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Acute and Chronic Toxicity of the Thiocarbamate Herbicide, Molinate, to the Cladoceran *Moina australiensis* Sars

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Fish kills have been reported in a Californian stream receiving rice culture discharge contaminated with thiocarbamate herbicides including molinate (Bailey 1993). Molinate (S-ethyl hexahydro-1H-azepine-1-carbothioate), commercial names Ordram, Higalnate, R-4572, or Sakkimol, is widely used in the rice growing fields of the Murrumbidgee irrigation area of southern New South Wales, Australia. Here, concentrations of up to 42 µg/L have been reported in lower drainage channels from rice fields (NSW Department of Water Resources, unpublished data), raising concerns of possible ecological impact of the herbicide.

Few data are available on the toxicity of molinate formulations to aquatic organisms for regulatory authorities to draw upon. In Australia, few toxicity data are available for cladocerans, and no data are available for native organisms. The endemic Australian cladoceran *Moina australiensis* Sars, is a relatively euryhaline species with a wide distribution in New South Wales, Victoria and Western Australia (Smirnov and Timms 1983), and has been used successfully as a toxicity testing organism by the New South Wales Environment Protection Authority for over 3 years (Julli 1993). Like other *Moina*, *M. australiensis* has a fast generation time, producing a first brood of up to 20 neonates at 4 days old, and 3 broods at 7 to 8 days old. The objectives of this study were to determine the acute and chronic effects of molinate on *M. australiensis*.

METHODS AND MATERIALS

M. australiensis was cultured and tested at 22.5°C in dechlorinated Sydney mains water of pH 7.2, conductivity of 148 µS/cm, and hardness of 36 mg/L as $CaCO_3$. Cultures were maintained in 2 L glass beakers and subjected to 50% water renewal 3 times weekly. Food was provided 3 times weekly at a concentration of 25,000 cells/mL of each of the unicellular algae *Raphidocellis subcapitata* (formerly named *Selenastrum capricornatum*) and *Ankistrodesmus* sp. Adults bearing eyed embryos were isolated one day prior to the commencement of the tests so that all neonates used in experiments were less than 24 hr old. Test vessels were 250-mL glass beakers, containing 200 mL test solution.

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For the acute 48-hr immobilization test, nominal molinate test concentrations were 0, 0.100, 0.375, 1.25, 4.25 and 15.0 mg/L, with 4 replicate beakers for each concentration and 5 neonates per beaker. Animals were transferred to fresh test solutions at 24 hr. A reference toxicant test (potassium dichromate, BDH AnalaR®) was run concurrently with the acute immobilization test, using concentrations of 0, 10, 15, 25, 38 and 60 µg/L Cr^{6+}, based on concentrations derived from Krassoi and Julli (in press). Animals were not fed for the duration of the acute tests.

In a separate chronic 8-d test, the effects of molinate on the reproduction of *M. australiensis* was assessed. The endpoints of this test were young production, and parental immobilization at the end of the test. Nominal molinate concentrations were 0, 20, 50, 150, 375 and 1000 µg/L, with 12 replicate beakers for each treatment and one neonate per beaker. Parental animals were transferred to fresh solutions at 48-hr intervals. Young were counted and removed daily. The algal food source mentioned above was provided at a concentration of 50,000 cells/mL of test solution on odd days (i.e., in fresh test solutions), supplemented with 25,000 cells/mL on even days.

Technical grade Ordram 960 (960 mg/L molinate) was obtained from ICI Australia Ltd. No carrier solvent was required in the preparation of stock solutions. For the acute test, molinate concentrations were measured twice at the beginning of renewal periods and once in 24-hr old solutions for all treatments. For the 8-d test, molinate concentrations were measured at the beginning of three renewal periods and once after 48 hr. This allowed an average measured:nominal ratio to be determined which was subsequently used to estimate actual molinate test concentrations.

Molinate was extracted using liquid-liquid extractions with dichloromethane based on USEPA method 508 (Graves 1989). Molinate concentrations were analysed using a Hewlett-Packard 5890 Series 2 Gas Chromatograph with N-P detector. Duplicate samples of the hexavalent chromium reference toxicant test were taken after 48 hr. Hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in acid solution (APHA 1992).

Conductivity of test solutions was measured with a EDT FE280 conductivity meter, pH was measured with a Jenco 6007 pH meter fitted with Ionode double junction electrode, and dissolved oxygen was measured with a Microelectrodes dissolved oxygen meter.

The 48-hr and 8-d EC50 (immobilization) values were determined by nonparametric analysis using a computer program based on the trimmed Spearman-Karber Method (Hamilton *et al.* 1977). For reproductive impairment in the 8-d test, the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) were determined using one-way ANOVA and Dunnetts test (SYSTAT 1992), following confirmation of homoscedasticity with Cochran's test (Dixon and Massey 1969). The statistical

power (1-β) of the ANOVA to detect a 25% reproductive impairment (at α=0.05) was estimated using DESIGN Version 2 computer program (Dallal 1988). The Inhibition Concentration of molinate causing a 25% reduction in young production (IC25) was calculated by linear interpolation using a bootstrap method to calculate 95% confidence interval approximations (Norberg-King 1993).

