquatic habitat could be used as a reliable and consistent index to its degree of contamination. The additional locations selected for sampling were the McNary and Deer Flat National Wildlife Refuges in Washington and Idaho, respectively.

Average residues of chlorinated hydrocarbons in water and sediments collected from the four refuges are shown in Tables C-3 and C-4. Water samples, which were collected from impounded water within each refuge, were filtered, and analyses were made of both the filtrate and the suspended material filtered from the water. Sediment samples were obtained by placing wide-mouth jars in refuge ponds to collect materials settling from the water over a 1- or 2-month period.

Analyses of water samples, which were collected periodically during the summer, showed that much greater concentrations of insecticides were present in suspended material than in filtered water. At all refuges, except Deer Flat, sediments collected continuously during the summer months in wide-mouthed jars contained lower concentrations of insecticides than did the suspended material in water samples.

The high residues in suspended materials and sediments at Deer Flat Refuge may be related to the scarcity of aquatic invertebrates in the refuge. Invertebrates found at Deer Flat were not comparable with those of the other refuges in abundance or forms present. Crustacea and insect larvae were noticeably scarce there. Evidence is increasing from various investigations that invertebrates are capable of metabolizing chlorinated hydrocarbons and that they can be a major factor in the elimination of the materials from water. Sampling at Tule Lake Refuge in 1962 suggested such a relationship. Suspended material in waste agricultural water entering the refuge contained an average of 20.3 ppm of DDT, while suspended material in water from marsh areas contained only 6.0 ppm. Sediments collected in wide-mouth jars contained an average of only 1.5 ppm of DDT. This decrease in residues could be related to the breakdown of the insecticides by invertebrates, which are numerous in Tule Lake.

Table C-5 gives average residues found in aquatic vegetation, invertebrates, and fish from the four refuges. Submerged plants at Deer Flat Refuge were highly contaminated with insecticides. and plankton at McNary Refuge contained relatively high residues. Average residues in whole fish at all refuges were relatively low. At Deer Flat Refuge, residues in fish were surprisingly low considering the high concentrations found in other components of the environment.

Although invertebrates may be capable of metabolizing chlorinated hydrocarbons, thus removing them from the water, low and apparently inconsequential residues have often created serious problems. Hunt and Bischoff (1960) have reported the death of western grebes and the spectacular accumulations of residues in wildlife after treatment of Clear Lake in California with .02 ppm of DDD.

Many deaths among several species of fish-eating birds at Tule Lake Refuge in 1960, 1961, and 1962 resulted from a contamination of the refuge with toxaphene in waste agricultural water. Fish collected there in 1961 contained up to 8 ppm of toxaphene, while residues in tissues of dead birds ranged from 3 to 650 ppm

Table C-3. Average residues of chlorinated organic insecticides in water from each of four National Wildlife Refuges. Analyses by paper chromatography

Refuges	Residues in ppm[1/]		
	Suspended Material	Filtrate	Total Sample
Tule Lake	6.0	.0002	.0005
Lower Klamath	7.3	.0002	.0003
McNary	9.3	.0002	.0003
Deer Flat	12.5	.0002	.0004

1/ Mainly DDT, but also includes dieldrin

Table C-4. Average residues of chlorinated organic insecticides in sediments from each of four National Wildlife Refuges. Analyses by paper chromatography

Refuges	Residues in ppm
Tule Lake	1.5
Lower Klamath	0.9
McNary	0.4
Deer Flat	40.0

Table C-5. Average residues of chlorinated organic insecticides in living substrates from each of four National Wildlife Refuges. Analyses by paper chromatography

Refuges	Residues in ppm[1/]		
	Aquatic Vegetation	Invertebrates	Fish
Tule Lake	1.0	0.4	2.5
Lower Klamath	1.1	2.5	1.5
McNary	0.8	5.2	3.3
Deer Flat	30.3	- [2/]	0.5

1/ Includes DDT, dieldrin, toxaphene and methoxychlor

2/ No comparable invertebrates found at Deer Flat

of DDT, and from 1 to 39 ppm of toxaphene. An unusual mortality of black-crowned night herons occurred in 1963 at Deer Flat Refuge; residues in dead birds were as high as 25 ppm DDT, 4 ppm dieldrin, and 5 ppm toxaphene. Such deaths, especially in fish-eating birds, have been widely reported and often have been associated with specific agricultural pest control practices. Rudd and Genelly (1955), for instance, reported the correlation between a serious mortality in egrets and herons and the use of dieldrin for control of rice leaf miner. Moore (1964) has shown that the fish-eating birds, in general, contain greater residues than any other group of birds. This undoubtedly results from their unique exposure to pesticide contaminations in aquatic habitats and in the food they eat.

Results of this work in the refuges show that pesticide residues were omnipresent in the environments sampled. The relative amounts of residues in different habitat components varied between refuges; the relationships between environment and insecticides were quite different at Deer Flat than at other refuges.

The significance of the behavior of insecticides in different areas and their specific effects on wildlife remain largely unknown. It is clear, however, that these relationships are complex and deserve further study.

Persistence of DDT on Forage Species (Richard E. Pillmore, William H. Robison, Richard A. Wilson)

The U.S. Forest Service conducted and coordinated a cooperative monitoring of its 1964 spruce budworm control project on the Salmon National Forest in Idaho. Approximately 525,000 acres were sprayed with DDT at a rate of 1 pound of DDT in one gallon fuel oil per acre. The Denver Wildlife Research Center studied the amount and persistence of DDT contamination in forage plants.

Because cooperating Idaho Game and Fish Department personnel considered the deer and elk summering in the Panther Creek Drainage most likely to remain in the treated area, this location was selected for study.

Forage sample collection sites were chosen along the drainage for accessibility and the presence of appropriate species of plants. Approximately 20 sampling plots having the above characteristics were established and samples of designated plants collected before the area was sprayed.

Following treatment with DDT, oil-sensitive spray cards indicated a deposit of 0.1 pound per acre or more on 10 of these sampling plots. Forage samples were taken from these 10 sites immediately after the spray application and at monthly intervals for the following 3 months. Equal quantities of similar plant parts of each species from all 10 collection sites were combined for each sampling period; the composite sample was air-dried and analyzed for pesticide residues (Tables C-6 to C-9). The rumen contents of nine deer, four collected before spraying and five collected about a month after the spraying, were also individually analyzed (Table C-10).

Table C-6. Residues on big sagebrush (Artesmesia tridentata) before and after treatment with DDT. Analysis was by gas chromatography (electron capture detector) (only nine collecting stations were sampled for big sagebrush).

Sample	Month	Residues in ppm			
		p,p'DDT	DDE	o,p'DDT & TDE	Total
Pre-spray	June-July	ND[1/]	ND	ND	ND
Post-spray	July	128.0	4.2	16.0	148.2
Post-spray	August	69.0	3.1	8.0	80.1
Post-spray	September	53.0	1.3	3.5	57.8
Post-spray	October	55.0	1.6	2.5	59.1
Check	July-October	ND	ND	ND	ND

1/ None detected

Table C-7. Residues on balsamroot (Balsamorhiza sagittata) before and after treatment with DDT. Analysis was by gas chromatography (electron capture detector).

Sample	Month	Residues in ppm			
		p,p'DDT	DDE	o,p'DDT & TDE	Total
Pre-spray	June-July	ND[1/]	ND	ND	ND
Post-spray	July	279.0	5.9	28.0	312.9
Post-spray	August	196.0	2.1	17.0	215.1
Post-spray	September	64.0	2.7	9.7	76.4
Post-spray	October	127.0	3.3	19.0	149.3
Check	July-October	ND	ND	ND	ND

1/ None detected

Table C-8. DDT Residues on wheat grass (Agropyron sp.) before and after treatment with DDT. Analysis was by gas chromatography (electron capture detector).

Sample	Month	Residues in ppm			
		p,p'DDT	DDE	o,p'DDT & TDE	Total
Pre-spray 1/	June-July	ND	ND	ND	ND
Post-spray	July	317.0	7.1	60.0	384.1
Post-spray	August	202.0	3.5	33.0	238.5
Post-spray	September	186.0	2.8	32.0	220.0
Post-spray	October	170.0	3.2	38.0	211.2
Check	July-October	ND	ND	ND	ND

1/ Only six collection stations represented for the pre-spray sample

At the time of spraying, the various plants sampled were in different stages of growth. Big sagebrush had produced considerable new vegetative growth, but no flowering shoots were noted. Balsamroot had almost completed flowering and the flower heads were rapidly consumed by livestock and game. Some leaf growth did occur, however, after the spraying. Wheatgrass had produced a conspicuous number of spikes and the grass clumps sampled were open. Many new Douglas-fir needles were eaten by the spruce budworm larvae leaving the twigs sparsely foliated. After the DDT application, production of new vegetative growth, which would be expected to reduce residues, was apparently less for wheatgrass and balsamroot than for big sagebrush (Tables C-6 to C-8). Because of the large leaf surfaces of balsamroot, it was anticipated that the highest residues would be found in this species; but, to the contrary, wheatgrass was found to have the highest residues (Table C-8). Douglas-fir needles contained the lowest residues (Table C-9).

From these data, it is obvious that the plants sampled retained a significant contamination for as long as three months following the DDT application. At this time, dead balsamroot leaves of gray or brownish color were on the ground; the standing wheatgrass was cured to a golden color; the big sagebrush carried its leaves and ripe seedhead, and the Douglas-fir needles persisted.

The rumen contents of the prespray deer collected, as with the prespray vegetation samples, contained no detectable residues. However, the rumen contents of the deer collected a month after the spray were contaminated (Table C-10).

Persistence and Effect of Parathion in Marsh Habitats (James O. Keith, in cooperation with Mir S. Mulla and Francis A. Gunther, University of California, Riverside, and Arthur F. Geib, Kern Mosquito Abatement District)

Two marsh areas were treated with parathion by aircraft to determine its effects on ducks, fish, and frogs, and to measure the persistence of residues in several samples of components of the environment. One area received a single treatment that resulted in residues of 0.85 pound per acre at water level, while another area was treated six times at weekly intervals with amounts resulting in residues of 0.05 pound per acre on the water for each treatment.

