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Acute Toxicity of Trichloroethylene to Saltwater Organisms

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Trichloroethylene (TCE) is a chlorinated aliphatic hydrocarbon primarily utilized for vapor-phase degreasing in the fabricated metals industry. Other applications include cold-metal cleaning and use in the manufacture of organic chemicals (Cogswell et al. 1982, Chemical Products Synopsis 1984). TCE enters the environment as a result of volatilization during its production and through its industrial uses [U.S. Environmental Protection Agency (EPA) 1980]. TCE has been detected in aquatic environments and organisms at part-per-trillion (pptr) concentrations (Pearson and McConnell 1975). Although TCE is indicated to be widely distributed, relatively limited data exist on the acute effects of TCE on aquatic organisms, especially saltwater species. Results of static acute tests of TCE with a saltwater alga, invertebrate, and fish are reported here to enhance the data base.

MATERIALS AND METHODS

The alga tested was the chain-forming diatom, Skeletonema costatum. The culture was obtained from the EPA Environmental Research Laboratory, Gulf Breeze, Fla., and maintained in stock culture at Bionomics Marine Research Laboratory (BMRL), Pensacola, Fla, according to procedures in U.S. EPA (1978).

Mysid shrimp, Mysidopsis bahia, were born in culture at BMRL and maintained for 3 days before testing. Mysids were reared in natural sea water generally following procedures in U.S. EPA (1978). During holding, temperature was maintained at 22±1°C and salinity at 19 ppt.

Sheepshead minnows, Cyprinodon variegatus, were hatched and reared for 4 to 6 days at BMRL. Sheepshead minnow eggs were spawned naturally in the laboratory in natural sea water basically following procedures in U.S. EPA (1978). During the 48-h period before test initiation, salinity was 19 ppt, and temperature was 22°C. Mortality was <1% during the same period. The fish were 5 to 6 mm total length and averaged 1.4 mg wet weight.

Test water for the algal test was synthetic seawater (Rila Marine Mix) adjusted to a salinity of 30 ppt and enriched with nutrients (U.S. EPA, 1978). Test water for shrimp and fish tests was natural seawater (20 ppt) filtered to 5 µm.

The alga, S. costatum, was tested in 125-mL flasks containing 50 mL of test solution or control water. Each flask was inoculated with approximately 2.0 x 10^4 cells/mL. The cultures were incubated at 20±1°C under 4,300 lux illumination. Test concentrations and controls were triplicated. Measurements of in vivo chlorophyll a were made using a Turner Model III fluorometer after 24, 48, 72, and 96 h of exposure. Cells counts were made after 96 h of exposure using a hemacytometer and Zeiss Standard 14-compound microscope.

Mysids and fish were tested in 1.6-L covered-glass dishes containing 1.0 L of test solution or control seawater. Ten shrimp or fish were tested per dish, and all treatments were duplicated. Shrimp were fed live (48-h old) brine shrimp nauplii on Days 0 and 2 during the test; fish were not fed.

All test organisms were definitively tested in water-soluble fraction (WSF) concentrations of 6.25, 12.5, 25, 50, and 100%. The 100% WSF solution was prepared by adding 1 part TCE to 1,000 parts dilution water (volume to volume) and stirring in a covered Erlenmeyer flask for 1 h. After allowing the solution to settle for 0.5 to 1 h, the WSF was siphoned into another container for distribution to the test containers.

Actual test concentrations of TCE were analytically determined at initiation and termination of the test, or when 100% mortality occurred in a treatment. On Day 0 of the algal test, 300 mL of each test concentration was prepared, and 100 mL was sampled in amber glass bottles with Teflon®-coating-lined screw caps. On Day 4 of the algal test, the triplicate test solutions were composited and 100 mL sampled. Composite 100-mL samples were removed from each test solution at initiation and termination of the shrimp and fish tests. The samples were analyzed on the day they were collected.

Based on the results of the tests, 24-, 48-, 72-, and 96-h LC50s or EC50s and 95% confidence limits were calculated, where possible. The computer program generated the LC- or EC50 values using the following statistical methods: moving average angle, probit, and binomial probability (Stephan 1977).

Water samples were transferred into a 250-mL separatory funnel/resin column apparatus prepared as follows. A small plug of glass wool was inserted into a 5-mL pipet and packed lightly into the tip. The pipet was then filled to the top graduation mark with polymeric resin (Mallinckrodt Amberlite® XAD-7), leaving about 5 cm of the pipet empty. Using silicone tubing, the pipet top was attached to the tip of a separatory funnel.

