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Azinphosmethyl Exposure to Grass Shrimp (*Palaemonetes pugio*) Life Stages with Emphasis on Larval Acetylcholinesterase Activity

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Azinphosmethyl is a nonsystemic, organophosphorus insecticide (OP) used to control insects and mites on a wide variety of crops (Labat-Anderson Inc. 1992). It is a derivative of thiophosphoric acid and produces toxicity by inhibiting the nervous system enzyme, acetylcholinesterase (AChE). The most recent estimates have put azinphosmethyl use in the US at 3 million lb active ingredient annually (EPA 1986). Fish kills have occurred in coastal South Carolina with water concentrations >7 µg/L after runoff from agriculture fields (Scott et al. 1990). Acute exposure of marine crustaceans to azinphosmethyl has been documented mostly with adult organisms (Labat-Anderson Inc. 1992; Moore 1988). Information is lacking regarding the effects of azinphosmethyl on larval and juvenile stages. Such information is important since, in the field, all developmental stages of a crustacean can be exposed to insecticide runoff.

The objectives of this research with azinphosmethyl were the following: 1) to conduct 96-hr acute toxicity bioassays in adult and two larval stages of the grass shrimp, *Palaemonetes pugio,* and; 2) to measure AChE activity in grass shrimp larvae following pulse dose exposures of azinphosmethyl during the larval life stage for assessment of sublethal effects and use of grass shrimp as an indicator species of OP exposure.

MATERIALS AND METHODS

Adult *Palaemonetes pugio* were collected from the western branch of Leadenwah Creek, a tidal tributary of the North Edisto River estuary in SC. This site has been extensively studied and is considered free from pesticide contamination (Scott et al. 1990). Seawater used for holding and toxicity tests was collected from Bohicket Creek, another tributary of North Edisto River also considered free from contamination. Adult shrimp were acclimated in 135-L tanks at 25°C, 20‰ salinity and 14-hr light:10-hr dark cycle. Shrimp were fed Tetramin® Fish Flakes and newly hatched *Artemia* (Buikema et al. 1980).

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To collect larvae, gravid females were placed in brooding traps (Key and Fulton 1993). An adequate number of larvae the same age were obtained from broods hatched at the same time from at least ten females. Larvae were pooled to minimize effects of genetic differences (Sandifer and Smith 1979). Larvae, to be exposed as 18-day old larvae, were segregated and reared in 8-L bowls under conditions similar to adults. All larvae were fed newly hatched *Artemia.*

Static renewal 96-hr bioassays, modified from Buikema et al. (1980) and McKenney (1986), were conducted on three age groups (newly hatched larvae, 18-day old larvae, and adults). All tests were conducted in a Revco® environmental chamber under conditions as described above. Five adult shrimp were exposed in 2-L glass bowls with three replicates for each of five azinphosmethyl concentrations and one control. Each exposure bowl contained 1 L of media which was renewed every 24 hr. Adults were not fed during the tests. The nominal concentrations for adult exposures were 0.20, 0.40, 0.80, 1.60 and 3.20 µg/L.

Newly hatched larvae (1 - 2 days old) were exposed in 4-L glass aquaria containing three compartmentalized plexiglass cages. Larvae were randomly placed one to each compartment for each of five azinphosmethyl concentrations and one control (30 animals/treatment). Each exposure aquarium contained 1 L of media which was renewed every 24 hr. Larvae were fed newly hatched *Artemia* after each water change. The nominal concentrations for newly hatched larvae exposures were 0.15, 0.30, 0.60, 1.20 and 2.40 µg/L.

Eighteen-day old larvae were chosen as the next life stage tested as this age was considered representative of the average age at metamorphosis to postlarvae in standards set forth by Tyler-Schroeder (1978). These larvae were exposed as described for newly hatched larvae. The nominal concentrations for 18-day old larvae exposures were 0.15, 0.30, 0.60, 1.20 and 2.40 µg/L.