No effects of treatments were noted on pinioned mallard ducks or caged bullfrogs (Rana catesbeiana) in either area. Caged and free-swimming mosquito fish (Gambusia affinis) suffered serious mortality under the heavy treatment, but were apparently unaffected by repeated exposure to the lower rate of

Table C-9. DDT residue on Douglas-fir needles (Pseudotsuga mensezii) before and after treatment with DDT. Analysis was by gas chromatography (electron capture detector).

Sample	Month	Residues in ppm			
		p,p'DDT	DDE	o,p'DDT & TDE	Total
Pre-spray	June-July	ND[1/]	ND	ND	ND
Post-spray	July	64.0	2.3	13.0	79.3
Post-spray	August	30.0	ND	ND	30.0
Post-spray	September	27.0	ND	ND	27.0
Post-spray	October	30.0	TR[2/]	5.3	35.3
Check	July-October	ND	ND	ND	ND

1/ None detected
2/ Trace

Table C-10. DDT residues in deer rumens after treatment of habitat with DDT. Analysis was by gas chromatography (electron capture detector).

Deer Number	Residues in ppm				
	DDE	p,p'DDT	o,p'DDT	TDE	Total
6	5.6	125.0	16.0	9.0	155.6
7	6.3	150.0	19.0	10.0	185.3
8	TR	16.0	2.4	8.6	27.0
9	3.2	53.0	7.6	12.0	75.8
10	3.1	130.0	4.0	15.0	152.3

applications. Gambusia populations quickly recovered from the 0.85 pound per acre treatment and soon had repopulated the pond.

Residues in water receiving 0.85 pound per acre decreased from 0.40 ppm on the day of treatment to 0.01 ppm after eight days; no residues were found in water on the fourteenth day after treatment. Initial residues in water receiving 0.05 pound per acre were 0.02 ppm and these disappeared within one week. The top 2 inches of bottom mud in the pond, treated with 0.85 pound per acre, contained 0.06 ppm on the day of treatment and these residues persisted for at least 22 days. Residues of 0.02 persisted in mud for only 1 day under the lighter treatment.

Submerged vegetation was contaminated at about 0.5 ppm for only 2 days under the heavy rate of application, but residues of about 0.5 ppm persisted on plants in the other pond through the 14th day after the sixth treatment. Residues on emergent vegetation ranged up to 14 ppm after the application of 0.85 pound per acre, and amounts of about 2 ppm persisted after 29 days. At 0.05 pound per acre initial residues on emergent plants were 1 to 5 ppm, but amounts fell to less than 0.1 ppm within 1 week.

Results suggest that these applications did not pose long-term hazards to ducks or frogs, or to mosquito fish populations. Residue analyses were largely exploratory to facilitate development of techniques. Chemical, bioassy, and cholinesterase inhibition analyses were made on most samples. Residues in most substrates did not persist or accumulate, but further work with residues in mud and on plants should be undertaken.

Fate and Persistence of DDT in a Forest Environment (James O. Keith and Edward L. Flickinger)

It has been shown repeatedly that chlorinated hydrocarbon insecticides can persist in aquatic environments and that they can be accumulated and transferred in aquatic food chains. These factors have often resulted in the serious and unexpected exposure of certain animals to concentrations of these insecticides. Aquatic ecosystems are especially susceptible to such involvements with pesticides, but the contamination of terrestrial environments can result in similar phenomena as evidenced by the DDT-earthworm-robin relationships reported by Barker (1958) and other workers. The implications of chlorinated hydrocarbons in food chains have not been studied in forest environments in the western United States, and such investigations seemed important considering the large areas treated each year for forest insect control.

In 1964, an application of 0.75 pound per acre of DDT to forest stands at Knox Mountain in northeastern California, for control of the white fir sawfly, presented an opportunity to evaluate the persistence of DDT in a forest habitat and its accumulation in forest wildlife. A program therefore was developed for the collection of samples from the treated area for residue analysis. Ten samples of each of 12 components were collected during each of 4 periods. The U.S. Forest Service provided funds for contract analyses of samples collected during the first three periods, and personnel from the Department of Biological Control of the University of California collected insect samples. The samples were analyzed by the Stoner Laboratories, Campbell, California. As a result of analytical procedures used, only DDT and its metabolite, DDE, were found in samples.

Table C-11 shows results of residue analyses of soil, plant, and animal tissues. Residues in insect samples expressed in ppm of DDT and DDE were combined as follows:

O-Day	1 & 2 Day	1 Month
206.2	83.7	1.9

In soil, litter, and plant material, DDT was consistently found in much greater concentration than DDE. In animal tissues, the greatest residues in pretreatment samples were of DDE, but much more DDT than DDE was found in O-Day samples. Progressively greater proportions of DDE were found in the 6-week and 3-month animal samples. In insects, the ratio of DDT to DDE was 6:1 in O-Day samples, 3:1 in the 1- and 2-day samples, and 1:1 in the

Table C-11. Residues in living and nonliving samples collected at Knox Mountain, California, after treatment with DDT. Analysis by Stoner Laboratories, Campbell, California, was for DDT and DDE [1/] on a wet weight basis.

Component	Period: Pre-treatment — One week prior to application	Post-treatment — 0-Day	Post-treatment — 6 Weeks	Post-treatment — 3 Months
Soil	.01	0.08	1.1	0.4
Litter	.08	19.1	6.9	6.1
Forb	ND [2/]	6.8	2.1	1.3
Grass	.04	20.4	6.0	2.4
Sagebrush	ND	16.3	1.4	0.3
Fir	.01	6.6	1.2	0.8
Robins	.40	0.6	2.8	0.2
Oregon Juncos	.40	2.6	1.5	6.0
Mt.Chickadees	.60	1.7	9.2	11.7
Wrights Flycatcher	.20	2.9	2.8	- [3/]
Western Tanager	.34	1.8	1.9	-
Deer Mice	ND	0.2	0.08	0.08

1/ Each figure represents the average of 10 samples.
2/ ND = None detected.
3/ Dash indicates no samples obtained

1-month sample. These findings suggest that the degradation of DDT proceeds differently when deposited on plant tissues than when ingested by animals.

Residues in all components increased markedly on the day of DDT application, but the trend in residue levels thereafter varied considerably between substrates. Residues in plant materials were greatest on the day of treatment and amounts then decreased progressively. In soil, residues increased only slightly after treatment, but amounts of insecticide in litter covering the forest floor greatly increased after DDT applications and remained high even after 3 months.

Dead and dying insects were collected in dropcloths for several days after spraying and later collections were made by sweeping foliage with insect nets. Residues in insects from dropcloths were very high. The analyses

showed that insects accumulate large quantities of DDT before dying and thereby become, for a short period, a highly concentrated source of DDT contamination for insectivorous animals. After 1 month, live insects still contained DDT residues, but in much smaller quantities.

Songbirds and deer mice were collected to depict the residues accumulated in wildlife. All birds contained residues before treatment, but no DDT was found in mice from pretreatment samples. Amounts of residues increased in birds after DDT applications. After 3 months, average residues in robins were less than before treatment. Wright's flycatchers and western tanagers had moved from the area after 3 months, but increased residue levels persisted in their bodies for at least 6 weeks. Juncos and chickadees appeared to be subjected to a real exposure to DDT, and residue levels were greatest 3 months after treatment. DDT appeared in deer mice after applications were made to the forest, but levels remained low through the first 3 months after treatment.

MISCELLANEOUS PESTICIDE WILDLIFE STUDIES AND OBSERVATIONS

Effects of Pesticides on White Pelican Populations (James O. Keith and Edward L. Flickinger)

A variety of techniques are used to evaluate the effects of pesticides on the dynamics of white pelican populations. These birds are rather continuously exposed to certain insecticides present in the fish they eat. Pelicans that feed often on fish in areas receiving waste agricultural water consistently contain insecticide residues. The birds have suffered mortality in certain areas after exposure to specific insecticides, and all pelicans examined have had relatively high levels of residues in their tissues.

Aerial photographs are used to determine the breeding population and productivity at each of three nesting colonies. Areas used by birds from each of these colonies during their annual movements have been documented through banding and color-marking young birds. Work began this year to determine insecticide residues in fish from the various areas used by birds. Samples of adults, eggs, and young from each breeding colony were also collected to find the degree of pesticide contamination in the birds themselves. The ultimate objectives of this work are to find where birds are exposed to insecticides and what effect their exposure has on the health, activity, and longevity of individuals, and on the productivity of subpopulations represented by the three breeding colonies.

Effects of Malathion on the Abundance and Food Habits of Songbirds (James O. Keith and Edward L. Flickinger)

A survey of foods eaten by songbirds at Tuolumne Meadows in Yosemite National Park has shown that several species could be affected by reduced food supplies after insecticide treatments. Pine siskins, mountain chickadees, Audubon warblers, hermit thrushes, and wood pewees were found to eat insects predominately; most stomachs from birds of these species contained 90 to 100 percent insect material. These species of birds were all less abundant on areas treated with malathion than on comparable check areas. On sprayed areas, numbers of these birds had decreased 74 percent 3 weeks after malathion applications, while on check areas numbers had decreased only 40 percent. Oregon juncos, whose diets included only 30 percent insects, increased on both areas, but were 50 percent more abundant on treated areas. The normal seasonal movements of various species certainly influenced their relative abundance on study areas; but malathion applications undoubtedly reduced available food, which appeared to restrict the abundance of insectivorous birds.

Removal of Pesticide Contamination from Rocky Mountain Arsenal Lakes (Mitchell G. Sheldon and D. Glen Crabtree)

Following high-level conferences between the Department of the Interior and the Department of Defense, the Army agreed to remove the aldrin and dieldrin contamination in the bottoms of the three industrial lakes at the Rocky Mountain Arsenal. This contamination

resulted from occasional equipment failures in a part of the plant at the Arsenal leased for the manufacture of agricultural insecticides. The leased installation, which recirculated the lake water for cooling purposes, had been responsible for the annual death of several thousand waterfowl over a period of 10 to 15 years. The Denver Research Center prepared a bottom sampling plan and analytical specifications for the determination of aldrin and dieldrin, and supervised the collection of approximately 800 samples, most of which were analyzed under contract by a private laboratory.

