Short-term Effects of Organophosphate Pesticides on Cholinesterases of Estuarine Fishes and Pink Shrimp

by

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The esterase-inhibiting insecticides (organophosphates and carbamates) are now produced and enter the environment in greater quantities than the chlorinated hydrocarbon insecticides (ANONYMOUS, 1972; ANONYMOUS, 1971). These pesticides act as nerve poisons by blocking synaptic transmission in the cholinergic parts of the nervous system (HEATH, 1961; KARCZMAR et al. 1970; KOELLE, 1963; METCALF, 1971; O'BRIEN, 1967). The disruption of nerve impulse transfers is caused by excessive accumulation of the neurotransmitter acetylcholine (ACh) which is normally broken down by the enzyme acetylcholinesterase (AChE, EC 3.1. 1.7 acetylcholine acetyl-hydrolase). The organophosphates and carbamates bind to the active site of the AChE and prevent breakdown of ACh (ALDRIDGE, 1971; FUKUTO, 1971; KOELLE, 1963; METCALF, 1971). AChE inhibitors probably cause death in higher vertebrates by blocking neurotransmission in the respiratory center of the brain or neuromuscular junctions of the respiratory apparatus (DeCANDOLE et al. 1953; HEATH, 1961; KOELLE, 1963), but this has not been confirmed for fish. Inhibition of AChE is also believed to be the mode of action of these pesticides on arthropods (HEATH, 1961; KOELLE, 1963; O'BRIEN, 1960; O'BRIEN, 1967).

The possible hazards of AChE inhibiting pesticides in the aquatic environment should not be ignored. Over one hundred AChE inhibiting pesticides are produced and over 200 million pounds are manufactured annually in the United States (CASIDA, 1964; ANONYMOUS, 1972; ANONYMOUS, 1971). Aquatic organisms show a broad range of response to organophosphate pesticides, depending on the compound, exposure time, water conditions, and species (EISLER, 1970a). Short-term lethal concentrations in water range from a few parts per trillion to several parts per million (EISLER, 1970b; ANONYMOUS, 1963; ANONYMOUS, 1970).

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Bulletin of Environmental Contamination & Toxicology,
Vol. 11, No. 5 © 1974 by Springer-Verlag New York Inc.

The organophosphate insecticides, with which we are concerned in this report, generally degrade more rapidly in the environment than do chlorinated hydrocarbon insecticides they are replacing. But, their presence and effects in the environment may be greater than expected because it may be necessary to apply the organophosphates more frequently and in greater quantity to control pests. The cholinesterases of vertebrates may remain inhibited for several weeks after exposure because of irreversible inhibition by extremely small quantities of dealkylated oxygen-analog metabolites of thiophosphates (COPPAGE and DUKE, 1972; HEATH, 1961; KOELLE, 1963; MACEK et al. 1972; O'BRIEN, 1960). Cumulative reduction of AChE by repetitive exposure has been demonstrated in some vertebrates (HEATH, 1961; KOELLE, 1963), and this may happen to fish subjected to similar repetitive exposure in the environment (HOLLAND and LOWE, 1966; ANONYMOUS, 1965; WEISS, 1958).

Recent studies have indicated AChE measurements are probably the best general index of organophosphate poisoning of fish in the environment (COPPAGE, 1972; MACEK et al. 1972; COPPAGE and DUKE, 1972). Also, if one considers the number of organophosphate compounds and the difficulty in detecting their highly toxic oxygen-analogs (McCULLY, 1972; PARDUE, 1971), AChE measurements in animals from the environment are probably the best general indicator of serious organophosphate pesticide pollution. The difficult task of detecting and interpreting residues alone in terms of effects on organisms is eliminated by measuring AChE in animals taken directly from the environment. A field study of three species of estuarine fishes from an area sprayed with organophosphate pesticide showed brain AChE inhibition was correlated with mosquito control operations with malathion (COPPAGE and DUKE, 1972). Brain AChE of fresh water fishes in ponds was also inhibited by application of Dursban® (MACEK et al. 1972). Inhibition of AChE in fish brains has been found below river outfalls of pesticide plants (COPPAGE, unpublished data; WILLIAMS and SOVA, 1966). Also, concentrations of malathion lethal to commercial shrimp may exist during mosquito control operations (CONTE and PARKER, 1971).

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We need more information on the relationship of AChE inhibition to poisoning and deaths of estuarine animals to aid in determining whether detrimental effects and "kills" in the environment are caused by organophosphate pesticide exposure. This report concerns AChE inhibitory effects of short-term laboratory exposures of four species of estuarine fishes and a commercial shrimp to lethal concentrations of malathion that may be found in the environment (CONTE and PARKER, 1971). In addition, AChE inhibitory effects of lethal exposure to naled, Guthion®, and parathion are reported for two of the fish species.

