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Persistence of the insect growth regulator Dimilin® in brackish water: a laboratory evaluation using larvae of an estuarine crab as indicator

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ABSTRACT: The persistence of Dimilin® (diflubenzuron), an insect growth regulator which interferes with chitin formation in the cuticle of insect larvae, has been studied using larvae of the estuarine brachyuran crab *Rhithropanopeus harrisii* (Gould) as test material. The results of the present investigation show that Dimilin breaks down relatively slowly in brackish water. It took about 8 weeks before a 10 ppb solution of Dimilin degraded to a level which did not affect survival of the crab larvae. Earlier it was shown (Christiansen et al., 1978) that nearly 100 % of *R. harrisii* larvae at each of the four zoeal stages died when molting to the succeeding stage after only 3 days of exposure to 10 ppb Dimilin. Hence, one should be extremely cautious in using Dimilin in estuarine areas where crab larvae occur.

INTRODUCTION

Environmental damage caused by insecticides throughout the last three decades has accelerated the attempts to develop agents which will affect target species without further destruction to the environment. The recent discovery of a group of chemicals with insecticidal activity which interferes with the formation of the insect cuticle may, according to a number of authors, be a step toward achieving this goal. These chemicals, including Dimilin®, inhibit formation of chitin in the cuticle of insect larvae and thus kill insects before they mature. Since vertebrates do not produce chitin, such compounds should be safe for higher animals. Dimilin (diflubenzuron) was released for sale in the United States in 1978. It is produced by the Thompson-Hayward Chemical Company (Kansas, USA) and is registered for control of the gypsy moth in the U.S. and is being considered for control of freshwater mosquitoes and cotton boll weevils.

Chitin is the most abundant organic skeletal component of many invertebrates, and is the characteristic polysaccharide of several major phyla among which is the Arthropoda (Dennel, 1960). Since the cuticle of all arthropods has much in common (Dennel, 1960), theoretically all arthropod larvae could be affected by Dimilin and related compounds. Dimilin, which has been tested both in the laboratory and in field experiments, shows relatively few adverse effects on nontarget species with the exception of some aquatic insects and small crustaceans (e.g. Miura & Takahashi, 1975; Mulla et al., 1975; Apperson et al., 1978). Marx (1977) mentions that some species of nontarget

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aquatic insects and small crustaceans are quite sensitive to Dimilin, which causes transitory declines in their populations. Christiansen et al. (1978) found severe effects on larvae of two estuarine crabs *(Rhithropanopeus harrisii* [Gould] and *Sesarma reticulatum* [Say]) when exposed to Dimilin. Lethal doses (7–10 ppb) for crab larvae were similar to those which are also lethal to several target species. The mode of dying resembled that described for insect larvae according to Christiansen et al. (1978). When larvae of the crab *R. harrisii* were exposed to 10 ppb Dimilin during the entire intermolt period (about 3 days) of the four different zoeal stages, nearly 100 % of the larvae died when molting to the succeeding stage. In the controls, 97 to 99 % survived. Christiansen et al. also found that first zoeal larvae treated with 10 ppb Dimilin on three different days during the intermolt period showed a greater sensitivity to the pollutant on the third day than on the other two; i. e., when the first layers of the new (pre-ecdysial) cuticle are formed. Costlow (1979) observed that zoeal larvae of *Menippe mercenaria*, the stone crab, could not survive 0.5 ppb Dimilin. The tolerance of the megalopa of *Callinectes sapidus*, the blue crab, was slightly higher, with less than 5 % survival at 3 ppb and 6 ppb. In a study on the ultrastructure of the cuticle of crab larvae exposed to 10 ppb Dimilin, Christiansen & Costlow (in preparation) have found deformation in both the pre-ecdysial and the post-ecdysial procuticle.

Since Dimilin has a rather high toxicity to the larvae of *Rhithropanopeus harrisii, Sesarma reticulatum, Menippe mercenaria* and *Callinectes sapidus,* we were interested in examining the persistence of this compound in brackish water. Factors affecting the stability of Dimilin in various types of freshwater and the persistence of Dimilin in the field (freshwater) have been studied by Schaefer & Dupras (1976) and Apperson et al. (1978) but, to our knowledge, nothing is known about the stability of Dimilin in estuarine waters. The purpose of this study has, therefore, been to examine the persistence of Dimilin in brackish water using larvae of the estuarine crab *R. harrisii* as test material.

A. W. Bourquin and P. H. Pritchard (United States Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida) have designed laboratory experiments to assess the fate of Dimilin in an estuarine environment. Their results are unpublished, but they have given us permission to report some of their results in our study.

MATERIALS AND METHODS

The experiments carried out in the present study were undertaken in July–October 1977 and July–August 1978. The methods described below apply to both years.

