Bull. Environm. Contam. Toxicol. 25, 477-481 (1980)

Toxicity and Molt-Accelerating Effects of Diflubenzuron on the Barnacle, *Balanus eburneus*

Gary Gulka, Cecilia M. Doscher and Norimitsu Watabe
Electron Microscopy Center and Department of Biology, University of South Carolina, Columbia, SC 29208

One class of insecticides, the benzoylphenyl ureas, interfere with chitin formation in arthropods, perhaps by blocking chitin synthetase, the polymerization enzyme. Favorable characteristics of these insecticides over other types include low biological magnification, effectiveness at low concentration and low persistence in soils (VERLOOP & FERRELL 1977). Diflubenzuron ([1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea]) is currently registered for a few uses; however, it is restricted from use within three miles of the coast in the Gulf coast states only (Thompson-Hayward Chem. Co., personal communication).

Concentrations of diflubenzuron of a few ppb (μg/L) or less affected survival and reproduction in a number of crustaceans, such as branchiopods, ostracods, copepods, mysidaceans and decapod larvae (CUNNINGHAM 1976, MIURA & TAKAHASHI 1974, NIMMO et al. 1979, CHRISTIANSEN et al. 1978, COSTLOW 1979).

This study examined various acute and chronic exposure effects of diflubenzuron on the barnacle, Balanus eburneus, a marine crustacean that undergoes frequent ecdysis as an adult on the order of every few days.

MATERIALS AND METHODS

Adult B. eburneus, 0.5 - 2.0 cm in diameter, collected with oyster shell substratum intact, were scrubbed clean and maintained individually in plastic compartmentalized trays. Technical grade, air-milled diflubenzuron (Thompson-Hayward Chem. Co.) was prepared in a 1 ppt stock solution with pesticide grade acetone as the carrier solvent (CUNNINGHAM 1976). Preliminary studies showed no mortality in acetone controls. The stock solution was stored at 4°C and renewed every two weeks. Barnacles were exposed to concentrations of 1 - 1000 ppb, diluted from the stock solution, in 28 - 31 ppt seawater. The water was renewed in the trays every other day, at which time the animals were fed equal amounts of 1 - 2-day-old Artemia nauplii and molts were recorded. Intermolt stages of barnacles were determined according to the methods of DAVIS et al. (1973).

0007-4861/80/0025-0477 $01.00

We observed the (i) molt-accelerating effects, (ii) intermolt stage at which death occurred, (iii) toxicity over a 28-day period and (iv) effects of shorter periods of exposure to diflubenzuron.

RESULTS AND DISCUSSION

Exposure to diflubenzuron over a 28-day period of both unfed and fed animals showed a dose-dependent mortality with increasing mortality occurring at higher concentrations (Fig. 1 a & b). Drastic mortality occurred during the second week of exposure. A greater degree of mortality occurred in the fed group than in the unfed group at the corresponding concentrations, and the minimum dosage that caused mortality was lower in the fed group (200 ppb) than in the unfed group (400 ppb). The greater mortality in the fed group may be caused by increased uptake of diflubenzuron due to feeding on contaminated food or may be the result of increased metabolic rate, such as the observed greater molting rate which is due to feeding. Mortality in the unfed group indicates that diflubenzuron can be readily absorbed through the exoskeleton.

In various trials, barnacles showed no mortality when exposed to 1000 ppb diflubenzuron for 24 and 48 h and then maintained in uncontaminated seawater for a total of 28 days. Exposure for 72, 96 and 144 h caused drastic mortality over the 28-day period, with greatest mortality again during the second week.

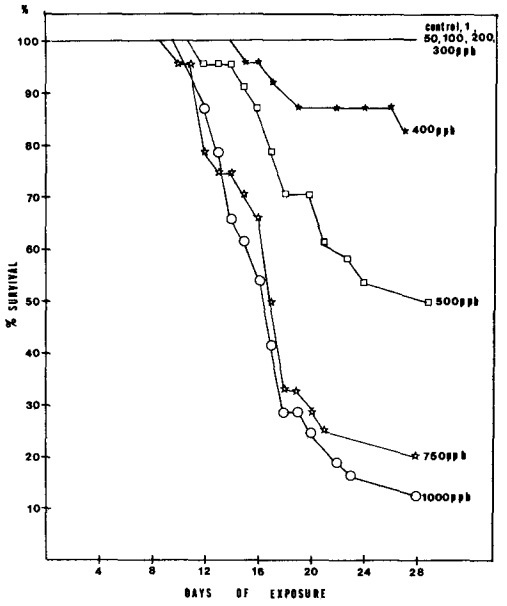
Barnacles that died during exposure were predominantly in stages D_2 to D_3 and A of the intermolt cycle. Thus, animals died during the process of shedding the old exoskeleton (D_2 to D_3) or shortly thereafter (A) (Fig. 2).

Diflubenzuron, at sublethal and lethal concentrations, caused a significant acceleration of the intermolt cycle (25 - 39%) in three separate trials in comparison to controls (Table 1). A possible explanation of these results may be related to the results of YU and TERRIERE (1977) in larval insects, in which diflubenzuron caused a reduction in the metabolism of β-ecdysone. Such an effect, they suggested, may cause an excess of the molting hormone at the time of the molt. A similar situation may exist in barnacles, in which diflubenzuron exposure leads to an excess of molting hormone, resulting in acceleration of the intermolt cycle.