MATERIALS AND METHODS

Inhibition of AChE activity was used as an indicator of poisoning in brains of spot (Leiostomus xanthurus; 65-150 mm total length), pinfish (Lagodon rhomboides; 65-125 mm), Atlantic croaker (Micropogon undulatus; 85-150 mm), and sheepshead minnows (Cyprinodon variegatus; 45-70 mm), and in the ventral nerve cord (VNC) of pink shrimp (Penaeus duorarum; 78-122 mm). The acetylcholine hydrolyzing enzymes from fish brains were characterized and assayed as previously described (COPPAGE, 1971). The assay was carried out with a recording pH-stat at pH 7 and 22° C. We mixed 2 ml of brain homogenate containing 5 mg of tissue per ml with 2 ml of 0.03 M acetylcholine iodide and measured the acetic acid liberated by enzymatic hydrolysis of ACh by titrating with 0.01 N NaOH. Shrimp VNC was assayed similarly, except temperature was 25° C and the homogenate contained 2 mg of VNC per ml. AChE activity of both shrimp and fish was measured as micromoles of ACh hydrolyzed per hour per mg of tissue in the reaction vessel. Each AChE assay sample consisted of pooled organs from 4 to 6 animals that survived pesticide exposure at a designated time.

In each test, 10 fish or shrimp were exposed in 3-5 replicates to technical grade pesticide in 8-liter acrylic plastic aquaria that received a mixture of flowing seawater (400 ml per minute) and pesticide from a common source. The pesticide was dissolved in acetone or benzene and infused into seawater by means of syringe pumps. Solvent infusion never exceeded 2.5 parts per million in the water and did not affect AChE activity. Pesticide concentration in the water was expressed in theoretical parts per billion (ppb), but was not verified by residue analysis because our chosen criteria for toxic effects were only death and AChE inhibition. In quadruplicate tests comparing

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different solvent (acetone vs. benzene) carrying the same quantity of malathion, there was no significant difference (Student's t-test, $P \leq 0.05$) in mortality or AChE inhibition. Temperature range was 18-23° C and salinity was 23-29 parts per thousand during the tests.

To determine the extent of AChE inhibition resulting from a near median kill, we assayed survivors in tests in which 40-60 percent of the test population was killed. The shrimp assayed had lost equilibrium (=moribund). Statistical comparisons of AChE activities of exposed animals were made with unexposed populations (Student's t-test, $P \leq 0.001$).

RESULTS AND DISCUSSION

AChE inhibition was great in surviving fish and moribund shrimp. Results of tests are summarized in Table 1.

TABLE 1.

AChE Inhibition in Fish and Shrimp by LC 40-60 of Organophosphates

Animal	Pesticide	Theoretical Conc. (ppb)	Hours Exposed	AChE Reduced (%) Mean	AChE Reduced (%) Range	Inhibition Significant at t 0.001
Spot	Malathion	1250	24	70	65-82	Yes
	Naled	75	24	85	82-89	Yes
	Guthion	20	24	96	93-98	Yes
	Parathion	10	24	88	87-89	Yes
Pinfish	Malathion	1000	24	88	87-89	Yes
	Naled	75	24	88	88-88	Yes
	Guthion	10	24	80	77-84	Yes
	Parathion	10	24	90	88-92	Yes
Croaker	Malathion	1000	24	86	79-90	Yes
Sheepshead Minnow	Malathion	200	24	96	90-99	Yes
Pink Shrimp (moribund)	Malathion	1000	48	75	72-82	Yes

Relatively consistent levels of AChE inhibition occurred in fishes even with different compounds and different species. The survivors of populations of fish in which 40-60 percent were killed by exposure to organophosphate pesticide had mean brain AChE reductions of 70-96 percent (Table 1). Mean AChE inhibitions in fishes were near or exceeded the "lethal threshold" of about 82 percent reduction indicated in a previous study of sheepshead minnows (COPPAGE, 1972), except inhibition of spot brain-AChE by malathion, which differs by only 12 percent. These inhibitions indicate that mean reductions in AChE activity of about 80 percent are critical in short-term organophosphate poisoning of the fishes tested and this may apply to fishes in general. Deaths may occur even at mean inhibition values of 70 percent in some cases, so the "lethal threshold" probably varies slightly among species. These specific levels of reduction of AChE show that it is unnecessary to rely on the dubious interpretation of residues alone to determine poisoning and cause of "kills" in the environment. Measurements of AChE activity and residue analysis or pesticide usage data would be especially helpful in cause and effect studies.

Reduction of activity of ACh hydrolyzing enzymes in the VNC of moribund shrimp was similar to that observed in fishes (Table 1). The large reduction (75 percent) of enzyme activity in moribund shrimp indicates that they too may be useful indicators.

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