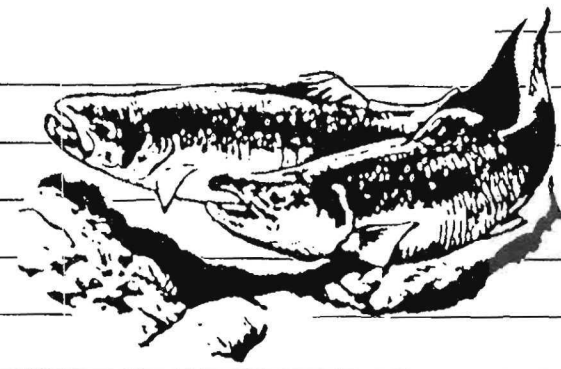
Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 90
DATE 1993



Cholinesterase Activity Useful in Detecting Freshwater Mussel Exposure to Organophosphate and Carbamate Pesticides

Determination of cholinesterase (ChE) activity is a well accepted method for diagnosing vertebrate exposure to organophosphate and carbamate pesticides. Cholinesterase activity that is inhibited by about 20% is considered indicative of exposure to ChE inhibitors. Brain ChE activity that is inhibited by 50% or more in dead animals is considered diagnostic of lethal anti-ChE poisoning. Responses and assessment tools for aquatic invertebrates exposed to ChE-inhibiting pesticides are not as well developed. In 1990, we investigated a die-off of freshwater mussels in central North Carolina. Using unperfected but repeatable techniques, we determined that cholinesterase activity in selected tissues was significantly inhibited at and downstream from the kill site compared to a reference site upstream. The inhibition of ChE activity in the mussels from the die-off site and the presence of nearby agricultural lands suggested that ChE-inhibiting pesticides may have caused the die-off. This led us to develop methods and criteria for evaluating exposure of freshwater mussels to these pesticides.

Adductor Muscle Is Tissue of Choice for ChE Assay

Nervous tissue and plasma, tissues normally used in vertebrate assessments of ChE activity, could not be easily obtained from mussels. We found that the mantle of the eastern elliptio *(Elliptio complanata)* had inconsequential ChE activity, whereas the adductor muscle had low but consistently detectable levels. The adductor muscle was easily identified and dissected and became our tissue of choice for ChE assays. Adductor muscle was placed in Tris buffer at a ratio of 1:10 and homogenized with a biohomogenizer (BioSpec Products). Other types of homogenizers did not work well with the fibrous muscle tissue. We centrifuged the homogenate, then extracted the sample for ChE assay from the clear layer between the pellet at the bottom and the frothy layer at the surface. To compensate for the low ChE activity in our samples, we used 200 µL of homogenate. This represented a 10-fold increase in the amount of homogenate normally used for vertebrate plasma and brain. These were the only modifications made to the standard colorimetric ChE assay as described by Hill and Fleming (1982).

ChE Activity Responds in Dose-related Manner

We exposed *E. complanata* to graded doses of aldicarb and acephate in a 96-h static test at 22° C. No mortality occurred at any concentrations, including the maximum concentrations of 320 ppm of acephate and aldicarb. Cholinesterase activity

was inhibited 20% by 0.01 ppm aldicarb and 1 ppm acephate. Above 5 ppm of either pesticide, no further reduction of ChE activity was notable in this species. We had anticipated that freshwater mussels would be very sensitive to organophosphate and carbamate pesticides and would die at low (<1 ppm) pesticide concentrations. In part, this hypothesis was based on the absence of other invertebrates and fish dying during the 1990 mussel die-off mentioned above. The absence of mussel mortality in our lab test presented us with an apparent contradiction between our experimental results that showed mussels were insensitive to representative carbamate and organophosphorus pesticides and our implication of ChE inhibitors in the die-off.