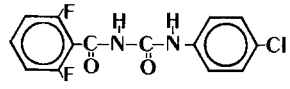

Fig. 1. Structural formula of Dimilin®

The compound Dimilin® or diflubenzuron (1-[4-chlorophenyl]-3-[2,6-difluorobenzoyl]-urea) (Fig. 1) was obtained from the Thompson-Hayward Chemical Company, Kansas, USA. It is a white crystalline solid which has a low solubility in water (0.2 ppm at 20 °C), but which is moderately soluble in organic solvents. Acetone was used as a carrier in the present investigation.

A solution of 1 ppt Dimilin was prepared by dissolving airmilled technical Dimilin in acetone. The 1 ppt solution was diluted in filtered seawater to 0.1 ppm Dimilin. Amounts of the 0.1 ppm solution were then added to filtered seawater to give a concentration of 10 ppb. The last-mentioned solution was stored in 15-l clear glass carboys at 24–26 °C under natural light conditions. The salinity of the seawater was 20 ‰ and the pH 6.5 in a newly prepared 10 ppb solution of Dimilin. A water sample from the area where ovigerous *Rhithropanopeus harrisii* crabs were collected had a salinity of approximately 17 ‰ and a pH of 6.9. This place, however, is a shallow water area in Neuse River, near Havelock, North Carolina, USA, where salinity and pH vary with tide and weather conditions.

The method for obtaining larvae was as described by Christiansen & Costlow (1975). Fifty newly hatched larvae of *Rhithropanopeus harrisii* were exposed to 10 ppb Dimilin the same day the solution was prepared. This concentration was selected because it had earlier been shown to be lethal to *R. harrisii* larvae (Christiansen et al., 1978). Further exposure of fifty newly hatched larvae was started 1, 3, 5, 7, 9, 12, 16, 21, 29, 42, 50 and 59 days after preparation of the 10 ppb solution. Two or three replicates were run at the same time, and larvae in different replicates always came from different female crabs. Filtered seawater was used as control medium, since Costlow (1977) has shown that acetone in concentrations up to 1 ppt does not affect larval development of *R. harrisii.* The zoeae were reared in small glass bowls (inside diameter 8 cm) with approximately 60 ml medium per bowl. Ten larvae were kept in each bowl, and number and stage of dead larvae were recorded daily. Every day all living larvae were transferred to clean bowls with replacement medium from the glass carboys containing the stored Dimilin solution. Thus, newly hatched larvae, which were exposed to a newly prepared 10 ppb solution, were exposed the next day to a one day old solution and the third day to a two days old solution and so forth. The age of the solution referred to in this study is the age the day exposure started on a series of newly hatched larvae. All larvae were fed daily with recently hatched *Artemia salina* nauplii from cysts obtained from the San Francisco area. Megalopa larvae were placed individually in plastic compartmented boxes to avoid cannibalism, and were maintained in the same box with daily changes of medium until the first crab stage. The larvae were reared in culture cabinets at a constant temperature of 25 °C and a photoperiod of 12 h light and 12 h dark. The day the last larva in an experimental series died, the number of living larvae in the corresponding control was counted. This number indicates the number of survival in the controls.

Glassware which came in contact with Dimilin was washed in acetone and rinsed, then washed with a non-toxic cleaning agent and rinsed again.

RESULTS AND DISCUSSION

In an earlier study, Christiansen et al. (1978) found that more than 95 % of *Rhithropanopeus harrisii* larvae died during molting to the second zoeal stage when exposed to 10 ppb Dimilin from hatching. No larvae survived beyond the second zoeal stage. This concentration was not only lethal for the larvae of the first zoeal stage, but also for the larvae of each of the three succeeding zoeal stages.

In the present study, 100 % of the larvae died in the zoeal stages both in a newly made 10 ppb solution of Dimilin as well as in solutions up to 42 days old. In comparison,

the survival in the controls was between 88 and 98 % when all exposed individuals were dead (Table 1). After between 42 and 50 days the compound had degraded to such an extent that some of the larvae survived to the first crab stage; i.e., 14 % survival in a 50 days old solution compared with 92 % in the control (Table 1). After 50 days, the chemical must have broken down quickly. As seen in Table 1, the survival of larvae to the first crab stage in a 59 days old solution increased to 95 % which was even higher than the 88 % survival in the control.

Mortality of larvae was always highest during molting to the second zoeal stage (Table 1). As mentioned earlier by Christiansen et al. (1978), all larvae looked completely healthy and swam and fed normally during the whole intermolt period until the shedding of the exuviae started. Most of the larvae were, however, unable to cast their molts completely and died within less than one or a few hours. In many specimens the dorsal spine was only partly freed of the old cast before the larvae died. The new cuticle of the exposed specimens also looked weaker than the new cuticle of the control individuals. Most of the specimens which survived beyond the first zoeal stage in solutions between 0 and 42 days old were abnormal with deformed swimming setae. Some also had deformed rostral and dorsal spines.

