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Susceptibility of Five Nontarget Organisms to Aqueous Diazinon Exposure

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Diazinon (O,O-diethyl O-[2-isopropyl-6-methyl-4pyrimidinyl] phosphothioate) is an organophosphorus insecticide widely used on various agricultural crops (e.g. fruit trees, corn, and tobacco) and is an active ingredient in many commercial fire ant repellents (EXTOXNET 1996) (Table 1). In 1990 and 1992, approximately 36,000 kg were applied to the Imperial Valley in California. Also, in 1990, approximately 295,000 kg of diazinon was applied to Central Valley, CA (Bailey et al. 1996). Almost 4 million kilograms are produced and used for professional pest control (40%), by private users in homes and gardens (40%), in agriculture (10%), and on turf (10%) in the United States alone (Robertson and Mazzella 1989). Because of its environmental chemistry, as well as its use in urban and agricultural settings, diazinon has a great potential to runoff these areas in either dissolved or sorbed phases (Larson *et al.* 1997). According to Miles *et al.* (1978), diazinon can enter the aquatic environment in soil bound form via leaching or direct soil erosion. Once in the aquatic environment, diazinon and other organophosphorus pesticides have the potential to affect ecosystems as far reaching as the marine environment (Galassi 1991).

The purpose of this research was to examine impacts of diazinon on nontarget organisms in aquatic environments. Data generated from such comparative aqueous toxicity experiments can be used for estimating ecological effects of future exposure, either accidental or intentional. Such data can also be used for risk assessment studies either prospectively or retrospectively (Moore *et al.* 1998a,b).

MATERIALS AND METHODS

In this research, the sensitivity of five freshwater aquatic organisms to diazinon was determined in aqueous laboratory exposures. A range of species was used to determine differential exposure responses (survival) for crustaceans, insects, and vertebrates. The experimental organisms were *Ceriodaphnia dubia* (waterflea), *Daphnia magna* (waterflea), *Hyalella azteca* (amphipod), *Chironomus tentans* (non-biting midge), and *Pimephales promelas* (fathead minnow). All test organisms were cultured in the University of Mississippi Aquatic Toxicology

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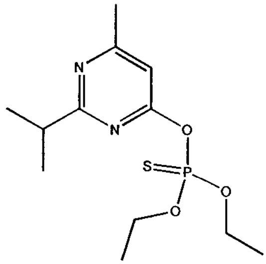
Table 1. Physical and environmental fate characteristics of diazinon.

Structure



Molecular weight (g/mol)	304.35
Water solubility (mg/L)[a]	40.0
Specific gravity (g/cm^3)[a]	1.117
Melting point (°C)[b]	120
Water persistence, $T_{1/2}$ (days)[b]	0.5 (acidic pH), 150-180 (neutral pH)
Soil persistence, $T_{1/2}$ (days)[b]	15-30

[a]Chemfinder, 1998
[b]EXTOXNET, 1996

Laboratory. Procedures for culturing *D. magna, P. promelas,* and C. *dubia* followed the methods of Peltier and Weber (1985). *H. azteca* culturing procedure methods followed those of de March (198 1). Methods used for *C. tentans* followed those of Townsend *et al.* (198 1).

Static aqueous toxicity experiments (48 hr) were conducted in incubators at 20+/- 1°C with a 16 hr light/ 8 hr dark photoperiod. Individual experiments were initiated by adding either ten *H. azteca* (425-1000 urn), ten *D. magna* (< 48 hr), six *C. tentans* (13-15 d), or 10 *P. promelas* (<24 hr) to each of three replicate 250-ml glass beakers per concentration of diazinon. *C. dubia* experiments were initiated by adding one organism (<48 hr) to each of ten replicate 25 ml plastic containers. Approximately 4 ml of glass beads (150-212 urn, Sigma Chemical Co., St. Louis, MO) were used as a substrate in *C. tentans* experiments to allow for tube building and to reduce stress on the organisms (Suede1 *et al. 1996). C. tentans* were fed 1 mL of Cerophyll™ per beaker at test initiation to decrease predation. Two, 1.4-cm diameter maple leaf discs were placed in each *H. azteca* test beaker to serve as a food source and substrate. *C. dubia* were fed 1 mL of a yeast/trout chow mixture and one drop of *Selenastrum capricornutum* algae daily. *D. magna* were fed 1 ml of *S. capricornutum* daily. *P. promelas* were fed 1 mL of newly hatched (<24hr) *Artemia salina* per day. After the 48 hr exposure, organisms were gently prodded with a dissecting probe and survival was determined by observation of organism responses. Water temperature,