Newly hatched grass shrimp larvae for AChE analysis were exposed to pulses of azinphosmethyl in 2-L bowls at three concentrations and a control for 15 days. The pulse exposures were for 6 hr/day every five days with each exposure occurring at a salinity of 10‰. This simulated field observations of rain events (lowered salinity) and tidal cycles (6 hr exposure) in SC. This exposure protocol was a modification of that developed by Key and Fulton (1993) and McKenney (1986). Exposures were performed on days 0, 5, 10, and 15 of the larvaes' life. At the end of each 6-hr pulse exposure, animals were placed in clean seawater at 20‰ salinity simulating salinity during times of no rain. Daily water changes (between exposures) were also made at 20‰ salinity. Three replicates of each concentration and control were used (40

animals/treatment). The nominal azinphosmethyl concentrations were 0.15, 0.60 and 2.40 µg/L. Larvae from each concentration and control were sampled (10 - 15 larvae/sample) for AChE analysis at the end of the 6-hr pulse exposure on day 0 and day 15. Whole body AChE activity was measured (as described in Key *et al. 1998 to be published in Aquatic Tox*) and reported as nmol product formed/mg wet weight/min.

For all tests, technical grade azinphosmethyl (phosphorodithioic acid 0,0-dimethyl S-[(4-oxo-1,2,3-benzotriazin3(4H)-yl) methyl] ester) was provided by Mobay Corporation. Pesticide grade acetone was used as a carrier (0.1%). Acetone was added to the control groups equal to the amount of carrier solvent used for the toxicity tests. For analytical assessment, a 10 µg/L azinphosmethyl spike was extracted and measured with an HP 5890A gas chromatograph (Scott et al. 1990). An azinphosmethyl recovery efficiency of 87 ± 5% was obtained. All concentrations presented in this paper are nominal.

Median Lethal Concentrations (LC50) with 95% confidence limits were determined using the Trimmed Spearman-Karber Method (Hamilton et al. 1977). Analysis of variance (ANOVA) was used to determine if significant group differences ($p \leq 0.05$) existed in AChE activity. Dunnett's procedure for comparison was used to determine significant differences from the control response for this measurement (Gad and Weil 1988).

RESULTS AND DISCUSSION

The 96-hr LC50 values for the three grass shrimp life history stages ranged from 0.38 µg/L for 18-day old larvae to 1.64 µg/L for adults (Table 1). Adult grass shrimp were significantly more tolerant to azinphosmethyl exposure than either the 18-day old larvae or newly hatched larvae after 96 hr. The 18-day old larvae were significantly more sensitive to azinphosmethyl than adults or newly hatched larvae (Table 1). The adult LC50 value was similar to adult grass shrimp LC50 values found in other studies. In 96-hr tests conducted by Moore (1988) on adult *P. pugio,* three LC50 tests yielded 1.20, 1.07, and 0.93 µg/L with a pooled LC50 of 1.05 µg/L. These values were slightly lower than the adult LC50 of this present study but were significantly higher than the 18-day old and newly hatched larvae LC50s. A related freshwater grass shrimp, *P. kadiakensis,* exhibited an LC50 of 1.2 µg/L (Mayer and Ellersieck 1986) similar to the adult LC50 of 1.64 µg/L in this present study.

Whole body AChE activities at day 0 and day 15 from the pulse exposure test are shown in Figure 1. AChE activity was significantly reduced ($p<0.05$) from control after 6 hr of exposure at all three concentrations tested (day 0, Figure 1). After four 6 hr exposures, only the 0.60 µg/L and

2.40 µg/L exposure groups were significantly lower ($p \leq 10.05$) than the control (day 15, Figure 1). For both time periods, there was a progressive reduction in AChE activity with increasing azinphosmethyl concentrations. Mortality did not occur in any concentration until day 1, 24 hr after the first exposure. Mortality on day 15, after the fourth 6 hr dose, ranged from 57% for the 2.40 µg/L concentration to 6% for the 0.6 µg/L concentration. It is crucial to note how these results point to the impact of sublethal azinphosmethyl exposures on crustacean larvae. The lowest concentration (0.15 µg/L) produced significantly depressed levels of AChE in the newly hatched larvae (day 0) after only 6 hr of exposure. This concentration was over three times lower than the newly hatched larval 96-hr LC50 (0.52 µg/L) and over 46 times lower than levels of >7 µg/L found in the field from agricultural runoff (Scott et al. 1990).

Table 1. LC50 values for adult, 18-day old and newly hatched larval *Palaemonetes pugio* continuously exposed to azinphosmethyl for 96 hr at 20‰ salinity.