Following the analytical work, the lakes were drained and 4 to 6 inches of soil removed. Areas of high concentrations of pesticides were resampled and 6 to 18 inches of additional soil removed.

In order to prevent recontamination of the lakes, a closed cooling system with cooling tower recommended by the Denver Center, has been installed and is now functioning. This facility includes a 650-million gallon sump into which the entire capacity of the cooling system may be dumped to permit cleaning or repair. The water may then be returned to the system or disposed of via the deep disposal well. This sump facility will prevent recontamination of the three lakes. The total cost to the Army of this decontamination operation was approximately $265,000.00.

Artificial Propagation of Sharp-tailed Grouse and Prairie Chickens (Lowell C. McEwen and Donald B. Knapp)

Prairie grouse are reared in captivity to provide birds of known history and condition for toxicity tests and to study effects of pesticides on reproduction. The first objective has been to successfully propagate the grouse without exposure to pesticides. Most of the problems encountered the previous 2 years were overcome in 1964 by improved facilities, techniques, and equipment. Sharp-tailed grouse propagation is summarized:

Fertile eggs	Eggs Hatched	%	Chicks Reared	%
312	239	76.6	148	61.9

In addition, 4 male and 6 female greater prairie chickens in cages produced a total of 41 fertile eggs. Of these, 24 were hatched and 17 were reared to maturity.

With each species, breeding pens contained two or three females and one or two males. Eggs were picked up daily, stored for no longer than 7 days, and artificially incubated and hatched. Chicks were started in indoor battery brooders and moved to outdoor pens at about 4 weeks of age.

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WILDLIFE STUDIES BY THE COOPERATIVE WILDLIFE RESEARCH UNITS

OHIO STATE UNIVERSITY

The Cycling of CL-36 Labeled DDT in a Marsh Ecosystem (Robert L. Meeks and Tony J. Peterle)

A two-year study to determine the fate of technical DDT in a freshwater marsh was begun in 1964 on a 4-acre study area near Lake Erie. After background samples were collected, a granular preparation of 3.9 millicuries of chlorine-36 ring-labeled DDT was applied to the area by helicopter at the rate of 0.2 pound technical DDT per acre. Plant, animal, soil, and water samples were collected at the following time intervals after application: 4 hours, 8 hours, 1 day, 3 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, and 4 months (to date).

Radioassay of 1,650 of the 2,000 samples collected to date has yielded the following tentative results:

The maximum DDT was released between 8 hours and 5 days after application with no definite peak noted. The granule size is known to affect the release rate. A finer granule would undoubtedly have had a more rapid release rate; however, the likelihood of drift from the aircraft would also have increased. Tadpoles, small carp, lesser duckweed, and bladderwort contained significant amounts of DDT after 4 hours. The DDT probably adhered to the plants and was either ingested by the organisms while in granular form or taken directly from undetectable amounts in the water through the epidermis or gills.

Uptake by both plants and animals was very rapid and extensive as indicated by the percentage of samples with significant amounts of DDT at the various sampling periods: 4 hours--9%; 8 hours--44%; 1 day--61%; 3 days--71%; 1 week--72%; 2 weeks--65%.

The highest concentration found was 245 ppm in algae at 3 days. Other highs were: at 4 hours, 45 ppm in lesser duckweed; at 8 hours, 26 ppm in bladderwort; at 1 day, 24 ppm in a tadpole; and at 1 week, 19 ppm in a small carp. Carp, crayfish, snails, tadpoles, lesser duckweed, algae, and bladderwort had consistently higher concentrations than other samples. The amounts of DDT associated with plants is important, regardless of their exact location when considered from the standpoint of ingestion by an organism. Snails had little or no DDT in their shells but usually significant amounts in their body tissues. This finding differs from some literature. With longer exposure to DDT, this differential in concentration may change.

The specific activity obtained made it possible to detect 7.8 picograms of labeled DDT with 95% efficiency when each sample was counted for 1 hour (after accounting for preparation efficiency and counterefficiency). This amount of labeled DDT would represent 0.14 microgram of technical DDT applied to the study area.

AUBURN UNIVERSITY, ALABAMA

Effects of Endrin-Arasan Treated Pine Seed on Wildlife (Bill Hamrick, Maurice F. Baker)

Pine seed commercially treated with 2 percent endrin, arasan, and aluminum coloring to repel birds and mammals was tested for toxic effects on penned quail and squirrels. The 2 percent used commercially in the southeast is double the recommended treatment.

Quail were force fed both treated and untreated seed of slash pine. One treated seed proved to be a lethal dose for a bobwhite. Five

out of 10 quail that were offered a mixture of treated and untreated seed died, and the remaining 5 showed symptoms of poisoning. One of the check birds in this test died, apparently by accident. Two of 3 quail, offered a choice of treated and untreated seed, died, and the other showed symptoms of poisoning. Quail offered aluminum colored but unpoisoned seed ate them as readily as the normal seed.

Five of 7 gray squirrels offered treated seed died, and the other 2 showed symptoms of poisoning. The squirrels that died cut from 30 to 136 seeds before death. The survivors cut 84 and 133 seeds.

These data suggest that the treatment does not provide the seed with much protection from these animals and is a potential hazard to them. Possible adverse effects of the field use of this treated seed cannot be projected from this study.

LOUISIANA STATE UNIVERSITY

Pesticide Residues in Water Birds

Specimens of approximately 50 water birds were collected along the lower Mississippi and Atchafalaya Rivers during 1964 by the Louisiana Cooperative Wildlife Research Unit in cooperation with Management and Enforcement personnel. The collection included egrets, gulls, herons, rails, terns, willets, and single specimens of yellowlegs, ibis, dowitcher, gallinule, and grebe. Pesticide residues in the vital organs and fat of the specimens were measured at the Feed and Fertilizer Laboratory, Louisiana State University.

DDT or its degradation products were present in the fat of 18 specimens, the levels ranging from 0.1 to 427 ppm (10 ppm or more in 5 of the 18). Dieldrin was detected in the fat of 6 specimens, the levels ranging from 0.1 to 0.8 ppm. The levels of endrin in 7 specimens ranged from trace amounts to 4.7 ppm. Detectable amounts of heptachlor epoxide (0.1 to 1.8 ppm), DDD (trace to 1.8 ppm), and a trace of aldrin were also found in several of the specimens. The findings further illustrate the almost universal presence in fish and wildlife of certain widely used, persistent chlorinated hydrocarbon insecticides.

SPORT FISHERY INVESTIGATIONS

by

Oliver B. Cope
Fish-Pesticide Research Laboratory
Denver, Colorado

Investigations on fish and pesticides underwent expansion during 1964 while moving toward the primary objective of the laboratory--to learn about the relationships of fish, pesticides, and the environment so that predictions can be made of the consequences when pesticides enter the aquatic situation. The inauguration of new work at new places brought the program closer to a balanced effort that should ultimately lead to the understanding we need to protect fishery resources from pesticidal harm and to use pesticides safely as tools in fishery management.

At the beginning of 1964, work was proceeding at Denver, Colorado; Tishomingo, Oklahoma; Marion, Alabama; Laurel, Maryland; La Crosse, Wisconsin; Jackson, Wyoming, and Beulah, Wyoming. During 1964, new activities began at Sandy Hook, New Jersey; Longview, Washington; Willard, Washington, and Seattle, Washington.

The work reported here is presented in the following categories: Laboratory Studies and Toxicology; Experimental Field Studies; Effects on Wild Populations.

LABORATORY STUDIES AND TOXICOLOGY

Fish Toxicity Tests at Denver

Bioassay work at Denver was directed toward testing new pesticides against bluegills, rainbow trout, and channel catfish, and also toward time-temperature studies with these species and insecticides and herbicides.

1. Results of bioassay tests with insecticides appear in Table 1, those for herbicides are in Table 2, and those for miscellaneous chemicals in Table 3.

2. Results of studies on the influence of time and temperature on the toxicities of insecticides to rainbow trout are presented in Table 4, and to bluegills in Table 5. With endrin, toxaphene, and lindane, toxicity increased with increase of temperature; this was not true with DDT or malathion.

Fish Toxicity Tests at Sandy Hook

Testing of 7 organochloride insecticides against a variety of fishes, grass shrimp, and mud snail was carried forward in the marine laboratory at Sandy Hook, N.J. Effects of salinity and temperature on toxicity were measured on these estuarine animals. It was found that higher temperatures accelerated mortality for the toxicants tested, except that Fundulus heteroclitus were most sensitive in the 20°-25° C. range and showed lowered sensitivity outside of this range. Fundulus were most sensitive at intermediate salinities, eels had increased sensitivities with increased salinity, and grass shrimp showed decreases in mortality with increased salinity. Exposures of Fundulus to LC_{75} (24-hour) concentrations of chlorinated hydrocarbon insecticides for periods of 15 to 2880 minutes showed negligible mortality when exposed less than 2 hours and held 21 days, relatively high mortality when exposed around 6 hours, and almost complete kill with exposures of 12 hours or greater.

Fish Toxicity Tests at Patuxent

The Fish-Pesticide Research Laboratory substation at Patuxent conducted studies on the effects of water hardness on the toxicities of herbicides to fish and tested new herbicides for toxicity to four species of fish.

