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# Acute Toxicity of the Pesticide Diazinon to the Freshwater Snail *Gillia altilis*

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Diazinon, or 0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl) phophorothioate, MW=276, is an organophosphorous pesticide. It has wide application under several trade names including Spectracide, Sarolex, Diazitol, and Basudin. Annually, in the United States, almost 4 million kg of diazinon are produced and used by professional pest control operators (40%), by private users in homes and gardens (40%), in agriculture (10%), and on turf (10%). It is used on over 75 food crops to control soil and leaf-eating insects. In households, it is used indoors to control such insects as flies and cockroaches and outdoors to control grubs and nematodes in lawns and gardens. Diazinon is available in formulations of up to 50% pure diazinon.

Due to its widespread use, contamination of aquatic environments with diazinon due to surface runoff is probable. Indeed, Miles et. al. (1978) have demonstrated that diazinon can accumulate and persist in organic soils for more than a year. It was also shown that diazinon can move from its soil bound form into the aqueous environment either via leaching or by direct soil erosion (Miles and Harris 1978). Marganian and Wall (1972) demonstrated that diazinon treatment of a marine salt marsh lead to a build up of diazinon in salt marsh sod and mud. Kanazawa (1975) found diazinon to be fairly persistent in water, degrading to 27% in 30 days as opposed to 0.1% in 7 days for Malathion, 2% in 28 days for Fenitrothion, and 1% in 9 days for Carbaryl. Faust and Gomaa (1972) reported the half life of diazinon in water, pH 7.4, to be 185 days. Because of this persistence in the environment, the toxicity of diazinon in the aquatic environment should be determined. Diazinon toxicity has been observed for several fish species. Cope (1965) reported $LC_{50}$ values after 24-h exposure for bluegills and rainbow trout of 0.052 and 0.380 mg/L respectively. Sastry and Malik (1982) reported an $LC_{50}$ value of 3.1 mg/L for a 96-h exposure of Channa punctatus to diazinon. We report here on the toxicity of diazinon to the snail Gillia altilis, a common invertebrate species that we have used before (Stewart and Robertson 1985).

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## MATERIALS AND METHODS

For all experiments performed, snails were collected, September through November, from the mouth of the ditch that drained into the Racquette River, Potsdam, New York. They were collected by hand and transported to the laboratory in 20-L containers filled with river water. Upon arrival the containers were aerated for approximately 8 h so as to assure no temperature shock. After this brief acclimatization period, the snails were placed in one of two holding tanks in the laboratory where they were fed Tetramin Staple Fish Food, lettuce, and algae from the river. The water in the holding tanks was tap water which was dechlorinated with sodium thiosulfate (Hartz Dechlorination Droplets) and aerated continuously. The snails were acclimated in the lab for at least 48 h before any experiments were performed. Water characteristics are in Table 1.

Table 1. Water Characteristics

| | River Water | Laboratory Water |
|---|---|---|
| Temp $^{o}C$ | 18.0 - 21.5 | 20.5 - 23.5 |
| pH | 6.6 - 6.9 | 6.7 - 6.9 |
| Hardness $CaCO_3$ mg/L | 20 - 22 | 22 - 35 |
| DO mg/L | 7 - 9 | 8 - 11 |
| Alkalinity $CaCo_3$ mg/L | 20 - 25 | 25 - 29 |

All exposures were carried out in 4-L rectangular glass aquaria which were aerated continuously. The water used in all experiments was tap water treated with Hartz Dechlorination Droplets at least 24 h before exposure. The temperature of the exposure system was maintained at approximately 22 $\pm$1.5 C. The diazinon (88.6% pure) used for all experiments was provided by CIBA-GEIGY Corp. Ethanol was used to prepare the stock solutions of 10 mM diazinon.

For the static exposures of 4 and 96 h, 1 L of the appropriate diazinon solution was placed in each tank. Twenty snails (average weight was 2.4 $\pm$0.7 g/snail) were exposed at each dose. After the appropriate exposure time, the snails were removed from the tanks and rinsed under running tap water to remove any residual diazinon from the shell. The snails were then placed for 1 week in the recovery system which consisted of 4-L glass jars filled with 1 L of conditioned water, continuously aerated. The water in the recovery jars was changed daily. During this 7-day period, dead snails were removed and counted at least every 24 h. Death was established by the failure of the snail to respond when its foot was prodded with a dissecting probe.