conductivity, dissolved oxygen, pH, alkalinity, and hardness were measured after test completion according to APHA (1992).

Water used for test dilutions was spring water collected at the University of Mississippi Field Station (UMFS) (Deaver and Rodgers 1996: Gillespie *et al.* 1996). Water was filtered to remove particulate matter using MFS 0.45-urn polymembrane filters. Hardness and alkalinity of filtered water were adjusted with $NaHCO_3$ and $CaCl_2$ to values between 60-80 mg/L as $CaCO_3$.

Diazinon stock solutions were prepared by dissolving granular Ortho Fire Ant Repellant™ (5% active ingredient diazinon) in one liter of Milli-Q deionized water. Nominal concentrations were then prepared for the aqueous toxicity experiments by adding stock solution and dilution water to either 250 ml beakers (200 ml test volume) or 25 ml plastic containers (20 ml test volume) depending upon the specific organism. Exposure concentrations ranged from 0.1 to 60,000 ug/L for the various experiments.

The EnviroGuard Diazinon Plate Immunoassay (Strategic Diagnostics Inc.) was used to determine measured diazinon concentrations in aqueous exposure vessels. Analytical ranges for diazinon were 0.8-25,000 ug/L. If experimental concentrations exceeded immunoassay analytical limits, dilutions were performed prior to analysis. Samples were analyzed at 450 nm with a microtiter plate reader spectrophotometer (Table 2).

Table 2. Pesticide concentrations in exposure chambers during experiments (n=2)

Pesticide	Nominal Concentrations (ug/L)	Mean Measured Concentrations (ug/L)	Standard Deviation	Average Recovery (%)
Control	0	0	0	0
Diazinon	0.80	1.19	0.488	146.9
	6.0	8.27	0.378	
	15.0	21.6	0.440	
	60.0	88.93	0.482	
	15,000	23,391.96	0.559	

Organism mortality and nominal diazinon concentrations were used to determine toxicity point estimates (LC50) using trimmed Spearman-Karber (Hamilton *et al.*, 1977). Analysis of variance (ANOVA) and Dunnett's multiple range test were used to calculate lowest observed effects concentrations (LOECs) and no observed effects concentrations (NOECs) by determining significant differences between control and treatment survival ($p \leq 0.05$). Insecticide concentrations and organism mortality were also used to calculate exposure-response slopes. These

exposure- response slopes demonstrated the response of exposed organisms per unit concentration in excess of the lower threshold for response (Moore *et al.* 1998 a, b).

Organism response slopes were used to determine relative toxicities of diazinon to different test species. These slopes were calculated using the linearized portion (between 20% and 80% mortality) of the exposure-response curves. Resulting data gave the exposure-response slope point estimate (% mortality/ug/L).

RESULTS AND DISCUSSION

The cladoceran C. *dubia* was the most sensitive organism exposed to diazinon with a mean 48 hr LC50 value of 0.92 ug/L and exposure-response slope of 300% mortality/ug/L (Table 3) (Figure 1). These findings are similar to those of Giddings *et* al. (1996) where cladocerans were the most sensitive species of zooplankton with reduced abundance at all treatment levels of diazinon. Bailey *et al.* (1997) reported a mean 96 hr LC50 of 0.44 ug/L with 24hr and 48hr LC50s within a factor of 1.1 to 2.0 of the 96hr calculations. Kuivila and Foe (1995) reported 24-96hr LC50s of 0.43 to 0.55 ug/L of diazinon contained in water samples from the Sacramento River. Seven-day experiments with water from the same river yielded 90-100% mortality in concentrations as low as 0.2 ug/L (Kuivila and Foe 1995).