In extensive tests on water hardness, it was concluded that water hardness did not significantly influence toxicity for the combinations

(Continued on p. 58)

Table 1. Toxicity measurements of various insecticides versus fish, 1964

Insecticide	Species	Wt. in grams	Temp., °F.	Estimated LC50, µg/liter 24 hrs	48 hrs	96 hrs
Aldrin, Tech	N. Z. Rainbow	3.52	55	36	31	31
Aldrin, Tech	Bluegills	1.1	75	9.6	7.4	5.2
Allethrin, Tech	Rainbow	0.90	55	20	19	19
Amer. Cyan. #52160	Rainbow	0.80	55	1,900	1,500	1,000
Aramite, Tech	Rainbow	0.90	55	730	390	320
Bayer 73, Tech	Rainbow	1.40	55	680	520	320
Bayer 9018, Tech	Rainbow	1.40	55	620	460	320
Bayer 29493, Tech	Rainbow	1.40	55	840	800	760
Bayer 37289, Tech	Rainbow	1.40	55	1,200	420	240
Bayer 37344, Tech	Rainbow	1.32	55	1,200	820	640
Bayer 37344, Tech	Bluegills	0.97	75	380	130	110
Bayer 38819, Tech	Rainbow	1.40	55	580	500	450
Bayer 41831, Tech	Rainbow	1.40	55	1,100	1,000	700
Ben Venue #35, Tech	Rainbow	0.80	55	8,000	3,500	3,000
Ben Venue #3C35 Tech	Rainbow	0.80	55	750	720	380
Ben Venue #52, Tech	Rainbow	0.80	55	700	540	480
Ben Venue #54, Tech	Rainbow	0.80	55	390	540	480
Chlordane, Tech	Rainbow	0.90	55	22	10	7.8
Chlordane	Bluegills	0.87	75	58	49	40
Diazinon, Tech	Rainbow	1.20	55	380	170	90
Diazinon, Tech	Bluegills	0.87	75	52	30	22
Dieldrin, Tech	N. Z. Rainbow	3.52	55	19	15	13
Dieldrin, Tech	Bluegills	1.1	75	5.5	3.4	2.8
DDD, Tech	Bluegills	0.87	75	56	45	42
DDVP, Tech	Bluegills	0.87	75	1,000	700	480

Insecticide	Species	Wt. in grams	Temp., °F.	Estimated LC_{50}, µg/liter 24 hrs	48 hrs	96 hrs
Dylox, Tech	Rainbow	1.65	55	28,000	3,200	1,400
Dylox, Tech	Bluegills	0.97	75	5,600	1,600	260
Dibrom, Tech	Bluegills	0.87	75	220	220	180
Dimethoate, Tech	Bluegills	0.33	75	28,000	9,600	6,000
Endrin, Tech	N. Z. Rainbow	3.52	55	1.8	1.2	0.86
Endrin, Tech	Bluegills	1.1	75	0.35	0.27	0.25
Ethyl guthion, Tech	Rainbow	1.40	55	49	23	19
Heptachlor, Tech	Rainbow	0.52	55	15	9	8
Kepone, Tech	Rainbow	1.2	55	66	38	20
Lindane, Tech	Rainbow	0.70	55	30	22	22
Ovex, Tech	Rainbow	0.90	55	860	720	620
Perthane, Tech	Rainbow	0.70	55	9	7	5
Phosdrin, Tech	Rainbow	0.90	55	34	17	12
Phosdrin, Tech	Bluegills	0.87	75	41	37	23
Sevin, Tech	Bluegills	0.87	75	3,400	2,500	2,000
Shell SD-7438, Tech	Rainbow	0.52	55	30	30	30
Shell SD-7438, Tech	Bluegills	1.2	75	250	250	250
Shell SD-9129, EC	Rainbow	0.52	55	12,000	7,000	4,900
Shell SD-9129, EC	Bluegills	1.2	75	23,000	8,700	4,000
Stauffer N-2790, Tech	Rainbow	1.65	55	110	32	19
Stauffer N-2790, Tech	Bluegills	0.97	75	45	24	6.2
Strobane, Tech	Bluegills	0.87	75	15	10	8.4
Union Carbide, UC 10854	Rainbow	0.52	55	240	200	180
Union Carbide, UC 10854	Bluegills	1.2	75	180	140	110
Union Carbide, UC 21149	Rainbow	0.52	55	1,000	560	560
Union Carbide, UC 21149	Bluegills	1.2	75	110	65	50

Table 2. Toxicity measurements of various herbicides versus fish, 1964.

Herbicide	Species	Wt. in grams	Temp., °F.	Estimated LC_{50}, µg/liter		
				24 hrs	48 hrs	96 hrs
Casoron, WP	Rainbow	1.32	55	23,000	22,000	18,000
Casoron, WP	Bluegills	1.20	75	22,000	20,000	10,000
Copper chloride, Tech	Bluegills	1.04	75	1,100	1,100	980
Copper sulfate, Tech	Bluegills	1.04	75	2,800	2,800	2,800
Dalapon, Tech	Bluegills	1.04	75	115,000	115,000	105,000
4(2-4) DB, Tech	Rainbow	2.64	55	13,500	7,000	5,400
Dead-X, EC	Rainbow	2.64	55	10,000	9,400	8,800
Dead-X, EC	Bluegills	1.04	75	14,000	13,000	9,200
2,6 dichlorobenzoic acid, Tech	Rainbow	2.64	55	148,000	140,000	140,000
2,6 dichlorobenzoic acid, Tech	Bluegills	1.04	75	120,000	120,000	120,000
Diuron, Tech	bluegills	1.04	75	12,000	7,400	4,000
Dylox, Tech	Bluegills	0.97	75	5,600	1,600	260
Dymid, WP	Bluegills	0.97	75	92,000	80,000	75,000
Dymid, WP	Rainbow	1.65	55	150,000	110,000	97,000
Fenac acid, Tech	Bluegills	0.87	75	61,000	50,000	41,000
Fenac sodium salt, WP	Bluegills	0.87	75	26,000	19,000	14,000
Hydram, Tech	Rainbow	1.65	55	540	290	200
Hydram, Tech	Bluegills	0.97	75	600	475	355
IPC, Tech	Bluegills	0.33	75	32,000	32,000	29,000
Simazine, WP	Bluegills	1.04	75	130,000	118,000	118,000

Table 2 (Cont.). Toxicity measurements of various herbicides versus fish, 1964.

Herbicide	Species	Wt. in grams	Temp., °F.	Estimated LC_{50}, µg/liter 24 hrs	48 hrs	96 hrs
Simazine, Tech	Rainbow	1.20	55	68,000	60,000	56,000
Sodium arsenite, Tech	Rainbow	2.64	55	100,000	60,000	26,000
Sodium arsenite, Tech	Bluegills	1.04	75	58,000	44,000	30,000
Stauffer R-1910, Tech	Rainbow	1.32	55	3,700	3,700	3,600
Stauffer R-1910, Tech	Bluegills	0.97	75	8,000	5,600	5,500
Stauffer R-4461, Tech	Rainbow	1.62	55	960	730	720
Stauffer R-4461, Tech	Bluegills	1.04	75	970	810	810
2(2,4,5)TP, Tech	Rainbow	2.64	55	23,000	21,900	14,800
2(2,4,5)TP, Tech	Bluegills	0.65	75	19,500	14,500	9,600
4(2,4)TP, Tech	Bluegills	1.04	75	20,000	15,500	8,600
Treflan, EC	Rainbow	1.32	55	14	11	10
Treflan, EC	Bluegills	1.20	75	23	20	18
Trefmid, WP	Rainbow	1.32	55	220	170	110
Trefmid, WP	Bluegills	0.97	75	920	660	345
Trifluralin, Tech	Bluegills	0.97	75	100	96	68
Trifluralin, Tech	Rainbow	3.52	55	210	130	86
Vernam, Tech	Bluegills	0.80	75	9,700	9,200	4,000

Table 3. Toxicity measurements of various chemicals versus fish, 1964.

Chemical	Species	Weight in grams	Temp., °F	Estimated LC_{50}, µg/liter		
				24 hrs	48 hrs	96 hrs
American Cyanamid CL 46676 (Avicide)	Rainbow	2.64	55	11,800	10,600	8,000
American Cyanamid CL 46676 (Avicide)	Bluegills	0.65	75	23,000	20,500	7,000
Roccal	Bluegills	0.39	75	550	510	310
Xylene	Bluegills	0.80	75	36,000	19,000	19,000
APO(TEPA) (chemosterilant)	Rainbow	0.80	55	No effect at 100,000 for 96 hrs		
Union Carbide UC 19786, Tech (miticide)	Bluegills	1.1	75	14	13	13
Union Carbide UC 20047A, Tech (miticide)	Bluegills	1.1	75	>10,000	6,500	4,400
Union Carbide UC 19786, Tech (miticide)	Rainbow	1.01	55	15	14	14
Union Carbide UC 20047A, Tech (miticide)	Rainbow	1.01	55	28,000	12,000	11,000

Table 4. Effects of time and temperature on the toxicities of four insecticides to rainbow trout of approximately 1 gram in average weight.

Temperature, °F	Toxicant	LC_{50}, µg/liter		
		24 hrs	48 hrs	96 hrs
35	Endrin	14.5	6.8	2.4
45	DDT	7.5	4.7	4.1
	Toxaphene	16.0	8.4	5.4
	Malathion	100	79	77
	Endrin	5.2	2.4	1.4
55	DDT	8.2	5.2	5.0
	Toxaphene	7.6	4.4	2.7
	Malathion	85	70	68
	Endrin	2.8	1.9	1.1
65	DDT	12.0	7.3	6.0
	Toxaphene	5.0	2.8	1.8
	Malathion	130	120	110
	Endrin	1.5	1.2	0.75

Table 5. Effects of time and temperature on the toxicities of four insecticides to bluegills of approximately 1 gram in average weight.

Temperature, °F	Toxicant	LC_{50}, µg/liter		
		24 hrs	48 hrs	96 hrs
45	endrin	6.2	1.6	0.7
	lindane	160	88	65
	dieldrin	54	34	16
	aldrin	130	26.4	9.7
55	endrin	3.2	1.4	0.7
	lindane	100	75	53
	dieldrin	40	26	18
	aldrin	36.8	12.5	7.7
65	endrin	1.4	0.7	0.4
	lindane	100	76	56
	dieldrin	24	18	14.5
	aldrin	16.4	8.3	6.2
75	endrin	0.8	0.6	0.4
	lindane	100	53	38
	dieldrin	14	11	9.3
	aldrin	9.3	6.7	5.6
85	endrin	0.3	0.2	0.2
	lindane	34	27	25
	dieldrin	10	8.4	7.1

tested, except in the case of silvex and black bullhead; here, toxicity in the hardest water was double that in the softest water.

