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Chemical Batch as a Factor Affecting the Acute Toxicity of the Reference Toxicant Potassium Dichromate to the Cladoceran *Moina australiensis* (Sars)

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The discharge of heavy metals such as chromium is a serious source of pollution to aquatic environments. Studies of the acute toxicity of hexavalent chromium have in the past been restricted to organisms not endemic to Australian ecosystems, which may demonstrate levels of sensitivity different to those of endemic organisms. Such a difference would have a direct bearing on Australian water quality criteria, which are based in part on ecotoxicological assessments.

This work is part of a program to establish baseline physiological and ecotoxicological data on endemic Australian species of Cladocera. *Moina australiensis* occurs commonly throughout Australia, and is euryhaline (Smirnov and Timms 1983). It is particularly useful for ecotoxicological testing as it produces a first brood of up to 20 neonates when only 4 days old.

Potassium dichromate has been widely accepted as a reference toxicant for acute toxicity tests using cladocerans (e.g. OECD 1987; Environment Canada 1990). The published 48-hr LC50 and EC50 values exhibit great inter- and intraspecific variation, which may be attributable to factors such as water hardness and salinity (Cowgill and Milazzo 1991a) alkalinity (Cowgill and Milazzo 1991b), temperature (Cairns *et al.* 1978), humic acid content (Stackhouse and Benson 1989) and genetic variability (Baird *et al.* 1989). This paper assesses another potential source of variability: the origin of commercial batches of potassium dichromate. Analytical reagent-grade potassium dichromate from Ajax, BDH and Mallinckrodt were tested. A significant difference in the toxicity of commercially available batches of reference toxicant may require setting uniform standards in inter- and intra- laboratory protocols for not only water quality variables, but also for the source or batch of reference toxicant to be used.

METHODS AND MATERIALS

Moina australiensis was cultured in 2 l beakers at 23°C in filtered Sydney mains water of pH 7.2, conductivity of 148 µS/cm, and hardness of 36 mg/L as $CaCO_3$. Cultures were subjected to 50% water renewals 3-times weekly, and fed 25,000 cells/mL of each of the unicellular algae *Raphidocellus subcapitata* (formerly *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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The 48-hr EC50 immobilization tests on each batch of potassium dichromate were run simultaneously and repeated 3 times using independently prepared stock solutions. Range finding tests were used to establish definitive test concentrations.

Three batches of potassium dichromate were tested; Ajax Univar®, BDH AnalaR® and Mallinckrodt AR®, each of which were randomly allocated to batch numbers. Batch 1 concentrations tested were 10, 15, 25, 37.5 and 60 µg/L, Batch 2 were 5, 10, 20, 40 and 80 µg/L, and Batch 3 were 10, 20, 30, 50 and 80 µg/L. Each concentration tested had 4 replicate beakers, each with 5 neonates. Measurements of pH, temperature, conductivity and percent saturation dissolved oxygen were made at 0 and 48 hr after test commencement.

Analysis of hexavalent chromium was initially carried out at 0 and 48 hours, but was then reduced to only 48-hr measurements as no difference was detected over time in the final concentration. Hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in acid solution (APHA 1980). Possible contaminants of all three batches were analyzed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

48-hr EC50 values were determined by non-parametric analysis using a computer program based on the trimmed Spearman-Kärber Method (Hamilton *et al.* 1977, 1978). EC50 estimates for batches were compared by one-way Analysis of Variance and Tukeys HSD multiple comparison tests (SYSTAT 1992), following confirmation of homoscedasticity with Cochran's Test (Dixon and Massey 1969). The overall mortality data for batches was probit transformed, and probit / log dose linear regressions and coefficient of determinations were computed. The homogeneity of slopes of the overall batch regressions were compared using analysis of covariance procedures (Zar 1974).

RESULTS AND DISCUSSION

There were no marked changes in pH values amongst test concentrations over the 48 hr of the tests. The median pH of the test solutions was 7.8 (7.3-8.2), and conductivity was 147 ±1µS/cm. Dissolved oxygen in the test solutions remained above 80% saturation throughout the test. Measured concentrations of Cr^{6+} remained above 90% of nominal concentrations, and were used for the EC50 determinations.

In all tests, Batch 2-sourced potassium dichromate consistently demonstrated a lower toxicity to *M. australiensis* than both Batches 1 and 3 (Table 1). The one way analysis of variance of EC50 values was significant (P=0.01). Tukey HSD test showed Batch 2 to be different to Batch 1 and 3, with no difference between Batch 1 and 3. There was a definite overlap of the EC50 95% confidence intervals between the latter two toxicant batches, but no overlap with the Batch-2 sourced potassium dichromate. Replication of the tests in time, provides confidence in the validity of the demonstrated differences between batches in the EC50 estimates.

Table 1. The 48-hr EC50 (µg/L) values (95% Confidence Interval) for three batches of potassium dichromate against *Moina australiensis*, repeated three times.

