Estuarine Pesticide Research—Bureau of Commercial Fisheries[1]

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Abstract

Pesticides that enter the estuarine environment can be accumulated by the fishery organisms that inhabit these fertile waters. Effects of pesticides on estuarine organisms and the fate of these chemicals in the estuarine environment are being studied at the Bureau of Commercial Fisheries (BCF) Pesticide Field Station, Gulf Breeze, Florida. Bioassay projects investigate the toxicity of new pesticides submitted by pesticide manufacturers and perform analyses requested by the Pesticide Review Board. Emphasis is placed on determining possible adverse effects of prolonged exposure (chronic studies) of fishery organisms to sublethal concentrations of selected pesticides. Ecological studies include the effect of a herbicide, Dichlobenil, on the ecology of an experimental pond. Experiments also are conducted to determine effects of sublethal levels of pesticides on the behavior of estuarine fish. For example, the capacity of fish to avoid water containing pesticides is tested in a special apparatus in the laboratory.

INTRODUCTION

THE BUREAU OF COMMERCIAL FISHERIES is vitally concerned about the harmful effects pesticides can have on estuarine organisms. Results of laboratory experiments on acute toxicity of pesticides to these organisms and other data indicate that residues of persistent pesticides are concentrated in the estuarine food web and may become toxic to predatory organisms. The evidence is clear that even low levels of pesticides (below those that cause mortality) can adversely affect the animals. In addition, commercial species are affected indirectly when their food organisms are killed or diverted to another habitat because of exposure to pesticides. This report discusses briefly the transport of pesticides to and within the estuarine environment and the Bureau's current research program to evaluate the effects of pesticides on estuarine fishery resources.

Pesticides can be classified according to their chemical structure or in reference to the animal or plant group which they are intended to control. Chlorinated hydrocarbon insecticides include such well-known chemicals as DDT and the aldrin-toxaphene group. These are persistent in water and sediments. Organophosphorous insecticides are much less persistent than the hydrocarbons and are degraded more rapidly in the environment. For this reason, they are usually preferred as control agents. Herbicides, such as 2.4-D, are now used in streams to control water hyacinths and are entering estuarine waters. Until 1964 more insecticides were sold than herbicides; but since then, sales in herbicides have taken the lead. Herbicides are generally less toxic to estuarine organisms than are insecticides.

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[1]Contribution No. 100

Pesticides found in the estuarine environment originate from three main sources: agricultural applications, applications to control noxious insects and effluents from industries that formulate pesticides. Often, pesticides applied to an agricultural crop to enhance food production reach the estuarine environment through "runoff" and erosion. Many tons of pesticides are applied on a world basis annually and more will be required if food production is to be increased. This increase in tonnage could have a profound effect upon many forms of life other than the target pest—for example, mortalities or such subtle effects as lowered rates of growth and reproduction in clams, oysters, crabs and fish. Pesticides used to control mosquitos, stable flies and other noxious insects reach and affect estuarine animals in a similar manner. Oysters in Santa Rosa Sound accumulated DDT that was used to control larvae of stable flies found in dead sea weed on the beach (Butler, 1968). Pesticides released from industrial manufacturers can cause isolated instances of gross pollution.

Pesticides can reach the estuarine environment through movement of water-borne materials, direct application and aerial transport. Undoubtedly, water transports most of the pesticides that enter estuaries. Water draining from fields treated with pesticides carries these chemicals in solution and attached to silt into streams and rivers that eventually flow into an estuary. Most chlorinated hydrocarbons are only slightly soluble in water but adsorb readily to suspended material such as silt particles and organic detritus. These suspended materials often flocculate when they enter the saline environment in an estuary and become part of the bottom sediment. Pelagic animals may eat pesticide-laden detritus or food organisms and transport the pesticide to other parts of the environment. Pesticides sprayed directly into estuarine areas to control noxious insects and plants create relatively high concentrations of the chemicals in the water. Aerial transport is probably the least effective mechanism for introducing pesticides in estuaries.