Between three and four weeks after the Dimilin solution was prepared, a degradation of the compound must have taken place. There was 100 % mortality of first zoeal larvae in a 21 days old solution, but in a 29 and 42 days old solution mortality was reduced approximately 25 % in the first zoeal stage. The compound was, however, still

Table 1. *Rhithropanopeus harrisii.* Survival of larvae exposed to Dimilin and effect of Dimilin on mortality of larvae in various stages. Concentration of a newly made solution of Dimilin (= Day 0) was 10 ppb. All larvae were exposed from hatching and the percentage indicates the averages of the replicate experiments

Age of solution when exposure started (days)	No. of replicates	Initial no. of larvae	Survival (%)		Days after hatching when all larvae in Dimilin were dead	Mortality (%) of Dimilin-exposed larvae in various stages				
			Control	Dimilin		I	II	III	IV	Megalopa
0	3	150	98.0[a]	0	12	95.3	2.7	2.0	–	–
1	3	150	92.7[a]	0	16	91.3	7.3	0.7	0.7	–
3	3	120	97.5[a]	0	8	99.2	0.8	–	–	–
5	2	70	94.3[a]	0	13	90.0	8.6	1.4	–	–
7	3	150	94.0[a]	0	12	90.7	9.3	–	–	–
9	2	100	93.0[a]	0	10	99.0	1.0	–	–	–
12	3	150	99.3[a]	0	10	97.3	2.7	–	–	–
16	3	150	94.7[a]	0	9	100	–	–	–	–
21	3	150	89.3[a]	0	9	100	–	–	–	–
29	3	150	93.3[a]	0	17	72.7	24.7	0	2.7	–
42	3	150	88.0[a]	0	12	75.3	24.7	–	–	–
50	3	150	92.0[b]	14.0[b]	–	45.3	33.3	4.0	1.3	2.0
59	3	150	88.0[b]	95.3[b]	–	1.3	2.7	0	0.7	0

[a] Percent survival when all exposed larvae were dead
[b] Percent survival to lst crab stage

very toxic after 42 days, since no larvae survived beyond the second zoeal stage (Table 1). A further reduction of the potency of the compound was observed between 6 and 7 weeks after preparation of the Dimilin solution. According to Table 1, the survival to the first crab stage in a 50 days old solution was 14 % which corresponds to the percent survival to the first crab stage of *Rhithropanopeus harrisii* larvae reared in 5 ppb Dimilin by Christiansen et al. (1978). About eight weeks after the solution was made, the concentration must have decreased to less than 1 ppb Dimilin. This was the lowest level at which Christiansen et al. (1978) found significant decrease in survival of *R. harrisii* larvae exposed to Dimilin.

Bourquin and Pritchard (personal communication) have studied the environmental fate of Dimilin employing a laboratory fate test system containing water and sediment from an estuarine salt marsh (Pritchard et al., 1979). They showed that Dimilin concentration (initially at 140 ppb) slowly decreased over a 3-week incubation period (25 ± 1 °C) giving an estimated half-life of more than 17 days. In sterile controls, a similar disappearance was observed indicating that most of the loss was due to abiotic factors. Dimilin was hydrolyzed to difluorobenzoic acid and p-chlorophenyl urea. The primary hydrolysis products were not further metabolized. In methyl parathion controls, the compound rapidly disappeared due to hydrolysis and biodegradation from the water column and was undetectable after three weeks incubation; this corresponded to an estimated half-life of 5–7 days. The degradation of Dimilin in these systems generally agrees with the detoxification observed in the crab larval experiments, i. e., a period of approximately 60 days. We found degradation, however, slower after 3 weeks in the crab experiments than Bourquin and Pritchard observed in their study.

Bourquin and Pritchard found only a small amount of Dimilin tightly bound to the sediment and most of it was extractable with solvent. Apperson et al. (1978), who have tested persistence of Dimilin in lentic habitats, did not find any residues in lake sediment. They also point out that the persistence of Dimilin can be attributed to its stability in waters of moderate temperature and pH. Schaefer & Dupras (1976) found that Dimilin is least stable when water temperature and pH are both relatively high. At 24 °C and pH 7.7, 87 % of the Dimilin added to tap water could be recovered after 9 days according to Schaefer & Dupras. This percentage is not far from that recovered after seven days (more than 90 %) by Bourquin and Pritchard in their test systems. The temperature used in the crab larval experiments is about the same (25 °C), and the pH measured slightly lower (6.5). Schaefer & Dupras (1976) also found that persistence of Dimilin was not greatly affected by sunlight or microorganisms.

Considering the high toxicity of Dimilin to estuarine crab larvae from exposure of only 3 days at 25 °C (see Christiansen et al., 1978), and the apparent lack of degradation of the compound within this time, one should be extremely cautious in using Dimilin for insect control in estuarine areas where crab larvae occur.

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