ACUTE EXPOSURE OF HEXACHLOROBENZENE TO SELECTED FRESHWATER ORGANISMS

by

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INTRODUCTION

Hexachlorobenzene is a commercially produced chemical of high annual tonnage production used as a feedstock for many chemical manufacturing processes. It is a chemical listed by the U.S. Environmental Protection Agency (EPA) in its "list of sixty-five" as a priority pollutant. Because of this, a numerical water quality criterion is needed as a guideline for the safeguard of the environment. The tests reported in this study are needed as a portion of the minimum data set required for derivation of numerical criteria. The tests are static, acute exposures with the following freshwater organisms: an amphipod (*Gammarus pseudolimnaeus*), snail (*Aplexa hypnorum*), leech (*Nephelopsis obscura*), hydra (*Hydra* sp.), and a stonefly (*Pteronarcys* sp.).

METHODS

Analysis of Hexachlorobenzene (HCB)

Water samples were collected for extraction by pipetting 50.0 mL from middepth near the center of each exposure chamber into a 125 mL glass bottle with a teflon-lined screw cap. The sample was extracted by adding 15.0 mL redistilled hexane (MCB Omnisolv, lot 5084) and stirring 45 min. After extraction, an aliquot of the hexane layer was taken for gas chromatographic (G.C.) analysis. In tests lasting 96 h, all exposure chambers were sampled at 0, 48 and 96 h while in 48 h tests all chambers were sampled at 0 and 48 h. Duplicate samples and spikes were prepared and analyzed at every sampling. The mean agreement of duplicate analyses was 96.0 ± 5.9 % (n=11); the mean spike recovery was 95.0 ± 9.0 % (n=8).

Analytical stock solutions of HCB were prepared by dissolving HCB (Aldrich Chemical Co., lot 010487, 97% purity) in acetone. The working standards were made in hexane, while the toxicant stock was prepared in N,N-dimethylformamide (Fisher Scientific Certified ACS Spectroanalyzed, lot 854701).

A Hewlett Packard model 5880A gas chromatograph with an electron capture detector was used for hexachlorobenzene analysis. Ultra-high purity hydrogen was used as the carrier gas and 95% argon-5% methane used as makeup gas. Duplicate injections of 1 µL were made into a 30 m SE-54 capillary column (J & W Scientific) using the following conditions: injector temperature = 250 C, detector temperature = 315 C; oven temperature profile: initial temperature = 55 C, initial hold = 1.00 min., program rate = 10 C/min., final temperature = 280 C, final time = 1.00 min. The retention time was 13.06 ± 0.02 min.

Analysis of N,N-Dimethylformamide (DMF)

Water samples were collected for analysis by collecting ≈10 mL of sample into a 13 mL screw-top test tube with teflon-lined cap. No sample preparation or preservation was used. DMF samples were collected and analyzed each day that HCB samples were collected. The mean agreement of duplicate samples was 98.4 ± 3.7 % (n=11); the mean spike recovery was 100.4 ± 6.0 % (n=11).

The analytical stock solution was prepared by dissolving DMF (Fisher Scientific Certified ACS spectroanalyzed, lot 854701) in deionized water. Working standards were prepared by diluting aliquots of the stock solution in deionized water. The analytical standards and stock solution were refrigerated at 4 C when not in use. Stock solutions and standards were prepared weekly to avoid bacterial contamination.

Samples were analyzed within 1 h of collection using a Perkin-Elmer/Hitachi 200 spectrophotometer at a wavelength of 225 nm. A laboratory water sample was used as a correction blank for all DMF samples because the water exhibited some absorbance at the previously mentioned wavelength.

