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EPA ENVIRONMENTAL RESEARCH BRIEF

Influence of an Insect Growth Regulator on Larval Development of a Marine Crustacean

Charles L. McKenney, Jr. and Edward Matthews*

Introduction

Increased understanding of the role of hormones in the regulation of a number of life processes of insects gained through intensive pioneer research during the first half of this century has introduced these hormones and their analogues as "third generation pesticides" used as biochemical biological control agents for insect pests (Williams, 1967, Slama et al., 1974). Of particular interest in strategies for insect control are certain hormones known as insect growth regulators, which are involved in the regulation of developmental and growth processes of insects (Bowers, 1982; Mian and Mulla, 1982; Staal, 1982; Jennings, 1983).

It has now been established that several hormones are involved in regulating the larval development of insects through the critical and complex process of metamorphosis (Hoar, 1975; Downer and Laufer, 1983) In addition to natural insect juvenile hormones, several major types of insect growth regulators have been studied to date (Mian and Mulla, 1982; Downer and Laufer, 1983). The most extensive group are the juvenile hormone analogues, which are substances of natural or synthetic origin that act as endogenous juvenile hormone and disrupt insect larval development.

When these insect growth regulators are used in pest control, residues of these compounds may enter the marine environment either by direct application toward aquatic-borne pests (such as mosquitoes) or indirectly through land-drainage or erosion from the adjacent pesticide-treated agricultural lands. With these usage patterns, it seems of particular interest to determine whether these compounds may affect marine or estuarine populations of closely related nontarget organisms. Substances that act as growth hormones for insects are found not only in insects, but also in other invertebrates (Tombes, 1970, Barrington, 1979). Of particular relevance are the crustaceans, the dominant marine member of the same phylogenetic group containing insects, Arthropoda. In fact, compounds with juvenile hormone activity in insects have been extracted from crustaceans (Schneiderman and Gilbert, 1958), suggesting that juvenile hormone may function in crustacean larval development and metamorphosis.

Since the mode of action of juvenile hormone in insects entails regulation of the complex process of metamorphosis during larval development, it is not surprising that larval stages of insect pests are routinely used as bioassay organisms in testing the activity of juvenile hormone analogues as potential insecticides (Downer and Laufer, 1983; Mian and Mulla, 1982) To best predict the environmental impact of this type of insecticide on estuarine biota, an analogous examination of their influence on the larval development of a ubiquitous and ecologically important estuarine crustacean appears appropriate For this purpose a series of studies were initiated to examine the effects of methoprene, a juvenile hormone analogue used in mosquito control, on developing larvae of the grass shrimp *Palaemonetes pugio*

Approach

To obtain larvae for the study, ovigerous grass shrimp (*Palaemonetes pugio*) were collected from shallow grass beds of Santa Rosa Sound, FL, and held in the laboratory in flowing, filtered (20 µm) seawater at 25 ± 1° C and 20 ± 1o/oo S, optimal temperature and salinity conditions for larval development of *P. pugio* (McKenney and Neff, 1979).

*The authors are with the U S Environmental Protection Agency's Environmental Research Laboratory, Gulf Breeze, FL 32561

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Upon release, larval grass shrimp were separated from the adult shrimp, using a flow-through hatching apparatus. Newly released *P. pugio* larvae were randomly distributed among replicate exposure conditions either individually in compartmented plastic boxes as previously described by McKenney and Neff (1981) or in groups of 10 in glass beakers as described by McKenney and Hamaker (1984). The exposure media was renewed daily for each exposure condition in each of the three replicates (6 larvae per replicate) in the plastic boxes. Larvae in the glass beakers were placed in a flow-through exposure system similar to that described by McKenney (1986) utilizing a proportional diluter described by Schoor and McKenney (1983).

