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# Toxicity of Chlorine and Other Chlorinated Compounds to Some Australian Aquatic Organisms

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Chlorine is widely used to disinfect drinking water and wastewater prior to discharge, and when used appropriately, its role in preventing the spread of waterborne infectious diseases is well established. Low levels of residual chlorine, however, can be harmful to aquatic life if drinking water or heavily chlorinated wastewater are discharged into the environment.

The US EPA (1986) and Environment Canada (CCME 1991) have established guidelines for the protection of aquatic life from the impact of exposure to residual chlorine, based on a large quantity of data detailing the variety of effects of chlorine in aquatic systems. These criteria have been supported by field studies conducted in the USA where it has been shown to exert both acute and sublethal effects at total residual chlorine (TRC) levels of less than 100 µg/L (Stewart *et al.* 1990; Szal *et al.* 1991 and Grizzle *et al.* 1988). Laboratory studies have shown acute and chronic effects at concentrations of total residual chlorine of less than 10 µg/L (Taylor 1993 and Szal *et al.* 1991). There is no water quality guideline specified for the management of chlorine in Australian conditions in the National Water Quality Guidelines for Fresh and Marine Water published by the Australia and New Zealand Environment and Conservation Council (ANZECC 1992).

Many water-treatment bodies in Australia add ammonia as well as chlorine to drinking water to lengthen the time that free chlorine remains available through the formation of chloramines. The two chemicals are added in the ratio of 3 parts $NH_3$ to 1 part $OCl^-$.

The NSW EPA is aware of a variety of practices where chlorinated waters may be released to the environment including, for example, during the emptying of water storages or swimming pools or in chlorinated effluents. It was decided to evaluate the potential local impacts of such activities.

This study was commenced, therefore, to obtain data on Australian organisms to support development of appropriate controls. The study included an evaluation of the toxicity of a mixture of chlorine and ammonia to assist in evaluating the potential impacts of drinking water on ecosystems.

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## MATERIALS AND METHODS

The animals used in this study were the Australian cladoceran *Ceriodaphnia dubia* and the Eastern king prawn *Penaeus plebejus* Hess. *Ceriodaphnia dubia*, which occurs throughout Australia, is taxonomically distinct from the North American *Ceriodaphnia dubia* Richard, but current taxonomic keys do not allow differentiation (Julli *et al.* 1990).

The Australian *C. dubia* were cultured in 2-L beakers at 23°C in filtered Sydney mains water of pH 7.2. Conductivity was adjusted to 500 µS/cm with seawater to provide optimal water quality conditions for this species. Cultures were subjected to 50% water renewal, three times weekly, and fed 25,000 cells/ml of each of the unicellular algae *Raphidocellus subcapitata* (formerly *Selenastrum capricornutum*) and *Ankistrodesmus* sp. at each renewal. Adults 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 beakers containing 200 mL test solution. each covered with cling film. Screw-top tubes which held 10 mL of test solution were assessed as an alternative test container to minimize chlorine evaporation, but chlorine levels dropped more rapidly than in the 250-mL beakers.

Chlorine was added to the test solutions as sodium hypochlorite(LR) and ammonia was added as ammonium sulfate (AR). Chemicals were purchased from the Fisons range and were the highest grade available.

Preliminary testing, according to standard toxicity testing protocols with renewal of test solutions every 24 hr, demonstrated the need to modify the standard 48-hr EC50 immobilization test with *C. dubia* (OECD 1987), due to the volatility of chlorine. As a result, for both chlorine and chlorine combined with ammonia, 1-hr EC50 immobilization tests and 24-hr EC50 immobilization tests with 6-hourly test solution renewals were performed. For ammonia, a 48-hr EC50 immobilization test with 24-hourly renewals was performed. Other aspects of the tests were performed according to standard laboratory protocols.

Ten-day lifecycle tests (OECD 1987) were performed using *C. dubia* for ammonia, chlorine and chlorine combined with ammonia, and brood size and timing of young production were observed. Tests were performed with 48-hr renewals for ammonia and 24-hr renewals for chlorine and chlorine combined with ammonia.

*Penaeus plebejus* were purchased from a commerical hatchery aged 15 d postlarvae. The animals were transferred into glass aquaria upon arrival and held in natural filtered seawater under flow-through conditions at 23°C±1°C for at least 10 d prior to use. The toxicity of these chemicals to this species was evaluated using a protocol based on the standard static renewal 96-hr LC50 protocol for *Mysid* shrimps (USEPA 1991). For the evaluation of ammonia toxicity, 24-hourly renewals of test solution were used. Again, adjustments were made to the exposure period and solution renewal interval in the standard 96-hr LC50 acute toxicity protocol after it became clear that the volatility of chlorine compromised the testing. The test with chlorine was conducted under flow-through conditions for 24 hr with 6-hourly replacement of stock toxicant solution. Time and space limitations at the Centre did not allow the repetition of the ammonia toxicity test using flow-through equipment.

