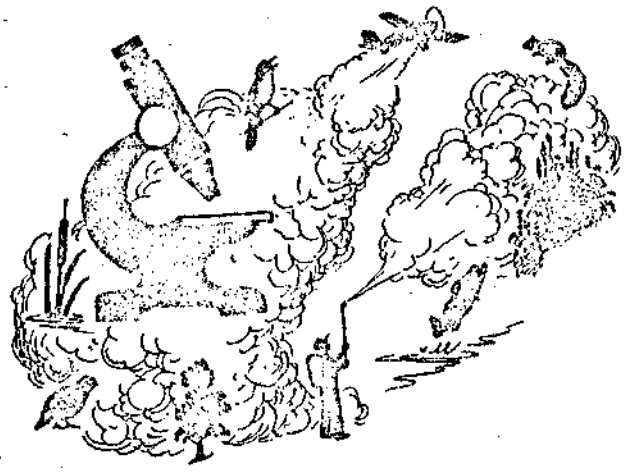
Item ID Number	02989 ☐ **Not Scanned**
Author	Butler, Philip A.
Corporate Author	
Report/Article Title	Commercial Fisheries Investigations
Journal/Book Title	
Year	1963
Month/Day	
Color	☐
Number of Images	16
Descripton Notes	Circular 167

PESTICIDE-WILDLIFE STUDIES

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



UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, Secretary
FISH AND WILDLIFE SERVICE
Circular 167

COMMERCIAL FISHERIES INVESTIGATIONS

by

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

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

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

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

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

Laboratory Studies and Toxicology

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

Phytoplankton

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

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

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

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

Crustacea

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

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

After exposure to lethal or near-lethal concentrations of toxic materials, crabs and shrimp often remain in a moribund condition for

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

Mollusks

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

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

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

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

In order to determine the recovery rate of oysters surviving a short-term exposure to pesticides, experimental oysters showing less growth than the controls at the termination of a screening experiment

were transferred to unpolluted running sea water. They were maintained there until their growth rate was restored to the same level as control oysters. This recovery period varied from 1 to 4 weeks and in most cases appeared to be independent of the severity of toxicity. These data indicate that for the chemicals tested, a single field application which might temporarily affect oyster growth would probably not have a lasting effect.

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

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

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

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

Fish

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

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

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

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

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

Future Considerations

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

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

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

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

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

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

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

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

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

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

(Continued)

*Tests performed in standing water aquaria.

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

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

*Tests performed in standing water aquaria.

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

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

(Continued)

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

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

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

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

(Continued)

*Tests performed in standing water aquaria.

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

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

(Continued)

*Tests performed in standing water aquaria.

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

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

*Tests performed in standing water aquaria.