RESULTS AND DISCUSSION

In all test solutions the conductivity was 145±2 µS/cm, and the pH of test solutions ranged between 7.5 in fresh test media to 8.4 in 48-hr old solutions, though there was no apparent dose effect. Dissolved oxygen concentrations remained above 80% saturation throughout the tests. Temperature of test solutions in all tests remained within 22.5°C±1.5°C

Measured concentrations of molinate averaged 72.4% (12.1 SD) of nominal concentrations in the 48-hr test, while for the 8-d reproductive impairment test, measured molinate concentrations averaged 76.4% (7.2 SD) of nominal concentrations. Degradation of molinate between renewal periods was not consistently evident. The above percentages were used in estimations of measured concentrations and subsequently used in EC50, LOEC, NOEC and IC25 calculations.

The 48-hr EC50 (immobilization) value was estimated to be 2.40 mg/L molinate (95% CI 1.42-4.18). There were no control mortalities. The majority of the mortalities of molinate exposed animals occurred between 24 and 48 hr of exposure. This 48-hr EC50 value is considerably lower than that reported for *Daphnia magna* of 14.9 mg/L (ICI Americas 1988, cited in Brandt *et al.* 1993).

The 8-d EC50 (immobilization) value for parental animals was estimated to be 0.30 mg/L molinate (95% CI 0.16-0.57). Most mortalities occurred in the first three days of the test.

Analysis of Variance rejected the null hypothesis that there were no differences in total young production over 3 broods between the concentrations tested ($P<0.001$). Based on Dunnetts test the No Observed Effect Concentration (NOEC) value and the Lowest Observed Effect Concentration (LOEC) value were 0.11 and 0.29 mg/L molinate, respectively.

The power (1-β) to detect a 25% impairment in reproduction (at α=0.05) was calculated to be 22%. This low power was in part due to the presence of a parental female in the control group which failed to produce young for the duration of the test, consequently increasing the group variance.

The Inhibition Concentration causing a 25% impairment in *Moina* reproduction (IC25) was estimated to be 0.15 mg/L molinate (95% CI estimate 0.10 - 0.20 mg/L).

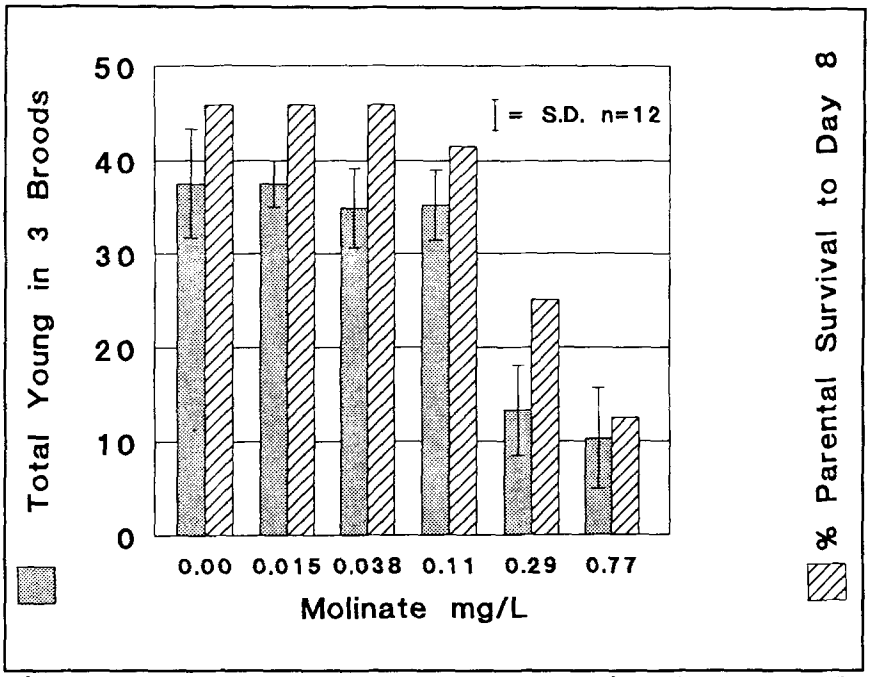

Figure 1. Number of young produced in three broods and survival by *Moina australiensis* when exposed for 8 days to various concentrations of molinate.

Measured concentrations of the Cr^{6+} reference toxicant remained above 85% of nominal in concentrations 15-60 µg/L, but decreased to 50% at the lowest concentration tested (10 µg/L). The 48-hr EC50 for chromium was 38.6 µg/L Cr^{6+} , which is well within the range reported previously (Krassoi and Julli, in press).

Based on the test results it would appear unlikely that the levels of molinate measured in the Murrumbidgee irrigation canals (42 µg/L) would cause either an acutely lethal or immobilizing effect on the test species, *M. australiensis*. However, field validation studies currently in progress (W Korth, CSIRO Division of Water Resources, *pers comm*) have shown that the canal waters are periodically toxic to cladocerans. Additional work is being conducted to elicit the factors responsible for this toxicity.

Effects on *M. australiensis* reproduction would also appear unlikely to occur at 42 µg/L molinate. During the 8-d test it was apparent that the algal food source was being affected by the molinate treatments. This was evidenced by a distinct yellowing of the settled algae on the bottom of test containers, the intensity increasing with molinate concentration. It is possible that the results of the cladoceran reproduction test were influenced by molinate-induced changes to the quantity and/or quality of the algal food source. Given that no toxicity data dealing with the effects of molinate on algae were found, it would seem

prudent to conduct such studies in order to more fully estimate molinate concentrations of environmental concern.

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