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Acute and Chronic Toxicity of Technical Picloram (4-amino-3,5,6-trichloropicolinic acid) to *Daphnia magna* Straus

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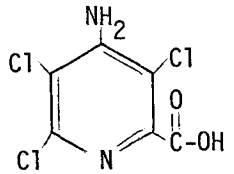
Technical picloram (4-amino-3,5,6-trichloropicolinic acid) is an active ingredient in Tordon® and Grazon® herbicides. Picloram has been shown to be effective in control of many woody plants and broadleaf weeds. Herbicides containing picloram have been used on industrial manufacturing and storage sites, rights-of-way (power and communication lines, pipelines, highways and railroads), pasture, rangeland and forest planting sites. 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 expand the toxicity database of picloram to aquatic invertebrates by estimating the acute and chronic toxicity of picloram to the freshwater invertebrate, Daphnia magna Straus.

MATERIALS AND METHODS

The test material used in this study was picloram, 4-amino-3,5,6-trichloropicolinic acid. Picloram has a molecular weight of 241.5 with the following structural formula:



The material used in this study had a purity of approximately 93%. The water solubility of picloram has been reported as 430 mg/L at 25°C (Weed Science Society of America 1983).

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

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The cladoceran, Daphnia magna Straus 1820, was the test organism used in this study. The brood stock was maintained in an environmental chamber set at 20°C±1°C with a light cycle of 16 hrs daylight (970-1250 lux)/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 (i.e., brood stock and chronic test organisms) were fed a diet of Selenastrum capricornutum Printz. The algae were fed at a rate equivalent to 1.25 mg dry wt/L of dilution water. 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 static acute data aided in selecting the appropriate concentrations for the chronic study.

The acute test consisted of exposing groups of 10 neonates to five concentrations (12.7, 20.5, 34.5, 57.0 and 94.4 mg/L) of the test material and a dilution water control. Test solutions of various concentrations were prepared using a one liter stock solution which had been adjusted to a pH of 8 with KOH solution. The five concentrations and the control were set in triplicate. Additionally, an extra beaker was set at the high, middle, low and control concentrations to avoid the risk of contamination while taking dissolved oxygen, pH and temperature measurements. The test beakers were maintained in a temperature controlled environmental chamber set at 20°C±1°C and a light cycle of 16 hrs daylight/8 hrs darkness.

The length of the acute test was 48 hrs. Mortality, defined as no movement when gently prodded, as well as dissolved oxygen, pH and temperature were recorded daily. The daphnids were not fed nor were the test solutions aerated during the test.

The chronic test was conducted in a manner similar to that reported by Gersich (1984) and Gersich et al. (1984). The study was designed to use a static renewal procedure with batchwise replacement of the test and control solutions on a Monday, Wednesday, and Friday basis. Test solutions of various concentrations (7.6, 11.8, 18.1, 29.6 and 46.9 mg/L) were prepared using a one liter stock solution that had been adjusted with KOH to a pH of 8.

The test vessels used in this study 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 the bottom of the beaker with a 1.0 mm mesh stainless steel platform. During the study each beaker contained the appropriate amount of food, dilution water and test material made up to 500 mL volume. 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.

The chronic test began by placing one neonate daphnid in each uniquely labelled tube. The daphnids remained in their respective 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 this study was 21 days. The critical endpoints were associated with reproduction, survival and growth. Data were collected on each of the endpoints every Monday, Wednesday and Friday, additionally pH, dissolved oxygen and temperature were also measured each renewal day.

High performance liquid chromatography (HPLC) was used to determine the picloram levels in the exposure vessels. The samples were filtered through a 0.45 µm Millipore® cellulose acetate/nitrate filter prior to injection into the reverse phase HPLC system. The mean filtration recovery was determined to be 100.7±1.7%. The eluate was monitored using a UV detector set at 280 nm. Relative standard deviations of 1.7-2.8% were obtained for replicate injections (n=10) of picloram standards at three levels (2.3 11.9 and 22.9 mg/L). On each Monday, Wednesday and Friday analyses were performed on all replicates from one particular test concentration and on one replicate from each remaining test concentration and the control.

The LC50 value and 95% confidence interval were determined for the 48 hr acute test. The LC50 value was based on analyzed concentrations. Thompson's moving average method was used to calculate the LC50 value (Thompson 1947). Thompson's moving average method was used because there were less than two concentrations at which the percent dead was between 0 and 100, therefore use of the probit method would not have given statistically sound results.

Data derived from the chronic study were analyzed using a two-tailed Dunnett's test (α=.05) (Winer 1971). Mean comparisons between test and control concentrations were performed on the following endpoints: percent survival, number of broods/adult, total young/adult and brood size/adult. The purpose of these comparisons was to gather data to be used in estimating 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

During this study the means and standard deviations of the following dilution water quality variables were: pH 8.1±0.1, conductivity 319±3 µmhos/cm, hardness 160±2 mg/L as $CaCO_3$ and alkalinity 69±2 mg/L as $CaCO_3$.