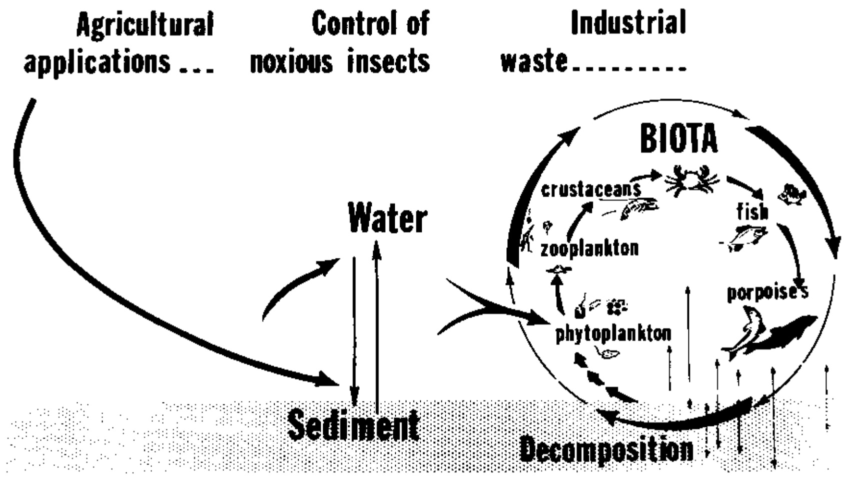

FIG. 1. Pesticides entering estuaries can become associated with water, sediment and biota.

In the estuary, pesticides become associated with one or more of three phases: water, sediment and biota (Fig. 1). Usually these chemicals are partitioned among all three phases, but accumulation by the biota is of particular interest. Organisms can obtain pesticides from the water and sediment. For example, phytoplankton can take up and concentrate pesticides directly from sea water. The pesticide is then transferred through the food web of the estuary. Residues of DDT in the sediment and organisms of a brackish marsh showed an increase in residues from phytoplankton to fish-eating birds (Woodwell, Wurster and Isaacson, 1967). Accumulation of DDT from organic detritus by fiddler crabs in a salt marsh was demonstrated by Odum, Woodwell and Wurster (1969). The particles of detritus contained residues thousands of times greater than the water, and crabs exposed to this material in the laboratory suffered loss of equilibrium. Other documentation of pesticides in estuaries is presented by Gray (1969). Thus, the potentially harmful effects of pesticides on estuarine fisheries is evident.

The Bureau of Commercial Fisheries began a research and monitoring program in 1958 at Gulf Breeze, Florida, to study the effects of pesticides on estuarine animals in the laboratory and to determine levels of polychlorinated pesticides in these animals in many of the nation's estuaries. Much information has been obtained on the relative toxicity of various insecticides to clams, fish, oysters, shrimp and other estuarine animals. Presently, investigators conduct pesticide research in three areas: bioassay, ecological studies and physiological studies. Also, we participate in a nationwide monitoring program.

BIOASSAY

As new pesticides are developed for commercial use, there is a need for determining acute and chronic toxicity of these chemicals to estuarine organisms. In addition to conducting routine acute tests, we have recently devoted much effort to long-term bioassays involving the chronic exposure of selected estuarine species to low-level pesticide pollution.

The acute toxic effects of various insecticides on shrimp, fish, oysters and other estuarine organisms have been evaluated in the laboratory. These short-term bioassays do not provide conclusive data, but they do provide information on relative toxicity. Table 1 shows an example of some recent tests. As a group, insecticides were generally more toxic to all organisms than the other pesticides but there are exceptions. The fungicide, Delan, and the experimental antifouling agent, ET-546, were extremely toxic to oysters. Results of these and other bioassay tests are sent to the Pesticide Registration Division of the U. S. Department of Agriculture to assist in certifying pesticides to be used in or near the coastal environment.