Invertebrate Toxicity Tests at Denver

Bioassay with aquatic insects and pesticides at Denver resulted in an enlargement of our catalogue of toxicities in the laboratory situation. During the year we established LC_{50} values for 24, 48, and 96 hours with 19 insecticides and 12 herbicides for the stonefly nymph, Pteronarcys californica, and numerous insecticides for the mayfly Baetis, the stoneflies Isoperla and Claassenia, the damselflies Amphagrion and Ophiogomphus, the snipefly Atherix, the cranefly Tipula, and a caddisfly of the family Limnephilidae. Some work was also done with Planaria and Daphnia. The order of toxicity for these invertebrates is very different than that for fish, and time is more important with the aquatic invertebrates than with fish.

Table 6 shows 1964 toxicity measurements with insecticides and nymphs of the stonefly Pteronarcys; values for herbicides appear in Table 7.

Table 6. Toxicity measurements of various insecticides versus nymphs of the stonefly Pteronarcys, tested at 60° F., 1964.

Insecticide	LC_{50}, µg/liter		
	24 hrs	48 hrs	96 hrs
Lindane	5.0	1.7	1.0
Allethrin	9.0	5.6	2.1
Pyrethrin	10.0	6.4	1.0
Bayer 37289	13.0	4.5	0.1
Bayer 37344	20.0	16.0	5.4
Ethyl guthion	23.0	8.0	2.0
DDVP	26.0	10.0	0.1
Ethyl parathion	28.0	11.0	5.1
Methoxychlor	30.0	8.0	1.4
Sevin	30.0	15.0	4.8
Bayer 41831	32.0	16.0	3.8
Strobane	40.0	7.0	0.5
Phosdrin	55.0	8.8	4.9
Baytex	130.0	39.0	4.4
Diazinon	150.0	74.0	25.0
Chlordane	170.0	55.0	15.0
Dylox	310.0	140.0	35.0
Dimethoate	510.0	140.0	43.0
Rotenone	2900.0	900.0	250.0

Table 7. Toxicity measurements of various herbicides versus nymphs of the stonefly Pteronarcys, tested at 60° F., 1964.

Herbicide	LC_{50}		
	24 hrs	48 hrs	96 hrs
Hydram	2,200 µg/l	700 µg/l	370 µg/l
Kuron	5,600 "	760 "	320 "
Esteron 99	8,500 "	1,800 "	1,600 "
Dead-X (95% Naptha)	11,000 "	5,000 "	2,000 "
Treflan	13,000 "	4,200 "	3,000 "
Casoron	42,000 "	8,400 "	6,600 "
Dichlorophenoxy Butyric Acid	56,000 "	48,000 "	15,000 "
Fenac (acid)	160,000 "	68,000 "	56,000 "
Fenac (sodium salt)	270,000 "	80,000 "	47,000 "
Sodium arsenite	160,000 "	110,000 "	45,000 "
Paraquat	>1,000 mg/l	>1,000 mg/l	>1,000 mg/l
Dalapon (sodium salt)	>1,000 "	>1,000 "	>1,000 "

Selective Breeding at Beulah, Wyoming

Preliminaries were done at the Fish Genetics Laboratory at Beulah, Wyoming, in connection with selective breeding studies to learn if changes in resistance of New Zealand strain rainbow trout to DDT can be measured. The assembly of testing, holding, and spawning facilities was undertaken toward the end of the year, and toxicity measurements with DDT and the New Zealand strain were made at Denver in preparation for exposures at Beulah.

Intermediary Metabolism Studies at Willard, Washington

A beginning was made at the Western Fish Nutrition Laboratory at Willard, Washington, on studies in intermediate metabolism in salmonids. Three staff members have begun work on normal metabolism prior to measuring effects of exposure to pesticides.

EXPERIMENTAL FIELD STUDIES

Malathion and Cutthroat Trout at Jackson, Wyoming

Phase II of the experiment involving feed and bath exposures of adult cutthroat to malathion began in the first quarter.

Brain cholinesterase activity followed a definite pattern throughout the year. Control fish exhibited a steady decline in activity with increase in size of the fish, and showed about half the activity of the fingerlings when

the experiment began in February 1963. After each treatment, brains of treated fish displayed declines in activity, followed by recovery over 30-day periods. The fish exposed to the most malathion sustained the most inhibition throughout the experiment. The fish showing the greatest decline in the November 1964 treatment had cholinesterase activities that were 41 percent of those of the control fish. Table 8 presents measurements of cholinesterase activity.

No significant differences among lots have yet appeared for growth rates or for day-to-day mortality. Histopathology studies show liver lesions appearing within 2 days after exposure and disappearing in the next 30 days. Apparently the changes seen in cholinesterase activity have little permanent physiological significance, according to Dr. Wood's cytologic evaluation.

Mirex and Bluegills at Marion, Alabama

A feeding experiment and a contact experiment were conducted at Marion, Alabama, with bluegills and Mirex, an insecticide with low acute toxicity for fish and high efficiency as an ant killer. Since it is widely used in the Southeastern U.S., our studies were aimed at measuring chronic effects on pondfish.

The feeding experiment featured weekly rations of Mirex in the diet to 13-gram bluegills in plastic pools. Each concentration, 5.0,

Table 8. Effects of exposure of cutthroat trout to malathion, Phase II; cholinesterase activity, means of 40 brains, expressed as micromoles of acetylcholine hydrolyzed per 2 mg. of brain tissue in 30 minutes at 25° C.

	I	II	III	IV	V
	Control	1.0 ppm bath	0.6 ppm bath	8 mg/kg in feed	4 mg/kg in feed
Pretreatment	.82 ± .16	.89 ± .11	.88 ± .11	.94 ± .11	.90 ± .13
2 days after treatment 1	.88 ± .11	.52 ± .14	.70 ± .12	.36 ± .16	.52 ± .22
30 days after treatment 1	.92 ± .10	.80 ± .14	.87 ± .12	.69 ± .10	.83 ± .12
2 days after treatment 2	.88 ± .09	.45 ± .11	.52 ± .11	.28 ± .12	.48 ± .19
30 days after treatment 2	.86 ± .15	.66 ± .09	.82 ± .10	.64 ± .10	.80 ± .15
2 days after treatment 3	.80 ± .09	.34 ± .11	.46 ± .12	.22 ± .13	.38 ± .18
30 days after treatment 3	.78 ± .13	.48 ± .10	.59 ± .08	.57 ± .11	.64 ± .12
2 days after treatment 4	.76 ± .12	.33 ± .13	.50 ± .14	.34 ± .15	.36 ± .18
30 days after treatment 4	.74 ± .09	.57 ± .13	.62 ± .12	.56 ± .14	.55 ± .10
2 days after treatment 5	.70 ± .12	.41 ± .11	.52 ± .12	.29 ± .15	.42 ± .17
30 days after treatment 5	.69 ± .13	.53 ± .09	.54 ± .11	.50 ± .09	.60 ± .12

3.0, 1.0, was fed weekly in two ponds each. When the experiment was terminated in December, no differences among lots were seen in total serum protein, electrophoresis patterns, microhematocrit, length or weight of fish. Whole-body residues of Mirex were highest in the fish fed 5 mg/K, averaging 3.39 ppm at 14 days; the fish fed 3 mg/K had 3.01 ppm, and the fish fed 1 mg/K had 1.05 ppm.

The experiment with Mirex and bluegills in earthen fish ponds had 2 ponds treated at 1 ppm, 2 at 0.0013 ppm, and 1 control. When the ponds were drained in November, the smallest average size, the lowest hematocrit percentage, and the lowest serum protein values were found in the control pond, but perhaps not significantly so.

Residues of Mirex in fish, through the 7-day samples, showed the highest accumulations in the highest-treated ponds. Rate of storage was extremely rapid in all treated fish. Residues of Mirex in bottom muds were lower than in the fish, but in the aquatic vegetation Mirex residues were much greater than those in fish.

Numbers of aquatic invertebrates sampled with plate samplers and dredges were approximately the same in all ponds.

Sodium Arsenite and Bluegills at La Crosse, Wisconsin

A study initiated in 1963 at La Crosse featuring long-term, sublethal exposures of bluegills to various amounts of sodium arsenite was continued in the laboratory in 1964 with work on residue analyses, histopathology, processing of bottom fauna and plankton, and assembling of data.

Analyses for residues of arsenic in whole fish showed accumulations in all fish. Adults and immatures stored arsenic at about the same rates, and the heavier the treatment, the higher the residues. Residues in organs of fish from a group that contained whole-body residues of 2.61 ppm at 16 weeks were: flesh, 1.36 ppm; skin and scales, 2.41; gills and digestive tract, 17.60; liver, 11.60; kidney, 5.89; ovaries, 8.39.

Arsenic residues in water collected at 16 weeks were correlated with treatment level, the largest being 9.04 ppm in the pond treated once a week at 0.69 ppm. In the bottom soils, the same pattern existed, with 109.9 ppm in the pond of heaviest treatment. Disappearance of arsenic was more rapid at low-treatment levels in water than in soils, compared with high-treatment levels.

No pathology appeared in fish in the first few weeks, but kidney and liver damage appeared thereafter, along with degenerative lesions in the ovaries. Nematodes, whose incidence was high in the pyloric caecae at the beginning of the experiment, disappeared after 2 weeks of exposure.

There was a reduction in bottom fauna in the heavily treated ponds, with numbers of organisms per sample amounting to less than half of those in the control and lightly treated ponds. There was a trend toward reduction of numbers of species with increase in concentration of sodium arsenite. Plankton samples showed depression of numbers of rotifers, cladocera, and copepods, especially in the heavily treated ponds.

