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Toxicity of Dimethoate to *Daphnia magna* and Freshwater Fish

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Due to their widespread distribution and toxic nature pesticides may have a serious impact on the aquatic environment and exert adverse effects on the associated organisms. Acute bioassay tests have been used to determine the actual impact of various pesticides on aquatic life and to formulate water quality standards using appropriate application factors (Verma et al. 1982).

The use of organophosphorus insecticides has largely increased as they are usually less toxic than the organochlorine insecticides. The organophosphorus insecticide used in this study, dimethoate [0,0-dimethyl-S-(2-oxo-3-aza-butyl)-dithiophosphate], is a contact and systemic insecticide effective against a broad range of insects on a wide range of crops. Therefore, the present investigation was designed to assess the acute and subchronic toxicity of dimethoate to two species of freshwater fish, guppy *(Poecilia reticulata)* and zebrafish *(Brachydanio rerio)*, and to determine chronic effects on survival and reproduction of the cladoceran *Daphnia magna* Straus.

MATERIALS AND METHODS

Dimethoate used in the toxicity tests was an emulsible concentrate containing 100 g dimethoate per liter [® Dimethoate Bayer 10% EC, Lot no. 7505]. Some tests with *Daphnia magna* were also conducted using high purity (min. 99%) dimethoate [Riedel-de Haën PESTANAL®]. Stock solutions of the pesticide were prepared by dissolving the appropriate amount in dilution water. Extraction of dimethoate from the test solutions was accomplished using methylene chloride and quantitation was done with a Carlo Erba HRGC 5300 Mega Series capillary gas chromatograph equipped with a flame ionization detector. The column was a 30-m fused-silica capillary column (0.32 mm I.D., 0.3 µm filmthickness) coated with polydimethylsiloxane (RSL-150 BP). Temperature programming : 50 °C followed by an increase of 20 °C/min to 200 °C. This temperature was held for 5 min. The detector temperature was 240 °C. The recovery of dimethoate [® Dimethoate Bayer 10%] when added to dilution water at

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concentrations from 1 to 7 mgL^{-1} was 87.1 ± 5.9% (n = 10). The recovery of pure dimethoate [Riedel-de Haën PESTANAL®] when added to dilution water at concentrations ranging from 5 to 21 mgL^{-1} was 100.8 ± 3.5% (n = 3). In general, more than 90% of the initially added amount of dimethoate was found after 48 h. Dimethoate was thus not volatilized and did not undergo any chemical change in this relatively short time period. This agrees with the slow degradation rate of dimethoate in river water as found by Eichelberger and Lichtenberg (1971).

The daphnids used in this study were obtained from a laboratory brood stock maintained in an environmental chamber set at 19 ± 1 °C and a light cycle of 14 h daylight and 10 h darkness. The daphnid stock culture was fed a diet of the green algae, *Chlorella pyrenoidosa*, and powdered commercial fish food (TetraMin®) on a daily basis. The daphnids used in all toxicity experiments were less than 24 h old. The zebrafish *(Brachydanio rerio)* were obtained from a commercial supply house. Before testing fish from a single stock of similar length and age were adapted to laboratory conditions in 200-L tanks filled with hard water for at least 12 days (T = 23 ± 2 °C). Laboratory reared guppies were also used as a test species. Fish were fed twice daily with TetraMin®, with food being withheld at least 24 h prior and during testing.

Dilution water used for stock cultures and tests was synthetic hard water ('Dutch standard water') having the following chemical composition (mgL^{-1} deionized water) : 100 mg $NaHCO_3$, 20 mg $KHCO_3$, 180 mg $MgSO_4.7H_2O$ and 200 mg $CaCl_2.2H_2O$. The water quality variables were as follows : pH 8.2 - 8.4 and hardness : ≈223 mgL^{-1} (as $CaCO_3$ + $MgSO_4$).

