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Effect of Diflubenzuron on an Estuarine Crustacean

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In a review of one of the newer insecticides, diflubenzuron (Dimilin, TH-6040, [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea]), MARX (1977) emphasized that its ability to impair chitin synthesis, leading to toxicity in insects, is useful for controlling several insect pests. Diflubenzuron, one of many chitin inhibitors, is registered for use against the gypsy moth, a pest destructive to forests. Applications are pending for registration by the U.S. Environmental Protection Agency for additional uses, including protection of cotton and soybean crops and as a mosquito larvicide (possibly in coastal waters). Additional advantages of diflubenzuron cited by MARX (1977) were: (i) low application rates to achieve control of target species; (ii) inability to move through the food chain, thereby averting concentration in higher carnivores; and (iii) degradation in the environment unlike some organochlorine pesticides.

Recent reports, however, indicated that diflubenzuron reduced populations of mayflies and cladocerans in field tests (MIURA & TAKAHASHI 1975) and killed cladocerans, clam shrimps, and tadpole shrimps at concentrations below 0.01 µg/L or less in 24 to 48 h in laboratory toxicity tests (MIURA & TAKAHASHI 1974). Reproduction also declined among brine shrimp, Artemia salina, exposed to 2.0 µg/L (CUNNINGHAM 1976).

Therefore, we believed it imperative to test effects of diflubenzuron on a marine crustacean, a vital link between primary producers and consumers in estuarine and marine food webs. Our tests showed that diflubenzuron is acutely and chronically toxic to a small, estuarine crustacean, Mysidopsis bahia.

METHODS AND MATERIALS

M. bahia was exposed to diflubenzuron in flowing saltwater; triethylene glycol was the carrier solvent. All tests were conducted in intermittent flows from a diluter (MOUNT & BRUNGS 1967) or continuous flow with the toxicant added by an infusion pump (BAHNER et al. 1975). We observed the mysids throughout a complete life cycle to assess such effects as (i) delay in formation of brood pouches, (ii) delay in release of young, (iii) decline in

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young produced per female (iv) reduced growth, (v) abnormal behavior, and (vi) death (NIMMO et al. 1977).

Diflubenzuron concentrations in water from the test aquaria were analyzed by liquid chromatography. Water samples were analyzed by extracting seawater with two 100-mL portions of 1:1 diethyl ether-petroleum ether (V/V). The combined extracts were concentrated to about 5mL. Each sample was analyzed with a Waters® liquid chromatograph under the following conditions: 30 cm x 3.0 mm (I.D.); Bondapak NH_2 column mobile phase, 2.5% 2-propanol in iso-octane at 2 mL/min; amount injected, 15 µL; UV detector set a 254 nm, range set at 0.01 AUFS. The detection limit is 0.4 µg/L. Recoveries from fortified seawater were greater than 87%. In some experiments, concentrations were below the detection limit. All concentrations, delivered by the same device, were believed to be within 20% of the intended.

RESULTS AND DISCUSSION

Diflubenzuron was acutely and chronically toxic to M. bahia: the calculated 96-h LC50 was 2.1 µg/L (95% fiducial limits, 1.6 to 2.7 µg/L at 24-25°C and 24-27 °/oo salinity. In a life-cycle experiment, the 21-day LC50 was 1.24 µg/L (95% fiducial limits, 0.84 to 1.8 µg/L at 24-26°C and 23-29 °/oo salinity). During the life-cycle studies, we found the reproductive success (number of young produced per female) to be a more sensitive criterion of effect than survival of adults. For example, only 13.5 young/female were produced in an estimated concentration of 0.075 µg/L, whereas 21.4 and 21.0 young/female were produced in controls (Table 1). As diflubenzuron concentrations increased, there was direct suppression of reproduction.

Some aspects of diflubenzuron create immediate concerns: (1) the acute and chronic effects to the crustacean, M. bahia; and (ii) difficulty in determining an accurate no-effect level because of high toxicity and lack of analytical techniques for concentrations below 0.4 µg/L in water. Also, concentrations required in field tests (MIURA & TAKAHASHI 1975) to control a target species, such as mosquitoes, affect mysids in the laboratory: the initial concentration required to control mosquitoes, calculated on an acre-foot of water, equals the 96-h LC50 for mysids. Further, assuming that diflubenzuron degrades with time in the environment (after several hours or, at most, a few days), it may be possible that populations of mysids or other aquatic arthropods could be exposed to sublethal concentrations. Diflubenzuron affected reproduction by a factor of 0.04 of the 96-h LC50. MARX (1977) in his review of the degradation of diflubenzuron stated that the premise that the chemical breaks down in a matter of hours or days is not universally accepted because the half-life has been found to be from less than a week to more than 16 weeks. An exposure to diflubenzuron that lasts 48 h (even though the chemical might be degrading) is roughly 15% of the entire life cycle of M. bahia.

The phylogenetic relationship of mysids to other crustaceans strengthens the concern of other investigators (METCALF et al. 1975) about the effects of this chemical on non-target species such as shrimp, crayfish, lobsters, and crabs. Diflubenzuron toxicity to the larval stages of three species of crabs has been demonstrated (J. COSTLOW, personal communication). Therefore, we suggest that continued studies address questions about the effects of diflubenzuron on commercially important aquatic species, especially larval or juvenile forms, and the potential for recovery of any affected populations of non-target arthropods after the chemical has reached non-detectable levels in their environment.

TABLE 1

Effect of Diflubenzuron on Reproductive Success of Mysidopsis bahia at 22-26°C and 22-28 °/oo salinity.

Nominal Test Concentrations	Seawater Control	TEG* Control	µg/L 0.075	0.25	0.50	0.75
Measured Test Concentrations	<0.4	<0.4	<0.4	<0.4	0.55	0.91
Total females	16	26	22	19	19	15
Juveniles produced	343	547	298	193	136	36
Juveniles per female $\bar{X}$	21.4	21.0	13.5**	10.2**	7.2**	2.4**

*Triethylene glycol control.

**Significantly different from controls at $\alpha = 0.05$ (Dunnett's test).

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