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Acute and Chronic Toxicity of Triclopyr Triethylamine Salt to *Daphnia magna* Straus

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The Garlon® herbicides have been shown to be effective in the control of annual and perennial broadleaf weeds and woody plants. These herbicides are used on rights-of-way (e.g. power lines, pipelines, highways, and railroads), industrial sites and non-crop areas and are additionally used in forest site preparation and for conifer release. Triclopyr [((3,5,6-trichloro-2-pyridinyl)oxy)acetic acid] is the active ingredient in Garlon® herbicides. The use patterns of various herbicides and other chemical agents may result in accidental introduction into natural waters. The objective of this study was to determine the acute and chronic toxicity of triclopyr triethylamine salt (triclopyr TEA salt) to the freshwater invertebrate, Daphnia magna Straus.

MATERIALS AND METHODS

Lake Huron water was used to culture and test daphnids. This water was carbon filtered, UV irradiated and adjusted to a hardness of ∿170 mg/L (as $CaCO_3$) with $CaCl_2$. The water was then autoclaved at 121°C and 124.1 kPa for 35 minutes.

The cladoceran, Daphnia magna Straus, was used as the test organism in this study. The brood stock was kept in an environmental chamber set at 20°C ± 1°C and a photoperiod of 16 hrs daylight/8 hrs darkness. Twenty-four hrs before testing, multiparous females were isolated and the neonates produced by these adults were used for acute and chronic testing.

All daphnids were fed a diet of Selenastrum capricornutum Printz. Feeding rate was the equivalent of 1.25 mg/L dry weight/day. The algal size and population distribution were measured with a Coulter Counter.

Acute testing procedures were based on the guidelines recommended by the ASTM Subcommittee on Safety to Aquatic Organisms (ASTM 1980). The 48 hr acute toxicity data was used to select the appropriate concentrations for the chronic study.

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The acute test consisted of exposing groups of 10 neonates to six concentrations (336, 480, 686, 980, 1400 and 2000 mg/L) of the test material, triclopyr TEA salt, and a control. The six concentrations and control were set in triplicate. In addition, an extra beaker was set at the high, middle, low and control concentration to avoid the risk of contamination while taking dissolved oxygen, pH and temperature measurements. The test beakers were placed in a temperature controlled environmental chamber set at 20°C ± 1°C and a photoperiod of 16 hrs daylight/8 hrs darkness.

The duration of the test was 48 hrs. Mortality, as well as dissolved oxygen, pH and temperature were recorded after 24 and 48 hrs of exposure. Daphnids were not fed nor were the solutions aerated during the test.

A static renewal procedure was used to conduct the chronic test (Gersich, In Press). With this procedure there was a batchwise replacement of test and control solutions at regular intervals (Monday-Wednesday-Friday).

The test vessels used in this study were 600 mL pyrex beakers. Each beaker contained five glass tubes (2.5 x 12.5 cm) with 363 µm mesh bottoms supported on a 1.0 mm mesh stainless steel platform. Each tube contained one daphnid during the study. The test and control beakers contained 500 mL of the appropriate amounts of test material, food and water. The beakers were held in a temperature controlled environmental chamber set at 20°C ± 1°C and a photoperiod of 16 hrs daylight/8 hrs darkness.

During the test the solutions were gently aerated to achieve 90-105% saturation. There were four replicates for each test concentration and the control, resulting in five daphnids/replicate or a total of 20 organisms/concentration. The daphnids were fed *S. capricornutum* at the equivalent rate of 1.25 mg/L dry weight at each renewal. The duration of the study was 21 days. Each Monday, Wednesday, and Friday reproduction, mortality, dissolved oxygen, pH and temperature were measured and recorded.

Triclopyr TEA salt concentrations used for the chronic test were 80.7, 149, 290, 574 and 1177 mg/L. High performance liquid chromatography (HPLC) was used to determine the triclopyr levels in the exposure aquaria. The samples were filtered through a 0.45 µm Millipore® cellulose acetate/nitrate filter prior to injection into an HPLC reverse phase system. The eluate was monitored using a UV detector at 280 nm. The relative standard deviations at the 95% confidence level for five replicate

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injections of the 20.2 mg/L and 121 mg/L triclopyr standard solutions were 2.4 and 1.3%, respectively. HPLC analysis was expressed as the concentration of triclopyr which was back calculated to triclopyr TEA salt and further adjusted to an as-received basis of the test material.

The LC50 values and 95% confidence intervals were determined for both the 48 hr acute and 21 day chronic tests. The LC50 value for the 48 hr acute test was based on nominal concentrations; whereas, the LC50 value for the 21 day chronic test was based on analyzed concentrations. Finney's method of probit analysis was used to calculate the LC50 values (Finney 1971).

Data derived from the chronic portion of the study were analyzed using a two-tailed Dunnett's t-test (α=.05) (Winer 1971). Mean comparisons between test and control concentrations were performed on the following endpoints: mean number of broods/daphnid, mean total young/daphnid, survival, mean brood size/daphnid and mean length. The purpose of these comparisons was to estimate the maximum acceptable toxicant concentrations (MATC). The MATC is an 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). The MATC is best estimated by integrating biological and statistical interpretations of the data.