The methods for the acute toxicity tests with daphnids and fish were based on the guidelines recommended by the Commission of the European Communities (Anonymous 1984). The daphnid static acute tests were conducted either in 50-mL glass beakers filled with 50 mL test solution (open system) or in 100-mL stoppered glass bottles containing 100 mL of test solution (closed system). In the open system five neonate (<24 h old) daphnids were used per beaker with four beakers for each test concentration and for the control. In the closed system ten daphnids were assigned to each of duplicate glass bottles at each dimethoate concentration. A control and at least five dimethoate concentrations were selected on the basis of a logarithmic expansion. The test vessels were kept in a temperature controlled room set at 19.5 ± 1 °C with a light cycle of 14 h daylight/10 h darkness. The duration of the daphnid acute tests was 48 h. The daphnids were not fed nor were the solutions aerated during testing. Mortality and immobilization of the test organisms as well as temperature, pH, and dissolved oxygen of the test solutions and the controls were recorded after 24

and 48 h of exposure. For acute tests with zebrafish and guppy, ten fish were randomly assigned to 3-L glass beakers filled with 3 L of test solution. The vessels were placed in a temperature controlled room at a temperature of 23 ± 2 °C. The duration of the fish tests was 96 or 168 h. The fish were transferred to new test medium after 48 and 96 h. The test solutions were gently bubble-aerated during the entire test period. Mortality was recorded after 24, 48, 72, 96, and 168 h of exposure. At each observation time dead fish were removed. Temperature, pH, and dissolved oxygen were measured at each renewal for all test concentrations. During acute testing the measured pH of the test solutions ranged from 7.4 to 8.4 while dissolved oxygen was always above 6 mgL^{-1}.

The chronic toxicity was determined from a 23-day renewal static test using ten neonate (<24 h old) daphnids at each dimethoate concentration. The daphnids were housed individually in 50-mL beakers filled with 50 mL test solution. The test medium was changed on days 2, 5, 7, 9, 12, 14, 16, 19 and 21. The daphnids were fed a diet of the green algae, *Chlorella pyrenoidosa*, and prepared fish food suspension (25 µL of TetraMin® extract following each water renewal). The algal feeding rate was $1x10^8$ cells per beaker following each medium renewal during the first week and $2-3x10^8$ cells per vessel during the remaining test period. The temperature was 20 ± 1 °C and no aeration was applied to the solution during testing. Reproduction was assessed by counting and discarding neonate daphnids each time the parent animals were transferred during the second week, and daily during the last week of the test. Adult survival, time to the first brood, number of neonates per female per day, and the number of 'eggs' and newborns produced by the adult daphnids were the parameters monitored during these tests. Temperature, pH and dissolved oxygen were recorded in two test vessels of each test concentration at each renewal.

For each set of data the daily LC50 and EC50 values and their corresponding 95% confidence intervals were estimated using the graphical method of Litchfield and Wilcoxon (1949). The reproductive data from the *Daphnia magna* life-cycle test were analyzed by analysis of variance and the one-tailed 'Student t' test.

RESULTS AND DISCUSSION

The calculated 48-h LC50 and 48-h EC50 values and the resulting No Observed Effect Concentrations (NOEC) for *Daphnia magna* during the acute tests with dimethoate are presented in Table 1. The emulsible concentrate appears to be slightly more toxic than the pure material. Similarly, Pickering et al. (1962) found that the toxicity of emulsible concentrates of organophosphorus insecticides to various warmwater fish species was generally higher than that of the technical materials in

Table 1. Summary of 48-h LC(EC)50 and 48-h NOLC(NOEC) values obtained with the *Daphnia magna* acute toxicity tests conducted with pure dimethoate (min. 99%) and an emulsible concentrate (10% EC).

