Bull. Environm. Contam. Toxicol. 20, 344-352 (1978)

Toxicity of Perchloroethylene, Trichloroethylene, 1,1,1-Trichloroethane, and Methylene Chloride to Fathead Minnows

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Static and flow-through fish toxicity tests were conducted on four chlorinated solvents: perchloroethylene (DOWPER*), trichloroethylene (HI-TRI*), 1,1,1-trichloroethane, and methylene chloride to demonstrate the significant difference between the static and flow-through toxicity values with highly volatile compounds. Flow-through tests provide information on toxic effects during continuous exposure of the organism to a constant concentration of a chemical while the static test provides one initial exposure of a nominal concentration. Oxygen is replenished, waste products removed, and fish are fed daily in the flow-through procedure. The static acute fish toxicity test is applicable when the compound is soluble in water and will remain in solution for a specified period of time. Because of the high volatility of the chlorinated solvents studied, the flow-through method was considered the appropriate test system.

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METHODS

Fish

Adult fathead minnows, Pimephales promelas Rafinesque were used in the toxicity tests. They were purchased from the White Bear Bait Company, White Bear Lake, Minnesota, and air freighted to our laboratory. Minnows were held in raw dechlorinated Lake Huron water at ∿12°C for at least 10 days prior to testing. They averaged 1.04 gm/fish in weight and 49.0 mm in total length.

0007-4861/78/0020-0344 $01.80

Water

Lake Huron water was used in the toxicity tests. The water is dechlorinated by an activated carbon filtering system, chilled to 12°C, and passed through an ultraviolet sterilizer prior to testing. Chemical characteristics of the water, analyzed according to AMERICAN PUBLIC HEALTH ASSOCIATION (1975), were: pH 7.8-8.0; 150 mg/ℓ total dissolved solids; 10 mg/ℓ chloride (Cl); 27 mg/ℓ calcium (Ca^{++}); 7 mg/ℓ magnesium (Mg^{++}), 0.1 mg/ℓ total phosphate (as P); 0.4 mg/ℓ organic nitrogen (as N); and 0.05 mg/ℓ ammonium nitrogen (as N).

Acute Toxicity Tests

The static and flow-through toxicity studies were conducted according to test methods described by the COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS (1975). Methyl or ethyl alcohol was used as the carrier solvent for all compounds except methylene chloride. The water solubility of methylene chloride was ∿20,000 ppm (DILLING, 1976) and did not require the use of a carrier solvent. A control containing the same amount of alcohol that is present in the highest chemical concentration and a Lake Huron water control were included in each test series. Dead or affected fish were counted daily and the dead fish removed.

In the static water fish toxicity tests the nominal concentration, or that amount of chemical initially added to the test chambers, was used to calculate the LC 10, LC 50, and LC 90 toxicity values. When a chemical with relatively low water solubility is tested, the nominal concentration of some aquaria in a test series may exceed its water solubility; therefore, the amount of chemical in solution in static test aquaria may be less than the nominal concentration even when a solvent carrier like ethanol is used to add the chemical. The highest concentration of ethanol used in any test solution did not exceed 0.5 ml/ℓ. Since these compounds are highly volatile, all aquaria were covered with SARAN WRAP* plastic film for the first twenty-four hours to help contain the material. Dissolved oxygen (D.O.) was monitored daily. At no time was it below 5.0 mg/ℓ. The tests were terminated at 96 hours.

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The flow-through toxicity tests differ from the static in that fresh solution containing the chemical is intermittently supplied to the aquaria throughout the testing period. A proportional diluter system similar to that described by Mount and Brungs (1967) was used to deliver the chemical. The chemical concentration was verified in each test chamber before and during the 96-hour test.

A carrier solvent is necessary to increase the rate of dissolution of the chemical in the mixing chamber. Methanol was used as the carrier solvent where necessary and did not exceed 0.3 ml/ℓ in the highest test concentration. This exceeds the maximum amount of carrier solvent of 0.1 ml/ℓ suggested by THE COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS (1975). Two control aquaria were maintained: one contained 0.3 ml/ℓ of methanol and the second, only dechlorinated Lake Huron water. At termination, affected fish were placed in freshwater aquaria to observe recovery.

Analytical

In the flow-through tests, concentrations of the compounds in test aquaria were analyzed by gas chromatography using a flame ionization detector (ARMENTROUT, 1975). A solution of the test chemical in methanol was used as the standard for the compounds analyzed in this study. Standards were prepared daily just prior to analysis. Peak area was used for GC calculations of compound concentrations in the aquaria. Parameters for GC analysis are shown in Figures 1-4 for all compounds of interest.