Diquat and Bluegills at La Crosse, Wisconsin

An experiment to measure chronic effects of the herbicide Diquat to adult and immature bluegills was carried on at La Crosse, Wisconsin, from May to August. Concentrations of 1.0 and 3.0 ppm of Diquat were used at various frequencies.

Samples of fish, water, plankton, bottom organisms, and mud were collected at intervals. Fish are being studied for residue content, pathology, and changes in blood constituents. Residues in water from pools are summarized in Table 9. Bottom organisms apparently were not reduced in numbers by Diquat.

Survival of adult and immature bluegills was not affected by Diquat. Growth of immature bluegills was not influenced by the herbicide, but adults had slightly less weight gain in treated pools than in the controls.

Casoron and Bluegills at Tishomingo, Oklahoma

The herbicide Casoron (dichlobenil) was studied at Tishomingo, Oklahoma, with respect to long-term effects on bluegills. The weed-killer was applied in earthen fishponds

Table 9. Residues of Diquat measured in water samples from pools at La Crosse, Wisconsin.

Pool No.	Concentration of Diquat (ppm)	Diquat Residues (ppm) Days after first application					
		3	10	21	42	84	168
1	1.0	0.57	0.16	ND[1/]	ND	ND	ND
6	1.0	0.65	0.13	ND	ND	ND	ND
5	3.0	2.52	1.03	0.12	ND	ND	ND
7	3.0	2.39	0.76	0.07	0.01	ND	ND

[1/] ND denotes none detectable.

at rates of 40, 20, 10, and 0 ppm as a wettable powder. Mortality up to about 25 percent occurred immediately in heavily treated ponds, but was lighter in other treated ponds.

Residues of Casoron in the waters declined rapidly after the 3-day sample, and only minute amounts were found in the water at 85 days. Concentrations of Casoron in bottom sediments at 7 weeks were 0.2 ppm or less. Regrowth of aquatic vegetation was inversely proportional to strength of treatment.

When the experiment was terminated in the field, the largest fish were found in one of the ponds treated at 40 ppm. At this time, immatures were found in all ponds, with the smallest numbers associated with high-treatment.

Casoron and Warm-Water Fish at Denver

A study of Casoron, bluegills, smallmouth bass, yellow perch, and green sunfish was made in one small pond near Denver. The formulation was granular, at 10 pounds per acre (0.6 ppm), in one application.

Residues in the water reached a peak at 16 days (0.32 ppm at the surface and 0.54 beneath the surface), and there was still 2 ppb at 166 days.

No acute mortality to fish was seen to result from exposure to Casoron. Residues of the herbicide were measured in all four species; bluegill and yellow perch, and green sunfish generally accumulated greater residues than did the black bass, but all species showed 4 - 8 ppm at 34 days.

Paraquat and Fish at Denver

The herbicide Paraquat was studied in a small pond near Denver to measure persistence and chronic effects on rainbow trout, green sunfish, bluegills, and channel catfish. Treatment was at 1 ppm; the peak of residue in the water was during the first day, and no Paraquat was detectable at 32 days.

No acute toxicity to fish was seen. Residues developed in all fish measured, with peaks of accumulation seen from 1 to 16 days, depending upon the species. No pathology attributable to Paraquat has been seen in the 40 specimens examined so far.

EFFECTS ON WILD POPULATION

Spruce Budworm Control

The U.S. Forest Service applied an experimental airplane treatment of 1/4 pound per acre of Cygon (dimethoate) in July to test its utility for spruce budworm control. The 1000-acre plot was located on the South Fork of

Iron Creek in the Salmon National Forest in Idaho. The spray pattern was complete, and the toxicant reached the streams in the area. Our staff was on hand and measured no effects on fish or aquatic invertebrates at the sampled stations.

GENERAL

1. Progress was made in the design of a fish-pesticide laboratory for Columbia, Missouri. Region III engineers had almost completed the detailed design work at the end of 1964. Well drilling was completed at the laboratory site, and the predicted flow of water is available.
2. Four new substations for pesticide work were established at existing Division laboratories. Staffing and development of facilities went forward at the Western Fish Disease Laboratory, Western Fish Nutrition Laboratory, Salmon-Cultural Laboratory, and the Sandy Hook Marine Laboratory.

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COMMERCIAL FISHERY INVESTIGATIONS

by

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Substantial progress was made by the Bureau of Commercial Fisheries in 1964 in assessing the impact of synthetic organic pesticides on the marine environment. The broad objective of this program is to determine to what extent fishery products are being damaged by the production or use of synthetic organic pesticides. Approximately half of the research projects were in the continuing program of ascertaining what concentrations of these chemicals have toxic effects on representative marine animals. The increased staff and budget made possible a broader investigation that emphasized the monitoring of pesticide residues in the environment, in the biota, and in processed food products.

A major portion of the investigations has been at the Biological Laboratory at Gulf Breeze, Florida. Projects concerned particularly with commercial fishery products were conducted at the Technological Laboratory in Pascagoula, Mississippi. One project, to determine the effects of programs for forest insect control on the salmon fishery, was conducted at the Biological Laboratory in Auke Bay, Alaska.

TECHNOLOGICAL LABORATORY PASCAGOULA, MISSISSIPPI

This project was initiated in July 1964 to survey pesticide residues in fish, shellfish, and other fishery products, and to determine if various processing techniques have any effect on residue levels. In addition, residue levels in fishery products at first point of landing are to be monitored to determine seasonal and areal differences in pesticide pollution.

The Technological Laboratory completed 74 residue analyses on fresh and processed fishery products. Two samples of processed oysters contained dieldrin at levels of 0.170 and 0.230 ppm; three samples of processed oysters contained endrin residues of 0.025 to 0.180 ppm. Three samples of shrimp and mullet contained trace amounts of BHC, and heptachlor epoxide. All other samples had residues only of DDT and its metabolites in measurable amounts. These data are summarized in table 1.

BIOLOGICAL LABORATORY, AUKE BAY, ALASKA

This laboratory has participated in a 4-year cooperative study to determine the effects of DDT on the biota in two watersheds which were sprayed experimentally to control the spruce budworm.

Field aspects of this study of the effects of a 1/4-pound-per-acre application of DDT were completed in August. After the spraying in June 1963, insect populations were completely annihilated, but 14 months later these populations had regained normal "prespray" levels

* This review is a digest of reports submitted by project leaders of the several individual studies.

Table 1.--Residues of DDT and its metabolites in fishery products. Samples are fresh except where indicated.

Region and kind of sample	Number of samples tested	Number of negative samples[1]	Range in residues in positive samples (ppm)
Gulf of Mexico Coast			
Fishmeal			
Processed	1	...	0.020
Oysters	5	...	0.020
Processed	14	8	0.014 - 0.070
Shrimp	6	5	0.063
Processed	6	1	0.020 - 0.070
Catfood			
Processed	3	...	0.094 - 0.127
Miscellaneous	11	4	0.020 - 0.098
Atlantic Coast			
Lobster tails and claws	1	...	0.289
Lobster heads	1	...	0.713
Cod fillets	5	...	0.041 - 0.232
Cod waste	5	...	0.072 - 1.650
Haddock fillets	9	9	...
Haddock waste	6	3	0.230 - 0.357
Tuna loins	1	...	0.681

[1] Less than 0.01 ppm

of abundance. The only persistent detectable effect of the spraying was a relatively high residual concentration in fish tissue of DDT (maximum 1.4 ppm) and its metabolites, DDE (maximum 2.6 ppm), and DDD (maximum 0.23 ppm). The physiological effects of these residues on the fish are unknown. Results of the 4-year study are being readied for publication.

BIOLOGICAL LABORATORY, GULF BREEZE, FLORIDA

The pesticide program at this laboratory has expanded during the year to include not only laboratory studies of acute and chronic toxicity levels but also the development of the methodology required for a national network to detect and measure pesticide pollution in shellfish areas.

Acute Toxicity

The laboratory techniques for evaluating the acute toxicity of pesticides to marine fauna have been fully described in earlier reports. Tests are conducted in flowing seawater, with the exception of those with phytoplankton. Stock solutions of most of the pesticides are made up in acetone, and test

animals are exposed to four or more dilutions. The effective concentration (affecting 50 percent of the test population), or EC_{50} value, is determined by graphical interpolation. In some tests, EC_{50} values were not determined because the solubility of the chemical limited the maximum concentrations that could be used. Salinity levels ranged from 17 to 27 parts per thousand and fluctuated with the tide during each experiment. Brief explanations of experimental conditions are given in the sections that follow.

Phytoplankton. The rate at which phytoplankton cells incorporate inorganic carbon into the cellular matrix can be precisely measured through the use of radioactive carbon. By mixing known amounts of C^{14} with two suspensions of phytoplankton, one of which contains a known concentration of pesticide, it is possible to measure the interference of the pesticide with growth in a stated period of time. This decrease in carbon fixation provides an index of productivity; the toxicities of various pesticides then may be compared. Table 2 summarizes the percentage decrease in productivity, as compared to controls, caused by the pesticides in 4 hours. Natural mixed phytoplankton communities were used in the tests.

Mollusca. Oysters, Crassostrea virginica, are so very responsive to environmental pollution that changes in their growth rates afford a good index of pesticide toxicity. Because small oysters grow at nearly uniform rates under similar conditions, it is possible to compare shell deposition (growth) in a control group with that in a group simultaneously exposed to a test chemical. The relative toxicity of a series of pesticides to oysters can then be expressed as EC_{50}, or the concentration of a chemical causing a 50-percent decrease in growth during a stated period--in these tests, 96 hours. Oysters surviving such treatment are transferred to unpolluted water and observations are made on the time required for growth rates to return to those shown by the controls. Data obtained in the current year are summarized in table 3.