Life Stage	LC50 (µg/L)	95% Confidence Limits µg/L
Adult	1.64	1.27 - 2.12
18-day old larvae	0.38	0.34 - 0.43
Newly hatched larvae	0.52	0.45 - 0.61

The AChE levels were lower for larvae at day 15 as compared to levels for day 0 larvae. At day 0, AChE activity was reduced by 85% in the 2.40 µg/L exposure group as compared to controls. At day 15, AChE activity was reduced by only 54% in the highest concentration as compared to controls. It is evident that younger larvae were more responsive to AChE inhibition than older larvae. The older day 15 larvae also exhibited overall lower AChE activity as compared to day 0 larvae since AChE activity will naturally decrease on a weight basis as larvae grow larger (Key *et al. 1998 to be published in Aquatic Tox*). The AChE levels at day 15 also suggests that recovery of AChE levels occurred between the four pulse doses. This may be evident in the highest concentration having similar AChE activities at both day 0 and day 15. For both larval age groups, the relationship between acute toxicity and AChE inhibition was directly correlated. As AChE inhibition increased toxicity to shrimp increased as well.

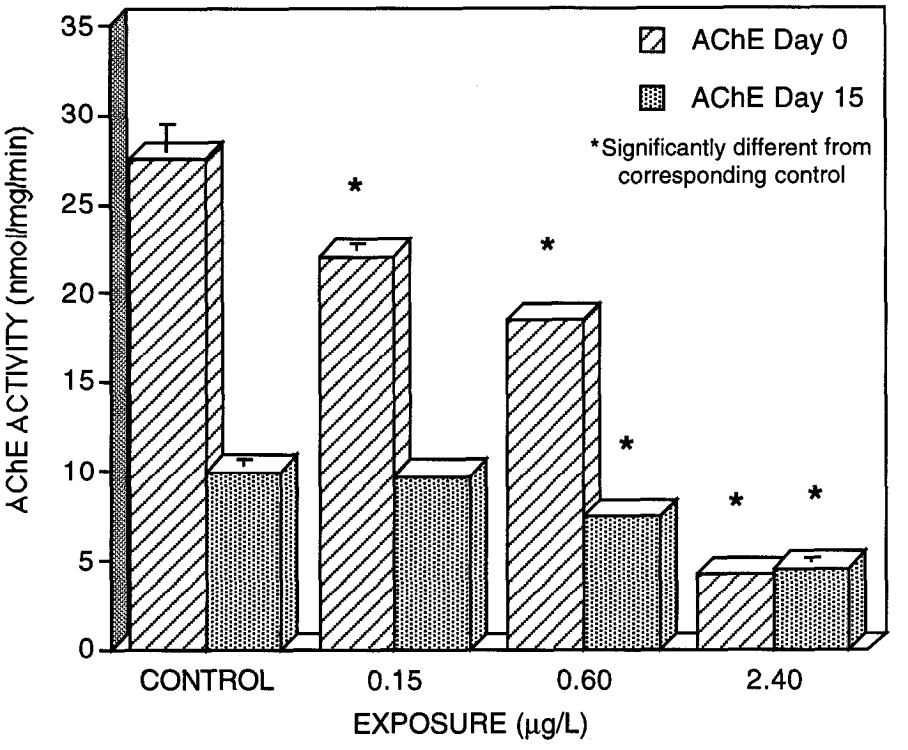

Figure 1, AChE activity in *Palaemonetes pugio* larvae at day 0 (one 6-hr pulse dose) and day 15 (four 6-hr pulse doses) after azinphosmethyl exposure at 10 ‰ salinity. The error bars represent the standard error of the mean.

Other studies (Coppage and Matthews 1974; Cripe et al. 1984) reporting AChE effects after azinphosmethyl exposure have been mainly with fish and basically showed what was presented in this research - azinphosmethyl significantly inhibits AChE levels. Those studies also concluded that analysis of AChE levels in aquatic animals would be a useful indicator of azinphosmethyl exposure. In this present study, the correlation between AChE activity and azinphosmethyl toxicity in grass shrimp larvae should point to increased use of *P. pugio* as an indicator species of OP exposure.

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