Use of Fish Brain Acetylcholinesterase to Monitor Pollution by Organophosphorus Pesticides

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The organophosphorus pesticides are toxic because they inhibit the enzyme acetylcholinesterase (AChE), which is essential to nerve conduction. Weiss (1,2,3) reported that very low concentrations of these pollutants could be detected by measuring the degree of inhibition of this enzyme in brains of exposed fish. He suggested that this technique be used to monitor pollution in natural waters (4,5). Recovery to normal

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Bulletin of Environmental Contamination & Toxicology,
Vol. 2, No. 3, 1967, published by Springer-Verlag New York Inc.

AChE levels after exposure to organophosphorus pesticides may take a month or more (3); it may be possible therefore to detect not only present but past pollution. The purpose of this study was to investigate the feasibility of using this technique to monitor organophosphorus pesticide pollution in estuarine waters.

Materials and Methods

This study was conducted at the Bureau of Commercial Fisheries Biological Laboratory, Gulf Breeze, Florida, between October 1965 and November 1966. Species selected were spot (*Leiostomus xanthurus*) and sheepshead minnows (*Cyprinodon variegatus*). Both species occur from Texas to New England and are generally abundant and easily obtained. Seven laboratories along the Atlantic and Gulf Coasts cooperated in this study by sending us fish from their areas periodically (about every 2 months) for AChE assay. Fish were frozen as soon as possible after capture and shipped in dry ice; we previously determined that enzyme activity is not affected by this treatment.

To determine AChE activity, we removed the brain from a fish and weighed it on a tared piece of aluminum foil. The brain was then homogenized in 4 ml of a phosphate buffer

(pH 7.2) of the following composition: 16.72 g $Na_2HPO_4 \cdot 12H_2O$ and 2.72 g KH_2PO_4 dissolved in 1 liter of distilled water. We diluted a 2-ml aliquot of the homogenate with sufficient buffer to make the tissue concentration 3.5 mg of tissue per ml of buffer. This material was incubated 4 hours at 25°C. We then added 1 ml of the diluted homogenate to 1 ml of 0.004 M solution of acetylcholine iodide and allowed the reaction to proceed 20 minutes at 25°C. We determined the residual acetylcholine colorimetrically at 525 mμ by the method of Hestrin (6) and calculated the level of activity on the basis of micromoles of substrate hydrolyzed per hour per mg of brain tissue. Since previous studies at this laboratory and by others (2) showed a decrease in activity per unit weight as fish-brain weight increases, we calculated a line of best fit for each species using data from 155 spot and 168 sheepshead minnows. This line was considered to represent 100% activity. In both species, the results of more than 80% of the analyses were between 90 and 110%; we consider these limits to represent normal variation. Groups that averaged less than 90% AChE activity were considered to have experienced significant exposure to some anti-cholinesterase agent.

Evidence of Pollution

We conducted AChE assays on 93 samples of spot and sheepshead minnows from 43 stations during this study; sample size ranged from 2 to 29 fish, and 1084 individual assays were performed. We found low enzyme activity in 17 samples (18.3%), but 13 of these were from only two areas (Table 1). The fish that showed low AChE activity (73% to 88% of normal) from South Carolina came from the vicinity of the Ashley River near Charleston. This river receives wastes from plants producing a variety of organophosphorus compounds. Williams and Sova (8) reported that Atlantic menhaden (Brevoortia tyrannus) and Atlantic croakers (Micropogon undulatus) from this river also exhibit low enzyme activity. Fish from the Galveston area with low activity (75% to 84% of normal), generally came from stations along the eastern edge of Trinity Bay. Samples from four other stations assayed as low as 77% to 85% of normal, but depression of enzyme activity in fish from these areas was not consistently present.

TABLE 1

Summary of Data on Organophosphorus Pesticide Monitoring
October 1965 - November 1966

Biological Laboratory	Fish Species[1]	Number Assayed	Number of Stations	Number of Samples	Number Positive[2]
Chesapeake Biological Laboratory, Solomons, Maryland	S	20	1	1	0
Gulf Coast Research Laboratory, Ocean Springs, Mississippi	S	35	3	4	1
	M	43	1	4	1
Bears Bluff Laboratories, Wadmalaw Island, South Carolina	S	111	8	9	4
	M	97	4	6	0
Virginia Institute of Marine Science, Gloucester Point, Virginia	S	25	1	2	0
	M	55	1	4	0
Bureau of Commercial Fisheries Biological Laboratories:					
Gulf Breeze, Florida	S	104	4	12	0
	M	194	1	15	0
St. Petersburg Beach, Florida	S	97	2	8	1
	M	57	2	4	0
Beaufort, North Carolina	S	39	1	3	1
Galveston, Texas	S	181	12	19	8
	M	26	2	2	1
		1084	43	93	17

1/S = spot, Leiostomus xanthurus; M = sheepshead minnow, Cyprinodon variegatus.

2/Less than 90% of normal AChE activity, presumably indicating pollution.

Potential Value of the Technique

This study indicates that organophosphorus pesticide pollution in estuaries along the Atlantic and Gulf coasts is not yet widespread, but should be followed, preferably by a technique that is dependable and not too costly. The techniques used by us are relatively straightforward and could readily be employed by other laboratories at moderate expense. The chief difficulty lies in the mechanics of handling and shipping the frozen samples.

We suggest several modifications of our procedures which would decrease the time required for AChE determinations without detracting from the value of the data. The 4-hour incubation period following homogenization of the brain tissue can be eliminated; a trained operator could then complete approximately 25 samples per day. Instead of analyzing individual fish, brains of 5 to 10 fish of uniform size from a station can be analyzed as a composite sample and yield a statistically valid datum. Additional fish species are presumably suitable for use as monitors; we are currently evaluating several species of the genus Fundulus for this purpose.

Use of these techniques for the periodic surveillance of estuarine fish populations will enable the biologist to recognize

incipient and chronic low levels of organophosphorus pollution in estuaries. Armed with such data, we can initiate remedial action before an environment is permanently destroyed.

Acknowledgments

We acknowledge the technical assistance of Donald C. Speed. We thank Dr. Robert F. Johnson for reviewing the manuscript.

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