Woodworth

# PESTICIDE-WILDLIFE STUDIES, 1963

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


**UNITED STATES DEPARTMENT OF THE INTERIOR**

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

## ABBREVIATIONS USED

| | |
|---|---|
| $EC_{50}$ | median effective concentration - the concentration of toxicant in the environment which produces a designated effect on 50 percent of the organisms exposed to it. |
| $ED_{50}$ | median effective dose - the amount of toxicant (usually measured in mg/kg) that produces a designated effect to 50 percent of the population of organisms receiving the dose. |
| $LC_{50}$ | median lethal concentration - the concentration of toxicant in the environment which kills 50 percent of the organisms exposed to it. |
| $LD_{50}$ | median lethal dose in amount of toxicant lethal to 50 percent of the animals to which it is administered under the conditions of the experiments. |
| mg/kg | milligrams per kilogram |
| ppb | parts per billion |
| ppm | parts per million |
| TLm | median tolerated limit - the concentration which produces mortality to 50 percent of the tested population in a given period of time. |
| µg/g | micrograms per gram. |
| µg/l | micrograms per liter. |



# COMMERCIAL FISHERY INVESTIGATIONS

by

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

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

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

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

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

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

## ACUTE AND CHRONIC TOXICITY STUDIES

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

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

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

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



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

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

### Phytoplankton

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

### Crustacea

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

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

Mollusks

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

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

Fish

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



Chronic exposure of fish to pesticides

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

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

Bioassay techniques

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

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

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

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

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

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

## RESIDUE STUDIES

### Uptake and retention of pesticides by shellfish

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

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

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

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



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

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

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

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

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

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

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

Accumulation of DDT in other marine animals

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

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

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

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



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

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

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

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

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

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

Effects of DDT in a tidal marsh

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

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

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

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

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



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

## FOREST INSECT CONTROL PROGRAMS

Hemlock looper

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

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

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

Spruce budworm

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

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

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

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

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

** Nymphal skins

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

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



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

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

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

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

## BENEFICIAL USE OF PESTICIDES

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

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

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

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

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

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

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

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

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

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



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

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

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

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

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

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

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

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

## FUTURE CONSIDERATIONS

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

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

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



## REPORTS

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

The following research reports have been approved for publication:

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

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

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

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



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

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

(continued)

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

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

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

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

2/ ne = no effect.

3/ ir = irritated.



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

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

(continued)

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

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

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

1/ ne = no effect.



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

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

(continued)

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

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

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

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

2/ ne = no effect

3/ ir = irritated



# SPORT FISHERY INVESTIGATIONS

by

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

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

## LABORATORY STUDIES AND TOXICOLOGY

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

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

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

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

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