Analyses of total chlorine and ammonia were conducted using a HACH DR2000 Portable Laboratory. The detection limit for both analytical methods was 0.01mg/L.

The LC50 and EC50 values were determined using the trimmed Spearman-Karber method (Hamilton *et al.* 1977,197s). The 3 brood lifecycle tests were analyzed using ANOVA with SYSTAT for Windows, Version 5 (1992) after ascertaining homogeniety of variances using Cochran's test (Snedecor & Cochran 1980). When the data were not homogeneous they were analyzed using Steels Many One Rank Test using TOXSTAT (Gulley *et al.* 1991).

## RESULTS AND DISCUSSION

Where ammonia toxicity was studied using ammonium sulfate in freshwater, pH ranged from 7.0 to 7.8 over all the acute tests and from 7.3 to 8.4 in the chronic test. Conductivity ranged from 468 to 2630 µS/cm in the acute tests and from 473 to 860 µS/cm in the chronic test. The large range in conductivity was due to the presence of ammonium sulfate.

**TABLE 1.** Toxicity Values for *C. dubia* and *P. plebejus* when exposed to chlorine and ammonia, alone and in combination.

| Type of Test | Chlorine (mgCl/L) | Ammonia (total mg$NH_3$/L) | Chlorine + Ammonia(mgCl/L+mg$NH_3$/L) |
|---|---|---|---|
| 1-hr LC50 (95%CL[1]) - freshwater[2] | 0.28 (0.26-0.31) | na[3] | 0.36+0.51 (0.34+0.49-0.37+0.54) |
| 24-hr LC50 (95%CL) - freshwater | 0.12 (0.11-0.13) | na | <0.063+0.09 |
| 24-hr LC50 (95%CL) - flowthrough marine[4] | 0.18 (0.16-0.19) | na | na |
| 48-hr LC50 (95%CL) - freshwater | na | 59 (52-67) | na |
| 96-hr LC50 (95%CL) - marine | na | 12 (9-15) | na |
| NOEC - freshwater | 0.048 | 51 | 0.011+0.017 |
| LOEC - freshwater | 0.066 | >51 | 0.033+0.048 |

[1] 95%CL - 95% confidence limits
[2] freshwater - tests conducted using the Australian *Ceriodaphnia dubia*
[3] na - not available
[4] marine - tests conducted using *Penaeus plebejus*

The 48-hr EC50 for C. *dubia* for ammonium sulfate was 230 mg/L $(NH_4)_2SO_4$ or 59 mg total $NH_3$/L (Table 1). No significant difference (P<0.05) was observed between treatments during the lifecycle test with *C. dubia* and so the NOEC was the highest test concentration of 200 mg/L ammonium sulfate or 51 mg/L total $NH_3$ (Table 1).

In the acute marine test of ammonia toxicity, pH ranged from 7.7 to 8.1 and salinity ranged from 31 to 33 parts per thousand. In the acute marine test of chlorine toxicity under flow-through conditions, pH was approximately 8.0 and salinity was approximately 36 parts per thousand.

The 96-hr LC50 for *P. plebejus* for ammonium sulfate was 45 mg/L; while as total $NH_3$ it was 12 mg/L (95% confidence limits - 9-15 mg/L total $NH_3$) (Table 1). The 24-hr LC50 under flow-through conditions for *P. plebejus* for sodium hypochlorite was 0.18 mgCl/L (Table 1). The 95% confidence limits were 0.16 mg/L and 0.19 mg/L.

In the acute tests to evaluate chlorine toxicity, pH ranged from 7.5 to 7.9 and conductivity ranged from 478 to 535 µS/cm. In the chronic test pH ranged from 7.6 to 8.3 and conductivity ranged from 475 to 530 µS/cm. In the acute tests of the toxicity of chlorine in combination with ammonia, pH ranged from 7.5 to 7.8 and conductivity ranged from 500 to 530 µS/cm. In the chronic test pH ranged from 7.6 to 8.0 and conductivity ranged from 480 to 560 µS/cm.

