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# Chronic Toxicity of Aniline and 2,4-Dichlorophenol to *Daphnia magna* Straus

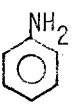
F. M. Gersich* and D. P. Milazzo

Health and Environmental Sciences, The Dow Chemical Company, 1702 Building, Midland, Michigan 48674

Data generated from daphnid chronic toxicity tests are used by various regulatory agencies for the development of water quality criteria. Two chemicals which are lacking reported chronic data are aniline and 2,4-dichlorophenol. Aniline is environmentally important because it is often found in dyes, varnish, rubber and chemical and gas plant wastes (McKee and Wolf 1963). The commercial importance of 2,4-dichlorophenol is associated with its use as a feedstock for herbicides and in the use of it's derivatives for such products as germicides, soil sterilants, antiseptics and moth proofing (U.S. Environmental Protection Agency 1980). The acute toxicity of 2,4-dichlorophenol to Daphnia magna has been reported by LeBlanc (1980); the toxicity of aniline to D. magna has been reported by Canton and Adema (1978) and Gersich and Mayes (1985). Chronic data for these chemicals are lacking for invertebrates. The objective of this study was to estimate the chronic toxicity of aniline and 2,4-dichlorophenol to Daphnia magna Straus, using a standard 21-day static renewal procedure.

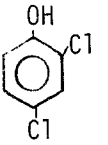
## MATERIALS AND METHODS

The test materials used in this study were aniline and 2,4-dichlorophenol. Aniline has a molecular weight of 93.1 (Verschueren 1983) with the following structural formula:




The aniline used in this study had a purity of approximately 99 mole % (reagent grade - Fisher Scientific). The water solubility of aniline has been reported as 34,000 mg/L (Vershueren 1983). The molecular weight of 2,4-dichlorophenol is 163.01 and the chemical has the following structural formula (Verschueren 1983):

*Send reprint requests to F. M. Gersich at the above address.




The 2,4-dichlorophenol used in this study was obtained from Kodak and had a purity of 99.9 mole % (as analyzed by gas chromatography). The water solubility of 2,4-dichlorophenol is reported as 4600 mg/L at 20°C (Verschueren 1983).

All test organisms were cultured and tested in Lake Huron water. This water was obtained from the Midland Water Treatment Plant (Midland, MI) prior to final chlorination and was adjusted to a hardness of about 170 mg/L (as $CaCO_3$) prior to autoclaving. The water was autoclaved at 121°C and 124 kPa for 35 minutes.

The cladoceran, Daphnia magna Straus 1820, was used as the test organism for these studies. The brood stock was maintained in an environmental chamber set at 20±1°C with a light-cycle of 16 h daylight (~1700-2400 lux)/8 h darkness. Twenty four hours before testing, multiparous females were isolated and the neonates produced by these adults were used in the chronic tests.

All daphnids (i.e., brood stock and test organisms) were fed a diet of the green alga, Selenastrum capricornutum Printz. The alga was fed to the brood stock at a rate equivalent to 2.50 mg dry wt/L of dilution water. The algal size and population distribution were measured with a Coulter Counter.

The chronic tests were conducted in a manner similar to that reported by Gersich (1984) and Gersich et al (1984). The studies were designed to use a static renewal procedure with batchwise replacement of the test and control solutions on a Monday, Wednesday and Friday basis. The test concentrations (10.6 to 170 µg/L) used in the aniline study were prepared by diluting aliquots of a stock solution (5 mg/500 mL of dilution water). The test concentrations for the 2,4-dichlorophenol study ranged from 0.38 mg/L to 6.0 mg/L and were prepared by diluting aliquots of an appropriate stock solution (~400 mg/L of dilution water). Dilution water controls were also used in these studies.

