Bull. Environ. Contam. Toxicol. (1986) 37:615–621

Bulletin of Environmental Contamination and Toxicology

Comparative Toxicity of Methanol and N,N-Dimethylformamide to Freshwater Fish and Invertebrates

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The organic solvents methanol and N,N-dimethylformamide (DMF) are used widely as industrial solvents. Methanol is used as a raw material for making formaldehyde, in antifreezes, and as fuel for stoves. DMF is used as a solvent for polyacrylic fibers and wherever a solvent with a slow rate of evaporation is required. Parker (1965) termed DMF as a "super solvent" because it does not change the inherent reactivity of many solutes. These solvents are also suggested by the American Society for Testing and Materials (ASTM) as preferred solvents in the preparation of stock solutions for acute toxicity tests. The ASTM further suggests a maximum concentration of 0.5 mL/L for these solvents in exposure water.

Although some toxicity data appear in the literature for methanol and DMF, there have been few studies which have been flow-through tests for a standard 48- or 96-h exposure period with measured toxicant concentrations. No data was found dealing with acute toxicity to midges. Only one DMF acute toxicity test was found for _Daphnia magna_, in which nominal concentrations were reported (LeBlanc and Surprenant 1983).

In this study, 96-h flow-through acute toxicity tests with methanol and DMF were conducted with three species of freshwater fish - rainbow trout (_Salmo gairdneri_), bluegill (_Lepomis macrochirus_) and fathead minnow (_Pimephales promelas_). Static acute toxicity tests (48 h) were conducted with DMF and two species of freshwater invertebrates - a midge (_Paratanytarsus parthenogeneticus_) and a daphnid (_Daphnia magna_). Median lethal (LC50) and median effect (EC50) concentrations for 96-h exposures were determined for fish and 48-h EC50's were determined for invertebrates.

MATERIALS AND METHODS

The exposure system for fish consisted of several modified proportional diluters (Mount and Brungs, 1967) with dilution factors ranging from 0.6 to 0.8. Each diluter cycle delivered 1.0 L of water and toxicant, where appropriate, to each of five test concentrations and a control. Each test concentration and control was duplicated by dividing the original volume equally using flow-splitting tanks (Benoit and Puglisi, 1973). The glass exposure tanks measured 20 x 35 x 9 cm, and contained 6.3 L of water. Diluters were cycled at a rate sufficient to yield 5 to 8 tank volumes/day. Daphnid exposures were conducted in 200 mL erlenmeyer flasks which were stoppered throughout the test. Five concentrations and controls were tested in quadruplicate. Midges were exposed in 90 mm diameter crystallizing dishes. Two toxicant solution volumes were replaced at the start of tests and the solutions remained unchanged for the duration of the tests. Five concentrations and a control were tested in duplicate. A photoperiod of 16-h light and 8-h dark was used for all tests. Fluorescent lamps provided a light intensity of 28-31 lumens for fish tests, 79-82 lumens for daphnid tests and indirect lighting provided 1.9 lumens for midge tests.

Water for all tests was untreated and obtained directly from Lake Superior and heated to mean temperatures ± s.d. of 12.7 ± 1.0°C for rainbow trout, 23.3 ± 1.7°C for fathead minnows, and 19.8 ± 2.3°C for bluegills. Temperatures were measured daily in each exposure tank. Daphnids were maintained in a 20.5°C water bath throughout the test. Midges were kept at room temperature (23°C). All water quality characteristics were measured using methods described by the American Public Health Association et al. (1975). EDTA hardness, total alkalinity and pH determinations were made on control, low, middle and high exposure concentrations at least once during each test except for hardness and alkalinity on the DMF test with rainbow trout, where measurements were made on dilution water only. Means and ranges for hardness and alkalinity were 46.4 (40.4-56.3) and 41.7 (30.0-46.0) mg/L as $CaCO_3$, respectively, for all tests. The pH ranged from 7.04 to 7.97 and were not toxicant concentration dependent. Dissolved oxygen concentrations were measured by the membrane electrode method, calibrated with modified Winkler determination at 24, 48, and 96 h on control, low, middle and high exposure concentrations except for the midge test which was only measured once in the control. Means and ranges for percent dissolved oxygen were 77.8 (61.0-95.9) for rainbow trout, 85.2 (78.1-98.0) for fathead minnows, 78.8 (54.3-88.9) for bluegills, 85.5 (77.5-90.8) for daphnids and 85.9 for midges.

Exposure samples containing methanol (Burdick & Jackson)[1] for bluegill and rainbow trout exposures and N,N-dimethylformamide (Burdick & Jackson, spectrograde) for rainbow trout exposures were analyzed by direct aqueous injection gas-liquid chromatography (Knuth and Hoglund, 1984). The percent recoveries of methanol-spiked exposure water for the rainbow trout and bluegill acute tests were 102.9 ± 3.6% (n=5) and 101.5 ± 2.7% (n=5),

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[1]Burdick & Jackson Laboratories, Inc., Muskegon, Michigan 49442.

respectively. The recovery of dimethylformamide from spiked rainbow trout test water was 100.2 ± 0.4% (n=6).