	Batch 1	Batch 2	Batch 3
Test 1	20.2 (17.1-24.0)	34.6 (29.4-40.6)	27.6 (23.7-32.2)
Test 2	24.7 (21.1-29.0)	38.6 (32.9-45.3)	23.9 (20.2-28.2)
Test 3	21.8 (18.4-25.8)	35.2 (29.5-41.9)	24.6 (21.7-27.9)
Overall EC50	22.5 (20.4-24.7)	36.1 (32.7-39.8)	24.5 (22.1-27.1)

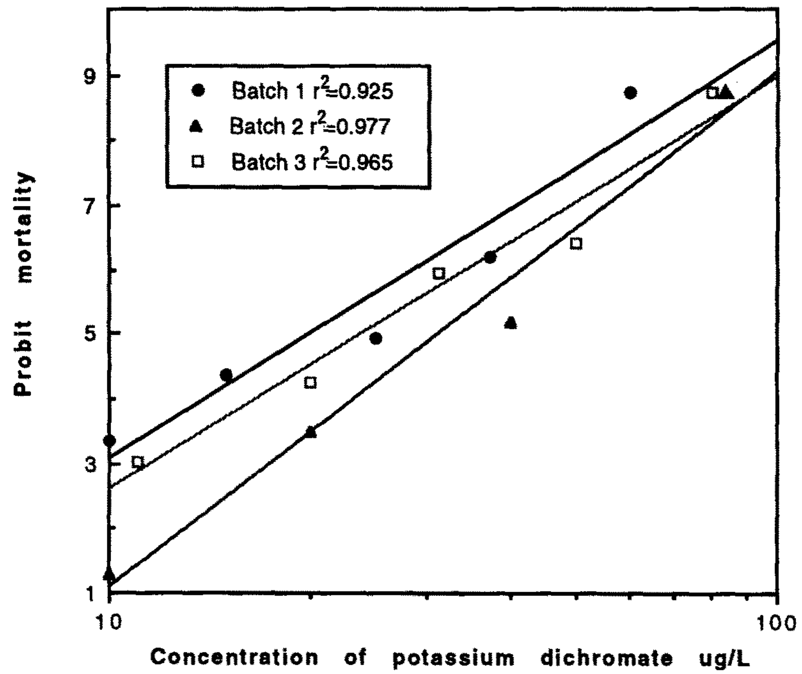

Figure 1. Acute toxicity of *Moina australiensis* to three batches of potassium dichromate.

The coefficient of determination values (r^2) of the dose response regression for each batch tested are given in Fig. 1. The r^2 for the overall estimate (combined Test and Batch) is degraded (0.911) relative to the individual Batch estimates. The equations for the linear regressions in Fig. 1 are $y = -3.38+6.43\log(x)$ for Batch 1, $y = -4.13+6.25\log(x)$ for Batch 2, and $y = -3.78+6.36\log(x)$ for Batch 3-sourced potassium dichromate. No significant differences were detected ($P>0.1$) between slopes of the probit mortality/ log concentration regression of all three Batches. Percentage mortality is consistently lower for Batch 2 over all concentrations tested.

The results from the ICP-AES analysis of the stock solutions of the three batches of potassium dichromate revealed no specific inorganic contaminants which could account for the higher toxicity of both the Batch 1 and 3 samples relative to that of Batch 2 (Table 2). Indeed the only differences appear to be higher quantities of aluminum, tin, cadmium, barium and iron in Batch 1 samples, and a higher quantity of calcium in Batch 3.

Table 2. Concentrations of elements (expressed in mg/L) in 50 mg/L stock solutions of the three batches of potassium dichromate, analysed by ICP-AES.

Batch	Ca	Ti	Cu	Mg	Fe	Mn	Si	Ni	Cd	Zn
1	0.042	<0.01	<0.01	0.019	0.010	<0.01	<0.1	<0.01	0.003	0.013
2	0.043	<0.01	<0.01	0.018	0.003	<0.01	<0.1	<0.01	0.001	0.011
3	0.075	<0.01	<0.01	0.016	0.001	<0.01	<0.1	<0.01	0.001	0.012

Batch	K	Na	Ba	Sr	Al	Mo	Sn	As	P	Cr
1	42	0.34	0.027	0.003	0.013	<0.01	<0.01	<0.02	<0.02	49
2	43	0.25	0.023	0.001	0.007	<0.01	<0.01	<0.02	<0.02	50
3	42	0.31	0.023	0.001	0.005	<0.01	<0.01	<0.02	<0.02	49

The lowest overall 48-hr EC50 value derived for *Moina australiensis* from the present experiments was 22.5 (20.4-24.7)µg/L (Batch 1). Thus, *M. australiensis* appeared to be more sensitive to hexavalent chromium than *Daphnia pulex* with a 48-hr LC50 of 180 (150-190) µg/L (Jop *et al.* 1987). The 24-hr LC50 to *Daphnia magna* was 140 (50-1800) µg/L (Bringmann and Kühn 1977), and 24-hr EC50 to *Daphnia carinata*, *Ceriodaphnia dubia* (New Zealand type) and *Simocephalus vetulus* was 423 (317-520) µg/L, 53 (39-79) µg/L and 154 (50-500)µg/L respectively (Hickey 1989).

The 24-hour EC50 for *M. australiensis* was found to be greater than the highest concentration of all batches tested. It is observed that the majority of mortalities occurred during the final 24 hours of the tests. The only reported daphnid of comparable sensitivity to *M. australiensis* is *Daphnia hyalina* with a 48-hr LC50 of 22 (32-15) µg/L (Baudouin and Scoppa 1974). Variation in these cited acute values may be due to factors such as differences in test conditions as length of test, hardness, pH, and conductivity, rather than or in addition to differing interspecific sensitivities.

Replication in time provides information on the precision of results, and avoids the advent of demonic and non demonic intrusion in the experimental design as described by Hurlbert (1984), and may reduce the often high variability between laboratories, as found by Dorn *et al.* (1987). Based on the data presented herein, a standardization of the batch of reference toxicant may be appropriate particularly in inter-laboratory ring tests, to reduce another potential source of variation.

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