The test vessels were 600 mL glass beakers, each containing five glass tubes (2.5 x 12.5 cm) with 363 µm mesh bottoms. The tubes were supported about 1.0 cm off of the bottom of the beaker with a 1.0 mm mesh stainless steel platform. During the studies each beaker contained the appropriate amount of food, dilution water and test material made up to a 500 mL volume. The test organisms were fed S. capricornutum at a rate equivalent to 2.50 mg dry wt/L of dilution water. The beakers were held in a temperature controlled environmental chamber set at 20±1°C and a photoperiod of 16 h daylight/8 h darkness.

The chronic tests began by placing one neonate daphnid in each uniquely labeled tube. The daphnids remained in their respectively labeled tubes for the duration of the study. Each test concentration and the controls had four replicates (4 beakers/concentration), resulting in 20 daphnids being exposed to each concentration.

The duration of each test was 21 days. The critical endpoints were associated with reproduction, growth and survival. Data were collected on each of the endpoints every Monday, Wednesday and Friday, additionally pH, dissolved oxygen and temperature were also measured and recorded on each renewal day.

Samples from the aniline chronic static renewal test were analyzed using an appropriate reverse-phase liquid chromatography method. The concentrations of 2,4-dichlorophenol were determined using high performance liquid chromatography. On each Monday, Wednesday and Friday during both studies analyses were performed on a replicate from each test concentration and the control.

Data derived from the studies were analyzed using a one-tailed Dunnett's test ($\alpha$=0.05) (Winer 1971). The Dunnett's procedure used simultaneously tested for heterogeneity of variances using Bartlett's test. If the variances were heterogeneous the Wilcoxon signed rank test was used to compare the means (Hollander and Wolfe 1973). Mean comparisons between test and control concentrations were performed on the following endpoints: percent survival, mean total young/adult, mean brood size/adult and mean dry weight/adult. The purpose of these comparisons was to estimate the maximum acceptable toxicant concentration (MATC). The MATC is defined as the estimated toxic threshold concentration falling between the highest concentration showing no effect and the next highest concentration showing a toxic effect when compared to the controls (McKim 1977).

## RESULTS AND DISCUSSION

The mean aniline concentrations derived from the analyzed test solutions are presented in Table 1. The mean analyzed concentrations ranged from 79 to 160% of the corresponding nominal values. The 160% value is associated with the difficulty in analyzing for aniline at low part per billion levels. The stability of aniline over the renewal period was examined by analyzing particular test solutions at 0 hours and once again prior to the next renewal. The concentrations of the test material in the same solutions at the beginning and end of the 48 hr renewal period ranged from 75 to 89%, with a mean and standard deviation of 81.7 ± 7.0% (N=6).

Table 1. The mean chronic data used to estimate the maximum acceptable toxicant concentration (MATC) for daphnids exposed to aniline for 21 days

| Mean analyzed concentration µg/L | Survival % | Mean total[a] young/adult | Mean brood[a] size/adult | Mean dry[a] weight/adult (µg) |
|---|---|---|---|---|
| Control | 100±0 | 90.3±12.9 | 24.0±1.5 | 867.5± 42.7 |
| 12.7±3.1 | 100±0 | 89.6±12.8 | 24.3±1.9 | 793.0± 58.9 |
| 24.6±2.9 | 80±41[b] | 89.7±9.4 | 23.4±1.7 | 810.3± 29.7 |
| 46.7±5.9 | 50±51[b] | 58.3±25.9[c] | 19.8±4.1[c] | 733.3±135.6 |
| 90.0±18.6 | 25±44[b] | 2.5±3.9[c] | 2.5±3.9[c] | 347.5± 23.1[b] |
| 168.6±7.5 | 0[b] | 0[c] | 0[c] | |

[a] The endpoints mean total young/daphnid and mean brood size/daphnid were based on N=total number of adults surviving at the end of the test in all replicates for a particular concentration. The endpoint mean dry weight/adult was based on determining the mean dry weight/adult alive at the end of the study/replicate and then averaging the four replicates per concentration.

[b] Mean significantly different from the control at $\alpha$=0.05 level, one-sided Dunnett's test

[c] Mean significantly different from the control at $\alpha$=0.05 level, Wilcoxon Test

During the aniline chronic study there was no control mortality. Throughout the test the dissolved oxygen measurements were all >90% saturation. The pH and temperature measurements ranged from 7.8 to 8.1 and 19.0 to 21.0°C, respectively.