RESULTS AND DISCUSSION

During this study the means and standard deviations of the following water quality variables were : pH 7.9 $\pm$ 0.1, conductivity 294 $\pm$ 25 µmhos/cm, hardness 149 $\pm$ 5 mg/L as $CaCO_3$ and alkalinity 56 $\pm$ 3 mg/L as $CaCO_3$.

The calculated 48-hr LC50 value of triclopyr TEA salt was 1170 mg/L (95% confidence interval: 1030-1340 mg/L). The no kill level was <336 mg/L and the 100% kill concentration was >2000 mg/L. There was 7% control mortality during the test. The dissolved oxygen measurements throughout the test were >60% saturation. The pH and temperature measurements ranged from 7.7-8.0 and 19.6°C-20.3°C, respectively.

The mean triclopyr TEA salt concentrations derived from the analyzed test solutions during the chronic portion of this study are presented in Table 1. All analyzed concentrations were within a range of 96-107% of the corresponding nominal values. The triclopyr TEA salt concentrations in the renewed test solutions (time 0) and in the same test solutions prior to the next renewal were found to be very similar (94.6-106.3% of the original concentration), thus attesting to the stability of the test material over the renewal period.

Table 1. Mean Analyzed Concentrations, Standard and Relative Standard Deviations of Triclopyr TEA Salt

Nominal Concentration mg/L	Mean Analyzed Concentration mg/L	Standard Deviation mg/L	Relative Standard Deviation (%)
Control	ND(6)	-	-
75	80.7	3.1	3.8
150	149.0	3.8	2.5
300	290.0	6.9	2.4
600	574.0	14.0	2.5
1,200	1,177.0	42.0	3.6

The 21 day LC50 value with its 95% confidence interval was 1140 (950-1590) mg/L. Although a 21 day LC50 value was determined, the data set did not meet the criteria established by the ASTM in that no observed mortality was >65% (ASTM 1980).

During the chronic test there was no control mortality. Throughout the study the dissolved oxygen measurements were >60% saturation. The pH and temperature measurements ranged from 7.8-8.1 and 19°C-21°C, respectively.

The chronic data used to estimate the MATC are presented in Table 2. Biological and statistical interpretation of the data indicates that the MATC lies between 80.7 and 149.0 mg/L. Another estimate as the MATC value, expressed as the geometric mean of 80.7 and 149.0, is 110 mg/L. The estimation of the MATC was based on data associated with the reproductive endpoints, mean total young/daphnid and mean brood size. These two endpoints both significantly differed from the control at the 149.0 mg/L level. The endpoints mean size and mean number of brood differed from the controls at the 290.0 mg/L level, whereas, percent survival differed from the controls at the 1177.0 mg/L level.

In 1974 a study was conducted to measure triclopyr residues in water and soil following a typical helicopter application of Garlon® 3A herbicide to a power line right-of-way (McKellar et al. 1982). The application rate was 11.2 kg/ha. Residues of triclopyr collected in the water and measured at regular intervals for 510 days, ranged from being non-detectable to 0.08 mg/L. The chronic toxicity estimate of triclopyr to *D. magna*, 110 mg/L, is approximately three orders of magnitude greater than the environmental concentration in water, following a maximum rate application.

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Table 2. The Chronic Data used to Estimate the Maximum Acceptable Toxicant Concentration of Daphnids Exposed to Triclopyr TEA Salt for 21 Days

Analyzed Concentration mg/L	Percent Survival	Mean Number of Broods/Daphnid	Mean Total Young/Daphnid	Mean Young/Brood	Mean Size (mm)
Control	100±0.0	4.2±0.4	103.5±10.1	24.7±1.4	4.03±0.04
80.7	100+0.0	4.2±0.4	100.7±13.6	24.0±2.6	4.00±0.03
149.0	100±0.0	4.1±0.7	76.4±11.8*	19.0±1.3*	4.02±0.02
290.0	100±0.0	3.7±0.6*	50.3±9.7*	13.6±1.0*	3.92±0.02*
574.0	95±10.0	3.6±0.6*	41.8±8.6*	11.9±1.4*	3.80±0.07*
1,177.0	45±19.1*	0*	0*	0*	3.09±0.09*

*significant at the $\alpha = .05$ level, 2-sided Dunnett's test

REFERENCES

American Society for Testing and Materials (ASTM). (1980) ASTM Standard E729-80: Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. ASTM, Philadelphia, Pennsylvania.

Finney, D J (1971) Statistical methods in biological assay. 3rd Ed. Cambridge University Press, Boston.

McKellar, R L, O E Schubert, B C Byrd, L P Stevens, E J Norton (1982) Aerial application of GARLON* 3A herbicide to a West Virginia watershed. Down to Earth 38(2):15-19.

McKim, J M (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Can. 34, 1148-1154.

Winer, B J (1971) Statistical principles in experimental design. McGraw Hill, New York.
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