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Comparative Toxicity of Two Dimilin Formulations to the Grass Shrimp, *Palaemonetes pugio*

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The benzoylphenyl ureas, which exhibit activity against larval stages of several insect species by interfering with the moulting process, were first introduced in 1972 (Verloop and Ferrell 1977). Dimilin® (common name diflubenzuron) is an example of this class of insecticide and is presently being produced by Duphar, B.V., Amsterdam, Holland. There are two major formulations of diflubenzuron, the technical grade (TG) and the wettable powder (WP-25). The toxicity of diflubenzuron on non-target invertebrates (both freshwater and marine species) is well documented in the literature. For the marine and estuarine crustaceans, however, almost all of the research has been conducted with the technical grade formulation instead of the field formulations - WP-25 or sand granule (SG). The difference between TG and WP-25 is that the former which contains pure form of diflubenzuron (DFB) is very insoluble in water and the latter contains additives which enhance the solubility or dispersion of DFB in water. These additives or "formulation factors" can increase or decrease the toxicity of the pesticide directly by altering its chemical nature or by affecting the solubility, which in turn could influence the absorption of the toxicant into the biologic system (Doull 1980). For example, Pickering et al. (1962) found that the emulsifiable concentrates of organophosphate insecticides were more toxic to fathead minnows and bluegills than the technical grade formulations. Similarly, the granular formulations of some herbicides were less toxic to five species of fish than the liquid formulations (Hiltibran 1967).

Apart from solubility, TG and WP-25 DFB differ in the mean particle size of the active materials. The mean particle size of DFB in TG is 10μ and for WP-25 it is 2μ (Verloop and Ferrell 1977). The particle size of the active ingredient in a DFB formulation has a considerable influence on the biological activity (Mulder and Gijswijt 1973; Maas et al. 1980). For example, Mulder and Gijswijt (1973) demonstrated that the effectiveness of DFB rapidly increases with decreasing particle size which should make WP-25 more toxic than TG. If this is true, it will be difficult to make accurate evaluation and comparison of data from marine toxicity studies that utilize TG and WP-25 DFB. The present investigation was designed to assess the toxicity of TG and WP-25 diflubenzuron to laboratory-reared larvae of the estuarine shrimp Palaemonetes pugio.

MATERIALS AND METHODS

Technical grade (TG) Dimilin contained 98.4% active ingredient, and the wettable powder (WP-25) contained 25% active ingredient of diflubenzuron. Both formulations were kindly supplied by Thompson-Hayward Chemical Company, Kansas, U.S.A. For the technical grade formulation whose solubility in water is extremely low (0.2 mg/l at 20°C), acetone was used as a solvent or carrier. The WP-25 formulation, on the other hand, contained talc (a solid diluent) and other ingredients (dispersing agents) which enhanced the solubility of diflubenzuron in water.

A stock solution of 1.0 g/L was prepared for both formulations of diflubenzuron by dissolving appropriate amounts in pesticide grade acetone (for TG) or directly in 20 ppt filtered seawater (for WP-25). For WP-25 at 1.0 g/L, Dimilin is in suspension; at the test concentrations of 5 µg/L or less however, it is in true solution (Frech Popp of Duphar B.V., Holland, pers. comm.). From these stock solutions, working solutions of 1.0 mg/L were prepared every other day when the solutions in the culture bowls were changed. The 1.0 mg/L solutions were appropriately diluted to give 0.1, 0.3, 0.5, 1.0, 2.5, and 5.0 µg/L active ingredient diflubenzuron (nominal concentrations). Ten µg/L acetone seawater solution and untreated 20 ppt filtered seawater served as controls for the TG and WP-25 formulations respectively.

Reproductively inactive adult grass shrimp (males and females) were collected from Calico Creek in Morehead City, North Carolina, between November and February (1982/1983) and allowed to acclimate to laboratory conditions of 20°C and a 12:12 day:night photoperiod for 2 to 3 weeks. After this period, the animals were stimulated to breed by increasing the temperature to 25°C and photoperiod to 14-h light:10-h dark. The shrimp were also fed (*ad libitum*) Artemia salina nauplii and fish twice a day instead of once. When spawning occurred, gravid females were removed from the "breeding tank" and placed individually in large Carolina Culture dishes (20 cm inside diameter) containing 20 ppt filtered seawater. The animals were then kept in a culture cabinet set at 25°C and 12-h light:12-h dark photoperiod. Animals were fed freshly hatched Artemia salina nauplii daily, except just prior to hatching of eggs, when they were fed twice a day to reduce incidence of cannibalism by the parents. The cysts of Artemia salina used in this study were obtained from the Great Salt Lake area. After hatching, the larvae from three or more females were pooled in order to provide sufficient larvae for the tests.

