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Chronic Toxicity of Biphenyl to *Daphnia magna* Straus

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The U.S. Environmental Protection Agency (EPA) issued a final test rule (1985) for biphenyl on the authority of Section 4(a) of the Toxic Substances Control Act (TSCA). Contained within this rule was the requirement for generating chronic daphnid toxicity data for biphenyl. Biphenyl is used primarily to produce dye carriers, heat-transfer fluids and alkylated biphenyls (Kirk-Othmer et al. 1979). As discussed in the rule, the use/disposal pattern for biphenyl suggests the potential for biphenyl to be released into the aquatic environment at significant concentrations through wastewater discharge (dye-carrier applications) or from leakage of heat-transfer fluids. The EPA based its testing requirements in part on the potential for biphenyl to produce chronic effects on aquatic vertebrates and invertebrates and because of detected concentrations of biphenyl in the environment.

The acute toxicity of biphenyl to Daphnia magna has been reported by LeBlanc (1980) and Dill et al. (1982). The 48-hr LC50 values reported by these researchers were 4.7 and 2.1 mg/L, respectively. To date, the chronic toxicity of biphenyl to fish and aquatic invertebrates has not been investigated. The objective of this study was to determine the chronic toxicity of biphenyl to D. magna. The daphnid chronic toxicity test is designed to estimate the maximum acceptable toxicant concentration (MATC). The MATC is defined as the concentration falling between the highest concentration showing no effect and the next higher concentration showing a toxic effect when compared to the controls. (McKim 1977).

MATERIALS AND METHODS

The test material used in this study was biphenyl (purity 99.5%). The physical/chemical properties of biphenyl have been previously reported by Verschueren (1983). A synopsis of these properties follows:

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Sample Name: biphenyl (diphenyl, phenylbenzene)
CAS No.: 92-52-4
Boiling Point: 254°C
Melting Point: 70°C
Specific Gravity: 1.18 at 0 to 4°C
Log P_{oct}: 3.16 to 4.09
Water Solubility: 7.5 mg/L at 25°C
Molecular Weight: 154.2
Molecular Formula: $C_{12}H_{10}$
Structural Formula:

The water supply for our laboratory is pumped from the upper Saginaw Bay of Lake Huron off Whitestone Point. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. The water, as it enters our facility is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the chronic test: pH 7.4 to 7.7, hardness (mg/L as $CaCO_3$) 73 to 78, alkalinity (mg/L as $CaCO_3$) 49 to 52 and conductivity (µmhos/cm) 160 to 170.

Testing was conducted with an intermittent-flow proportional diluter, similar to that described by Mount and Brungs (1967). The diluter was equipped with an automatic pipette, which at the beginning of each cycle was triggered to inject the appropriate amount of test material into the toxicant mixing chamber. The toxicant mixing chamber was equipped with a recirculating pump which provided mixing for at least 3 min before the solution was delivered to the toxicant cells. The diluter had a dilution factor of ~0.50. At each cycle 500 mL of test solution or control water was delivered to each flow-splitting dilution chamber. These chambers, which were randomly positioned on the diluter, diverted ~125 mL to each of four replicate test chambers at each test concentration and the control during the acute test. During the chronic study, the 125 mL delivered from the splitter cells to each replicate was split five ways into each of the five tubes contained within a replicate beaker. The diluter was set to cycle every 30 min resulting in a minimum of 15 volume replacements in each beaker per day.

The cladoceran, Daphnia magna Straus, 1820, was used as the test organism in this study. The daphnids were cultured in the laboratory from parthenogenetic females. On the day before testing began, reproductively mature females were isolated. The young produced by these adults were removed and used for acute and chronic testing within 24-hr. All brood stock daphnids are maintained on a diet of the

green alga, Selenastrum capricornutum, fed at rate of ~1.25 mg dry wt/L of water on a Monday, Wednesday and Friday basis. The S. capricornutum was cultured under sterile conditions in medium described by Cowgill et al. (1985).

