Sources of error in the use of fish-brain acetylcholestrinase activity as a monitor for pollution[1]

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Recurring pollution of natural waters from the manufacture and use of pesticides has accentuated the need for suitable monitoring methods. The determination of fish-brain acetylcholinesterase (AChE) activity has been proposed as a means of detecting organophosphorous pollution (4) and has been used for monitoring purposes (5, 6).

Weiss (1, 2, 3) demonstrated that a 40-70% inhibition of fish-brain AChE is usually lethal. He detected inhibition from concentrations of phosphate insecticides as low as 1 ppb over a 15 day exposure period and 0.1 ppb over a 30 day exposure period. Williams and Sova (5) detected inhibition in brains of two species of fishes collected downstream from suspected sources of organophosphorous pollution. Holland et al. (6) evaluated this monitoring method and reported AChE inhibition in fish from polluted waters. AChE inhibition data have been used as evidence implicating agricultural chemical plants in

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[1]Supported by grants ES 00086-03 and UI 00348-01 from the U. S. Public Health Service.

Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 1, 1969, published by Springer-Verlag New York Inc.

fish kills (7). Nicholson (7) suggested that a 10% depression in AChE activity be used as a criterion for evaluating water quality relative to carbamate and organophosphorous contamination.

We studied the practicality of using fish-brain AChE activity as a monitoring method and examined potential sources of error in the method.

Materials and Methods

Bluegill sunfish (Lepomis macrochirus), 5-7 cm long, were seined from ponds having no detectable O-P insecticidal contamination, as determined by gas chromatographic analyses. Individual brains were removed, weighed on tared aluminum foil, and homogenized in 10 ml of 0.2 M Tris buffer (pH 8.2) containing 0.2 M NaCl and 0.02 M $MgCl_2$. Additional buffer was added to give a final tissue concentration of 4 mg/ml.

Reaction mixtures containing 4 mg of tissue (1 ml of stock brei) and 4 μM of acetylcholine bromide (1 ml of 0.004 M AChBr) were incubated for 15 min at 25 C. The reaction was stopped with 2 ml of alkaline hydroxylamine. Color was developed with one ml of 2:1 aqueous HCl and one ml of 0.37 M $FeCl_2$, and read at 540 mμ on a Beckman Model B Spectrophotometer. Specific activities were calculated from a regression equation and expressed as μ M AChBr hydrolized/mg brain tissue/hour.

Experiments and Results

The reliability of the technique was illustrated by assaying 10 aliquots from a pooled homogenate of 3 brains. The range in specific activity was 0.08 and the standard deviation 0.024.

TABLE 1

Acetylcholinesterase Activities for Three Bluegill Populations on Different Days

Population	Day	Specific Activity		
		Range	Sample Mean	Population Mean
A	1	2.41-2.70	2.54	
	2	2.10-2.87	2.69	
	3	2.98-3.30	3.14	2.79
B	1	2.64-3.10	2.92	
	2	2.47-2.84	2.67	
	3	2.95-3.33	3.15	2.91
C	1	2.90-3.15	3.03	
	2	2.70-2.93	2.84	
	3	2.95-3.18	3.12	3.00

Three populations of bluegills were studied to determine the variation in AChE activity within and among populations. Brains of 10 freshly-killed fish from each population were

tested on 3 different days. The maximum difference in mean specific activity for the 3 populations was 7% (Table 1). Within population variation in the 3 tests was 19, 9, and 15%; maximum difference between populations on a given day was 16%, but 19% on different days.

Various regions of the brain differ in specific activity, e.g., inferior lobe (3.93), optic lobes (2.84), cerebellum (1.90), cerebral hemispheres (1.73), medulla (2.75).

A total of 140 bluegills was collected at one time from a pond and used to study effects of prior treatment upon AChE determinations. Normal activity for the population was 2.64 (Fig. 1 A).

Three groups of 20 fish each were frozen at -75 C. After 16 hr, frozen brains were removed from one group. The second group was thawed 2 hr at room temperature, and the third group of fish was thawed 2-3 min in a water bath at 45 C. The mean specific activities of these groups were 2.36, 2.62 and 2.51 respectively (Fig. 1 B, C, D). Analysis of variance and Duncan's New Multiple Range Test showed that the 2.36 value differs significantly from the others.

Twenty freshly excised brains were frozen in 2 ml of Tris buffer, and 20 brains were frozen on tared aluminum foil. These brains showed mean specific activities of 2.71 and 2.67 respectively (Fig. 1 E, F). Brains without inferior lobes had a mean specific activity of 2.47 (Fig. 1 G).