Higher Temperatures Increase Toxicity and Alter Behavior

We exposed mussels to 0 and 5 ppm acephate and aldicarb for 96 h at 21, 24, 27, and 30° C. Cholinesterase activity of adductor muscle within treatment groups was not altered by increasing tank temperatures to 30° C (Fig. 1). However, 75% of the mussels exposed to acephate and 33% exposed to aldicarb died at 30° C. No mortality occurred in controls at 30° C or in any groups maintained at lower water temperatures. Increased water temperature, and possibly lower dissolved oxygen (DO) levels, seemed to interact with pesticide exposure to increase mortality in *E. complanata*, a factor which needs to be considered in the investigation of field die-offs.

We observed mussels daily for siphoning action and foot extension. If a mussel was open (which indicated siphoning activity), we tapped its shell with a probe and recorded the number of taps required for complete shell closure. Sublethal concentrations of acephate and aldicarb, independently or interacting with high water temperatures, decreased the shell closure responsiveness of mussels (Fig. 2).

ChE Activity Takes 12 Days to Recover

To assess the rate of recovery of ChE activity after a significant depression, we exposed mussels to 0 and 5 ppm acephate and aldicarb at 21 and 27° C. After 96 h of exposure, we transferred all mussels to clean tanks maintained at the same temperatures and sacrificed three mussels from each treatment group at days 0 (the day of transfer), 2, 6, 12, and 24. We analyzed mussels for ChE activity and plotted the recovery of ChE activity over time. At day 0, ChE activity of all dosed mussels was significantly depressed (Fig. 3). Cholinesterase activity in aldicarb-exposed mussels recovered to control levels in about 12 days. Depression of ChE activity in acephate-exposed mussels was detectable ≥24 days following exposure. Temperature did not influence recovery rates.

ChE Activity Shows Promise for Monitoring Mussel Exposure to Organophosphate and Carbamate Pesticides

Cholinesterase activity in adductor muscle of *E. complanata* is easily measurable and is significantly inhibited by aldicarb and acephate concentrations at least one to two orders of magnitude below those that caused mortality under our most stressful test conditions. Cholinesterase activity is unaffected by water temperature, and inhibition is detectable for several days following pesticide exposure. In studies not reported here, we concluded that the Asian clam (*Corbicula* sp.) is not a suitable surrogate species for *E. complanata* for assessing anti-ChE poisoning.

We suggest that ChE activity be routinely measured in mussel die-offs that have an unknown etiology or in which ChE-inhibiting pesticides are suspected. In addition, we believe it is feasible to monitor the exposure of mussel populations to ChE-inhibiting pesticides by regular measurement of ChE activity. A pilot monitoring program conducted by the Raleigh Ecological Services office is based on the following recommendations: (1) Sample only abundant species of mussels whose populations are not of national concern; (2) sample a minimum of three mussels per site per sampling period and assay samples individually; (3) sample at 10–12-day intervals to maximize detection of exposure while minimizing sampling effort; (4) place samples on ice at the time of collection and store frozen; (5) assay reference samples concurrently with monitoring samples and adjust for differences between runs by standardizing against reference samples; and (6) record water temperature and DO at the time of collection, particularly if dead mussels are seen.

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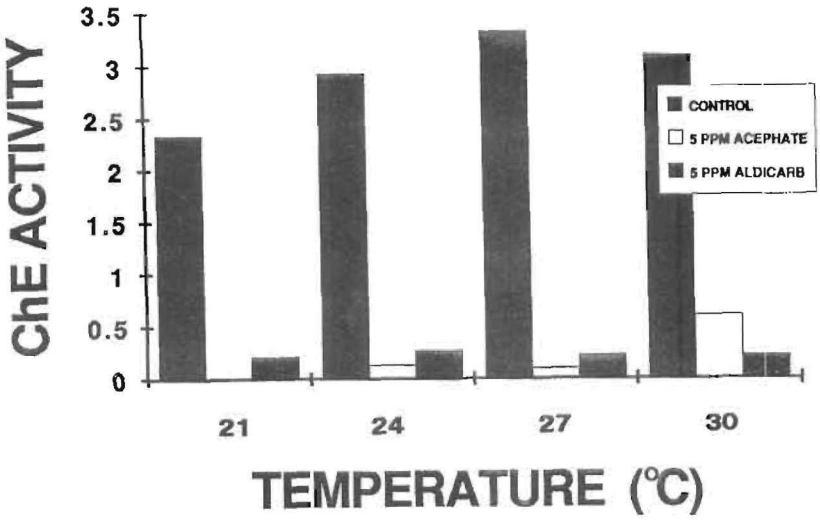