Larvae exposed in boxes were individually reared through completion of metamorphosis in a range of nominal concentrations of the juvenile hormone analogue methoprene Both technical grade (R,S)-methoprene, isopropyl [2E,4E]-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate, and the single isomer (S)-methoprene, isopropyl [2E,4E,7S]-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate as contained in the formulation Altosid Liquid Larvicide (ALL) were used in these static exposures For the flow-through studies larvae were exposed only to (S)-methoprene in ALL All methoprene concentrations referred to throughout this paper are nominal concentrations In static exposures, daily records were maintained on survival and molting frequency Molting rate was determined by the presence of the cast exuvia for each larva Observations for individual larva were ended upon completion of larval development through metamorphosis to the postlarval form (Broad, 1957). Similarly, survival and developmental duration to completion of metamorphosis were recorded for all larvae in the flow-through exposures.

On days corresponding with the intermolt of various larval stages, 8 larvae for each exposure concentration were individually sealed in all-glass syringes with fully oxygenated media from the appropriate exposure condition; for each larva, oxygen consumption and ammonia excretion rates were determined using methods described by McKenney (1982) and Shirley and McKenney (1987) Each larva was then briefly rinsed in distilled water and placed in an oven to dry at 60° C for 48 hours The weight of each larva was subsequently determined to the nearest 0 1 µg on an electronic microbalance O:N ratios were calculated for each larva as the ratio of atoms of oxygen consumed to atoms of nitrogen excreted

Growth rates of larvae were determined, using their respective dry weights and formulae described by McKenney (1986). In addition, bioenergetic terms for daily production (P) and maintenance (R) costs were derived from the caloric equivalents of daily growth and respiration rates of the larvae as described by McKenney (1982) These values were used to derive net growth efficiency values (K_2) (Winberg, 1971) or larvae under the various methoprene exposure conditions by the formula

$$K_2 = \frac{P}{(P+R)}$$

Values for the various biological responses are presented as means ± standard errors. Significant differences between control and exposed larvae were determined by analysis of variance with arcsine transformation to stabilize variable survival percentage data (Zar, 1984) and William's procedure for multiple comparisons between means (Williams, 1972).

Results and Discussion

During exposure to technical grade concentrations of the isomeric mixture (R,S)-methroprene and equivalent concentrations of the single isomer (S)-methoprene in a liquid formulation (Altosid Liquid Larvicide), differential survival values were found through the complete larval development of *Palaemonetes pugio* (Figure 1). No larvae survived completion of metamorphosis while exposed to 1000 0 µg methoprene/l in a static-renewal system, regardless of the isomeric form Even though larval survival was significantly reduced by exposure to 100.0 µg/l of (R,S)-methoprene and not by this concentration of (S)-methoprene, an analysis of variance of the entire data set showed no significant difference in larval survival between the two isomers for exposure concentrations from 0.1 to 1000 0 µg/l

An examination of the survival of discrete larval stages of grass shrimp during exposure to (R,S)-methoprene showed that larval toxicity to 100 µg/K was stage specific with the second and final larval stages responsible for significant mortality (Table 1). This apparent stage-specific sensitivity to juvenile hormones and their analogues is seen during insect larval development (Downer and Laufer, 1983) and accounts for the predominant use of the final, premetamorphic larval instar of insects during efficacy testing of these compounds as insecticides (Mian and Mulla, 1982, Downer and Laufer, 1983).

Further examination of this phenomenon as it exists in the response of crustacean larvae to insect growth regulators resulted in a study demonstrating differential survival rates of early and final larval stages of *P. pugio* when exposed to (S)-methoprene in a flow-through system (Figure 2). Larval viability during the first two larval stages was reduced by four days exposure to concentrations of 31 and 62 µg methoprene/l, but not for exposure during the final larval stage. An analysis of variance of the 96-hour survival patterns of these larval stages separately exposed to concentrations of (S)-methoprene from 31 to 250 µg/l revealed no significant differences in sensitivity between these larval stages.

The type of exposure system used to evaluate the effects of insect growth regulators on crustacean larvae can influence the results of these studies Survival of *P. pugio* through total larval development was several orders of magnitude more sensitive to methoprene in a flow-through exposure system than when using a static-renewal exposure system. A concentration of 100 µg/l(S)-methoprene produced no significant larval mortality in a static-renewal system (Figure 1), while a concentration of 8 µg/l reduced the number of larvae completing metamorphosis from 82 to 28 percent in a flow-through system (Table 2). It is also interesting to note in the latter study that the developmental duration of *P. pugio* larvae was prolonged by methoprene exposure, suggesting a similar hormonal function of juvenile-hormone-like compounds in crustaceans as seen in insects.

