Bull. Environm. Contam. Toxicol. 28, 7–10 (1982)

Acute Toxicity of Tetrachloroethylene and Tetrachloroethylene with Dimethylformamide to Rainbow Trout (*Salmo gairdneri*)

P. J. Shubat, Steven H. Poirier, Michael L. Knuth, and L. T. Brooke

Center for Lake Superior Environmental Studies, University of Wisconsin—Superior, Superior, WI 54880

Researchers using solvent carriers to facilitate the solubility of hydrophobic compounds have questioned the effects of the carriers on toxicity. ROESIJADI et al. (1976) reported that acetone used as an additive at a concentration of 158 mg/L had no apparent effect on the 96 h LC50 of Aroclor 1254 to the marine shrimp Paleomonetes pugio. STRATTON et al. (1980) and DALELA et al. (1979) demonstrated solvent carrier effects to Anabaena and the freshwater teleost, Labeo rohita, respectively. Both researchers advised that solvents used as carriers can interact with the toxicant being investigated and that solvent concentrations be kept as low as possible. If a solvent carrier is used, STRATTON et al. (1980) recommended determination of a test organism's sensitivity to the solvent and further, to the solvent toxicant combination (STRATTON & CORKE 1981).

Dimethylformamide (DMF) has been suggested by the AMERICAN SOCIETY FOR TESTING AND MATERIALS (1980) as a preferred solvent in the preparation of stock solutions for acute toxicity tests. The ASTM further suggests a maximum solvent concentration of 0.5 mL/L (472 mg/L DMF). The acute toxicity of DMF to rainbow trout had been previously determined as 9860 mg/L for a 96 h LC50 value (University of Wisconsin-Superior, unpublished data), and no effect was observed in fish exposed to concentrations less than 7700 mg/L.

In this study, two acute toxicity tests were conducted with tetrachloroethylene (TCE). DMF was used as an additive in one of the tests and was proportionally diluted with the toxicant.

MATERIALS AND METHODS

Rainbow trout (Salmo gairdneri Richardson) from Fattig Fish Hatchery, Brady, Nebraska, were held for 25 days before testing with TCE and for an additional 30 days before testing with TCE/DMF. Fish were held in 12°C Lake Superior water and were fed trout pellets from Glencoe Mills, Inc. until 24 hours before testing. Average fish weights at the time of testing were 3.20 g for the TCE test and 5.86 g for the TCE/DMF test. Tests were conducted with a proportional diluter (MOUNT & BRUNGS 1967) having a 0.65 dilution factor. Five toxicant concentrations and a lake water control were tested in duplicate. Exposure tanks con-

0007-4861/82/0028-0007 $01.00

taining 6.3 L of water received additions of 0.5 L every 10 min (TCE) or 12 min (TCE/DMF). Flourescent lights provided a 16 h photoperiod of 28 ft.-candles.

Ten fish were randomly assigned to each exposure tank and observed for loss of equilibrium and mortality. Criteria for death were immobility when gently prodded and no opercular movement. Observations were made at 1, 3, 6, 12, and 24 h, and at daily intervals thereafter until the test was terminated at 96 h. Lake Superior water was used in the tests and temperatures were maintained at 11.6 ± 0.2°C (TCE) and 12.2 ± 0.3°C (TCE/DMF). Measurements were made for dissolved oxygen, pH, total alkalinity, and hardness (EDTA) in accordance with Standard Methods (APHA 1975).

A solution of toxicant was generated with a system similar to the apparatus described by VEITH & COMSTOCK (1975). A 400 mL volume of toxicant solution was released from the reservoir into the diluter at each diluter cycle. During the TCE/DMF test a 3100 mg/L concentration of DMF was maintained in the toxicant reservoir by adding 1.3 mL DMF to the reservoir each cycle. Paired tanks were analyzed daily for TCE or TCE and DMF.

Exposure samples containing tetrachloroethylene (Aldrich Chemical Co., 99% pure) were extracted with hexane and analyzed on a Tracor 550 gas chromatograph equipped with a Ni^{63} electron capture detector. A 1.83 m x 4 mm i.d. glass column packed with 3% OV-101 on 80/100 mesh Chromosorb® W was used. Argon-methane (95:5) was the carrier gas and maintained at a 50 ml/min flow rate. The column, inlet, and detector were operated at 65°, 200° and 300°C, respectively. The recovery of TCE from spiked Lake Superior water was 89.9% ± 6.2% (n=23). Exposure samples containing dimethylformamide (BURDICK & JACKSON, spectrograde) were analyzed directly on a Beckman model DB-G UV spectrophotometer at a wavelength of 200 nm.

LC50 values were calculated by the trimmed Spearman-Karber method (HAMILTON et al. 1977).

RESULTS AND DISCUSSION

Concentrations of TCE in the two tests were similar (Table 1). TCE concentrations in the toxicant reservoir averaged 140 mg/L (TCE) and 120 mg/L (TCE/DMF). A DMF concentration of 3100 mg/L in the toxicant reservoir did not appear to affect TCE solubility.

