

Effects of Deltamethrin and Ethanol on Survival, and Mechanical Response of *Daphnia spinulata*

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Deltamethrin ((S)- α -cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) (DM) is a powerful biocide, extensively used in domestic pest control and in some agricultural practices (Leahey 1985). DM is soluble in ethanol (ET) but not in water.

Species of the genus Daphnia are the most frequently used zooplankton organisms in toxicological bioassays (De Wolf et al. 1988; Day and Kawshik 1987; Gersich et al. 1986; Stratton and Corke 1981). Daphnia spinulata (Biraben 1917) is a native Argentine cladoceran, which is widespread in freshwater environments of the Buenos Aires province (Olivier 1960). This area has most of the urban concentrations and the greatest number of industries, agricultural, and cattle centers of Argentina.

The purpose of this study was to evaluate the effects of DM and its solvent on individuals of D. spinulata.

MATERIALS AND METHODS

The biological material of this study was provided by the National Institute of Fisheries Investigation and Development (Buenos Aires, Argentina), from an artificial pond culture. In our laboratory, the water fleas were mass cultured in artificial pond water (APW) (pH 7.4 ± 0.2 ; total hardness as CaCO_3 86.5 ± 3.3 mg/L;

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conductivity 241 ± 8.7 umhos/cm; dissolved oxygen 7.75 ± 0.15 mg/L) (Alvarado and Johnson 1966) at 24 °C for 4 wk. Seventy-two hours prior to the experiments 260 newborn (<24 h old) daphnids were selected from the mass culture and acclimatized in a chamber set at 20 °C in APW with 12 h L-D photoperiod. Prior to test, the water fleas were fed Scenedesmus sp at a concentration of 5×10^5 cells/mL, and during the experiments they were starved.

A 2.5 mg/L stock solution was prepared using 99 % purity DM powder as active ingredient (a.i.). The DM a.i. was provide by US Environmental Protection Agency (LOT N° 605).

DM was added to APW in 10 mL ET. Appropriate dilutions of the stock solution were made in order to get the following nominal concentrations of DM: 0.31, 0.62, 1.25, 2.50, 5.00 and 10.00 ug/L. For experiments with the solvent, an ET stock solution (10 mL of ET/L of APW) was also made. The assayed solvent concentrations were: 1.25, 2.50, 5.00, 10.00, 20.00 and 40.00 uL/L. The ET used was anhydrous and was purchased from Merck Laboratories (Germany). To maintain the ET and DM concentrations, covered glass bowls were utilized in all experiments. All test solutions were renewed every 24 h. Both in DM and in ET experiments, 10 individuals were placed in each glass bowl with 100 mL of test solution. Control experiments were also run. All experiments were conducted in duplicate at 20 °C and 12 h L-D.

Every 24 h the following parameters were recorded: a) mortality and b) mechanical response. In the second case, the individuals were stimulated by a gentle prodding with a microneedle and the number of antennal impulses were recorded during 15 sec as escape response. Antennal impulses were counted under stereoscopic microscope. Death was considered when individuals did not respond to a gentle prodding.

Mechanical response data were analyzed by one-way ANOVA in conjunction with Dunnett's test (Steel and Torrie 1960).

RESULTS AND DISCUSSION

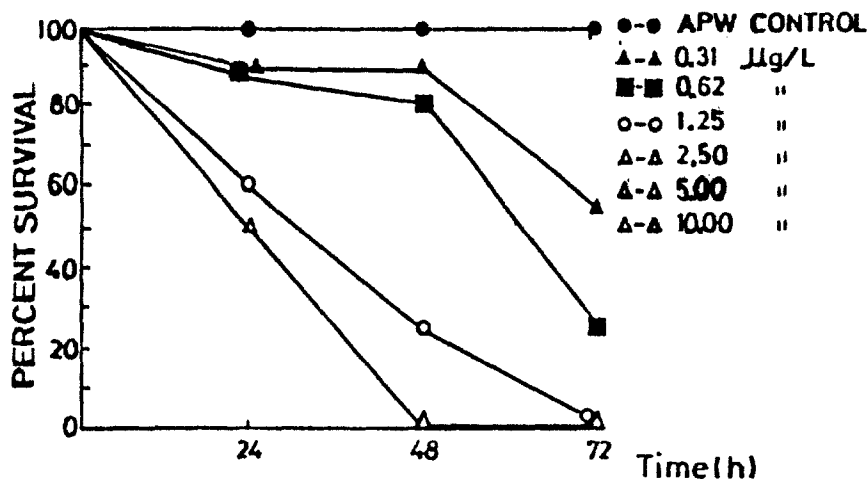


FIGURE 1. Survival curves for *D. spinulata* exposed to several concentrations of deltamethrin. At 2.50, 5.00 and 10.00 µg/L the individuals showed the same response. (n= 20).