Lethal Concentrations and Confidence Intervals

Results are reported in terms of the concentration producing death to 10%, 50%, and 90% of the fish (LC 10, LC 50, and LC 90) after exposure for a specified period of time. The 95% confidence limits are reported for each LC value. A computer program of Finney's probit analysis (FINNEY, 1952) was utilized to calculate the LC values, the confidence limits, and the slope of the regression curve.

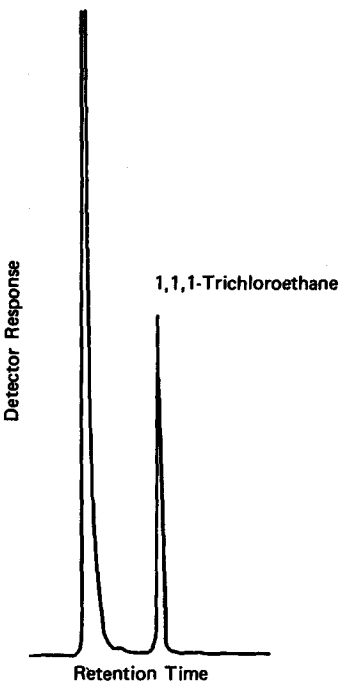

Figure 1: Chromatographic Parameters for 1,1,1-Trichloroethane:

¼" O.D. x 2 mm I.D. 6' 0.4% carbowax E1500 on carbopack A glass column on-column injection 110°C isothermal, 150°C injection port temp., 300°C detector temp. F.I.D., Flame range 1 x 4 atten. 20 mℓ/min. N_2 carrier gas flow 1 microliter sample size

Hewlett Packard 5710 GC.

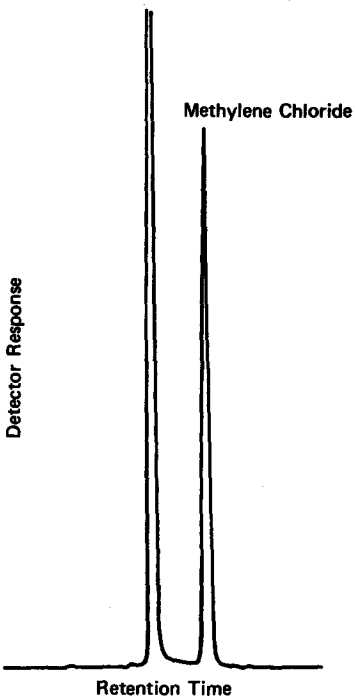

Figure 2: Chromatographic Parameters for Methylene Chloride:

¼" O.D. x 2 mm I.D. 6' 0.4% carbowax E1500 on carbopack A glass column on-column injection 60°C isothermal, 150°C injection port temp., 250°C detector temp. F.I.D., Flame range 1 x 16 atten. 20 mℓ/min. N_2 carrier gas flow 1 microliter sample size.

Hewlett Packard 5710 GC.

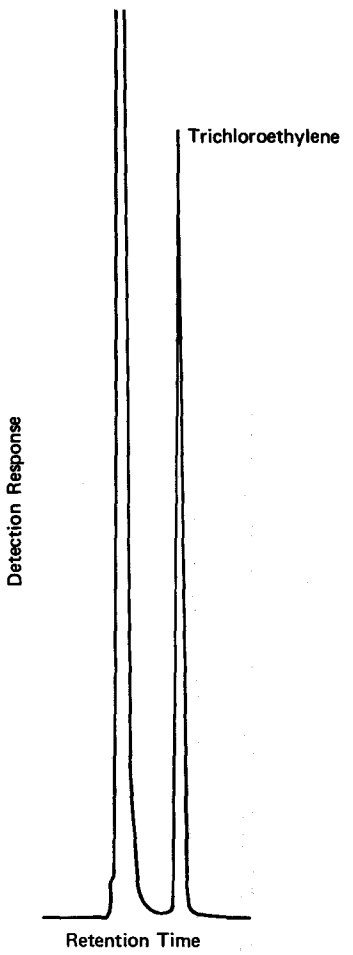

Figure 3: Chromatographic Parameters for Trichloroethylene:

¼" O.D. x 2 mm I.D. 6' 0.4% carbowax E1500 on carbopack A glass column on-column injection 150°C isothermal, 150°C injection port temp., 250°C detector temp. F.I.D., Flame range 1 x 8 atten. 20 mℓ/min. N_2 carrier gas flow 1 microliter sample size

Hewlett Packard 5710 GC

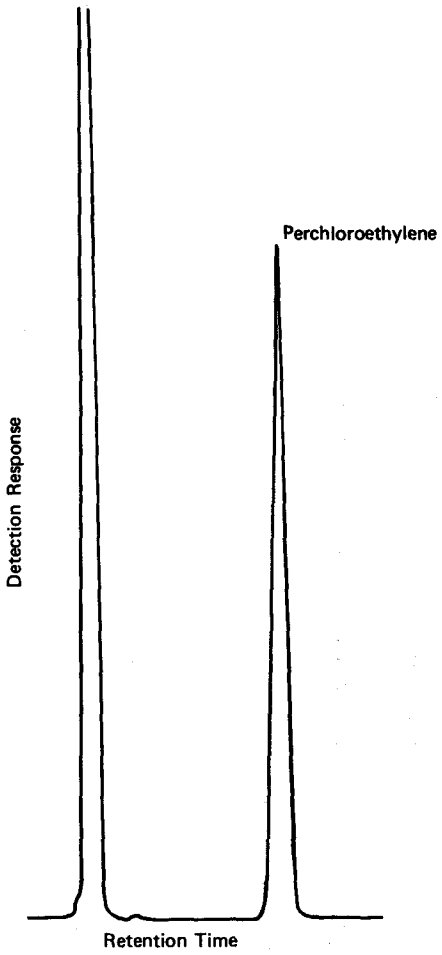

Figure 4: Chromatographic Parameters for Perchloroethylene:

¼" O.D. x 2 mm I.D. 6' 0.4% carbowax E1500 on carbopack A glass column on-column injection 150°C isothermal, 150°C injection port temp., 250°C detector temp. F.I.D., Flame range 1 x 2 atten. 20 mℓ/min. N_2 carrier gas flow 1 microliter sample size

Hewlett Packard 5710 GC

RESULTS AND DISCUSSION

The static and flow-through results for the compounds tested are summarized in Table I. As expected, the flow-through toxicity values of all compounds were less than the static toxicity values. In flow-through tests LC 50 values varied from 50% to 86% of the static test values.

TABLE I

Comparison of Acute Flow-Through and Static Fish Toxicity LC 50 Values

Compound	Hour	mg/ℓ Flow-Through[a]	mg/ℓ Static[b]
1,1,1-Trichloroethane	96-hr	52.8 (43.7-77.7)[c]	105 (91-126)
Methylene chloride	96-hr	193 (140.8-277.8)	310 (262-391)
Trichloroethylene	96-hr	40.7 (31.4-71.8)	66.8 (59.6-74.7)
Perchloroethylene	96-hr	18.4 (14.8-21.3)	21.4 (16.5-26.4)

[a]Calculated using the measured concentration in the water.
[b]Calculated using the nominal water concentration (amount added at start of the test).
[c]95% confidence limits.

Perchloroethylene was the most toxic of the chlorinated solvents tested. The 96-hour LC 50 value for this compound in the static acute toxicity test was 21.4 mg/ℓ, and with the flow-through acute toxicity test was 18.4 mg/ℓ. In order of decreasing toxicity, trichloroethylene 96-hour LC 50 value was 66.8 mg/ℓ for the static acute toxicity test, and 40.7 mg/ℓ for the flow-through acute toxicity test. 1,1,1-Trichloroethane in the static acute toxicity test 96-hour LC 50 value was 105.0 mg/ℓ and 52.8 mg/ℓ for the flow-through acute toxicity test.