Test chemical	Test system	48-h LC50 (95% C.I.) (mgL^{-1})	48-h EC50 (95% C.I.) (mgL^{-1})	48-h NOLC (mgL^{-1})	48-h NOEC (mgL^{-1})
Dimethoate (min.99%)	Open	1.7(1.5-2.0)	1.5(1.3-1.9)	0.6	0.4
	Closed	2.0(1.7-2.4)	1.8(1.6-2.1)	0.9	0.9
Dimethoate (10% EC)	Open	0.83(0.55-1.26)	0.74(0.65-0.84)	0.4	0.4
		0.83(0.41-1.68)	0.56(0.48-0.66)	0.4	0.4
	Closed	0.83+	0.78(0.68-0.89)	0.6	0.6
		1.26+	0.80(0.70-0.92)	0.4	0.4
		1.6(1.0-2.6)	0.88(0.76-1.02)	0.6	0.5

NOLC = No Observed Lethal Concentration
NOEC = No Observed Effect Concentration with E = complete immobilization
+ No confidence interval calculated

acetone. A similar result was obtained for the toxicity of synthetic pyrethroid insecticides to rainbow trout (Coats and O'Donnell-Jeffery 1979). The higher toxicity of the dimethoate emulsible concentrate might be due to the contribution of the emulsified solvent xylene to the total toxicity. Bringmann and Kühn (1977, 1982) reported 24-h LC50 and 24-h EC50 values of xylene for *Daphnia magna* of 150 and 165 mgL^{-1} respectively. Other *Daphnia magna* toxicity data with xylene included a study by Bobra et al. (1983) who reported 48-h LC50 values ranging from 3.2 to 9.5 mgL^{-1} and a study of Hermens et al. (1984a) who reported a 48-h EC50 value of 14 mgL^{-1} (*m*-xylene).

The differences between 48-h LC50 and 48-h EC50 values are generally quite small. Also, the results obtained in open test vessels agree very well with those obtained in closed bottles suggesting that no volatilization of test material has occurred. The lowest NOEC value from these studies was 0.4 mgL^{-1} which was obtained for the dimethoate emulsible concentrate. Frear and Boyd (1967) evaluated the acute toxicity of various pesticides to *Daphnia magna* and they reported a 26-h LC50 value of 2.50 mgL^{-1} for dimethoate. In a study on the joint effects of a mixture of different chemicals on mortality and inhibition of reproduction of *Daphnia magna*, Hermens et al. (1984b) reported a 48-h LC50 of 6.4 mgL^{-1} for dimethoate. Our results with pure dimethoate are slightly lower than their published data.

The results of the semi-static toxicity tests with zebrafish

and guppy are summarized in Table 2. Zebrafish appears to be significantly more susceptible towards dimethoate than guppy.

Table 2. Median lethal (LC50) and No Observed Lethal Concentration (NOLC) values associated with the guppy (number of replicates:3) and zebrafish (number of replicates:2) acute toxicity tests conducted with an emulsible dimethoate concentrate (10%).

Species	LC50 (mgL^{-1}) (95% C.I.)			NOLC (mgL^{-1})		
	48-h	96-h	168-h	48-h	96-h	168-h
Zebrafish	7.5 (6.1-9.7)	6.8 (5.6-8.3)	6.2 (5.3-7.4)	3.1	3.1	3.1
	8.2 (7.0-9.6)	7.8 (6.9-8.9)	7.0 (5.7-8.5)	5.3	5.3	5.3
Guppy	15.7 (13.5-18.3)	13.0 (10.9-15.4)	-	8.8	6.4	-
	10.4 (9.1-11.9)	10.4 (9.1-11.9)	-	6.8	6.8	-
	11.2+	11.2+	-	5	5	-