Modifications in the energy metabolism of grass shrimp larvae occurred with exposure to methoprene

Figure 1. **Comparative survival percentages of *Palaemonetes pugio* from hatch through completion of metamorphosis while exposed in a static-renewal system to a range of concentrations of (R,S)- and (S)-methoprene. Asterisk denotes significant differences from the control (0) survival ($P<0.05$).**

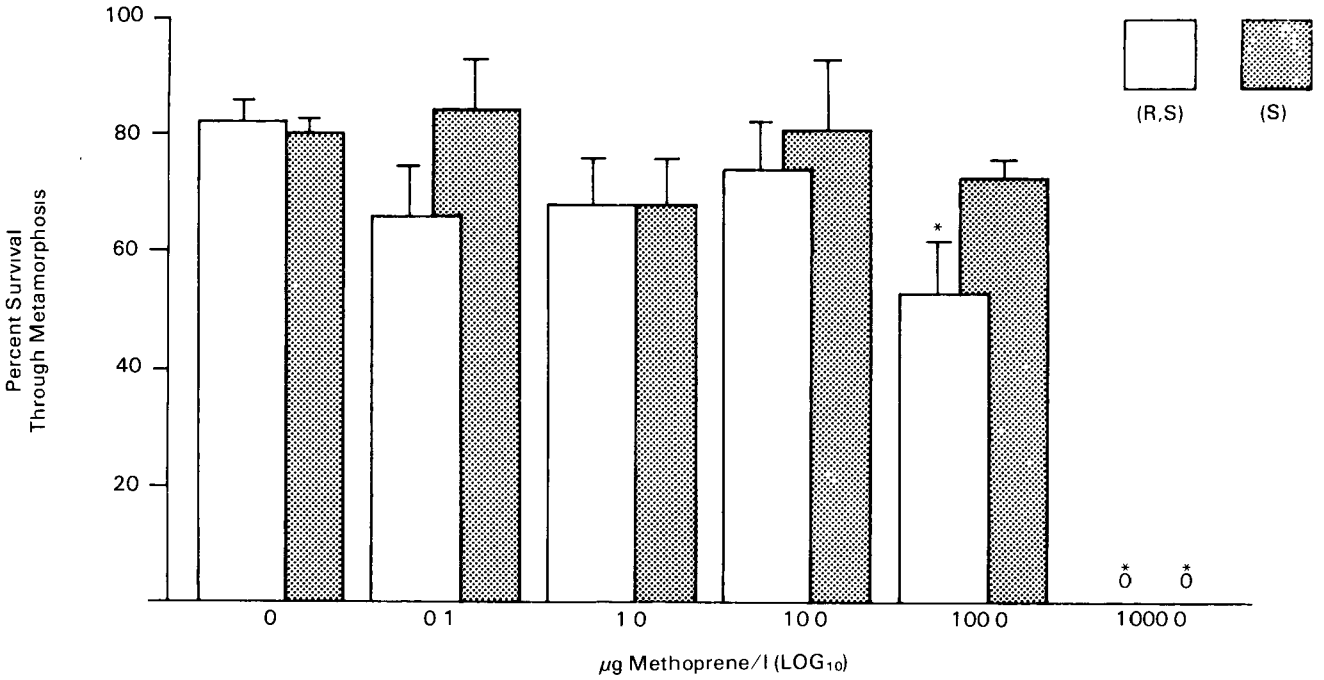

Table 1. Survival Percentage of Discrete Larval Stages of *Palaemonetes pugio* Reared Through Larval Development to Completion of Metamorphosis While Exposed in a Static-Removal System to a Range of Concentrations of (R,S)-Methoprene.