TABLE II

Chlorinated Solvents Flow-Through Fish Toxicity Studies

Compound	Hrs.	mg/ℓ Effective Concentration (EC) Value[a] EC 10	EC 50	EC 90	Lethal Concentration (LC) Value LC 10	LC 50	LC 90	Slope
1,1,1-Trichloroethane	24	10.5 (8.0-11.5)[b]	12.1 (10.9-13.5)	14.1 (12.9-18.3)				
	48	10.0 (7.8-10.9)	11.5 (10.4-12.8)	13.2 (12.1-17.3)				
	72	9.0 (6.7-10.0)	11.1 (10.0-12.6)	13.8 (12.3-13.8)	34.1 (20.8-41.2)	55.4 (46.2-82.7)	89.9 (67.0-254.7)	6.09 (2.47-9.70)
	96	9.0 (6.7-10.0)	11.1 (10.0-12.6)	13.8 (---)	30.8 (18.8-37.6)	52.8 (43.7-77.7)	90.8 (66.4-245.9)	5.45 (2.41-8.48)
Methylene chloride	24	68.5 (44.2-86.7)	112.8 (99.8-150.8)	220.1 (175.1-335.4)	122.0 (72.7-160.8)	268.0 (213.0-346.6)	589.0 (432.6-1077.4)	3.74 (2.32-5.17)
	48	66.3 (42.6-79.7)	99.0 (83.2-121.5)	147.6 (120.5-249.7)	94.0 (50.7-130.4)	265.0 (202.5-369.7)	746.3 (494.7-1712.1)	2.84 (1.76-3.93)
	72	66.3 (42.6-79.7)	99.0 (83.2-121.5)	147.6 (120.5-249.7)	67.3 (32.3-98.9)	232.4 (172.4-337.6)	802.0 (497.4-2132.6)	2.38 (1.47-3.29)
	96	66.3 (42.6-79.7)	99.0 (83.2-121.5)	147.6 (120.5-249.7)	51.2 (22.5-78.2)	193.0 (140.8-277.8)	722.1 (447.4-1947.1)	2.22 (1.38-3.07)

Trichloroethylene	24	15.2 (10.0-18.3)	23.0 (19.8-27.4)	36.2 (30.3-51.2)	34.7 (24.4-41.4)	52.4 (44.3-65.7)	79.1 (63.7-131.6)	7.16 (3.79-10.54)
	48	16.9 (11.6-19.6)	22.7 (19.7-27.3)	30.6 (26.0-49.2)	27.7 (17.3-35.0)	53.3 (43.1-75.5)	102.6 (73.3-238.0)	4.51 (2.39-6.63)
	72	15.5 (10.0-18.2)	22.2 (18.9-27.3)	31.8 (26.2-56.0)	20.9 (11.9-26.1)	39.0 (31.8-57.5)	72.6 (51.7-109.2)	4.73 (2.15-7.31)
	96	13.7 (8.5-16.6)	21.9 (18.4-28.5)	34.9 (27.3-70.9)	17.4 (9.0-22.9)	40.7 (31.4-71.8)	95.0 (59.0-419.9)	3.47 (1.60-5.35)
Perchloroethylene			Plotted TE_m[c]					
	24		14.4		15.1 (9.1-18.5)	23.5 (19.5-28.2)	36.6 (30.0-59.1)	6.65 (3.35-9.94)
	48		14.4		13.9 (7.8-16.7)	19.6 (15.9-22.8)	27.6 (23.6-42.7)	8.56 (3.67-13.44)
	72		14.4		13.2 (7.5-16.0)	18.9 (15.3-22.1)	27.1 (23.1-41.2)	8.18 (3.69-12.67)
	96		14.4		13.2 (7.5-16.0)	18.4 (14.8-21.3)	25.6 (22.0-38.0)	8.90 (3.88-13.91)

[a]The effective concentration. Concentration producing an adverse effect. In this case the effect noted was loss of equilibrium.
[b]95% confidence limit.
[c]TE_m - median tolerance effect is obtained by a logarithmic plot of the data. Insufficient data was available to obtain a computer plot by Finney's probit analysis.

The least toxic of the chlorinated solvents studied was methylene chloride with a 96-hour LC 50 value of 310.0 mg/ℓ for the static acute toxicity test and 193.0 mg/ℓ using the flow-through technique.

The fish were observed for the following effects: loss of equilibrium, melanization, narcosis and swollen, hemorrhaging gills. Concentrations producing one or more of these observable effects in 50% of the fish (EC 50) ranged from 11.1 mg/ℓ for 1,1,1-trichloroethane to 99.0 mg/ℓ for methylene chloride. Fish affected during the exposure were transferred to static freshwater aquaria at the end of the 96-hour exposure period. Only those fish which were severely affected by high concentrations of the chemical did not recover. However, short exposures to these compounds at the sublethal level seem to produce only reversible effects. Table II shows a comparison of the loss of equilibrium EC values and LC values found in this study.

ACKNOWLEDGEMENTS

The authors wish to thank D. N. Armentrout, G. U. Boggs, T. D. Lickly, and L. S. Chatterton for their analytical support duirng this study.

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