The 1-hr EC50 for *C. dubia* for sodium hypochlorite was 0.59 mg/L or 0.28 mgCl/L (Table 1). The 24-hr EC50 for *C. dubia* for sodium hypochlorite was 0.26 mg/L or 0.12 mgCl/L. During the lifecycle test with sodium hypochlorite all animals in the two highest concentrations died within 4 days. The LOEC was determined as 0.066 mgCl/L and the NOEC was 0.048 mgCl/L (Table 1). The observed effect was death prior to the production of any young. There were no significant effects on young production.

The 1-hr EC50 for *C. dubia* for sodium hypochlorite combined with ammonium sulfate was 0.36 mgCl/L+0.51 mg total $NH_3$/L (Table 1). In the 24-hr test all animals except the controls died within 12 hr of exposure commencement and the EC50 for *C. dubia* for sodium hypochlorite combined with ammonium sulfate was <0.063 mgCl/L+0.09 mg total$NH_3$/L (Table 1).

During the lifecycle test with sodium hypochlorite combined with ammonium sulfate all animals in the highest concentration died within 3 d. The LOEC was determined as 0.033 mgCl/L+0.048 mg total$NH_3$/L and the NOEC was 0.011 mgCl/L+0.017 mg total$NH_3$/L (Table 1). The observed effect was death prior to the production of any young. There were no significant differences in young production between treatments.

No test was performed with *P. plebejus* with chlorine combined with ammonia as combined discharges do not occur to the marine environment.

Studies conducted on northern hemisphere cladocerans have shown them to be very sensitive to chlorine exposure. Taylor (1993) found that under flow-through conditions the 24-hr LC50 was approximately 0.005 mg/L. total residual chlorine (TRC) for *C.*

*dubia* Richard (the North American species). Other studies have shown that *Daphnia magna* are acutely affected by chlorine at concentrations ranging from 0.002 to 0.080 mg/L (USEPA 1984). This study indicates that the Australian waterflea which was tested was not as sensitive to chlorine for short periods of exposure (1 hr) while it has similar sensitivity to other waterfleas studied when exposed to chlorine over a longer time period (24hr).

The lowest concentration of total chlorine without ammonia present causing an effect in sublethal tests with the cladoceran species tested was 0.066 mg/L. The lowest concentration that caused an effect of the mixture of total chlorine with ammonia in the ratio used in chloramination was 0.033 mg/L.

As no water quality guideline exists for chlorine in Australian conditions, the procedures specified by the OECD for determining environmental concern levels when a database is limited (OECD 1992) were used to determine an appropriate interim control level for chlorine in Australian conditions. Environmental concern levels are the concentrations in the ambient system that if exceeded suggest action is required. More data are required to refine the figure to a full guideline value. They are not equivalent to water quality criteria and, in some instances, may be more protective. The OECD procedures detail that where chronic (or sublethal) data are available on the species listed in the minimum premarketing dataset the lowest NOEC available should be divided by 10 to determine the concentration in the environment which should act as a trigger level for some action. Based on these mechanisms for establishing environmental concern levels (ECLs) and the toxicity results from this work, the concentration of total chlorine in the presence of ammonia that should be protective in freshwater is 0.003 mg/L or 3 µg/L. This figure is in line with the water quality guidelines already established based on toxicity values for northern hemisphere species. The Canadian guidelines (CCME 1991) recommend that total residual chlorine should not exceed 2 µg/L, while the USEPA water quality criteria (USEPA 1986) recommend that total residual chlorine should not exceed 11 µg/L as a four-day average and 19 µg/L as a one-hour average more than once every three years. Both these figures are based on a large database of information on species resident in the northern hemisphere.

In marine systems, overseas studies have shown that invertebrate species such as amphipods, hermit crabs and shrimps are sensitive to chlorine exposure at concentrations ranging from 0.090 to 0.687 mg/L (USEPA 1984).

Only acute studies were completed on the prawn *P. plebejus.* The OECD recommend that to derive an environmental concern level, if acute toxicity data only are available, the lowest acute LC50 or EC50 should be divided by 100 or 1000, depending on the amount of data available. In this case, 100 was used due to the large quantity of data available on overseas species to add to the Australian data collected in this study. Based on the 24-hr LC50 figure listed above, the environmental concern level for chlorine in marine situations would be 0.0018 mg/L or 1.8 µg/L. This figure is also in line with those determined in the northern hemisphere. The USEPA water quality criterion for chlorine in marine and estuarine systems states that the concentration of chlorine produced oxidants should not exceed 7.5 µg/L as a four-day average and 13 µg/L as a one-hour average more than once every three years (USEPA 1986).

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