A flow-through acute toxicity test was conducted to aid in the selection of biphenyl concentrations to be used in the chronic test. Specifications of this test can be seen in Table 1. The acute toxicity test consisted of exposing groups of 10 neonate daphnids to five concentrations of the

Table 1. Specifications associated with standard procedures for performing flow-through acute and chronic tests with daphnids

Condition	Acute Test	Chronic Test
Temperature	20 ± 1 °C	20 ± 1°C
Photoperiod	16-hr light/8 dark	16-hr light/8 dark
Source of Organisms	laboratory reared	laboratory reared
Diet	NA	Selenastrum capricornutum 2.50 mg dry wt/L of dilution water twice daily
Test Vessel	400-mL beaker	600-mL beaker
Observations	D.O., pH, temperature mortality 0, 24, 48-hr	D.O., pH, temperature mortality, offspring M-W-F
Effect Criteria	mortality-immobility	reproduction, mortality, growth (as weight)
Length of Test	48-hr	21-d

test material, a carrier control (acetone 0.1 mL/L) and a water control. The five test concentrations and the controls were set in triplicate, resulting in 30 neonate daphnids being exposed to each concentration. The test vessels were maintained in a temperature controlled water trough set at 20 ± 1°C. Dissolved oxygen, pH and temperature were measured in the high, middle, low and control concentrations daily. The duration of this test was 48-hr. The lowest test concentration in

the acute test which resulted in either mortality or sublethal effects was used as the highest concentration in the chronic test.

Specifications associated with the flow-through chronic test can be seen in Table 1. The test vessels for this study were 600-mL glass beakers. Each beaker had a 2.5 cm^2 notch cut in the lip to facilitate water drainage from the vessel. Each test beaker contained five glass tubes (2.5 x 12.5 cm with 363 µm mesh bottoms) which were set on the bottom of the beaker. The test unit design was such that the test and control solutions entered the tops of the tubes and eventually passed through the mesh bottom and exited through the notch cut in the beaker. The beakers were maintained in a temperature controlled water trough.

The chronic test began with the placement of one neonate daphnid (<24-hr old) in each of the five tubes contained within a beaker. The tubes were given unique labels and each daphnid remained in a uniquely labeled tube for the entire study. There were four replicate beakers for each concentration (5 concentrations) and for the water and carrier controls (acetone 0.2 mL/L), resulting in 20 daphnids being exposed to each concentration. The duration of the chronic test was 21 d.

Each test beaker contained approximately 400 mL of the appropriate amounts of test material, food and dilution water. The daphnids were fed a diet of S. capricornutum at the rate of ~2.50 mg dry wt/L of dilution water twice daily. Each Monday, Wednesday and Friday the young produced by each adult were counted and discarded and adult survival was recorded. Dissolved oxygen, pH and temperature were measured daily from a high, middle, low and control replicate.

The concentration of biphenyl in the test and control solutions was analyzed using reverse-phase high performance liquid chromatography. For each analysis a 50-µL aliquot of the test solution or standard was injected on a DuPont Zorbax ODS column (4.6 mm x 25 cm). The eluent was a mixture of 80% CH_3CN/20% H_2O (V/V) with a flow rate of 1.0 mL/minute. Biphenyl was detected using a UV detector set at 254 nm. The linearity of the detector was assessed by making biphenyl standards in dilution water. The biphenyl levels in the samples were quantified using an external standard technique, in which a response factor for a particular analysis day was calculated and applied to the samples to determine the biphenyl concentrations. The test material was used to make all analytical standards and fresh standards in dilution water were prepared each analysis day.

Analyses for the acute test were performed at 0, 24, and 48-hr. Analyses during the chronic test occurred at a minimum on days -1, 0, 7, 14, and 21. On each sampling day, samples were taken from two replicates from each test concentration and the controls. Additional samples were taken on days 2, 5, 13 and 19.

The LC50 and 95% confidence intervals were determined for the 48-hr acute test using probit analysis (Finney 1971). The LC50 values were based on analyzed concentrations.

Data derived from the chronic portion of this study were analyzed using analysis of variance followed by Dunnett's t-test ($\alpha = 0.05$) (Winer 1971). Mean comparisons between test and control concentrations were performed on the following parameters at the end of the test: percent survival, mean total young/daphnid, growth (as weight) and mean brood size. The purpose of these comparisons was to estimate the maximum acceptable toxicant concentration (MATC).

RESULTS AND DISCUSSION

Biphenyl was found to have a linear response at 254 nm using the analysis system described over the concentration range of 0.011 mg/L to 0.898 mg/L ($r^2 = 0.999$). The instrumental precision was found to range from 1.7% at 0.03 mg/L to 0.5% at 0.61 mg/L. The method precision ranged from 4.9% at 0.03 mg/L to 2.3% at 0.61 mg/L. The mean concentration values used to determine toxicity values (LC50, MATC, etc.) were rounded to two decimal places.