Table 2.--Percentage decrease in productivity of phytoplankton during 4-hour exposure to different pesticides

Pesticide	Percentage decrease	Pesticide	Percentage decrease
Herbicides		Insecticides	
		Carbamate	
Dalapon, Na salt	0.0	Zectran	0.0
N-Serve	15.0		
Shell SD7961	0.0	Organophosphorus	
Silvex, polyglycol butyl ether ester	77.7	Ronnel	89.3
Sodium TCA	0.0	Shell SD8447	7.2
2,4,5-T, polyglycol butyl ether ester	89.4	Shell SD8448	10.0
Tordon 22K	8.4	Nematocide	
Tordon 101	0.0	Nellite	0.0
Veon 245	0.0		
Zytron	58.8		

Table 3.--Concentration of pesticides in sea water causing a 50% decrease in oyster-shell growth, EC_{50}, in 96 hours

Pesticide	Average water temperature (°C.)	96-hour EC_{50} (ppm) or percentage decrease in growth at 1.0 ppm [1/]	Recovery period (weeks)
Fungicides			
Dexon	21	ne	...
Difolatan	20	0.034	7+
Herbicides			
Acrolein	21	0.055	1
Ametryne	27	14%	1
Atrazine	28	ne	...
Dalapon, sodium salt	31	ne	...
Diquat	27	ne	...
Hydram	24	ne	...
Knoxweed 42	29	44%	1
N-Serve	10	0.28	1
Paraquat	20	ne	...
Prometone	28	ne	...
Prometryne	27	19%	1
Shell SD7961	12	ne	...
Silvex, polyglycol butyl ether ester	30	23%	1
Stauffer R-1910	24	ne	...
Stauffer R-4461	24	0.45	1
2,4,5-T polyglycol butyl ether ester	13	0.14	1
Tordon 101	30	ne	...
Veon 245	29	ne	...
Vernam	29	ne	...
Zytron	27	0.33	3
Insecticides			
Carbamate			
Zectran	9	ne	...
Chlorinated hydrocarbon			
BHC (45% gamma isomer)	27	0.36	1+
BHC (45% gamma isomer)	14	1.0	1
DDE	12	0.014	4
DDT + Strobane [2/]	26	0.022	3+
DDT + Toxaphene [2/]	26	0.030	4+
Strobane	14	0.02	3
Strobane	25	0.059	2
Strobane + Methyl parathion	23	0.026	3

Table 3.--Concentration of pesticides in sea water causing a 50% decrease in oyster-shell growth, EC_{50}, in 96 hours (continued)

Pesticide	Average water temperature (°C.)	96-hour EC_{50} (ppm) or percentage decrease in growth at 1.0 ppm 1/	Recovery period (weeks)
Organophosphorus			
Amer. Cyanamid 43,913	12	0.20	2
Amer. Cyanamid 52,160	13	35%	3
Amer. Cyanamid 52,160	28	0.042	2
Meta-Systox R	21	ne	...
Phorate (Thimet)	21	0.64	1
Ronnel	13	0.17	...
Shell 4072	16	0.60	1
Shell SD7438	17	0.10	...
Shell SD8447	17	ne	...
Shell SD8448	23	0.40	1
Shell SD9129	21	ne	...
Stauffer N-2790	25	0.33	1
Stauffer R-5092	30	ne	...
Nematocide			
Nellite	22	ne	...
Sea lamprey larvicide			
TFM	22	ne	...

1/ ne = No effect at 1.0 ppm

2/ Insecticide combinations currently being used in agriculture

Crustacea. Shrimp, the most valuable commercial fishery resource in the United States, spend part of their life span in estuarine areas susceptible to pesticide pollution. Since they are rather closely related to insects, the insecticides are particularly toxic to them. These chemicals may kill shrimp quickly or paralyze them so that they lose their sense of balance. Tests are conducted for 24 and 48 hours, in flowing seawater. In table 4 the pesticides are evaluated in terms of EC_{50}, the concentration causing death or loss of equilibrium within the stated period.

Fish. The sensitivity of estuarine fish to pesticides varies unpredictably with the species. The younger specimens are most quickly affected and, therefore, juveniles are used in the tests. Since not all species are available throughout the year, no comparative data are presented in this report. Summaries of experimental data on fish are given in table 5.

Table 4.--Concentration of pesticides in sea water causing 50% mortality or loss of equilibrium, 24- and 48-hour EC_{50}, to adult shrimp

Pesticide	Kind of shrimp[1/]	Average water temperature (°C.)	24-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm [2/]	48-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm [2/]
Defoliant				
DEF	B	30	0.028	0.028
Fungicides				
Dexon	B	14	ne [3/]	ne [3/]
Difolatan	B	15	—	—
Herbicides				
Acrolein	B	14	0.19	0.10
Ametryne	B	28	ne	10%
Atrazine	B	27	20%	30%
Dalapon, Na salt	B	29	30%	40%
Diquat	W	28	ne	ne
Diuron	B	29	ne	ne
Fenuron	B	29	10%	10%
Hydram	B	30	10%	30%
Knoxweed 42	B	29	40%	0.48
N-Serve	B	28	ne	ne
Paraquat	B	15	ne	ne
Prometone	P	26	ne	ne
Prometryne	P	26	ne	ne
Silvex, polyglycol butyl ether ester	B	30	0.28	0.24
Sodium TCA	B	27	ne	ne
Stauffer R-1910	B	30	ne	ne
Stauffer R-4461	B	30	10%	10%
2,4,5-T, acid	B	28	ne	ne
2,4,5-T, polyglycol butyl ether ester	B	28	10%	20%
Tordon 101	B	27	ne	ne
Veon 245	B	28	ne	ne
Vernam	B	28	ir	20%
Zytron	B	27	0.0015	0.0003

Table 4.--Concentration of pesticides in sea water causing 50% mortality or loss of equilibrium, 24- and 48-hour EC_{50}, to adult shrimp (continued)

Pesticide	Kind of shrimp[1/]	Average water temperature (°C.)	24-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm [2/]	48-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm [2/]
Insecticides				
Carbamate				
Zectran	B	27	0.0068	0.0052
Chlorinated hydrocarbon				
DDE	B	28	0.052	0.028
Organophosphorus				
Ronnel	B	27	0.015	0.0052
Shell SD7438	B	27	0.028	0.0024
Shell SD8447	P	26	0.42	0.28
Shell SD8448	P	26	0.28	0.032
Shell SD9129	B	21	0.32	0.069
Stauffer N-2790	B	29	0.0024	0.0019
Stauffer R-5092	B	28	0.0032	0.0028
Nematocide				
Nellite	B	27	ne	ne

1/ B = Brown shrimp, Penaeus aztecus; P = pink shrimp, P. duorarum; W = white shrimp, P. setiferus.

2/ ne = No effect at 1.0 ppm; ir = irritated at 1.0 ppm (see footnote 3 for exception).

3/ No effect at 0.1 ppm, the highest concentration that could be tested because of the limited solubility of the chemical.

Table 5.--Concentration of pesticides in sea water causing 50% mortality, 24- and 48-hour EC_{50}, to juvenile fish

Pesticide	Species of fish [1/]	Average water temperature (°C.)	24-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm [2/]	48-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm [2/]
Defoliant				
DEF	S	27	0.32	0.24
Fungicides				
Bayer 47531	S	13	0.032	0.032
Chemagro 2635	S	21	0.15	0.032
Chemagro 4497	S	24	0.032	0.032
Dexon	C	21	ne	ne
Difolatan	K	20	0.28	0.032
Herbicides				
Acrolein	K	21	0.28	0.24
Ametryne	S	28	ne	ne
Atrazine	S	28	ne	ne
Dalapon, Na salt	K	20	ne	ne
Diquat	K	19	ne	ne
Fenuron	S	25	ne	ir
Hydram	S	25	10%	20%
Knoxweed 42	S	25	ne	ne
Neburon	S	25	0.32	0.32
N-Serve	S	16	ne	ne
Paraquat	K	19	ne	ne
Prometone	S	26	ne	ne
Prometryne	S	28	ne	ne
Shell SD7961	S	16	ne	ne
Silvex, polyglycol butyl ether ester	S	16	0.41	0.36
Sodium TCA	M	28	ne	ne
Stauffer R-1910	S	25	ne	ne
Stauffer R-4461	S	25	0.32	0.32
2,4,5-T polyglycol butyl ether ester	S	16	0.32	0.32
Tordon 101	M	28	ne	ne
Veon 245	S	27	ne	ne
Vernam	S	28	ne	ne
Zytron	S	27	0.32	0.32

Table 5.--Concentration of pesticides in sea water causing 50% mortality 24- and 48-hour EC_{50}, to juvenile fish (continued)

Pesticide	Species of fish 1/	Average water temperature (°C.)	24-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm 2/	48-hour EC_{50} (ppm), or percentage mortality at 1.0 ppm 2/
Insecticides				
Carbamate				
Zectran	C	12	ne	ne
Chlorinated hydrocarbon				
DDE	S	12	ne 3/	ne 3/
DDT	C	9	1e 3/	0.005
DDT	L	21	0.028	0.018
Endrin	L	27	0.00079	...
Telodrin	S	13	0.0024	0.0003
Organophosphorus				
Amer. Cyanamid 43,913	S	13	ir	20%
Amer. Cyanamic 52,160	S	13	ne	ir
Bayer 37289	S	13	ir	0.32
Bayer 41831	C	9	ir	ir
CO-RAL	C	12	10%	0.28
Dibrom	L	21	0.13	...
Dylox	C	13	ne	ne
Ethion	C	12	0.42	0.069
Ronnel	S	13	0.42	0.32
Shell 4072	S	14	1e	1e
Shell SD7438	S	17	0.32	0.32
Shell SD8447	S	17	ir	ir
Shell SD8448	S	19	ir	1e
Shell SD9129	K	20	ne	ne
Stauffer N-2790	S	24	0.28	0.24
Stauffer R-5092	S	26	0.020	0.020
Nematocide				
Nellite	S	21	ne	ne
Sea lamprey larvicide				
TFM	S	19	ne 3/	ne 3/

1/ S = Spot, Leiostomus xanthurus; C = Sheepshead minnow, Cyprinodon variegatus; K = Longnose killifish, Fundulus similis; M = Striped mullet, Mugil cephalus.