Concentration (µg/l)	Larval Stage						
	I	II	III	IV	V	VI	VII
0.0	100	100	98	98	98	95	96
0.1	100	100	100	100	91	97	91
1 0	100	100	98	91	92	90	93
10.0	100	100	100	94	96	94	92
100.0	100	92[a]	100	94	100	90	79[a]
1000.0	17[a]	44[a]	100	100	100	100	100

[a]Significantly less than the control (0.0) survival at that larval stage ($p<0.05$).

concentrations which prevented successful development through completion of metamorphosis (Table 3 a,b). Respiration rates of larvae were significantly elevated as early as 24 hours after exposure to these concentrations of methoprene For unexposed larvae, O.N ratios suggested a shift from predominant usage of lipid as major metabolic substrate to a significant increase in protein as the energy source during premetamorphic larval stages Similar energy substrate patterns toward greater reliance on protein catabolism just prior to metamorphosis have been observed during the larval development of other marine crustaceans (Capuzzo and Lancaster, 1979; Anger, 1986) and may represent a physiological prerequisite necessary for successful completion of metamorphosis. However, lipid catabolism remained dominant for premetamorphic larvae of *P. pugio* exposed to methoprene, as indicated by their significantly higher O.N ratios (Table 3b). Modification of this premetamorphic energy utilization pattern by methoprene exposure could indicate an important physiological mechanism of toxicity of these substances to developing crustacean larvae.

The earliest and most sensitive response of grass shrimp larvae to methoprene exposure was a retardation of their growth rates (Table 4 a,b). Reduced net growth efficiency values suggest that retarded larval growth rates resulted from less assimilated energy being available for tissue production. As indicated by elevated respiration rates of methoprene-exposed larvae, proportionally more of the physiologically available energy was channeled into energy required for metabolic maintenance. In that juvenile hormones are thought to also play a functional role in the regulation of insect energy metabolism (Downer and Laufer, 1983), these metabolic and bioenergetic responses of

Figure 2. **Comparative 96-hour survival percentages of *Palaemonetes pugio* larvae after exposure to (S)-methoprene during first larval stages and final premetamorphic larval stage. Asterisk denotes significant differences from the control (0) survival ($P<0.05$).**