The resin column then was rinsed to remove possible contaminants by adding 100 mL of acetone to the separatory funnel and adjusting the stopcock to control the flow of acetone through the resin at approximately 5 mL/minute. Following the acetone cleanup, the column was prepared for use by displacing any acetone remaining on the resin with a 10-mL distilled water rinse. The flow of water through the resin was stopped by closing the separatory funnel stopcock when the water level was approximately 5 mm from the top of the resin.

After addition of the water sample to the apparatus, the stopcock was opened and adjusted to provide a flow-rate through the column of approximatly 2 mL/min. The sample was allowed to run completely through the column, leaving the resin dry.

A 10-mL aliquot of nanograde acetone then was added to the separatory funnel and allowed to elute the sample from the resin at about 2 mL/min. A graduated 25-mL concentrator tube was placed under the tip of the resin trap to collect the acetone rinse. The resin trap was allowed to run dry. The 10-mL sample in the concentrator tube contained the trichloroethylene in solution and was diluted as necessary with nanograde n-hexane and analyzed using gas chromatography.

Extracts were analyzed on a Hewlett Packard Model 5880A gas chromatograph equipped with a Nickel-63 electron capture detector and 1.8-m-x-2-mm glass column using the following:

Temperature (°C):	Injection port--100 Oven--25 Detector--100
Column packing:	SP-2250 (1.5%) plus SP-2401 (1.95%) on 100/120-mesh Supelcoport
Gas and flow rate:	Argon/methane (95%/5%) at 15 mL/min

The mean percentage recovery of three concentrations of TCE added to seawater was 81% with standard deviation of ±12%.

RESULTS AND DISCUSSION

The concentration of TCE that dissolved in seawater at 20 ppt or 30 ppt after 1 h of stirring ranged from 232 to 595 ppm. This was within the range of concentrations which could be expected based upon the reported water solubility of 1,000 ppm TCE at 20°C. As expected, based on a vapor pressure of 77 mm Hg, TCE concentrations decreased rapidly from seawater solution through volatilization during the tests. Concentrations of TCE decreased by more than 75% during the first 24 h of exposure, as indicated by four samples, and were less than 3% of initial concentrations after 96 h (Table 1).

Because TCE concentrations rapidly decreased with time and because greater than 90% of all shrimp and fish mortalities occurred during the first 24 h, LC50s were calcuated using both average measured concentrations from test initiation and test-

Table 1. Results of chemical analyses of TCE in seawater during 96-h static toxicity tests

Percent water-soluble fraction	Measured concentration (mg/L; ppm)								
	Test Initiation			Test Termination			Average		
	A	B	C	A	B	C	A	B	C
Control	ND	0.06	ND	ND	ND	ND	--	--	--
6.25	17	11	28	ND	0.31	ND	8.5	5.6	14
12.5	24	24	67	ND	0.18	0.01	12	12	34
25	70	51	155	ND	0.01	0.07	35	26	78
50	129	132	237	ND	5.6*	59*	64	69	148
100	300	232	595	ND	28*	119*	150	130	357

A = Results of Skeletonema costatum test.
B = Results of Mysidopsis bahia test.
C = Results of Cyprinodon variegatus test.
ND = Not detected.

*Measurements after 24 hours when 100% mortality observed.

termination samples, and also using initial measured concentrations. The 96-h LC50 values, calculated using initial concentrations, ranged from 27 ppm for mysids to 150 ppm for the diatom (Table 2). The 96-h LC50 values, calculated using averages of initial and final TCE concentrations, were 52 to 63% of the initial concentration values.

Only three other LC50 or EC50 values have been reported for TCE to saltwater organisms prior to this work. Pearson and McConnell (1975) reported LC50 values of 16 ppm and 20 ppm, respectively, for the fish, Limanda limanda, and barnacle nauplii, Elminius modestus, and an EC50 of 8 ppm for the unicellular alga, Phaeodactylum tricornutum. Borthwick (1977), although unable to calculate LC50 values, reported mild intoxication in grass shrimp at 2 ppm and in sheepshead minnows at 20 ppm. These effects subsided after a few hours, but recurred with daily renewals of test solutions. Similar observations were noted in the mysid and sheepshead minnow tests reported here, but at higher concentrations. Sheepshead minnows were observed spinning in the average TCE concentration of 357 ppm, and mysids swam erratically in average test concentrations greater than or equal to 26 ppm. These early effects were followed by death (within 24 h).