The data used to estimate the chronic value (MATC) for aniline are presented in Table 1. Interpretation of the data indicates that the MATC lies between 24.6 and 46.7 µg/L. Another estimate of the MATC value expressed as the geometric mean of 24.6 and 46.7 is 33.9 µg/L. The determination of the MATC was based on the endpoints: survival, mean total young/adult, mean brood size/adult and mean dry weight/adult, all of which significantly differed from the controls at the 46.7 µg/L level. The chronic data from this study can be used for formulating an acute/chronic ratio. Dividing the chronic value generated during this study (33.9 µg/L) into the daphnid acute LC50 value (170 µg/L) generated by Gersich and Mayes (1985) results in a daphnid acute/chronic ratio for aniline of approximately 5. Acute/chronic ratios play an important role in the development of water quality standards.

The mean 2,4-dichlorophenol concentrations derived from the analyzed test solutions are presented in Table 2. The mean analyzed concentration ranged from 89 to 113% of corresponding nominal values. The test material was found to be stable over the renewal period. The concentrations of the test material in the same solutions at the beginning and end of the 48 h renewal period ranged from 78 to 90% with a mean and standard deviation of 85.5 ± 4.2% (N=6).

During the 2,4-dichlorophenol chronic study there was no control mortality. Throughout the test the dissolved oxygen measurements were all >90% saturation. The pH and temperature measurements ranged from 7.4 to 8.3 and 19.0 to 21.0°C, respectively.

The data used to estimate the chronic value (MATC) for 2,4-dichlorophenol are presented in Table 2. The control organisms in the 2,4 dichlorophenol study exhibited larger broods and were slightly heavier when compared to the aniline study controls. Interpretation of the data indicates that the MATC lies between 0.74 and 1.48 mg/L. Another estimate of the MATC expressed as the geometric mean of 0.74 and 1.48 is 1.05 mg/L. The estimation of the MATC was based on the endpoints: survival, mean total young/adult, and mean brood size/adult; all of which significantly differed from the controls at the 1.48 mg/L level. Dividing the chronic daphnid value reported in this study (1.05 mg/L) into the acute LC50 value (2.6 mg/L) reported by LeBlanc (1980) results in an acute/chronic ratio of approximately 2.5 for 2,4-dichlorophenol.

The data generated from this study will be used in a future publication to evaluate the utility of a short-term (14-day) static renewal test. Fourteen-day tests conducted at 24°C, with temperature acclimated organisms have the potential to save a significant amount of manpower when conducting chronic daphnid tests.

Table 2. The mean (±S.D.) chronic data used to estimate the maximum acceptable toxicant concentration (MATC) for daphnids exposed to 2,4-dichlorophenol for 21-days.

| Mean analyzed concentration mg/L | Survival (%) | Mean total[a] young/adult | Mean brood[a] size/adult | Mean dry[a] weight/adult (µg) |
|---|---|---|---|---|
| Control | 100 | 128.5±20.1 | 32.0±4.2 | 1007.5±60.1 |
| 0.37 | 95±22 | 143.7±15.7 | 35.3±4.5 | 1251.3±108.7 |
| 0.74 | 100 | 119.3±16.7 | 29.1±3.5 | 1192.0±78.7 |
| 1.48 | 85±35* | 54.9±22.1* | 18.8±4.0* | 995.3±172.2 |
| 2.96 | 5±22* | 0* | 0* | 0* |
| 5.94 | 0* | 0* | 0* | 0* |

[a] The endpoints: mean total young/daphnid, and mean brood size/daphnid were based on N=total number of adults surviving at the end of the test in all replicates for a particular concentration. The endpoint, mean dry weight/adult was based on determining the mean dry weight/adult alive at the end of the study/replicate and then averaging the four replicates per concentration.

* Mean significantly different from the control at $\alpha$=0.05 level, one-sided Dunnett's test

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