The larvae were reared in small Carolina Culture bowls (inside diameter = 8 cm) containing approximately 50 mL solution per bowl. Ten larvae were maintained in each bowl, and for each solution, five such bowls were prepared, giving a total of

50 larvae per test concentration. Each experiment was conducted at least three times. Every day the number of dead larvae and exuviae were noted. These were then removed and living larvae were fed a few drops of freshly hatched *Artemia salina*. On alternate days, living larvae were transferred to clean bowls containing fresh solutions of the appropriate concentration of diflubenzuron. The process was repeated until the larvae metamorphosed into postlarvae. After metamorphosis, the number of postlarvae and the date of metamorphosis were noted for each bowl.

Statistical analysis was performed on the data using standard analysis of variance techniques. All percentages of survival to the postlarval stage were arcsin transformed prior to analysis but the results are reported in the percent scale. Tests of significance were made at the probability levels of $p = 0.05$ and $p = 0.01$ although subsequent reference to the term "significant" indicates $p \leq 0.05$. The 72-h and 96-h LC 50's were calculated using a computer program for probit analysis.

RESULTS AND DISCUSSION

The survival of *Palaemonetes pugio* larvae during the first 15 days (i.e., up to stage VIII zoea) and to the postlarval stage, was significantly ($p < 0.001$) reduced with increasing concentrations of both the technical grade (TG) and the wettable powder (WP-25) formulations of diflubenzuron (Tables 1 and 2). At concentrations of 2.5 and 5 µg/L, none of the larvae survived to day 15. One hundred percent mortality occurred on days 13-14 and days 5-6 for larvae exposed to 2.5 and 5.0 µg/L diflubenzuron respectively. This clearly indicates that concentrations of diflubenzuron > 2.5 µg/L are lethal to *Palaemonetes pugio* larvae upon chronic exposure. Lethal concentrations are defined here as those in which less than 10% of the larvae survived to the postlarval stage (Christiansen et al. 1978; Bookhout et al. 1979; Epifanio 1971).

Two-way ANOVA performed on the results showed that there is no significant difference (Cochran's $C = 0.295$, $p > 0.05$) in the way TG and WP-25 affect the survival of the larvae up to day 15 and to postlarvae. Also there is no significant interaction between the formulation of diflubenzuron and test concentration (Cochran's $C = 0.192$, $p > 0.05$). For the two diflubenzuron formulations, between 81 and 98% of the larvae that survived to day 15 eventually metamorphosed successfully to the postlarval stage at concentrations 0 - 1.0 µg/L, except for TG at 1.0 µg/L where only 55% metamorphosed successfully. This decrease (cf 81% in WP-25) cannot readily be explained especially because in comparing the survival from hatch to postlarvae there is no significant difference between TG and WP-25.

Table 1. Survival of *Palaemonetes pugio* larvae in seawater control and 6 concentrations of WP-25 diflubenzuron.

Concentration µg/L	Hatch to Day 15 No	%	Day 15 to Postlarvae No.	%	Hatch to Postlarvae No.	%
Seawater Control	145	96.7	137	94.5	137	91.3
0.1	128	85.3	113	88.3	113	75.3
0.3	115	76.7	113	98.3	113	75.3
0.5	120	80.0	111	92.5	111	74.0
1.0	81	53.3	65	81.8	65	43.3
2.5	0	00.0	0	00.0	0	00.0
5.0	0	00.0	0	00.0	0	00.0

Table 2. Survival of *Palaemonetes pugio* larvae in acetone control and 6 concentrations of TG diflubenzuron.

Concentration µg/L	Hatch to Day 15 No	%	Day 15 to Postlarvae No.	%	Hatch to Postlarvae No.	%
Acetone Control	144	96.0	126	87.5	126	84.0
0.1	134	89.3	109	81.3	109	72.7
0.3	129	85.7	120	93.0	120	80.0
0.5	102	68.0	99	83.9	99	66.0
1.0	88	58.7	48	54.5	48	32.0
2.5	0	00.0	0	00.0	0	00.0
5.0	0	00.0	0	00.0	0	00.0

Duration of development from hatching to the postlarvae for both TG and WP-25 was not significantly ($p > 0.05$) affected by increasing diflubenzuron concentration (excluding 2.5 and 5.0 µg/L), Fig. 1. Also for each concentration, there is no significant difference in the effect of diflubenzuron on the duration of larval development that can be attributed to the formulation used. When the 72-h and 96-h LC 50's are computed, it was observed that there was no significant difference (Chi square, $p = 0.05$) in the acute toxicity of TG and WP-25 to larvae of the grass shrimp (Table 3).