+ No confidence interval calculated

Again, the dimethoate emulsible concentrate is more toxic than the pure compound: a dimethoate concentration of 26 mgL^{-1} (pure compound) did not cause mortality or any adverse effects on the exposed guppies within 96 h. Our results with guppy and zebrafish fall well within the range of LC50 values (8.6-370 mgL^{-1}) reported by Canton and Slooff (1979) for dimethoate toxicity to fishes. Verma et al. (1982) conducted bioassay trials with various pesticides to the freshwater teleost Singii *(Saccobranchus fossilis)* and obtained a 96-h LC50 of 4.6 mgL^{-1} for dimethoate (Rogor® 30% Emulsible Concentrate). In a study on the toxicity of some insecticides to guppy, Gupta et al. (1984) reported a 96-h LC50 of 19 mgL^{-1} for dimethoate (Rogor® 30% Emulsible Concentrate). Using an arbitrary application factor of 0.024 (Verma et al. 1982) a presumable safe concentration of 0.19 mgL^{-1} is obtained for dimethoate. This safe concentration is much lower than the value of 2.7 mgL^{-1} derived from the 168-h test with zebrafish using the NOLC x LC25/LC50 value as a water quality criterion (Canton and Slooff 1979).

The results of the life-cycle reproduction test with *Daphnia magna* exposed to dimethoate (pure and emulsible concentrate) are summarized in Table 3. In all tests no mortality occurred

in the controls. As appears from Table 3, differences in adult survival (LC50 values) after 23 days between daphnids exposed to pure dimethoate and an emulsible dimethoate concentrate are not significantly different. The same is also true for the 23-d EC50 values. Chronic NOLC of NOEC (E = immobilization)

Table 3. A summary of the *Daphnia magna* life-cycle test results for dimethoate (all concentrations in mgL^{-1}).

Parameter	Test chemical			
	Dimethoate (10% Emulsible Concentrate)		Dimethoate (pure compound, min. 99%)	
	Test 1	Test 2	Test 1	Test 2
23-d LC50 (95% C.I.)	0.13 (0.10-0.16)	0.15 (0.10-0.21)	0.23 (0.17-0.30)	0.11 (0.09-0.13)
23-d EC50 (95% C.I.)	0.11 (0.08-0.14)	0.15 (0.10-0.21)	0.19 (0.15-0.24)	0.11 (0.09-0.13)
23-d NOLC	0.047	0.076	0.17	0.08
23-d NOEC	0.047	0.076	0.10	0.08
Limits *	0.023[a] - 0.047[b]	0.042[a] - 0.076[b]	0.095[a] - 0.170[b]	0.060[a] - 0.124[b]
Geometric mean of limits	0.033	0.056	0.130	0.085

* These are the MATC (Maximum Acceptable Toxicant Concentration) values based on reproduction (cumulative number of young/female daphnid).

[a] Not significantly different ($\alpha = 0.05$) from the controls (the NOEC = No Observed Effect Concentration).

[b] Significantly different ($\alpha = 0.05$) from the controls (the FOEC = First Observed Effect Concentration).

values are generally lower for the emulsible dimethoate concentrate, as was also observed during acute testing. Statistically significant differences between the mean cumulative number of young produced in the controls and in the test concentrations were used to define the limits of the MATC values and the Geometric Mean of Limits (Nebeker, 1982). These limits are the actual test concentrations which do or do not inhibit the reproduction of *Daphnia magna*. For the pure dimethoate, the MATC values based on reproduction are very similar to the 23-d NOEC values while they are about two times lower than the 23-d NOEC for the emulsible concentrate. The emulsible dimethoate concentrate affects the reproductive performance of *Daphnia magna*

at a lower level than the pure dimethoate itself (0.023 and 0.042 mgL^{-1} versus 0.095 and 0.060 mgL^{-1} respectively). Hermens et al. (1984b) reported a 16-d EC50 (50% inhibition of reproduction) value of 0.31 mg dimethoate per liter for *Daphnia magna*, a factor three higher than in this study.

An emulsible concentrate (10%) of the insecticide dimethoate appears to be more toxic to *Daphnia magna* and to two freshwater fish species than the pure compound. As a result of the present investigation, a dimethoate concentration of 0.033 mgL^{-1} would be considered safe. This value was derived from a 23-d *Daphnia magna* life-cycle test with reproduction as criterion.

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