The mean biphenyl concentrations derived from the analyzed test solutions during the acute test are presented in Table 2. All analyzed concentrations were within a range of 63.3 to 97.6% of nominal. The calculated 48-hr LC50 value for biphenyl was 0.36 mg/L (95% confidence interval: 0.28 to 0.47 mg/L). During the 48-hr test the no observable effect level was 0.04 mg/L and the 100% kill concentration was > 0.96 mg/L. There was no mortality in the acetone controls and 3% mortality in the water controls over the 48-hr test period. No sublethal effects were observed during this test. The dissolved oxygen (D.O.) measurements throughout the test were all >90% saturation. The pH and temperature measurements ranged from 7.4 to 7.9 and 20.5 to 20.7°C, respectively. A summary of the acute test is presented in Table 2.

The mean biphenyl concentrations derived from the analyzed test solutions during the chronic test are presented in Table 3. The mean analyzed concentrations ranged from 90.3 to 132% of the corresponding

nominal values. Over the course of the 21-day study the variation of analyzed solutions within a concentration was very small. During the 21-d chronic study there was no mortality observed in either the acetone or water controls. Throughout the test the dissolved oxygen measurements were >90% saturation (range 8.4 to 8.8 mg/L). The pH and temperature ranged from 7.4 to 7.8 (pH within a concentration remained within ± 0.3 units) and 20.0 to 20.6°C, respectively.

The data used to estimate the MATC are presented in Table 3 and 4. As can be seen in Table 4 there were no statistically significant differences between the controls based on survival, mean brood size, mean number of young per adult and mean dry weight. For the purpose of estimating the MATC the data derived from the water controls were compared to those of the various treatment groups (Table 3). Interpretation of the chronic data indicates that the MATC lies between 0.17 and 0.32 mg/L and is 0.23 mg/L expressed as the geometric mean of these two concentrations. The no observed effect concentration was 0.17 mg/L. The estimation of the MATC was based on data associated

Table 2. A summary of the 48 hr flow-through acute test for Daphnia magna exposed to biphenyl

Mean Analyzed Concentration (mg/L)	Number of Organisms Exposed	% Dead (#Dead) 24-hr	% Dead (#Dead) 48-hr
0.96 ± 0.17	30	30(9)	87(26)
0.48 ± 0.05	30	7(2)	57(17)
0.24 ± 0.03	30	0	40(12)
0.09 ± 0.04	30	0	7(2)
0.04 ± 0.01	30	0	0
Acetone - Control	30	0	0
Water - Control	30	0	3(1)

LC50 Values and 95% C.I.

24-hr 1.3 (1.0-3.9) mg/L - Probit Analysis
48-hr 0.36 (0.28-0.47) mg/L - Probit Analysis

with survival and reproduction (mean brood size and mean total young per adult). These endpoints both significantly differed from the controls at the 0.32 mg/L concentration. No other toxic effects were observed. During the study the first young born in the controls were observed on days 8 and 9. Additionally, the average number of young per adult in the controls exceeded 40 and there were no ephippia produced by any of the test organisms.

Table 3. The mean chronic data used to estimate the maximum acceptable toxicant concentration (MATC) for daphnids exposed to biphenyl under a flow-through regime for 21-d

Mean Analyzed Concentration (mg/L)	Survival (%)	Mean Brood Size/Adult	Mean Total Young/Adult	Mean Dry Wt/Adult (µg)
0.56 ± 0.13	0*	0*	0*	0*
0.32 ± 0.05	20*	10.1 ± 1.8*	34.0 ± 10*	135 ± 92
0.17 ± 0.03	100	18.6 ± 0.5	67.2 ± 3.1	274 ± 76
0.07 ± 0.02	100	18.2 ± 0.4	64.7 ± 2.2	285 ± 88
0.03 ± 0.01	100	18.2 ± 0.2	67.5 ± 2.5	280 ± 48
Water Control	100	18.9 ± 0.4	63.1 ± 2.6	217 ± 52

*Mean significantly differs from the control at the $\alpha = 0.05$ level; two-tailed Dunnett's t-test

Table 4. The mean chronic data used to compare the similarity of the acetone and water controls in the biphenyl 21-d chronic daphnid test*

Concentration	Survival (%)	Mean Brood Size/Adult	Mean Total Young/Adult	Mean Dry Wt/Adult (µg)
Acetone Control.	100	18.6 ± 0.3	67.0 ± 2.3	238 ± 34
Water Control	100	18.9 ± 0.4	63.1 ± 2.6	217 ± 52

*No significant differences between any of the means at the $\alpha = 0.05$ level, two-tailed Dunnett's t-test.

Dividing the chronic value generated during this study (0.23 mg/L) into the acute value (0.36 mg/L) results in an acute/chronic ratio of 1.6. Based on the acute/chronic ratio calculated for biphenyl it would be unlikely to observe chronic invertebrate effects much below levels that are acutely toxic.

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