Table 3. Toxicity point estimates and slopes for 48 hr aqueous diazinon exposure.

Organism	LC50 and confidence intervals (ug/L)	LOEC (ug/L)	NOEC (ug/L)	Exposure-response slope (%mortality / ug/L)
C. dubia	0.92 (0.83-1.09)	0.8	0.6	300
D. magna	2.39 (1.63-3.42)	1.5	0.8	25.6
H. azteca	15.07 (12.08-18.87)	11.0	7.5	4.76
C. tentans	52.47 (36.71-73.76)	37.5	30	1.02
P. promelas	15,940 (12,090-19,600)	12,500	6,000	0.005

D. magna, another microcrustacean, was the second most sensitive species with a mean 48 hr LC50 of 2.39 ug/L and an exposure-response slope estimate of 25.64% mortality/ug/L. The ACQUIRE database (1994) reported a 48 hr LC50 of 0.96 ug/L and a 21 d LOEC of 0.32 ug/L. EXTOXNET (1996) reported a 48 hr EC50 of 0.96 ug/L. ACQUIRE (1994) reported a 48 hr EC50 of 1.1 ug/L and a 21d LOEC of 0.32 ug/L. 96 hr LC50s of 0.47 and 0.41 ug/L were noted by Bailey *et* al. (1996). Meier *et al.* (1976) reported a 96 hr LC50 of 2.0 ug/L. Fernandez-Casalderrey *et al.* (1995) concluded that sub-lethal concentrations of 0.25 and 0.30 ug/L delayed the time of first reproduction and depressed growth rate. A 24 hr LC50 of 0.86 ug/L, as well as growth depression at sub-lethal concentrations above 0.05 ng/L was noted by Sanchez *et al.* (1998).