2/ ne = No effect at 1.0 ppm; ir = irritated at 1.0 ppm; 1e = lost equilibrium at 1.0 ppm (see footnote 3 for exceptions).

3/ Maximum concentration tested, 0.1 ppm; effects noted refer to this concentration.

Chronic Toxicity

Mass mortalities in animal populations due to acute pesticide poisoning can usually be detected, explained, and in large measure prevented. Of more concern is the possibility that chronic, low levels of pesticide pollution in the estuary are causing equally serious but unnoticed changes in the fauna or the environment. Several laboratory studies have been completed under controlled conditions which provide data that help interpret the effects of chronic pollution.

Crustacea. Shrimp are particularly susceptible to the toxic effects of endrin; a concentration of 0.6 ppb will kill half of an experimental population in 24 hours. At one tenth of this concentration, shrimp will survive 10 days; further dilution to a level of 0.025 ppb will permit 15 percent of a population to survive 2 months. Survivors of the 2-month exposure contained whole body residues of less than 5.0 ppb of endrin.

Similar experiments with a small series of blue crabs showed that they can survive a 5-month exposure to DDT. Preliminary screening tests showed that a DDT concentration of 1.0 ppb will kill crabs in 8 days. At a concentration of 0.25 ppb, however, experimental crabs survived and grew as well as control animals. A crab that died after 4 months of exposure to 0.5 ppb of DDT had a body residue of 5.36 ppm of DDT and its metabolites.

Oysters. The biological accumulation of some pesticides by the oyster can be of extraordinary magnitude within a few weeks. DDT, for example, may be stored by the oyster during a 40-day exposure at levels 70,000 times greater than the 0.1-ppb concentration in the surrounding water. Oysters also concentrate such common pesticides as endrin, 2,4-D esters, and others, but to a much smaller extent.

A series of laboratory experiments was conducted to isolate the sites of DDT storage in the oyster. Earlier work had demonstrated that approximately 65 percent of the DDT residue was stored in the digestive tract, associated glands, and the gonads. More detailed analyses now show that a major part is stored in the gonads. It is reasonable to postulate that significant amounts might be stored in the gametes themselves. Here the residues would be in a critical position to affect the viability of the gametes and the course of larval development. Whether they do in fact still remains to be determined, but these studies clearly demonstrate the localization of DDT in the gametes of the spawning oyster.

Mature oysters were exposed to 1.0 ppb of DDT for 12 days. Appropriate analyses then showed that the whole oyster of either sex contained from 14 to 20 ppm of DDT. Eggs and sperm isolated and analysed separately contained equally large residues. The storage of DDT in the eggs is expected, in view of their high yolk content. DDT is typically stored in fatty tissues, and the whole body of the oyster is only 3 to 5 percent fat. The high residue in the spermatozoa needs to be explored further.

The logical sequel to this work is the culturing of contaminated larvae. This study was attempted but failed for technical reasons. In view of the work of others, showing 100 percent mortality in oyster larval cultures within 6 days on exposure to only 1.0 ppm of DDT, the probability that larvae containing 20 to 30 ppm of DDT can develop into normal animals is open to doubt.

Fish. Field observations have indicated that many species of animals now show a marked resistance to specific pesticides. Characteristic of these is the well-known example of the DDT-resistant house fly. It is important to know whether a fish can grow accustomed to a particular pesticide gradually, and whether, in the process, it may suffer some somatic or chromosomal damage.

Juvenile specimens of the spot were exposed to Telodrin, a highly toxic chlorinated hydrocarbon insecticide. Preliminary screening showed that a concentration of 0.025 ppb would kill the fish in 10 days. The fish were

able to survive 5 months, however, at a concentration of 0.01 ppb. At the end of this exposure period, no differences were apparent in growth or histology between control and experimental fish. At the end of the experiment the treated fish were just as sensitive as unexposed fish to previously determined lethal concentrations of the chemical.

In a second series, spot were exposed to a concentration of 0.05 ppb of endrin for 8 months. Double this concentration is usually lethal within 5 days. At the end of the exposure period, no changes in growth rates or histology were detected. Curiously, when exposed fish from the experiment were re-exposed to previously determined lethal concentrations of endrin, they were twice as sensitive as the control fish.

These experiments indicate that the development of resistant strains is best explained by the process of natural selection. Experiments were undertaken on this process. Small populations of the sheepshead minnow were exposed to sufficiently high levels of DDT so that fewer than 10 percent survived. These presumably resistant fish were permitted to breed under natural conditions. Their offspring, when tested for DDT resistance, were much more sensitive than the parent stock. A possible explanation of this unexpected result is that the germ plasm of the parents had been sensitized during the original exposure. These data do not refute the theory that resistant stocks are developed by natural selection. They do indicate that relatively large original populations are necessary before this process can be effective.

Development of Methodology for Monitoring

Mollusks. Earlier investigations at this laboratory demonstrated that oysters are particularly efficient in removing the chlorinated hydrocarbon pesticides from the environment and storing them as long as the concentration of the pollutant in the environment stays constant or increases. When the environmental pollution decreases, residues in the oyster gradually decrease. Under experimental conditions, the oyster stores pesticides present in the water at concentrations as low as 10 parts per trillion.

Consequently, an intensive investigation has been made of methods for using the oyster and other mollusks to monitor pesticide pollution of the marine environment. To establish the relationship between environmental pollution and pesticide residues, oysters and mussels were exposed to pesticides under controlled laboratory conditions. Twelve mussel samples and 53 oyster samples showed that, in general, the oyster was more effective in recording changes in environmental pollution levels. Oysters also are more adaptable to changing salinity, and consequently are the organism of choice in establishing monitor stations.

Wire bags containing oysters and mussels were placed at five stations along the salinity gradient in local estuaries. These oysters have been sampled at 15- and 30-day intervals and changes in DDT residues recorded during the year. A total of 161 analyses of mussels and clams, and 342 oyster analyses revealed that these bioassay animals indicate not only the different levels of pollution at different geographical locations but seasonal changes as well. As a result, a program is underway to establish oyster bioassay stations on Pacific, Atlantic, and Gulf coasts of the United States; these stations will be visited periodically by cooperating agencies. Analyses of this type will help identify sources of pesticide pollution and the extent to which fishery resources are being contaminated.

Crustacea. Twelve miscellaneous samples of crabs, shrimp, and other crustacea were examined for residues of chlorinated hydrocarbon pesticides. Four samples were processed shrimp caught in the vicinity of the Mississippi River delta; they contained no detectable residues. The remainder contained negligible amounts of DDT and its metabolites. One sample of ghost shrimp, Callianassa jamaicensis, collected at the site of a fish kill in Florida contained 6.13 ppm of DDT.

In controlled laboratory experiments, both crabs and shrimp stored relatively small amounts of endrin and DDT as residues. The crustacea, in general, appear to be unsatisfactory as monitoring animals.

Fish. The methodology of using resident fish populations as indicators of pollution from the chlorinated hydrocarbon pesticides in estuaries is being evaluated. Stations in upper and lower areas of the Pensacola Bay estuary were sampled at 1-month intervals during the year by collecting two different species of fish. Typically, residues of DDT and its metabolites were twice as great in the 1963 year class of fish as in the 1964 year class, and approximately four times as high in midsummer as in the winter. It was found necessary to composite samples of 10 or more fish to average out individual variations.

During the year, 152 regular and random samples, representing 19 species of fish, were analyzed. Six samples, mostly menhaden, contained small amounts of dieldrin and endrin. These and all other samples of freshly caught fish contained residues of DDT and its metabolites at levels ranging from 0.01 to 13.7 ppm. Moribund or dead fish obtained from sites of fish kills in Pensacola Bay and from the South Carolina coast contained DDT residues ranging from 0.30 to 5.72 ppm.

Pinfish, Lagodon rhomboides, were exposed to DDT under laboratory conditions to compare their tolerance to DDT in the water with residues accumulated in their tissues. Although DDT residues of 13 ppm or more have been observed in apparently healthy fish, an exposure to a concentration only a thousandth of this (0.01 ppb) kills 100 percent of an experimental group in 48 hours.

Estuarine pollution by the organophosphorus pesticides is less persistent in the environment and not as readily detected as the residues of the chlorinated hydrocarbons. Evidence exists, however, that these chemicals cause measurable decreases in cholinesterase enzyme levels in fish brain tissues. These decreases are quantitatively related to the amount of pollution experienced by the fish. Initial studies have established normal enzyme levels in two species of fish. These values appear to be nearly constant throughout the year and in different geographical areas.

Under laboratory conditions, significant and reproducible decreases in enzyme activity can be caused by exposure of fish to some pesticides at concentrations as low as 0.5 ppb. This depression in activity may persist 1 week or longer. Routinely used, this test gives promise of being a good indicator of organophosphorus pesticide pollution in the marine environment.

Miscellaneous. Random samples of water, silt, and vegetation were analyzed for DDT to determine their suitability in monitoring the environment. Eight of 25 samples were negative; the remainder contained negligible DDT residues.

It has become apparent that a systematic and periodic collection of samples is necessary to an understanding of the importance of pesticide residues found at a given location. Analyses made at regular intervals show whether the contamination is increasing or decreasing, and indicate possible sources of the pollution.

FUTURE CONSIDERATIONS

The Bureau of Commercial Fisheries research program on pesticides in 1964 has demonstrated certain facts which are summarized and stressed at this point:

1. Many marine animals have a sharply defined threshold response to pesticide concentrations. This may permit the use of some urgently needed control chemicals in restricted areas without endangering nontarget animals.

2. It is possible that pesticide residues which are not acutely toxic may have subtle long-lasting effects on the animal or its progeny. Research in this area should be

augmented to discover the extent to which present management methods are harming the environment beyond repair.

3. The oyster has been demonstrated to be extremely sensitive in detecting low levels of chlorinated hydrocarbon pesticides in the estuarine environment. Its use to monitor seasonal pollution should be expanded to include a majority of our important shellfish areas.

PUBLICATIONS

The following papers were published or in press in 1964.

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