Methanol was monitored in the fathead minnow acute exposure by using Rhodamine B dye at 27.0 µg/L as a tracer added to the stock solution. Actual methanol concentrations were then calculated by the known ratio of dye to methanol. Rhodamine B was analyzed using a Baird-Atomic model SFR100 spectrofluorimeter with excitation and emission wavelengths of 554 and 578 nm, respectively. All other exposure samples containing dimethylformamide for fathead minnows, bluegills, midges and daphnia tests were analyzed directly on a Beckman model DB-UV spectrophotometer at a wavelength of 200 nm.

Juvenile rainbow trout were obtained from Fattig Hatcheries, Brady, NE and bluegills from the Lake Mills Fish Hatchery, Lake Mills, WI. They were held in 530-L fiberglass holding tanks at their respective test temperatures and fed a commercial formulation of dry food. Fathead minnow tests were conducted using 28-to 32-day-old fish reared at the U.S. EPA Environmental Research Laboratory-Duluth (Duluth, MN) test facility. Fathead minnow larvae and fry were fed live San Francisco Bay brine shrimp. Groups of 10 rainbow trout and bluegills or 20 fathead minnows were randomly assigned to the exposure tanks at the start of each test. Fish were not fed 24 h before or during a test. Average weights of exposed fish were obtained by weighing control fish from each test (Table 1). Death was the primary endpoint, although effects on equilibrium, behavior and coloration were noted. Dead fish were counted and removed at 1, 3, 6, 12 and 24 h after initial exposure and at 24-h periods thereafter.

Midges and daphnids were cultured at the ERL-Duluth laboratory, and brood stocks were fed a mixture of blended commercial fish food and yeast (Anderson, 1980). Twenty-four h before starting a test, third instar midges, ten in number, were randomly transferred to each exposure dish. Each dish contained 200 mL of food and lake water in a ratio of 2.5 mL:1 with a fine layer of acid washed sand for case building. Midges were examined for normal movement and case building after 24-h acclimation. Prior to adding toxicant, water was siphoned off to a depth of 0.5-1 cm, leaving an undisturbed layer of food that had settled.

Groups of five daphnids <24-h old, were counted and randomly assigned to exposure flasks at the start of the test. Complete immobilization of the daphnids was used as the test endpoint. Observations on midge and daphnids were made at 3, 6, 12, 24 and 48 h.

Determination of LC50 and EC50 and their 95% confidence intervals were made using the Trimmed Spearman Karber method (Hamilton et al., 1977). Mortality data from duplicate exposure tanks were combined before LC50 and EC50 determinations were made. Procedures or methods not specified followed the basic flow-through or static acute toxicity test method described by the U.S. Environmental Protection Agency (1975).

RESULTS AND DISCUSSION

The 24-, 48- and 96-h LC50 and EC50 values and their 95% confidence limits were calculated for the fish tests along with the 24 and 48-h values for the invertebrate tests (Table 1). There was relatively little change in the LC50 and EC50 values between 24- and 96-h. No controls died in any of the tests.

Rainbow trout and bluegills exposed to methanol were affected immediately in the two highest exposures and remained affected throughout the tests with most mortalities occurring within 3 h after initial exposure. The 96-h LC50's and their 95% confidence limits for rainbow trout and bluegills were 20,300 (19,800-20,700) and 15,400 (13,500-17,600) mg/L, respectively. Fathead minnows exposed to methanol were affected in the two highest exposures throughout the test with most mortalities occurring within the first 12 h. The 96-h LC50 and its 95% confidence limits for fatheat minnows was 29,400 (28,500-30,400) mg/L.

Fish exposed to DMF showed the same pattern of death and stress as with methanol. Rainbow trout and bluegills were stressed and showed mortalities within 3 h after initial exposure and deaths continued throughout the test with all fish dead in the highest concentration at 96 h. The 96-h LC50's and their 95% confidence limits for rainbow trout and bluegills exposed to DMF were 9,800 (9,000-10,700) and 7,100 (6,700-7,500) mg/L, respectively. Fathead minnows exposed to DMF showed mortalities immediately after initial exposure with 89.7% dead in the highest exposure at 96-h. The 96-h LC50 and its 95% confidence limits for fathead minnows exposed to DMF was 10,600 (10,400-10,800) mg/L.

Midges and daphnids exposed to DMF were affected immediately after initial exposure with deaths occurring in all exposures for midge and in the three highest exposures for daphnids. The 48-h EC50's and their 95% confidence limits for midges and daphnids were 36,200 (32,700-40,100) and 14,500 (13,300-15,900) mg/L, respectively.

Although methanol toxicity has been studied for a long time, wide ranges of toxicity have been reported. Weigelt *et al.* (1885) found that a 2-h exposure to 10,000 mg/L in tap water was tolerated by an unidentified species of trout without apparent injury. Powers (1917) reported that 250 mg/L methanol killed goldfish (*Carassius auratus*) within 11 to 15 h in distilled water. Liebmann (1960) cited threshold concentrations for fish narcosis as high as 31,000 mg/L. Dawson *et al.* (1970) listed the critical lethal concentration of methanol for fish at 240 mg/L and Gillette *et al.* (1952) reported that at less than 8000 mg/L methanol, all creek chubs (*Semotilus atromaculatus*) tested were able to survive 24 h.