Exposure Conditions

The water supply for organism acclimation and testing was the municipal water supply for the City of Superior, WI. The water was dechlorinated by charcoal filtration and sodium sulfite addition. Water temperature was controlled using a constant temperature water bath except for the *Hydra* test which was run at a controlled room temperature. All acute toxicity tests were conducted with duplicate controls and exposure treatments except for the *Hydra* test which had triplicate chambers. The tests consisted of three toxicant treatments, plus a control and solvent control except for the stonefly test which had only had two toxicant treatments. Nominal concentrations used were 5 $\mu g \cdot L^{-1}$ (approximate saturation level), 50 $\mu g \cdot L^{-1}$ (10 times approximate saturation) and 100 $\mu g \cdot L^{-1}$ (20 times approximate saturation) except for the stonefly test which were 5 $\mu g \cdot L^{-1}$ and 100 $\mu g \cdot L^{-1}$. A control and (DMF) solvent control were run with each test. In the solvent control chamber the DMF concentrations were nominally equal to the amount in the highest (100 $\mu g \cdot L^{-1}$) exposures, except for the leech test where they were nominally equal to the middle exposure concentration.

Test chambers were of varying sizes. The test with the leech was conducted in 30 x 30 x 30 cm chambers containing 14 L of test solutions. Amphipod and snail tests were conducted together in 19 x 16 x 21 cm chambers containing 4 L of test solution with two leaf discs (*Betula* sp. and *Populus* sp) 20 mm in diameter. The stonefly test was also conducted in the 19 x 16 x 21 cm chambers containing 4 L of test solution with two twigs ($\approx$8 cm long x 1 cm diameter) placed on the bottom for cover. *Hydra* tests were conducted in 100 mL beakers using 80 mL of test solution. The number of organisms per test chamber consisted of 5 for all tests except for the amphipod test which had 10 per chamber.

Test chambers were conditioned for at least 48 h before the test began with the concentrations to be tested. (This was not done for the *Hydra* test). Test solutions were gently aerated for all tests, with the exception of the *Hydra* test. Dissolved oxygen was measured in control, solvent control, low and high exposures at the initiation of each test and every 48 h thereafter (Table 1). Total hardness (EDTA), total alkalinity and pH were measured at least once during each test on control, solvent control, low and high exposures (Table 1) in accordance with Standard Methods (APHA 1980). Water temperatures were recorded daily with mean temperatures calculated (Table 1). Dissolved oxygen (mean percent saturation) ranged from 75.9 to 107.7 % for all the tests; mean total (EDTA) hardness ranged from 47.4 to 49.7 $mg \cdot L^{-1}$ as $CaCO_3$; mean total alkalinity ranged from 39.6 to 42 $mg \cdot L^{-1}$ as $CaCO_3$; and arithmetic means of pH ranged from 6.96 to 7.58.

Loading requirements were met as stated by ASTM (1980) for all tests except for the leech which was 1.06 g/L. Tests were begun by placing the test organisms in the exposure chambers within 30 min after the toxicant was added except for the *Hydra* test in which the test organisms were allowed to acclimate to test chambers for 24 h before adding toxicant.

The criteria for death were lack of movement, absence of respiratory movement and lack of reaction to gentle prodding. Because death was not easily determined for *Hydra*, only an EC_{50} was determined. The *Hydra* EC_{50} was based on whether the foot was attached or not attached to the bottom or walls of the test chambers. This was determined by application of a gentle suction with an eyedropper to each animal. Any animals that were dislodged by the suction or which were already free-floating were considered affected. Exposures were

TABLE 1. Test Water Characteristics for Static Acute Toxicity Tests with Hexachlorobenzene. Values are Means ± Standard Deviation, Ranges and Sample Sizes

Species	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Conductivity (mhos/cm)	Temperature °C
Leech (*Nephelopsis obscura*)	75.9±3.2 (70.5-79.8) n=12	47.5±2.9 (43.4-50.0) n=4	39.6±2.3 (37.6-42.8) n=4	6.96±0.21 (6.73-7.21) n=8	100±3 (97-103) n=4	16.6±0.3 (16.0-16.8) n=50
Amphipod (*Gammarus pseudolimnaeus*)	78.2±7.5 (68.0-88.0) n=12	49.5±0.6 (48.6-50.1) n=4	41.5±1.3 (39.6-42.4) n=4	7.20±0.40 (7.14-7.24) n=8	97±0 (97-97) n=4	16.6±0.2 (16.2-16.8) n=50
Snail (*Aplexa hypnorum*)	78.2±7.5 (68.0-88.0) n=12	49.5±0.6 (48.6-50.1) n=4	41.5±1.3 (39.6-42.4) n=4	7.20±0.40 (7.14-7.24) n=8	97±0 (97-97) n=4	16.6±0.2 (16.2-16.8) n=50
Stonefly (*Pteronarcys* sp.)	107.7±1.8 (104.6-110.7) n=12	47.4±0.4 (46.9-47.7) n=3	40.8±0.8 (40.0-41.6) n=3	7.58±0.09 (7.49-7.68) n=4	86±0 (86-86) n=4	13.1±3.2 (10.0-18.9) n=40
Hydra (*Hydra* sp.)	101.2±6.3 (93.5-108.7) n=8	49.7	42	7.13±0.69 (6.49-7.83) n=8	-	24.6±0.4 (22.9-25) n=12

