ORGANOPHOSPHATE-INDUCED ENZYME INHIBITION AND ACUTE TOXICITY IN EMBRYONIC *PALAEMONETES PUGIO*

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ABSTRACT

A common and ecologically important inhabitant of estuarine ecosystems is the grass shrimp, *Palaemonetes pugio*. Since the reproductive period in these animals corresponds to the peak agricultural growing season, all developmental stages of grass shrimp are at risk for pesticide exposure. Organophosphorus compounds (OPs), which produce toxicity via the inactivation of acetylcholinesterase (AChE) enzyme, are widely used as agricultural insecticides. Earlier research has shown that 24-h EC_{50} values for AChE inhibition are highly predictive of 96-h LC_{50} values in adult and larval grass shrimp. The goal of this study was to examine the relationship between OP-induced enzyme inhibition and acute toxicity in embryonic grass shrimp. The widely used OP insecticide, chlorpyrifos, was used as a model toxicant. Embryos used in the AChE bioassay were exposed for 24 hours in 100 ml Erlenmeyer flasks. Each flask contained 100 embryos and 100 ml of the appropriate treatment solution. Twenty-four well tissue culture plates were used in the 96-h acute toxicity tests. Each well contained one embryo and 2 ml of the treatment solution. The chlorpyrifos 24-h EC_{50} value was 0.49 µg/L (95% C.I. = 0.35 - 0.77), while the 96-h LC_{50} value was >40.00 µg/L. These findings suggest that the relationship between OP-induced AChE inhibition and mortality in grass shrimp embryos is less clear-cut than that reported for adult and larval organisms. Furthermore, developing embryos appear to be more tolerant of reduced levels of AChE activity.

INTRODUCTION

Acetylcholinesterase (AChE) is a nervous system enzyme whose primary function is to terminate nerve impulse transmission at cholinergic synapses by hydrolyzing the neurotransmitter acetylcholine (ACh). When AChE is inhibited, there is a build up of acetylcholine in the synaptic cleft. This accumulation of ACh leads to a blockade of nerve impulses. In invertebrates, this blockage induces the typical pattern of nerve poisoning: restlessness, hyperactivity, tremors, convulsions, and paralysis (Ware, 1989). Inhibition of AChE occurs when an organism is exposed to a cholinesterase-inhibiting insecticide (i.e. organophosphate and carbamate insecticides).

Grass shrimp, *Palaemonetes pugio*, fulfill a vital role in energy transfer and ecosystem stability by accelerating the breakdown of detritus and serving as a prey item for numerous commercially and recreationally important fish and crustaceans. Embryos are brooded externally on a female's abdomen for about two weeks prior to hatching and are thus directly exposed to any contaminants that might be present in the water column.

A study by Key (1995) examined the relationship between acute toxicity and AChE inhibition in larval and adult grass shrimp exposed to three organophosphorus insecticides: azinphosmethyl, malathion, and chlorpyrifos. Key (1995) determined that six out of the eight 24-h EC_{50} values were predictive of the subsequent 96-h LC_{50} values. A correlation (r = 0.93) provided further support for this relationship. The objective of this study was to use the model toxicant, chlorpyrifos, to examine the relationship between OP-induced AChE inhibition and acute toxicity in embryonic grass shrimp.

MATERIALS and METHODS

Experiment # 1: AChE Bioassay

- Stage VI embryos were pooled from 3 - 4 females.
- 100 embryos were exposed in 125 ml Erlenmeyer flasks to 100 ml of the appropriate treatment solution.
- Treatments: 0.125, 0.250, 0.500, 1.00, and 2.00 µg/L.
- Test conditions: 20 ppt salinity, 20°C, and 12-h light:12-h dark.
- A Median Effective Concentration (EC_{50}) was calculated using a linear interpolation method: The Inhibition Concentration Approach (Norberg-King, 1993).

Experiment # 2: Acute Toxicity of Chlorpyrifos

- Stage V embryos were pooled from two females.
- A twenty-four well tissue culture plate was used as the dosing chamber for each treatment.
- Each well contained 1 embryo and 2 ml of the appropriate treatment solution (n=24).
- Treatments: 0.3125, 0.625, 1.25, 2.50, 5.00, 10.00, 20.00, and 40.00 µg/L.
- Test conditions: 20 ppt salinity, 27°C, darkness.
- An 80% renewal of each treatment solution was done every 24 hours.