The necessity for long-term or chronic tests on the effects of sublethal concentrations of pesticides was clearly demonstrated in the results of two experiments reported by Lowe (1970). Oysters (*Crassostrea virginica*) exposed to a mixture of DDT, toxaphene and parathion continuously for 9 months weighed less than control oysters after 6 weeks but did not show a statistically significant difference until the 22nd week. In another experiment, mortalities in blue crabs (*Callinectes sapidus*) due to mirex adsorbed on bait, a chemical used to control fire ants, did not occur until 5 to 15 days after the animals were exposed. These effects would not have been detected with short-term (acute) bioassays.

TABLE 1

Relative Toxicity of Selected Pesticides to Shrimp, Fish and Oysters
Rank in Toxicity is Shown in Parentheses: (1) = Most Toxic
and (11) or (12) = Least Toxic (from Lowe, 1969)

Pesticide	Shrimp 48-hour EC_{50}[1]		Fish 48-hour EC_{50}[2]		Oysters 96-hour EC_{50}[3]	
	ppm (mg/liter)		ppm (mg/liter)		ppm (mg/liter)	
Insecticides						
Dursban	0.0002	(1)	0.0032	(2)	0.27	(8)
Parathion	0.0002	(2)	0.015	(4)	22% decrease at 1.0 ppm	(10)
DDT	0.0006	(3)	0.0028	(1)	0.010	(3)
Toxaphene	0.0042	(4)	0.028	(5)	0.038	(4)
DDT + Toxaphene + Parathion	--		--		0.05	(5)
Landrin	0.0042	(5)	3.2	(7)	No effect at 1.0 ppm	(11)
Abate 4-E	0.020	(6)	No effect at 1.0 ppm	(9)	0.17	(6)
Herbicides						
Igran	No effect at 1.0 ppm	(9)	20% mortality at 1.0 ppm	(8)	1.0	(9)
Weed-B-Gon (2,4-D + 2,4,5-T formulation)	No effect at 1.0 ppm	(10)	Irritated at 100.0 ppm	(10)	0.19	(7)
Fungicide						
Delan	0.15	(7)	0.010	(3)	0.0086	(2)
Miscellaneous compounds						
Corexit 7664 (oil-spill remover)	No effect at 1000.0 ppm	(11)	No effect at 1000.0 ppm	(11)	26% decrease at 32.0 ppm	(12)
ET-546 (arsenical antifouling agent)	10% mortality at 10.0 ppm	(8)	0.15	(6)	0.0021	(1)

[1]48-hour EC_{50} = Concentration of pesticide in sea water causing 50% mortality or loss of equilibrium to juvenile penaeid shrimp.

[2]48-hour EC_{50} = Concentration of pesticide in sea water causing 50% mortality to juvenile killifish (Fundulus similis).

[3]96-hour EC_{50} = Concentration of pesticide in sea water causing 50% decrease in oyster shell growth.

The chemical analysis of residues is an important part of the bioassay program and other programs concerned with quantitative studies of pesticides. Our chemical assay project provides analyses for research projects at this station and for the National Monitoring Program. Details of our procedure for preparing and analyzing marine samples are available in a manual prepared by A. J. Wilson, Jr. (no date). A flow chart of the general procedure is shown in Fig. 2.

ECOLOGICAL STUDIES

A pollutant may be defined as any substance added to the environment which has a measurable and generally detrimental effect upon the environment. Thus, pollution such as the introduction of pesticides in estuaries is an ecological problem—interaction of the pesticide with biotic and abiotic factors of the environment. Therefore, we can no longer limit our studies of pesticides to any single factor in the environment. Investigations must also be made with populations of organisms which are interacting with their physical, chemical and biological environment.

