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Toxicity of Abate® to Green Frog Tadpoles

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Wetlands are essential habitats for many beneficial species of plants and animals. However, when wetlands are in proximity to urbanized areas, they may be breeding areas for mosquitoes which may become nusiance species and vectors for numerous zoonoses and epizootics. Public health officials, therefore, sometimes call for measures to control these populations of mosquitoes. Because many control measures affect non-target organisms, mosquito control may result in a conflict between maintaining the benefits of wetlands and reducing the negative affects of pests. This is partilcularly true for many U.S. Fish and Wildlife Service National Wildlife Refuges along the Atlantic and Pacific coasts that were established years ago to provide habitat but now are surrounded by urbanization.

In response to this concern, the U.S. Fish and Wildlife Service has sought ways of controlling mosquitoes that are both efficacious and comparatively safe to non-target species. Potential control methods include various biological control agents (e.g. *Gambusia affinis, Bacillus thuringiensis, Lagendium giganteum*), juvenile growth inhibitors (e.g. methoprene) and several pyrethroid and organophosphorus pesticides (e.g. pyretrhin, temephos, fenthion, malathion). Temephos [O,O'-(thiodi-4, 1-phenylene) O,O,O',O'-tetramethyl phosphorothioate)] is a widely used organophosphorus pesticide to control mosquitoes (Mu Lee and Scott 1989), but it is lethal to fish and non-target invertebrates. Field and laboratory studies also indicate that temephos may affect the behavior and survival of fiddler crabs *(Uca pugnax)* (Ward and Busch 1976, Ward et al. 1976). Little, if anything, has been published about the toxicity of temephos to amphibians.

The purpose of this paper is to determine the median lethal concentration of an emulsifiable formulation of temephos, Abate® 4-E (American Cyanamid Co., hereafter referred to as Abate) and to study its effects on the behavior and cholinesterase activity in green frog *(Rana clamitans*) tadpoles. Abate contains 44.6% temephos and 55.4% inert ingredients listed as petroleum distillates on the label.

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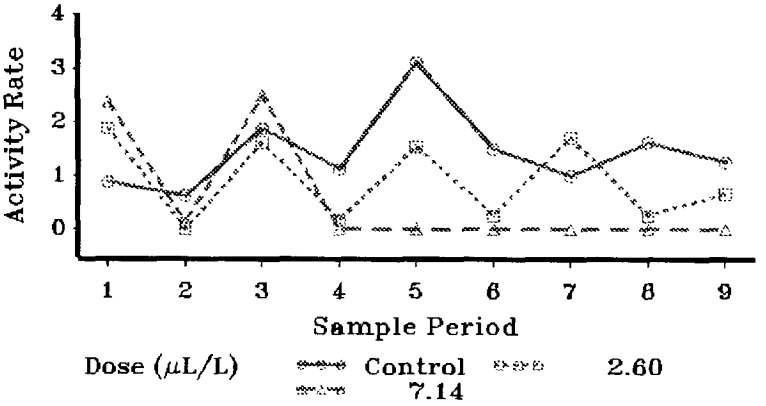

Figure 1. Activity (number of swimming bouts/tadpole/2 min) of green frog tadpoles exposed to Abate for 96-hr two sample periods per day.

METHODS AND MATERIALS

The bioassay generally followed ASTM (1988) guidelines for 96-hr static renewal tests. Green frog tadpoles (stage 25-27, Gosner 1960) were collected from unsprayed wetlands located at Patuxent Wildlife Research Center, Laurel, MD and kept in aerated pond water within an environmental chamber (temperature 20-21° C, 16:8 light:dark photoperiod) for 1 wk prior to testing. Tadpoles were then acclimated to reconstituted soft water (RSW, hardness 40-48 mg $CaCO_3L^{-1}$, ASTM 1988) by successively diluting pond water with RSW over an 8 d period. Four tadpoles were placed in each of 4-L glass bell jars containing 2 L of soft water and one of six geometrically arranged concentrations of Abate ranging from 1.86-10 µLŽL^{-1}. Each concentration was run in duplicate. Water was replaced with fresh solutions of Abate and RSW every morning. Tadpoles were fed a half pellet (ca 0.5 g) of rabbit chow (alfalfa) every other day.

Twice each day starting approximately 15 min after water was renewed, we sampled the activity level of tadpoles by counting the number of swimming events (active propulsion for >= 1 cm distance) during 2 min divided by the number of living tadpoles. Movement caused by another tadpole was not counted as an activity. Death was determined by lack of movement including signs of respiration when tadpoles were prodded with a glass rod. All dead tadpoles were collected as soon as possible, placed in glass jars for each treatment, and frozen at -20 °C. Survivors were anesthetized by chilling and then frozen.