Diflubenzuron should have the most drastic effects on crustaceans that undergo frequent ecdysis, especially if exposure is only very limited in scope. This includes a number of lower crustaceans and crustacean larvae. Very little is known about possible concentrations of diflubenzuron to be expected in coastal waters as well as degradation. It is thus difficult to predict potential effects of diflubenzuron on coastal waters.

That barnacles die from exposure during the process of shedding the old exoskeleton is in agreement with effects of this

(a)



(b)

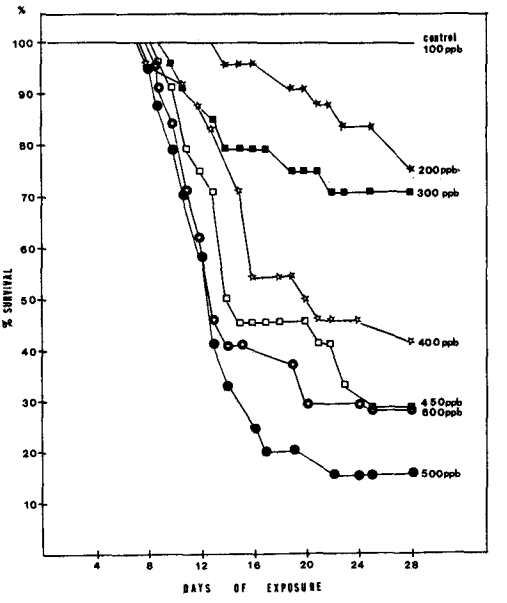


FIGURE 1. Toxicity of diflubenzuron to barnacles exposed for 28 days to concentrations of 1 - 1000 ppb. (a) Unfed animals. (b) Fed animals.

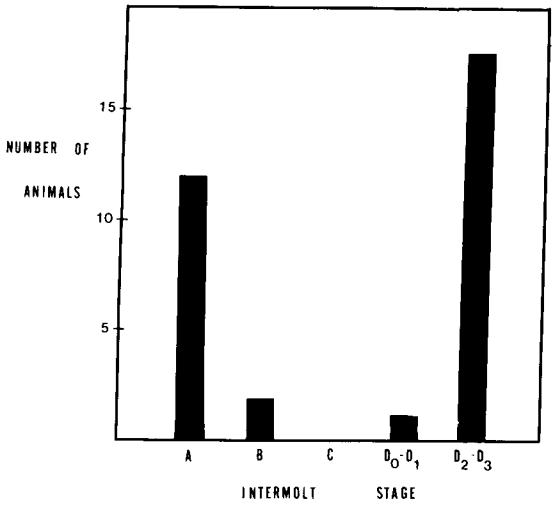


FIGURE 2. The intermolt stage at death of various barnacles exposed to diflubenzuron.

TABLE 1. Molt-accelerating effect of diflubenzuron over a 28-day period in three separate trials (I-III) at varying concentrations. Molting rate is expressed as number of molts per 28 days in diflubenzuron. Only animals that survived for the 28 days were used in the calculations (numbers in parentheses). Differences between experimental and control were significant at the 0.05 level, t test.

	Concentration	Molts / 28 days in diflubenzuron	Molts / 28 days Control
I	100 ppb	4.6 ± 1.3 (24)	3.6 ± 1.0 (24)
	200	4.8 ± 2.2 (18)	
	300	4.8 ± 1.9 (17)	
	400	5.0 ± 1.2 (10)	
	500	4.0 ± 0.6 (8)	
II	100 ppb	6.1 ± 1.3 (28)	4.6 ± 1.2 (28)
III	50 ppb	5.8 ± 1.6 (35)	4.3 ± 0.9 (18)

insecticide on insects (MULDER & GIJSWIJT 1973). Histopathological effects on the crustacean exoskeleton in progress in this laboratory are also similar to effects observed in insects.

ACKNOWLEDGEMENTS

We thank Thompson-Hayward Chem. Co. for the gift of diflubenzuron. This study was supported in part by NSF grant PCM 77-01528 and PCM 77-27866 and a fund from the Electron Microscopy Center, University of South Carolina. This is Contribution No. 343, Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina.

REFERENCES

CHRISTIANSEN, M.E., J.D. COSTLOW and R.J. MONROE: Mar. Biol. 50, 29 (1978).

COSTLOW, J.D.: In Marine Pollution: Functional Responses, p. 355. New York: Academic Press (1979).

CUNNINGHAM, P.A.: Environ. Entomol. 5, 701 (1976).

DAVIS, C.W., U.E.H. FYHN and H.J. FYHN: Biol. Bull. 145, 310 (1973).

MIURA, T. and R.M. TAKAHASHI: Environ. Entomol. 3, 631 (1974).

MULDER, R. and M.J. GIJSWIJT: Pestic. Sci. 4, 737 (1973).

NIMMO, D.R., T.L. HAMAKER, J.C. MOORE and C.A. SOMMERS: Bull. Environm. Contam. Toxicol. 22, 767 (1979).

VERLOOP, A. and C.D. FERRELL: In Pesticide Chemistry in the 20th Century, p. 237. Washington: American Chemical Society (1977).

YU, S.J. and L.C. TERRIERE: Pestic. Biochem. Physiol. 7, 48 (1977).