Ecological studies at this station include observations on the effects of herbicides on the primary productivity of a coastal pond. Although much is known about the capacity of herbicides to eliminate undesirable plants, little is known on how they affect the biogeochemistry of the ecosystem. To determine this effect, Walsh and Heitmuller (1969) introduced a herbicide (Dichlobenil) into the water of a small (0.15 ha.) pond and used a similar but larger (0.91 ha.) pond for comparative purposes. *Chara vulgaris* and *Potamogeton pectinatus* were the dominant hydrophytes in both ponds. About 1 month after treatment, all of the *Potamogeton* and about 80% of the *Chara* were eliminated. The number of blue-green algae increased as the vascular hydrophytes died. Four months after treatment, the pond appeared as it did before treatment.

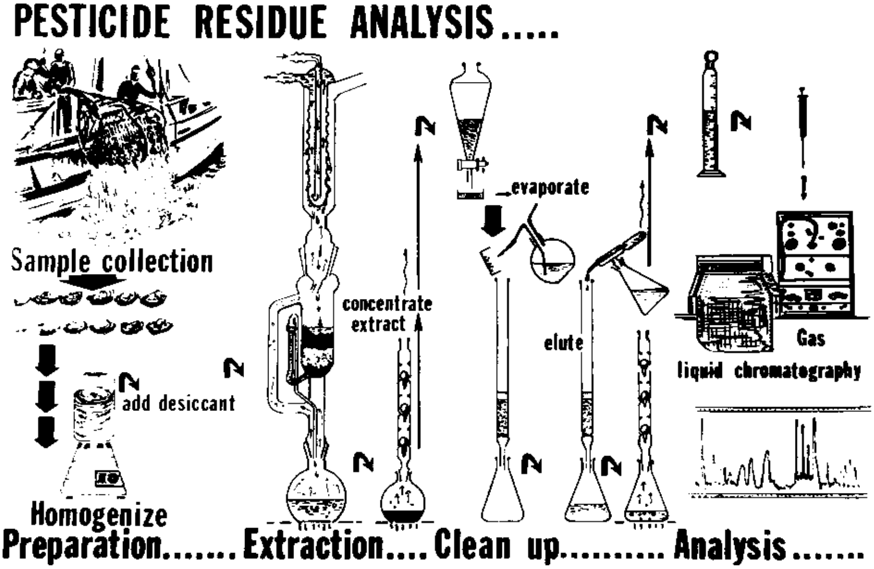

FIG. 2. Analytical procedure for determining pesticide residues in marine organisms.

Other work with populations of organisms concerns the effect of pesticides on protozoans. These organisms, with algae and bacteria, form the broad basis of the aquatic food web. Also, as predators of bacteria, ciliates have an important role in regeneration of nutrients. Yet, few data are available on the sensitivity of ciliates to pesticides. N. R. Cooley (1969) found that the density of a population of *Tetrahymena pyriformis* grown in the presence of 10 ppm DDT was 26% less than that of an untreated "control" population. *Paramecium multi-micronucleatum* and *Paramecium bursaria* were sensitive to the chemical (Gregory, Reede and Priester, 1969). Work is in progress to determine how toxic effects of various insecticides are produced and which metabolic processes are affected.

The presence of pesticides in the water of an estuary could adversely affect the behavior of populations of resident fish or those that spend only a portion of their life cycle in these fertile nursery grounds. For example, if fish avoided water that contained pesticides, they might not be able to reach water of a particular salinity required for spawning or they could be forced out of the estuary to search for better quality water. Hansen (1969) investigated the capacity of sheepshead minnows, *Cyprinodon variegatus,* to avoid pesticides.

These fish avoided water containing DDT, endrin, Dursban® and 2,4-D. They did not avoid test concentrations of malathion or Sevin®. Fish exposed simultaneously to two different concentrations of the four pesticides avoided previously, avoided the highest concentration of 2,4-D but preferred the higher concentrations of DDT. Investigators are now determining the effect of DDT on the salinity preference of fish.