Fig. 1. Cholinesterase activity (mMol min^{-1} g^{-1}) in adductor muscle of the freshwater mussel *Elliptio complanata* after a 96-h exposure to 5 ppm acephate or aldicarb. Mussels were maintained at different water temperatures during the exposure to the pesticides.

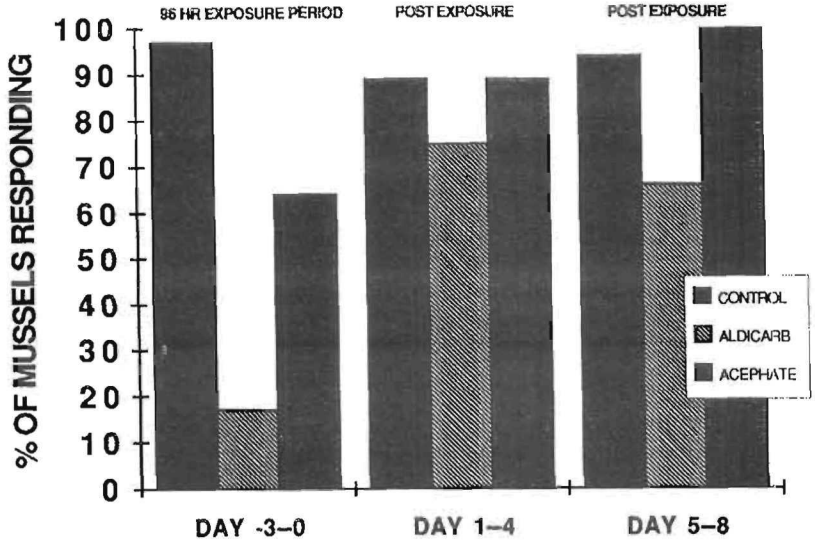

Fig. 2. Shell closure response of the freshwater mussel *Elliptio complanata* to gentle tapping during and following a 96-h exposure to 5 ppm aldicarb or acephate. Mussels were maintained at 27°C during the test.

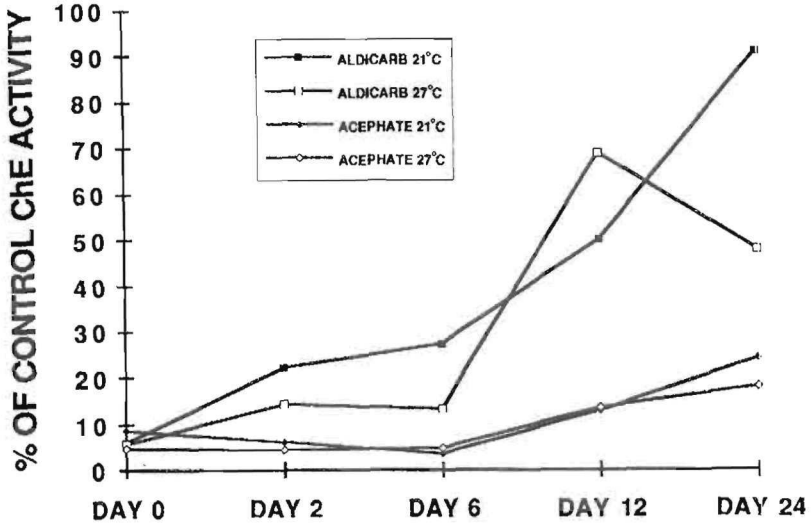

Fig. 3. Recovery of cholinesterase (ChE) activity in adductor muscles of the freshwater mussel *Elliptio complanata* following a 96-h exposure to 5 ppm aldicarb or acephate. During the exposure and recovery periods, mussels were maintained at either 21 or 27°C.