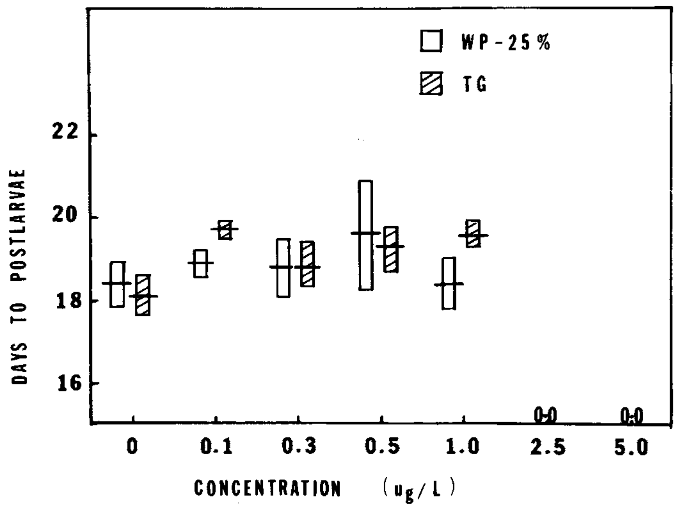

Figure 1. Effect of two formulations of diflubenzuron (WP-25 and TG) on duration of development of Palaemonetes pugio from hatch to postlarvae. The mean (+SE) of three replicate experiments are shown.

Table 3. Calculated LC 50's (μg/L) of technical grade (TG) and wettable powder (WP-25) formulations of diflubenzuron.

Time (h)	Formulation	LC-50	Fiducial Upper	Limits Lower
72	TG	2.95	3.30	2.66
	WP-25	2.83	3.27	2.49
96	TG	1.84	2.08	1.64
	WP-25	1.39	1.54	1.27

The results of the present investigation are different from those of Mulder and Gijswijt (1973). They observed that the toxicity of diflubenzuron with different particle size, increased as the particle size of the active ingredient decreased. For example at 0.3 μg/L, the mortality (%) of Pieris brassicae was 6, 43 and 100 when the mean particle size of diflubenzuron used was 15, 7 and 4 μ respectively. Since the particle size of diflubenzuron in WP-25 is much smaller (2 μ) than that obtained for TG in acetone (10 μ) (Verloop and Ferrell 1977), it is to be expected that our results will show significant differences. However, this is not the case. One possible explanation for these differences is that the highest test concentration used in the present investigation (5.0 µg/L) is far below the water solubility (0.2 mg/L ≡ 200 µg/L) of diflubenzuron at 20°C. Thus, there was probably no crystallization when acetonic solution of the TG formulation was added to seawater as suggested by Maas et al. (1980) and Cunningham (1982). Probably at concentrations greater than the water solubility, some differences in the biological activity between TG and WP-25 diflubenzuron may occur.

The results of Mulder and Gijswijt (1973) substantiate, to some extent at least, the above speculation. At 0.01 mg/L (10 µg/L), the results of Mulder and Gijswijt (1973) did not show any significant increase in effectiveness of diflubenzuron with decreasing particle size. One might conclude then that as long as the concentration of diflubenzuron is < 10 µg/L (which is the concentration most often used in laboratory studies with Dimilin) there will be no difference in the efficacy of the compound, irrespective of the formulation used.

Using the same diflubenzuron formulation (granular formulation) with different carriers and particle sizes (of the granules, not the active ingredient of diflubenzuron), Mulla and Darwazeh (1975) observed differential toxicity to mosquito larvae. Differences have also been demonstrated for pesticide formulations with similar solubilities. The technical grade and encapsulated formulations of parathion have similar solubilities, but the encapsulated formulation was shown to be 45-60% and 19-35% less toxic than the technical grade in static and chronic tests respectively (Jarvinen and Tanner 1982). On the other hand, although the solubility of technical grade and encapsulated formulations of diazinon are different, their toxicities (both acute and chronic) to fathead minnow were the same (Jarvinen and Tanner op. cit.). These results show that the effects of formulation and carriers on the toxicity of pesticides are complex and there is no one explanation for the differences observed when they do occur.

In conclusion, we have demonstrated that the two formulations of diflubenzuron commonly used in laboratory toxicity tests (TG and WP-25) are similar in their efficacy as determined by both acute and chronic toxicity tests with grass shrimp larvae. On the basis of this similarity and that observed for Rhithropanopeus harrisii (see Christiansen and Costlow 1980 and Wilson and Costlow, *unpublished report*, 1985), it is inferred that within the range of concentrations used in laboratory studies, there should be no significant difference in the toxicity of diflubenzuron formulations. Thus data obtained from other marine toxicity studies with TG formulation of diflubenzuron (see Cunningham 1982) cannot be dismissed as "ecologically meaningless". The warning issued by Maas et al. (1980) regarding the use of the technical grade formulation of diflubenzuron in acetone therefore needs to be modified. Also valid comparisons can now be made between data obtained from studies with TG and

WP-25 diflubenzuron and conclusions made from studies with TG are applicable to WP-25 without the need for a "correction factor".

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