PHYSIOLOGICAL STUDIES

Sublethal concentrations of pesticides can cause subtle changes in the physiological processes of estuarine organisms. These changes are difficult to detect, yet they can be just as important as more evident effects from lethal doses. We are presently investigating the effects of pesticides on the blood of crustaceans and certain enzyme systems of fishes.

Nimmo and Blackman (1969) conducted physiological studies to determine the effect of DDT on the protein composition of shrimp blood and the distribution of this chemical in the shrimp. Preliminary investigations were made with grass shrimp *(Palaemonetes pugio)*, but white shrimp *(Penaeus setiferus)* and pink shrimp (*Penaeus duorarum*) were used in most studies. Pink shrimp exposed to 0.1 part per billion (ppb) of DDT showed a gradual decrease in the blood protein thought to be hemocyanin. Accumulation of DDT in shrimp was measured by analyzing whole-body residues. Residues were greatest in the hepatopancreas, least in the muscle.

Metabolic activity in fish, as in other animals, is dependent on enzyme activity and pesticides can interfere with this activity. Specifically, organophosphate pesticides inhibit cholinesterases, which are enzymes that function in the transmission of nerve impulses and ion transport. Holland, Coppage and Butler (1967) used the degree of inhibition of cholinesterase in brains of fish to measure organophosphorus pesticide pollution along the Atlantic and Gulf coasts. Coppage (1969) made laboratory tests to determine the relation of the toxicity of several organophosphate pesticides to *in vivo* inhibition of cholinesterase of sheepshead minnows. The enzyme activity of fish exposed to several pesticides was compared with normal enzyme activity in unexposed fish. Under test conditions, the number of fish killed by the organophosphates was proportional to the inhibition of cholinesterase in the brains of the fish, i.e., the greater the amount of inhibition, the larger the number of fish killed.

MONITORING PROGRAM

The Bureau of Commercial Fisheries has entered into cooperative agreements with state conservation agencies, and university and federal marine laboratories in 15 coastal states to establish a pesticide monitoring program. Shellfish were chosen as "indicator" organisms because they are sessile and readily concentrate pesticides from their environment, yet the chemical is flushed out of their tissues at a uniform rate when the pesticide is no longer present in the environment. Samples of oysters, clams or mussels, depending upon the estuary, are collected from about 170 stations at 30-day intervals and are analyzed for 10 or more pesticides. The samples are analyzed either by the collecting agency

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or at Gulf Breeze. More than 5,000 samples have been analyzed since July 1965.

The amounts of pesticides in shellfish vary according to the region in which the shellfish were collected and reflect the amounts of pesticides applied to crops within the particular drainage basin (Butler, 1966). For example, DDT residues in oysters from Willapa Bay, Washington, are low (20 ppb or less) and the drainage basin consists of forested slopes with little agriculture. Oysters from the south coast of Texas, however, contained 500 ppb. The basin in which these oysters were located drains an area that produces three crops a year and is treated heavily with pesticides.

PRESENT KNOWLEDGE AND FUTURE GOALS

Hayne, Duke and Sheets (1969) summarized the present state of knowledge about pesticides in estuaries as follows: "There is increasing apprehension based upon accumulating evidence of toxicity at low concentrations of chlorinated hydrocarbon and other pesticides and a growing body of facts about disturbance of the physiology at sublethal levels of these chemicals. An ambient level sufficient to promote body storage at measurable levels exists almost generally, at least for DDT. But there are very few known facts about the biologically effective levels in estuaries or about the populational effects of these levels. It is fair to say that we know and understand the problem only well enough to outline the areas where we need to know more."

Based on our knowledge to date, future research at this station will emphasize: (1) long-term tests on the effect of sublethal concentrations of pesticides on the physiology of estuarine organisms in sensitive stages of their life history, (2) investigations of the dynamics of pesticides in estuaries and (3) special studies of the movement and magnification of pesticides in estuarine food webs.

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