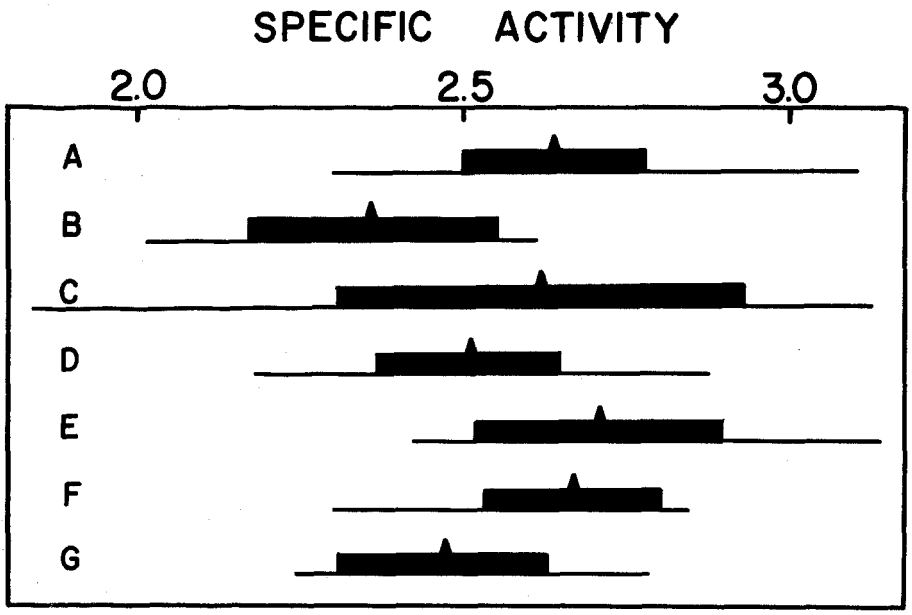

Figure 1. Brain acetylcholinesterase activities in 7 groups of 20 bluegills. A - Controls; B-F - freezing and thawing procedures; G - without inferior lobe. Line represents the range; bar, standard deviation; pointer, mean.

Brains from moribund fish exposed 30 min in 750 ppb and 13 hr in 20 ppb parathion showed mean specific activities of 1.97 (range 0.64-1.73, SD 0.64) and 1.14 (range 1.61-2.35, SD 0.24) respectively.

Discussion and Conclusions

Our data show that a monitoring method based upon a 10% depression in fish-brain AChE activity can lead to erroneous conclusions, even under carefully controlled conditions. A 10% inhibition was found in brains of unthawed fish. Fish for

monitoring purposes are usually shipped on dry ice, and the brains are removed without thawing. Presumably the reduced activity is not effected by freezing, but rather by inclusion of ice and extraneous tissues, or possibly loss of the inferior lobe, which is loosely attached, and has a high specific activity. Brains removed from fish thawed for 2 hr at room temperature, though not significantly inhibited showed increased variation.

Published accounts suggest that death occurs in fish when AChE levels drop to 40-70% of normal activity. This generality has obvious exceptions, as demonstrated in our experiments. Fish that became moribund in 750 ppb parathion showed only 25% AChE inhibition; those that became moribund in 20 ppb parathion showed 57% inhibition. Other tests conducted in our laboratory show that mortality and recovery from organophosphorous poisoning are not necessarily related to the degree of AChE inhibition. Test specimens experiencing over 90% inhibition may fail to develop pronounced symptoms of O-P poisoning, and recover completely when removed to fresh water.

Differences within and among populations indicate that AChE activity of a species fluctuates with time. Furthermore, the confusing relationship between mortality and the degree of AChE inhibition jeopardizes logical interpretation of data, i.e., the degree of AChE inhibition is not always related to the amount of inhibitor present or to the length of exposure. Also,

the cholinesterases are inhibited by more substances than any other group of enzymes (8).

We conclude, therefore, that a 10% depression of fish-brain acetylcholinesterase activity based on present handling procedures may be an impractical criterion for monitoring organophosphorous pollution. If used with caution, the method can support other types of data. Standardization of sample handling procedures prior to analysis and use of standard laboratory strains of fish for monitoring purposes instead of natural populations might be helpful. We are presently investigating inhibition-mortality relationships in O-P poisoning.

Literature Cited

1. WEISS, C. M., Ecology. 39:194 (1958)
2. WEISS, C. M., Sewage and Ind. Wastes. 31:580 (1959)
3. WEISS, C. M., Trans. Amer. Fish. Soc. 90:143 (1961)
4. WEISS, C. M., J. Water Pollution Control Fed. 37, 647 (1965)
5. WILLIAMS, A. K., and C. R. SOVA, Bull. Environ. Contamination and Toxicol. 1:198 (1966)
6. HOLLAND, H. T., D. L. COPPAGE, and P. A. BUTLER, Bull. Environ. Contamination and Toxicol. 2:156 (1967)
7. NICHOLSON, H. P., Science. 158:871 (1967)
8. DIXON, M., and E. C. WEBB, Enzymes, p 355 (1964), Academic Press, New York