To confirm nominal Temephos concentrations in water, we extracted 10 ml samples of water from a 10 µLŽL^{-1} and a 1.86 µLŽL^{-1} solution immediately after mixing. Temephos in water was extracted in triplicate with methylene chloride and the extracts were pooled, concentrated, and cleaned through a florisil

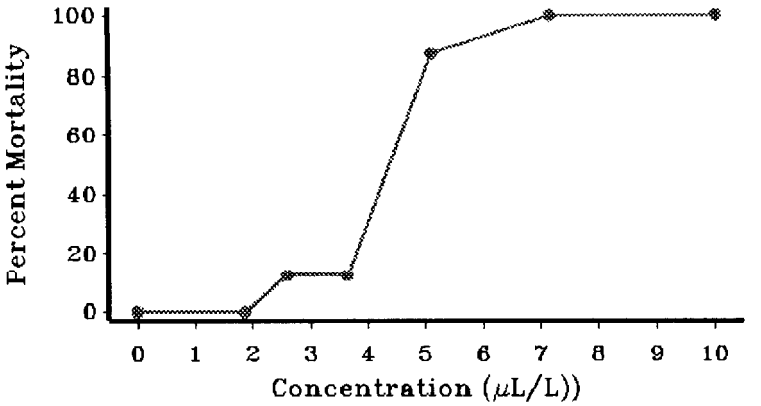

Figure 2. Percent mortality of green frog tadpoles exposed to Abate for 96-hr. Eight tadpoles per concentration.

column and analyzed via gas chromatography on a DB-1 capillary column. Detection limits were 0.08 μLŽL^{-1}. Analyzed values were ± 12% of nominal concentrations.

Within 2 wk, the intestinal coils of the tadpoles were removed and the remaining whole bodies were homogenized using a tissue grinder and analyzed for butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activities using the method of Ellman et al. (1961); activity rates of control tadpoles were used in lieu of specific activities. Acetylthiocholine iodide and γ–butyrylthiocholine iodide were used as substrates for AChE and BChE, respectively. To obtain repeatable activity rates with slopes > zero we had to wait 5 min before taking the first spectrophotometric reading and used 200 and 400 μL of tissue homogenate for AChE and BChE. Each sample was run at least in duplicate or until two readings were within 10% of each other and then averaged.

Differences in activity levels among concentrations of Abate were compared with analysis of variance after data were log-transformed; *a posteriori* comparisons were made with Tukey's test. The LC_{50} and associated statistics were calculated using logit analysis (Proc CATMOD, SAS 1987) which is more appropriate than probit analysis when multiple organisms are used per test chamber. The responses of BChE and AChE to dose were tested with regression analysis.

RESULTS AND DISCUSSION

Activity levels varied with concentration of Abate and time (Fig. 1). Tadpoles in control chambers tended to be more active in the morning (odd numbered sample periods) than in the afternoon. However, there was no general trend through time (r^2=0.019, p=0.582). Differences between morning and afternoon activity levels increased for tadpoles at intermediate concentrations of Abate (e.g. 2.60 μLŽL^{-1}).

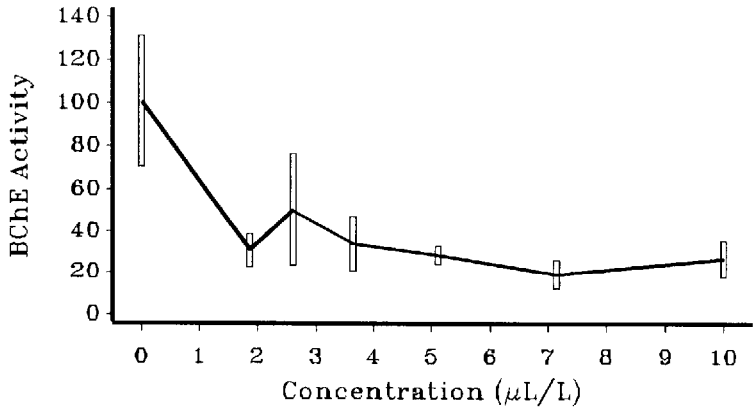

Figure 3. Mean (± S.E.M.) activity of BChE expressed as percent of control activity in green frog tadpoles exposed to Abate for 96-hr.

generated either through photocatalytic degradation within hours or metabolically (Eto 1974, Lores et al. 1985). The enhanced bimodality in activity at the intermediate concentrations compared to controls suggests that tadpoles have to be exposed to or metabolize temephos to these breakdown products in order to display symptoms but can recover with further degradation of temephos. Tadpoles at high dose levels (e.g. 7.14 µL•L^{-1}) nearly ceased moving by the afternoon of the second day of treatment. These tadpoles lay on the bottom of the jars and respired laboriously. Differences in activity rates were dependent on dose ($p = 0.0001$), sample period ($p = 0.0001$) and the interaction between dose and sample period ($p = 0.026$). Specifically, tadpoles at 5.10, 7.14, and 10.0 µL•L^{-1} were less active than controls ($p < 0.05$).