Experiment # 3: Time to Hatch

- Embryos that survived the 96-h exposure period in experiment # 2 were dosed daily until hatching.
- The time to hatch was determined for each treatment.
- Kruskal-Wallis One Way Analysis of Variance on Ranks were performed to determine if there were significant differences among treatments with respect to time to hatch.
- Dunn's Method was used to compare the treatments to the control.

RESULTS

Experiment # 1: AChE Bioassay

- 24-h EC_{50} for AChE inhibition in the embryos was 0.49 µg/L (95% C.I. = 0.35 - 0.77 µg/L) (Table 1).

Table 1: Embryonic chlorpyrifos 24-hr EC_{50} value and mean % AChE inhibition for each treatment.

Treatment (µg/L)	Mean % AChE Inhibition
Control	0
0.125	0
0.25	11.7
0.5	41.2
1	64.4
2	72.2
24-h EC_{50} = 0.49 µg/L (95% C.I. = 0.33 - 0.77)	

RESULTS

Experiment #2: Acute Toxicity of Chlorpyrifos

- In the acute toxicity tests, mean mortality after 96 hours was less than 50% at the highest concentration tested (40.00 µg/L).

Table 2. Percent mortality for each treatment following a 96-h exposure

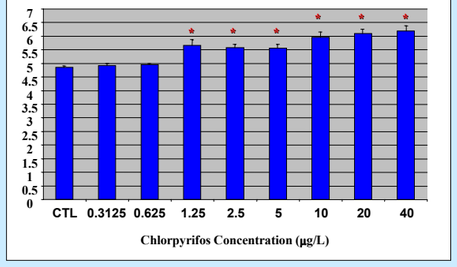
Treatment (µg/L)	Rep 1	Rep 2	Rep 3	MEAN
Control	0	8.33	0	2.78
0.3125	0	0	4.17	1.39
0.625	4.17	0	0	1.39
1.25	45.83	0	4.17	16.67
2.50	33.33	8.33	0	13.89
5.00	62.5	8.33	0	23.61
10.00	37.5	0	8.33	15.28
20.00	50	0	0	16.67
40.00		0	4.17	2.09

Experiment # 3: Time to Hatch

- A delay in hatching was observed as the chlorpyrifos concentrations increased (Figure 1).

Figure 1: Mean # of Days to Hatch for Embryos Exposed to Chlorpyrifos

* Denotes a significant difference from the control



CONCLUSIONS

- Embryonic grass shrimp AChE activity was inhibited by chlorpyrifos. The 24-h EC_{50} value was **0.49 µg/L** (0.35 - 0.77 µg/L) (Table 1). Key (1995) noted similar AChE sensitivity in both larval (newly hatched 24-h EC_{50} = **0.56 µg/L** and 18-Day Old 24-h EC_{50} = **0.28 µg/L**) and adult (24-h EC_{50} = **0.56 µg/L**) grass shrimp.
- In the acute toxicity tests, little mortality was observed at concentrations >80 fold higher than the 24-h EC_{50} for AChE inhibition.
- Although Key (1995) observed a strong relationship between OP-induced AChE inhibition and mortality for larval and adult grass shrimp, there was no clear-cut relationship in the embryos.
- Developing embryos appear to be more tolerant of reduced levels of AChE activity than larvae or adults.
- Chlorpyrifos exposure caused an increase in hatching times at concentrations similar to those causing significant AChE inhibition (Figure 1).

REFERENCES

Key, P.B., 1995. The lethal and sublethal effects of malathion, azinphosmethyl, and chlorpyrifos exposure on the grass shrimp, *Palaemonetes pugio*, with emphasis on larval life cycle pulse exposure. PhD. Dissertation. University of South Carolina, SC, p. 124.

Norberg-King, T.J., 1993. A linear interpolation method for sublethal toxicity: The inhibition concentration (ICp) approach. National Effluent Toxicity Assessment. Technical report 03-93.

Ware, G., 1989. The pesticide book. Thomson, Fresno, CA, p. 336.