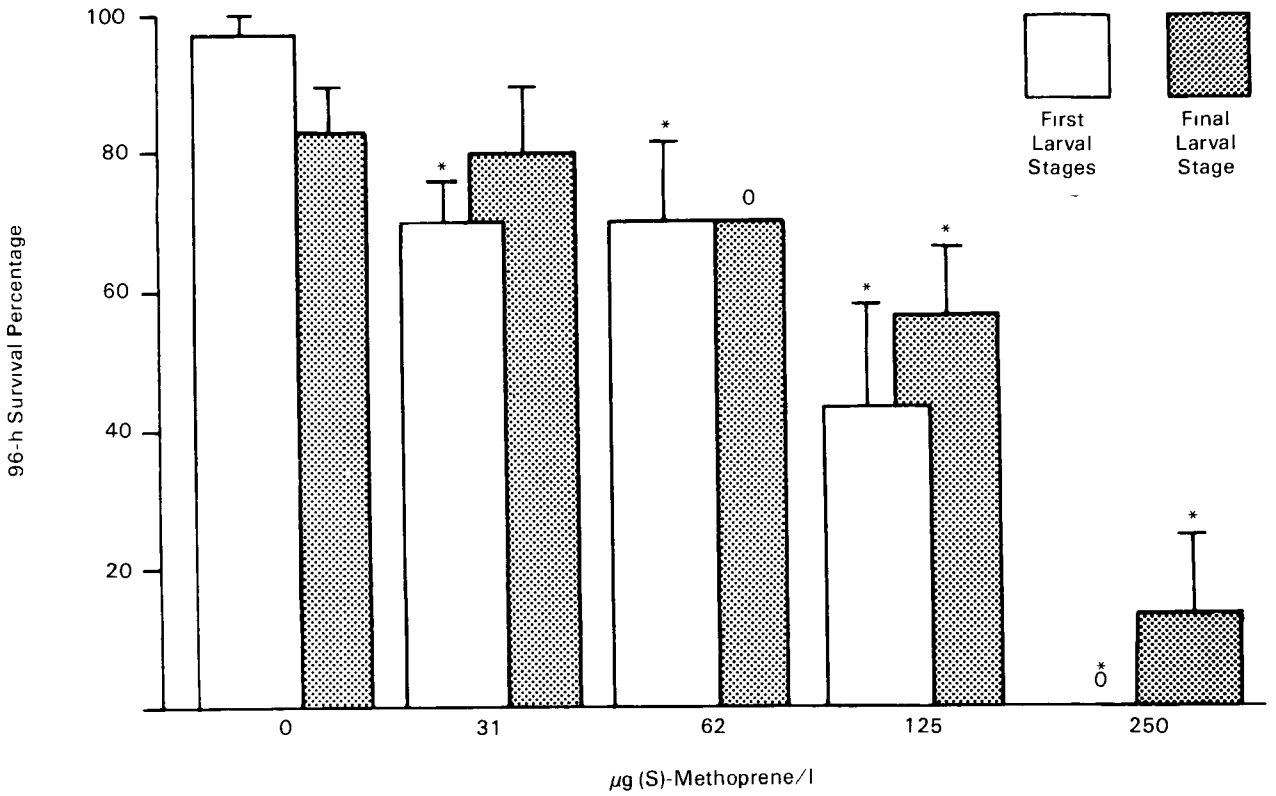

Table 2. Survival Percentage and Developmental Duration of *Palaemonetes pugio* Reared Through Larval Development to Completion of Metamorphosis While Exposed in a Flow-Through System to a Range of Concentrations of (S)-methoprene. Each Value Represents the Mean ± Standard Error.

Concentration (µg/l)	Survival Percentage	Developmental Duration in Days
0	82 ± 4	18.8 ± 0.7
8	28[a] ± 6	18.5 ± 0.4
16	8[a] ± 3	17.5 ± 0.7
32	7[a] ± 4	20 5 ± 0.5
62	2[a] ± 2	29.0[a] ± -

[a]Significantly different from the control (0 0) value ($p < 0.05$).

crustacean larvae to an insect growth regulator with juvenile-hormone activity may suggest similar endocrine control of these functions in this closely related group of organisms.

Conclusions

Larval survival, growth, and energy metabolism of an estuarine shrimp (*Palaemonetes pugio*) were altered by exposure to low µg/l concentrations of an insect growth regulator (the juvenile hormone analogue, methoprene) Larvae were several orders of magnitude more sensitive to methoprene in a flow-through exposure system than in a static-renewal exposure system.

The first two larval stages and the final premetamorphic larval stage were more sensitive to methoprene toxicity than the intermediate larval stages As indicated by reduced net growth efficiency values, elevated metabolic maintenance demands of exposed larvae were related to retarded larval growth rates. A premetamorphic shift in substrate utilization patterns, thought to be a physiological prerequisite for successful metamorphosis in marine crustaceans, was altered by exposure to methoprene concentrations that prevented completion of larval development through metamorphosis.

These findings support an analogous functional approach in the selection of an appropriate testing procedure to

Table 3a. The Influence of Continual Exposure to a Range of Concentrations of (R,S)-Methoprene on the Energy Metabolism of Various Aged *Palaemonetes pugio* Larvae. VO = Weight-Specific Oxygen Consumption Rate (µg 02 mg^{-1} Dry Wt h^{-1}); VN = Weight-Specific Ammonia Excretion Rate (µg NH_3-N mg^{-1} Dry Wt h^{-1}); and O:N = Ratio of Atoms of Oxygen Consumed to Atoms of Nitrogen Excreted.