By 24 hr of exposure, half of the tadpoles at 10 µLŽL^{-1} had died. At the end of 96 hr, the percent mortality for each concentration was: control - 0%; 1.86 µLŽL^{-1} - 0%; 2.60 and 3.64 µL•L^{-1} - 12.5%; 5.10 µL•L^{-1} - 87.5%; and 7.14 and 10 µL•L^{-1} - 100% (Fig. 2). The jar ($p= 0.805$) and jar by dose interaction ($p=0.903$) effects were not significantly different from zero, indicating that these effects were trivial. The dose-response curve for Abate could be described as: logit(mortality) = -8.1329 - 1.917* Concentration (µLŽL^{-1}), LC_{50}=4.24 µL•L^{-1}, S.E. on slope= 0.594 µLŽL^{-1}, df=6, $p=0.0017$. This median lethal concentration is slightly higher than that found for salmonids (Mayer and Ellersieck 1986) and about the same as found by Tsai (1978) for other species of fish.

BChE and AChE responded very differently to these levels of Abate. The activity rate (± SD) for BChE in control tadpoles was 0.979 ± 0.415 mol min^{-1} g^{-1} of tissue which is consistent with values published for some fish species (Magnotti et al. 1994). The activity of this enzyme, expressed as percent of control value, declined precipitously with concentration of Abate, even at 1.86 µL•L^{-1} of Abate ($r^2= 0.577$, $p = 0.0001$) (Fig. 3). In contrast, AChE (mean activity rate in controls = 18.28 ± 2.96 mol min^{-1} g^{-1}) increased with concentration of Abate ($r^2= 0.348$, $p=0.0001$) (Fig. 4).

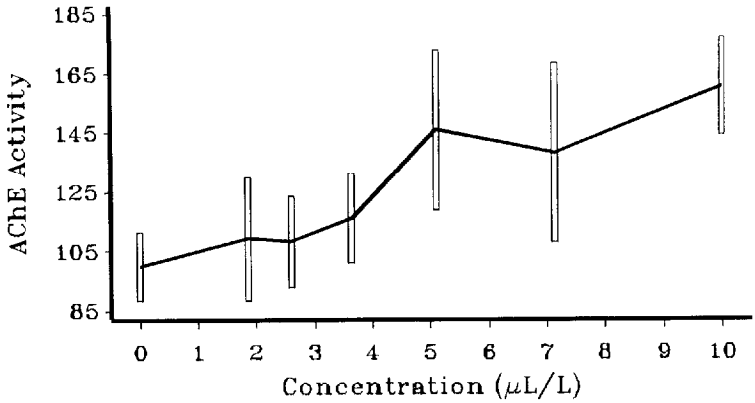

Figure 4. Mean (± S.E.M.) AChE activity as percent of control in green frog tadpoles exposed to Abate for 96-hr.

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BChE is produced by muscle and liver tissue in fish but its function is uncertain. The amount of enzyme present, or its activity rate, varies considerably among fish species and even tissues within a species (Kozlovskaya et al. 1993). AChE is the principal neurotransmitter in animals and tends to be concentrated in brain tissue. BChE is substantially more sensitive to cholinesterase-inhibiting pesticides than AChE in fish (Magnotti et al. 1994), birds (Thompson et al. 1991) and mammals (Ecobichon and Comeau 1973). Thus greater depression in the activity of BChE compared to AChE was expected. However, the increase in AChE with concentration of Abate demands further interpretation.

Typically, organophosphorus (OP) pesticides depress AChE activity (Hill and Fleming 1982, de Llamas et al. 1985). In fact, AChE depression compared to controls is often used as a diagnostic of OP poisoning (Ludke et al. 1975) . Rarely, animals may respond to low, sublethal, levels of OP exposure by increasing cholinesterase activity. Thompson et al. (1991) observed increased carboxylesterase activity in house sparrows *(Parus domesticus)* treated with demeton-S-methyl. Thompson and Walker (1994) suggested that this elevated cholinesterase could have come from the liver. Although the majority of AChE is in nervous tissue, Kozlovskaya et al. (1993) reported AChE activity in muscle and liver of fish. The parent compound of temephos has relatively low toxicity and must be metabolized to sulfone or sulfoxide forms to be lethal. Therefore, one possible answer to the diverging activities of AChE and BChE upon exposure to Abate is that green frog tadpoles are inefficient in metabolizing the pesticide. The more sensitive BChE was probably inhibited by the amount of temephos that was metabolized, but the AChE levels were unaffected. Tadpoles at the higher dose levels were stressed for long periods of time - sometimes for more than a day - and this stress could have stimulated the nervous system to produce more acetylcholine and AChE. Alternatively, tadpoles that died during the study laid for variable periods of time before they

were frozen and these delays may have affected AChE in some way that is presently unclear. Further studies are planned to examine this situation.

Label instructions on Abate prescribe application rates of 0.5 to 1.5 ounces per surface acre (ca. 244 mlŽha^{1}). This complicates hazard assessments based on concentration because water depths vary among wetlands. However, in an associated study, we determined that, following label instructions, application to a series of constructed wetlands with a mean depth of 0.5 m would result in a concentration of 0.02-0.05 µLŽL^{-1} which is two orders of magnitude less than the LC_{50} for this species. Because we renewed the Abate concentration each day, whereas temephos degrades rapidly under field conditions (Lores et al. 1985), tadpoles are probably at even less risk than suggested by these data.

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