Test conditions for the two tests were very similar, differing slightly in cycle length (the TCE test tanks received 10.6 volume additions per day, the TCE/DMF test tanks received 9.3) and in average temperature by 0.6°C. Mean values and standard deviations for water chemistry parameters of the TCE test were: dissolved oxygen, 81.6 ± 5.2% saturation; pH, 7.13 ± 0.02; total alkalinity, 46.9 ± 0.6 mg/L as $CaCO_3$; and hardness (EDTA), 44 ± 1 mg/L as $CaCO_3$. Values for the TCE/DMF test parameters were: dissolved oxygen, 80.7 ± 5.4% saturation; pH, 7.23 ± 0.01; total alkalinity, 46.5 ± 0.5 mg/L as $CaCO_3$; and hardness (EDTA), 46 ± 1 mg/L as $CaCO_3$.

TABLE 1

Concentrations (mg/L) of TCE and DMF for the two tests (mean ± standard deviation)

EXPOSURE TANK	TCE TEST	TCE WITH DMF TEST	
	TCE	TCE	DMF
control	<0.001 ± 0.000	<0.004 ± 0.004	0
A	2.41 ± 0.223	2.23 ± 0.459	75.8 ± 13.9
B	3.69 ± 0.203	3.53 ± 0.845	122 ± 12.2
C	6.39 ± 0.649	5.95 ± 1.41	220 ± 11.2
D	11.2 ± 0.197	11.3 ± 0.936	326 ± 16.3
E	17.3 ± 1.02	16.4 ± 1.47	513 ± 17.0

One fish died in a TCE/DMF control chamber after 24 h of exposure and one death occurred in a TCE control chamber after 72 h of exposure. No cause of death was determined.

Responses of the fish in the two tests were similar (Figure 1). In both tests, 81% of the deaths occurred within the first three hours of exposure. TCE tested alone yielded a 96 h LC50 of 4.99 mg/L with a 95% confidence interval of 4.73-5.27 mg/L. TCE tested with DMF yielded a 96 h LC50 of 5.84 mg/L with a 95% confidence interval of 5.05 - 6.76 mg/L.

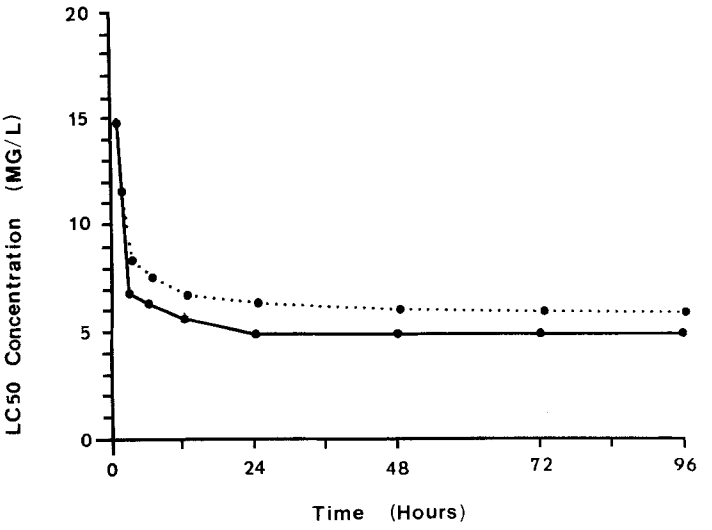

Figure 1. LC50 concentrations (mg/L) at various exposure intervals for exposures of TCE (———) and TCE with DMF (····) to rainbow trout.

Fish exposed to TCE with DMF were larger than those exposed to TCE alone, which may explain the slightly greater tolerance observed in the test. KUMARAGURU & BEAMISH (1981) also observed increased tolerance with an increase in body size in tests conducted with permethrin and rainbow trout.

When it is necessary to conduct tests with solvents as carriers of the toxicant it is desirable to keep all solvent concentrations constant. This is not always feasible when the test concentrations are proportionally diluted. Data from this study demonstrate that the solvent carrier dimethylformamide, when proportionally diluted and used at or below the maximum recommended level, has no apparent effect on the acute toxicity of tetrachloroethylene to rainbow trout.

ACKNOWLEDGEMENTS

This study was supported by a cooperative agreement between the University of Wisconsin-Superior and the U.S. Environmental Protection Agency (CR 806864020). This paper has been approved for publication by the Director of the Center for Lake Superior Environmental Studies as Publication No. 44 of the Center series.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: Standard Methods for the Examination of Water and Wastewater. 14th ed, Washington, D.C., APHA: 1975.

AMERICAN SOCIETY FOR TESTING AND MATERIALS: Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (1980).

DALELA, R.C., S.K. BANSAL, A.K. GUPTA, AND S.R. VERMA: Water, Air, and Soil Pollution 11, 201 (1979).

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON: Environ. Sci. Technol. 11, 714 (1977).

KUMARAGURU, A.K. AND F.W.H. BEAMISH: Water Res. 15, 503 (1981).

MOUNT, D.I. AND W.A. BRUNGS: Water Res. 1, 21 (1967).

ROESIJADI, G., S.R. PETROCELLI, J.W. ANDERSON, C.S. GIAM, AND G.E. NEFF: Bull. Environ. Contam. Toxicol. 15, 297 (1976).

STRATTON, G.W., R.E. BURRELL, M.L. KURP, AND C.T. CORKE: Bull. Environ. Contam. Toxicol. 24, 562 (1980).

STRATTON, G.W. AND C.T. CORKE: Bull. Environ. Contam. Toxicol. 27, 13 (1981).

UNIVERSITY OF WISCONSIN - SUPERIOR, unpublished data, 1978.

VEITH, G.D. AND V.M. COMSTOCK: J. Fish. Res. Board Can. 32, 1849 (1975).