Benville and Mauck (1971) with more recent and comparable data to ours, found the 96-h TL50 of methanol to be 19 mL/L (recalculated to 15,000 mg/L using the specific gravity of methanol) for rainbow trout. We obtained a 96-h LC of 20,100 mg/L for rainbow trout.

Compared to methanol, toxicity information on DMF is relatively recent with the oldest comparable research being Benville and Mauck (1971), who found the 96-h TL50 for rainbow trout to be 12 mL/L or 11,300 mg/L. This compares quite well with our 96-h LC50 of 9,800 mg/L for rainbow trout. The most recent and most comparable research was that of Cardwell (R.D. Cardwell, Environgenics Systems Co., El Monte, CA, unpublished manuscript) who obtained 96-h LC50's for three species of fish. They found the 96-h LC50's for fathead minnows, bluegills and brook trout (*Salvelinus fontinalis*) to be 10,410, 6,300 and 8,366 mg/L, respectively. Our data compares very well, with 96-h LC50's of 10,600, 7,100 and 9,800 mg/L for fathead minnows, bluegills and rainbow trout, respectively. Bluegills were found to be the most sensitive species of fish tested followed by rainbow trout, with fathead minnows the least sensitive in both studies.

Our daphnid test results show good agreement with the results of LeBlanc and Surprenant (1983). They obtained a 48-h LC50 for DMF of 13,000 µL/L (12,278 mg/L) in a toxicity test in which the concentrations were unmeasured. We obtained a 48-h LC50 of 14,500 mg/L.

Our results for DMF toxicity to the fish and invertebrate species tested indicated that *Daphnia magna* and the midges were less sensitive to DMF than the three fish species tested. No toxicity data for midges could be found in the literature. *Paratanytarsus parthenogeneticus* is notably more tolerant than the other species tested, with a 48-h LC50 of 36,000 mg/L.

Researchers using solvent carriers to facilitate the dissolution of hydrophobic compounds should be aware of the effects of the carriers on the test organisms and the effects of the compound-solvent combination. Belousov (1969) observed damage to the bronchial epithelium of goldfish when exposed to 100 mg/L DMF and Cardwell (unpublished manuscript) reports the maximum acceptable toxicant concentration for fathead minnows was between 5 and 11 mg/L for DMF. One study (Shubat *et al*. 1982), has shown that DMF, when used at or below the maximum recommended level of 0.5 mL/L (472 mg/L), has no apparent effect on the acute toxicity of tetrachloroethylene to rainbow trout.

Acknowledgments. This study was supported by a cooperative agreement between the University of Wisconsin-Superior and the U.S. Environmental Protection Agency (CR806864020). This paper has been approved for publication by the Director of the Center for Lake Superior Environmental Studies as publication no. 57 of the Center series.

Table 1. Median lethal (LC50) and median effect (EC50) concentrations (mg/L) and their 95% confidence limits for various test species exposed to methanol and N,N,-dimethylformamide (DMF)

Species	Average weight ± s.d. (g)	LC50 24-h	LC50 48-h	LC50 96-h	EC50 24-h	EC50 48-h	EC50 96-h
				Methanol			
Rainbow trout	0.813 ± 0.136	20,300 (19,800-20,700)	20,100 (19,500-20,700)	20,100 (19,500-20,700)	13,200 (*)	13,200 (*)	13,000 (12,800-13,400)
Fathead minnow	0.126 ± 0.036	29,700 (29,000-30,500)	29,700 (29,000-30,500)	29,400 (28,500-30,400)	29,700 (29,000-30,500)	29,700 (29,000-30,500)	28,900 (27,000-30,100)
Bluegill	3.07 ± 1.38	19,100 (17,400-21,000)	19,100 (17,300-21,100)	15,400 (13,500-17,600)	16,100 (14,500-18,000)	16,000 (14,400-17,900)	12,700 (11,800-13,700)
				N,N,-dimethylformamide (DMF)			
Rainbow trout	5.08 ± 1.97	10,600 (*)	9,800 (9,000-10,700)	9,800 (9,000-10,700)	10,600 (*)	9,800 (9,000-10,700)	9,800 (9,000-10,700)
Fathead minnow	0.047 ± 0.022	11,400 (10,800-12,000)	10,700 (10,400-11,000)	10,600 (10,400-10,800)	11,400 (10,800-12,000)	10,700 (10,400-11,000)	10,600 (10,400-10,800)
Bluegill	0.912 ± 0.350	7,500 (7,200-7,800)	7,500 (7,200-7,800)	7,100 (6,700-7,500)	7,500 (7,200-7,800)	7,500 (7,200-7,800)	7,100 (6,700-7,500)
Midge	-	-	-	-	46,800 (43,200-50,800)	36,200 (32,700-40,100)	-
Daphnid	-	-	-	-	26,300 (23,400-29,600)	14,500 (13,300-15,900)	-

* Due to no partial mortalities or effect, confidence limits could not be obtained.

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Received September 13, 1985; accepted December 13, 1985