A 96 h static renewal bioassay was also performed. The term static renewal is used to indicate that the diazinon solutions

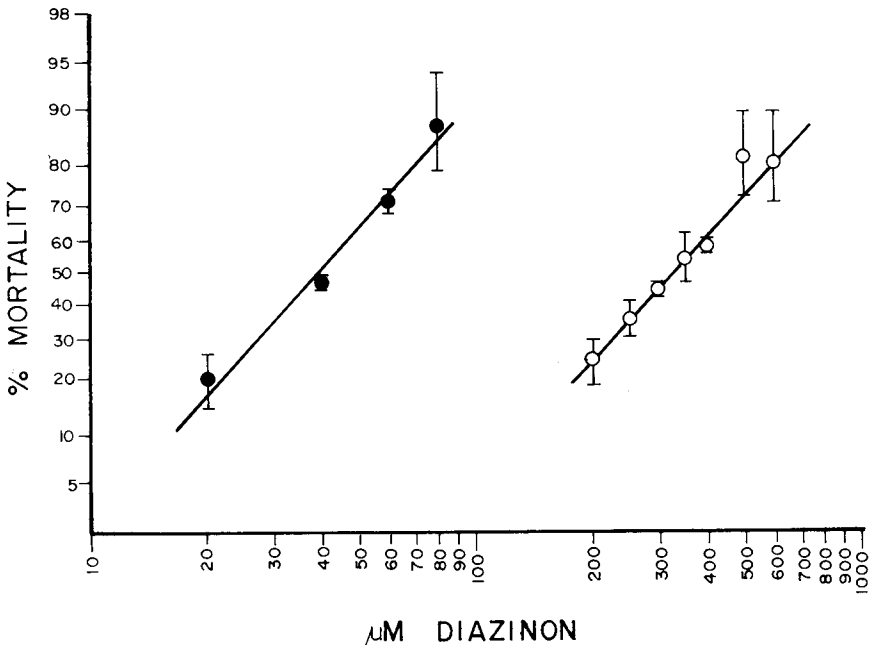


Figure 1. Dose-Response Curves. Closed circles represent the response of snails exposed to diazinon for 96-h. Open circles represent the response of snails exposed to diazinon for 4 h. In both cases the data plotted was pooled from three separate experiments.

were changed every 24 h during the 96-h exposure. At each 24- h interval, the snails were removed from the tanks and rinsed with conditioned water. The tanks were cleaned and refilled with fresh solutions of diazinon, and the snails were replaced. This 96-h static renewal bioassay was performed to try to more closely simulate a flow-through system.

In all experiments, controls were exposed to 1 L of conditioned water. Also, since diazinon was dissolved in ethanol, snails were also exposed to the highest doses of ethonol used for both the 4- and 96 h exposures, 0.1 and .01 M ethanol respectively.

RESULTS AND DISCUSSION

Mortality data between 15 and 85% for three separate 4-h static exposures to Diazinon were pooled and are plotted in Figure 1. The line was fit by eye and the predicted $LC_{50}$ value obtained for the 4-h exposure to Diazinon was 340 μM (93 ppm).

Figure 1. also shows the pooled results for three separate 96-h static exposures to diazinon based on nominal calculations. Again, the mortality data between 15 and 85% were plotted and the

predicted $LC_{50}$ value obtained was 40 µM (11 ppm). This value is considerably less than value of 340 µM (93 ppm) obtained for a 4-h static exposure, indicating as expected, greater toxicity with increasing exposure time.

On one occasion we performed the 96-h static renewal bioassay to compensate for possible diazinon losses due to diazinon metabolism, adsorption or decomposition. Our results showed an increase in toxicity and indicate that if a flow- through system were used, the 96-h $LC_{50}$ value would be lower than what we report here.

This present study determined the toxicity of diazinon to the freshwater snail Gillia altilis. Based on nominal calculations the $LC_{50}$ values for static 4- and 96- h exposures were 340 µM (93 ppm) and 40 µM (11 ppm), respectively. These values are greater than what has been reported for other freshwater species, $LC_{50}$ of 0.19 µM and 1.38 µM respectfully for bluegill and rainbow trout after a 24-h exposure (Cope 1965) and 11 µM for Channa punctatus after a 96-h exposure (Sastry and Malik 1982).

Kanazawa (1978) examined the uptake and bioconcentration of diazinon in freshwater fish and snails and reported that the fish bioconcentrated diazinon to 5 to 10 fold greater levels than did the snails studied. This could be due to physiological factors; differences in the exposed surface area/body weight; differences in the gill surface area and/or flow rate over the gill surface area caused by differences in respiration rates of the species involved; differences in the amount ingested orally. The difference could also be due to differences in metabolism, excretion or sites of action of diazinon once absorbed. Finally, the size of the storage sites (lipid content) could result in large differences between the species. Diazinon has been shown to bioconcentrate more in fatty fishes (Sequchi and Asaka 1981).

It is important to note that we chose this species of snail, Gillia altilis because we thought it to be a good indicator species for environmental pollutants. In previous work (Stewart and Robertson 1985) we reported on the sensitivity of the species to the chlorinated hydrocarbon pesticide, pentachlorophenol. There, we reported that it was considerably more sensitive to pentachlorophenol than other species studied. This is obviously not the case for Diazinon and again points out the problems one encounters when one tries to identify a unique species that one could use to identify all environmental contamination problems.

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