checked every 24 h for death and/or effect.

Test organisms were acquired from a variety of sources. Amphipods were collected from the Eau Claire River, Douglas County, WI, and leeches from a private pond near Superior, WI. Snails were received from the U.S. EPA Environmental Research Laboratory, Duluth, MN, and _Hydra_ from the Lemberger Co., Oshkosh, WI. Stoneflies were collected from the Blackhoof River, Carlton County, MN. Recommended procedures for care, handling, and acclimation of test organisms were followed (ASTM 1980).

The organisms were acclimated to the test water and exposure temperature for a minimum of 96 h prior to test initiation.

RESULTS

Mortalities and effects did not exceed 10% of the organisms in any of the control or hexachlorobenzene exposures. Therefore no LC_{50} or EC_{50} values could be determined.

Adult leeches (mean length, 81 ± 5 mm; mean weight, 2.97 ± 0.50 g) were exposed to three concentrations (1.2 ± 0.6, 35.3 ± 10.3 and 73.2 ± 12.8 $\mu g \cdot L^{-1}$) of hexachlorobenzene, plus a control and solvent control (Table 2), in duplicate. No leeches were affected or dead after 96 h in any of the exposure or control chambers.

Adult amphipods (mean length, 11 ± 1 mm; mean weight, 0.031 ± 0.008 g) were exposed to three concentrations (2.0 ± 0.7, 41.5 ± 5.4 and 77.6 ± 8.4 $\mu g \cdot L^{-1}$) of hexachlorobenzene, plus a control and solvent control (Table 2), in duplicate. No more than 5% of the amphipods were affected or dead after 96 h in any of the exposure or control chambers.

Adult snails (mean weight, 0.038 ± 0.011 g) were exposed to three concen-

TABLE 2. N,N-Dimethylformamide Concentrations ($mg \cdot L^{-1}$) in the Solvent Control and Low, Medium, and High Hexachlorobenzene Exposure Concentrations for the Various Test Organisms

	Solvent Control	Exposure Concentrations Low	Medium	High
Leech (*Nephelopsis obscura*)	109	9.6	93	171
Amphipod and snail (*Gammarus pseudolimnaeus* and *Aplexa hypnorum*)	179	8.4	94	180
Stonefly (*Pteronarcys* sp.)	182	13.0	174	-
Hydra (*Hydra* sp.)	171	55.0	154	168

trations (2.0 ± 0.7, 41.5 ± 5.4 and 77.6 $\mu g \cdot L^{-1}$) of hexachlorobenzene, plus a control and solvent control (Table 2), in duplicate. No more than 10% of the snails were affected or dead after 96 h in any of the exposures. No controls were dead.

Stonefly nymphs (mean length, 18 ± 2 mm; mean weight, 0.114 ± 0.038 g) were exposed to two concentrations (3.3 ± 0.7 and 69.7 ± 4.3 $\mu g \cdot L^{-1}$) of hexachlorobenzene, plus a control and solvent control (Table 2), in duplicate. No more than 10% of the nymphs were affected or dead after 96 h in any of the exposures. No controls died.

Adult Hydra were exposed to three concentrations (21.6, 35.9, and 65.9 $\mu g \cdot L^{-1}$) of hexachlorobenzene, plus a control and solvent control (Table 2), in triplicate. No Hydra were affected after 48 h in any of the exposure chambers or the solvent control chambers. The controls had 5% affected.

REFERENCES

American Society for Testing and Materials (ASTM). 1980. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E729-80. 1916 Race St., Philadelphia, PA 19103.

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