Table 2. Calculated 96-h LC50 and EC50 values for saltwater organisms exposed to TCE in static, unaerated seawater*

Organism	EC50 or LC50 (and 95% confidence limits) (mg/L; ppm) Initial measurements	Average measurements
Diatom	150 (139-162)	95 (79-143)
Mysid	27 (19-36)	14 (12-26)
Fish	99 (83-118)	52 (43-64)

*Calculations using initial TCE concentration measurements and average of initial and final measurements.

The LC50 and EC50 values reported here for sheepshead minnow and diatom lie within the range of values reported for TCE in freshwater (i.e., 21.9 to 100 ppm) by Canton and Adema (1978), Alexander et al. (1978), and U.S. EPA (1978), but were much greater than all other values reported for TCE in saltwater (Table 3). Differences in saltwater test results could be attributed to differences in species sensitivity, differences in the type of test system employed, use or non-use of solvent, and/or differences in test temperature. Use of a flow-through system by Pearson and McConnell (1978) in their fish test and stoppered containers for their static tests may be responsible for the greater toxicities observed relative to those measured in the sheepshead minnow and diatom static tests presented herein. However, despite the more vigorous test conditions employed in the invertebrate test by Pearson and McConnell (1978), they reported a higher LC50 value for E. modestus (20 mg/L) than observed herein for the mysid M. bahia (14 mg/L). This certainly confirms that mysids are one of the more sensitive organisms tested to date with TCE.

Differences between TCE test concentrations at which mild intoxication symptoms were observed in test organisms in this study and by Borthwick (1977) may possibly be explained on the basis of carrier solvent synergism and/or temperature effects. Although the grass shrimp P. pugio is generally considered to be a less sensitive organism than the mysid M. bahia, Table 3 shows that Borthwick (1977) reported symptomatic behavior of intoxication in P. pugio at a concentration more than 10 times lower (i.e., 2 mg/L) than observed in this study with M. bahia (>26 mg/L). Variation in toxicity between studies can be lowered by testing with the same species. This is the case with the intoxication symptoms for C. variegatus observed in this study and also those reported by Borthwick (1977). This study reported symptoms at an average TCE concentration of 357 mg/L; the latter

Table 3. Comparison of saltwater toxicity test results reported for TCE (mg/L)

	This Study (Average Concentration)	Other Studies
1. Acute LC_{50}		
Fish	52 (*C. variegatus*)	16 (*L. limanda*)[a]
Invertebrate	14 (*M. bahia*)	20 (*E. modestus*)
2. Diatom Algae EC_{50}	95 (*S. costatum*)	8 (*P. tricornutum*)
3. Intoxication Effects (i.e., "spinning," erratic behavior)		
Fish	357 (*C. variegatus*)	20 (*C. variegatus*)[b]
Invertebrate	$\geq$26 (*M. bahia*)	2 (*P. pugio*)

[a]Pearson & McConnell (1975)
[b]Borthwick (1977)

reported them at 20 mg/L. Some of this difference is almost certainly due to the higher temperature (30°C) employed in the Borthwick (1977) study compared to this study (22°C). In addition, the use of a solvent (triethylene glycol) to make the TCE more miscible with the test medium may have potentiated the effects of TCE in some tests.

Enhanced toxicity of TCE and its metabolic products has been reported in man and experimental animals with ethanol (U.S. EPA, 1980). Even the specific solvent utilized may alter the toxicity. Canton and Adema (1978) reported LC50s for *D. magna* generated among three laboratories of 41 to 100 ppm. No mention was made of the use of solvents. However, when solvents were utilized in tests with *D. magna*, LC50 values of 85.2 ppm and 18 ppm were determined using acetone and triethylene glycol, respectively (U.S. EPA 1978, Leblanc 1980). These data on *D. magna*, therefore, suggest a possible increase in TCE toxicity when triethylene glycol is added to enhance its solubility in the test solution.

Since TCE volatilizes from the water so rapidly, it is important to assess the possible consequences of solvents and temperature on the toxicity of TCE. This seems especially true in light of the apparent inversion in relative sensitivity between grass shrimp and mysid tested with TCE. That is, the typically more tolerant grass shrimp tested with solvent and at a higher relative temperature was more sensitive to TCE than the generally acknowledged more sensitive mysid tested at a lower temperature without the aid of a solvent.

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