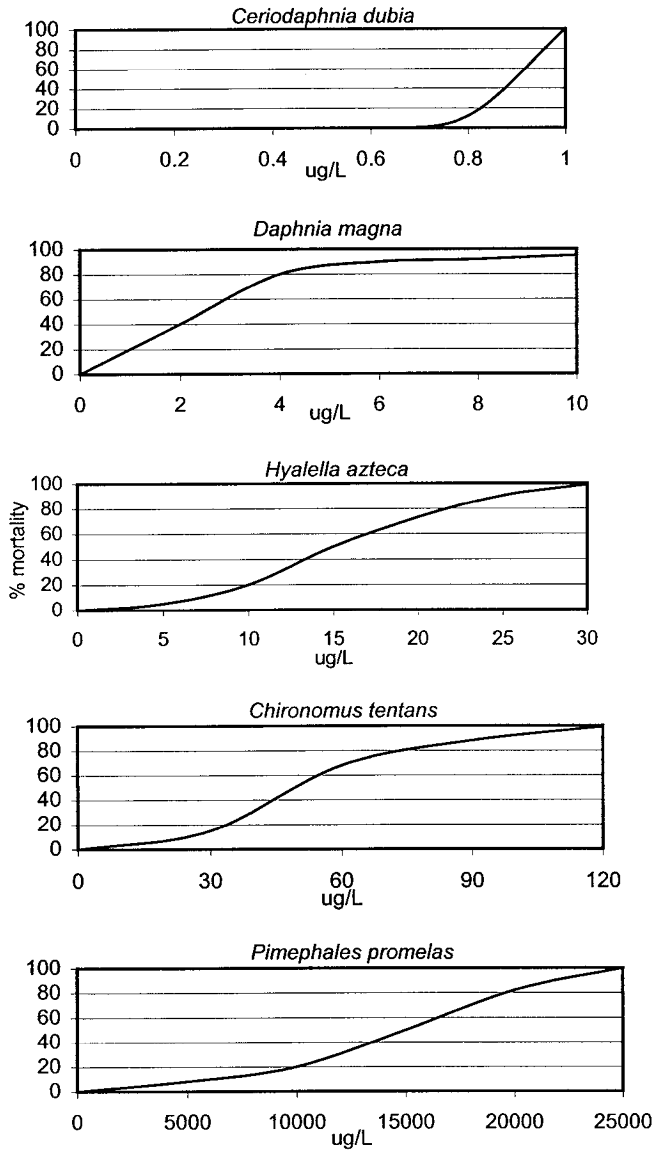


Figure 1. Diazinon 48-hr exposure-response slopes

The amphipod *H. azteca* was the next most sensitive organism. Calculated point estimates gave a mean 48 hr LC50 of 15.07 ug/L and an exposure-response estimate of 4.76% mortality/ug/L. Of the few outside data found, ACQUIRE (1994) reported a mean 48hr LC50 of 22 ug/L. Collyard *et al.* (1994) reported 96 hr LC50s according to the age of the organism. In intermediate age classes (2 - 24 d), LC50s ranged from 3.8 - 4.6 ug/L.

Next in decreasing sensitivity was the non-biting midge C. *tentans.* Organism mortality data yielded a mean 48 hr LC50 of 52.74 ug/L and an exposure-response point estimate of 1.02 % mortality/ug/L. A 96hr LC50 of 0.03 ug/L was reported in the ACQUIRE database (1994). Giddings *et al.* (1996) reported a LOEC for the tribe Chironomini, the tribe for *C. tentans,* of 54 ug/L. Stevens (1991) noted a 24 hr LC50 of 13 ug/L in the midge *Chironomus tepperi.*

P. promelas, a fish species and the only vertebrate tested, was the least sensitive species exposed to aqueous concentrations of diazinon. *P. promelas* had a mean 48 hr LC50 of 15,940 ug/L and an exposure-response slope of 0.0048 % mortality/ug/L. ACQUIRE (1994) reported a 25d LOEC of 3.2 ug/L and a 48/96 hr EC50/LC50 of 6,970 ug/L. Jarvinen and Tanner (1982) noted 96 hr LC50s of 7,800 and 6,900 ug/L. Norberg-King (1989) reported a NOEC of 16.5 ug/L and a LOEC of 37.8 ug/L for early life stages of *P. promelus* as well as an NOEC of 83.8 ug/L for 32 d old fish.

The test organisms used represented a range of a typical freshwater ecosystem. The five organisms greatly differed in physiology and niches occupied which allowed for comparisons of the effects of diazinon on diverse trophic levels. Invertebrates were dramatically more sensitive than the vertebrate as all the invertebrates were at least 200 times more sensitive to diazinon exposure than was the vertebrate species, *P. promelas.* Since the test compound is an insecticide, it was hypothesized that diazinon would most adversely affect the insects (C. *tentans).* This was not the case as *C. dubia,* a freshwater crustacean, was the most sensitive species being approximately 12 times more sensitive than the next most sensitive species, *D. magna* (Table 3). Although C. *tentans* is not a target organism for diazinon applications, it was the only true insect included in the study.

These data are significant because comparable concentrations of diazinon are found in the environment to affect non-target organisms. In a 1997 study, concentrations of diazinon of up to 1.1 ug/L were found in agricultural runoff from the San Joaquin River (Domgalski *et al.* 1997). In the current laboratory study, *C. dubia* populations were reduced by 50% at a concentration of 0.92 ug/L. It is important, therefore, to examine how aqueous exposure to diazinon affects non-target organisms. Greater understanding of diazinon and its toxicity allows for predictions of potential environmental damage either prospectively or retrospectively. Subsequent studies can focus on joint toxicity of diazinon and

other commonly used insecticides such as chlorpyrifos since multiple pesticides may be used in single applications.

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