Concentration (µg/l)	Early Larvae - 1-day old		
	VO	VN	O:N
0	10 7 ± 0.5	0.086 ±0.015	136 ± 21
8	12.8 ± 0.8	0.104 ± 0.012	116 ± 15
16	12.4 ± 1.1	0.089 ±0.012	137 ± 15
32	14.4[a] ± 1.4	0.125 ± 0.028	122 ± 16
62	14.0[a] ± 2 0	0.189[a] ± 0.090	120 ± 33
125	15.4[a] ± 1.0	0.278[a] ± 0.049	57[a] ± 7
250	23.4[a] ± 3.6	0.308[a] ± 0.047	64[a] ± 16

Concentration (µg/l)	Intermediate Larvae - 9-days old		
	VO	VN	O.N
0	6.0 ± 1.0	0.070 ± 0.036	120 ± 30
8	5.7 ± 0.3	0.035 ± 0.008	152 ± 26
16	6.0 ± 0.7	0.097 ± 0.058	159 ± 64
32	8.7[a] ± 0 9	0.071 ± 0.011	124 ± 20
62	9.4[a] ± 1.4	0 100 ± 0 028	89 ± 16

[a]Significantly different from the control (0) ($p < 0.05$)

***Table 3b. The Influence of Continual Exposure to a Range of* Concentrations of (R,S)-Methoprene on the Energy Metabolism of Various Aged *Palaemonetes pugio* Larvae. Symbols are as described in Table 3a.**

Concentration (µg/l)	Premetamorphic Larvae - 16-days old		
	VO	VN	O·N
0	5.7 ± 0.5	0.235 ± 0 042	25 ± 4
8	5.7 ± 0.3	0.130 ± 0.051	72[a] ± 21
16	4.0[a] ± 0.2	0.099[a] ± 0 042	73[a] ± 22

Table 3b (continued)

Concentration (µg/l)	Postlarvae - 19-days old		
	VO	VN	O:N
0	4 4 ± 0.5	0 252 ± 0 028	16 ± 2
8	4.7 ± 0.2	0 112 ± 0.040	66[a] ± 14
16	4 5 ± 0.4	0 154 ± 0 031	32[a] ± 10

[a]Significantly different from the control (0) ($p < 0.05$)

Table 4a. The Influence of Continual Exposure to a Range of Concentrations of (R,S)-Methoprene on Growth of Various Aged *Palamonetes pugio* Larvae. DW = Dry Weight (µg); WG = Daily Weight Gain (µg Day^{-1}); and K_2 = Net Growth Efficiency. Each Value Represents the Mean ± Standard Error.

Concentration (µg/l)	Early Larvae - 1-day old		
	DW	WG	K_2
0	33 ± 2	8 ± 0 5	68 ± 1
8	25[a] ± 1	2[a] ± 0.1	37[a] ± 1
16	25[a] ± 2	3[a] ± 0.2	42[a] ± 2
32	22[a] ± 2	1[a] ± 0 1	14[a] ± 1
62	22[a] ± 2	1[a] ± 0.1	19[a] ± 2

Concentration (µg/l)	Intermediate Larvae - 9-days old		
	DW	WG	K_2
0	236 ± 36	77 ± 12	83 ± 2
8	135[a] ± 14	37[a] ± 4	81 ± 1
16	150[a] ± 28	43[a] ± 8	81 ± 2
32	88[a] ± 11	19[a] ± 2	69[a] ± 2
62	56[a] ± 14	7[a] ± 2	57[a] ± 4

[a]Significantly less than the control (0) ($p < 0.05$).

evaluate potential environmental hazards of a new type of pesticide The results of these studies suggest that the use of similar crustacean larval testing procedures would be appropriate in such assessments of insect growth regulators in the marine environment

Table 4b. The Influence of Continual Exposure to a Range of Concentrations of (R,S)-Methoprene on Growth of Various Aged *Palaemonetes pugio* Larvae. Symbols are as described in Table 4a.

Concentration (µg/l)	Premetamorphic Larvae - 16 -days old		
	DW	WG	K_2
0	334 ± 36	17 ± 2	44 ± 2
8	345 ± 51	49[a] ± 7	69[a] ±1
16	415 ± 72	65[a] ± 11	77[a] ± 1
Concentration (µg/l)	Postlarvae - 19-days old		
	DW	WG	K_2
0	474 ± 30	58 ± 4	71 ± 2
8	409 ± 53	24[a] ± 3	52[a] ±1
16	511 ± 65	37[a] ± 5	58[a] ± 2

[a]Significantly different from the control (0) ($p < 0.05$).

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