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The acute toxicity of picloram to Daphnia magna was estimated by determining the 48 hr LC50 value. The calculated 48 hr LC50 value and 95% confidence interval of picloram were 68.3 (63.0-75.0) mg/L. The no kill and 100% kill levels were found to be 34.5 and 94.4 mg/L. During the acute test the dissolved oxygen measurements were >60% saturation, while pH and temperature measurements ranged from 7.2-8.2 and 20.0-20.9°C, respectively.

The mean picloram concentrations derived from the analyzed test solutions are presented in Table 1. All analyzed picloram concentrations were within a range of 90.5-98.0% of the corresponding nominal values. The stability of the test material over the renewal period was examined by analyzing the acute test solutions at 0 and 48 hrs. The ratios of the same solutions over the 48 hr time period ranged from 105 to 112% with a mean and standard deviation of 106.8±2.9%. These data attested to the stability of the test material over the renewal period.

The chronic data used to estimate the MATC are presented in Table 1. Interpretation of these data indicates that the MATC lies between 11.8 and 18.1 mg/L. Another estimate of the MATC value expressed as the geometric mean of 11.8 and 18.1 is 14.6 mg/L. The determination of the MATC was based on the reproductive endpoint, mean total young/adult. This endpoint significantly differed (α=.05) from the control at the 18.1 mg/L level. The endpoint, mean brood size/adult also statistically differed from the controls at the 18.1 mg/L level. The mean brood size/adult for the controls and the daphnids exposed to 18.1 mg/L of the test material were 21.8±0.3 and 19.4±0.4, respectively. Because of the small variance associated with the brood size endpoint these two values were determined to be significantly different; however, we feel that such a small difference would not reflect an important decrease in population productivity. To determine which endpoint, mean total young/adult or mean brood size/adult, is more relevant to the objectives of chronic studies, consideration must be given to how these values are determined. It is not inconceivable that at a particular test concentration total young could significantly differ from the controls whereas brood size would not. Mean brood size is calculated using both total young and the number of broods. Although total young could be significantly reduced, if the number of broods were also reduced it is feasible that the endpoint mean brood size would not reflect the decrease. If one of the primary goals of daphnid chronic studies is to estimate the effect of a test material on population productivity, then the endpoint mean total young/adult is more appropriate than mean brood size.

Another endpoint examined during this study was mean dry weight/adult (Table 1). The dry weight data was not statistically analyzed nor was it used to estimate the MATC. The control mean dry weight/adult determined for this study was 600 µg, which was slightly less than the 700-800 µg normally experienced for healthy brood stock adults. Daphnids in this study were fed S. capricornutum at a rate of 1.25 mg dry wt/L of dilution water

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

Analyzed Concentration $\bar{x} \pm SD$ mg/L	Survival %	Mean Total Young/Adult	Total Number of Broods/Adult	Mean Brood Size/Adult	Dry Weight/Adult µg[a]
Control	95	82.8 ± 6.2	3.8 ± 0.3	21.8 ± 0.3	600
7.6 ± 0.24	100	84.0 ± 5.9	3.8 ± 0.3	22.4 ± 0.5	500
11.8 ± 0.34	95	84.0 ± 5.8	3.9 ± 0.3	21.5 ± 0.2	500
18.1 ± 0.56	90	70.9 ± 3.3*	3.7 ± 0.2	19.4 ± 0.4*	500
29.6 ± 0.70	100	36.0 ± 5.6*	3.1 ± 0.3*	11.8 ± 0.7*	500
46.9 ± 1.34	60*	13.5 ± 4.4*	1.8 ± 0.3*	7.6 ± 1.7*	300

*Mean significantly different from the control at the $\alpha = 0.05$ level, 2-sided Dunnett's test.

[a]Weight data was not statistically analyzed.

every Monday, Wednesday and Friday. It may be more appropriate to follow the recommendations of Goulden et al. (1982) that when feeding on a Monday, Wednesday and Friday basis a feeding rate of 2.50 mg dry wt/L of dilution water should be used.

During the chronic study a 5% control mortality was observed. The pH and temperature measurements throughout the test ranged from 7.9-8.3 and 19.5-20.5°C, respectively. The dissolved oxygen measurements were all >60% saturation.

Published data on the acute and chronic effects of picloram to aquatic invertebrates is lacking. However, the Herbicide Handbook Committee (1983) have reported that picloram has a low order of toxicity to wildlife and fish. The acute and chronic data generated from this study corroborate this conclusion.

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