The DM experiment data indicate a high sensitivity of *D. spinulata* to the toxic effect of this pesticide (FIGURE 1). At 24 h of exposure, 50 % of the individuals died in the concentration range between 2.50 to 10.00 µg/L. At 48 h the mortality increased up to 1.25 µg/L. At 72 h, 45 % of the individuals were dead in the lowest assayed concentration.

Data obtained from ET experiment (FIGURE 2) indicate no significant mortality at 24 and 48 h of exposure. At 72 h, 20 % of mortality was recorded between 5.00 to 40.00 µL/L.

Since ET was used as DM solvent, the above mentioned concentrations must be found at 1.25, 2.50, 5.00 and 10.00 µg/L of DM. At 72 h, all exposed organisms were dead at these DM concentrations. Consequently, we conclude that the lethal effect is attributable to a DM rather to a ET effect.

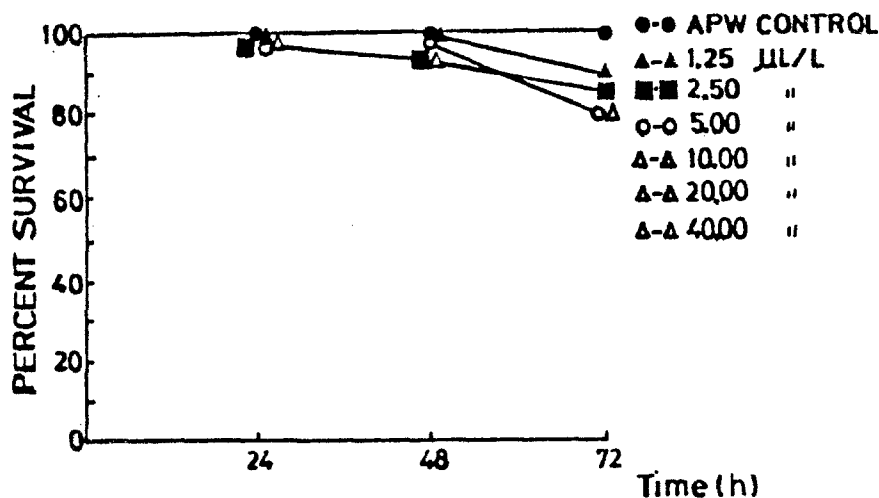


FIGURE 2. Survival curves for *D. spinulata* exposed to several concentrations of ethanol. At 10.00, 20.00 and 40.00 uL/L the individuals showed the same response. (n= 20).

TABLE 1. Response to mechanical stimulus in individuals exposed to deltamethrin. Results are the mean (\bar{X}) values of the number of antennal impulses/15 sec from independent determinations (n= 5-10). a: no significant difference in comparison with control ($p \leq 0.5$; Dunnett's test); b: individuals with spastic movements and erratic swimming; c: n= 5; d: all individuals were dead prior to 48 or 72 h.

ug/L	48 h		72 h	
	\bar{X}	SD	\bar{X}	SD
0.00	40.00	3.22	26.50	3.00
0.31	38.04 <u>a</u>	3.04	18.60 <u>a</u>	2.72
0.62	43.00 <u>a</u>	3.37	<u>b</u> , <u>c</u>	
1.25	<u>b</u> , <u>c</u>		<u>d</u>	
2.50	<u>d</u>		<u>d</u>	
5.00	<u>d</u>		<u>d</u>	
10.00	<u>d</u>		<u>d</u>	

The results of the response to mechanical stimulus in DM experiments are expressed in TABLE 1. This parameter was observed up to 48 h of exposure; at this time, no significant differences between control and exposed animals were recorded at 0.31 and 0.62 ug/L ($p \leq 0.5$;

Dunnett's test). The survivors of 1.25 ug/L showed spastic movements, which were not antennal impulses, and erratic swimming.

It must be born in mind that DM has a high primary action on the central nervous system of the arthropods, which causes motor disturbances (Miller and Salgado 1985). The survivors at 1.25 ug/L showed a degree of intoxication, which causes a motor inability to get an effective response to mechanical stimulus.

TABLE 2. Response to mechanical stimulus in exposed individuals to ethanol. Results are the mean (\bar{X}) values of the number of antennal impulses/15 sec from independent determinations (n= 10).

uL/L	48 h		72 h	
	\bar{X}	SD	\bar{X}	SD
0.00	40.00	3.22	26.50	3.00
1.25	33.30	3.85	27.25	3.04
2.50	24.43	3.24	25.25	2.37
5.00	36.20	2.28	18.50	2.52
10.00	30.70	2.28	18.75	3.48
20.00	32.10	2.71	19.88	2.90
40.00	34.00	2.49	23.43	2.96

In ET experiments no significant difference between control and exposed individuals was found ($p \leq 0.5$; Dunnett's test) with respect to the response to a mechanical stimulus (TABLE 2). Therefore, an inhibitory effect on an effective response can be attributed to a DM action.

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