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Acute Toxicity of Inorganic Chloramines to *Daphnia magna* in Two Types of Dilution Water

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Inorganic chloramines are used as a disinfectant in drinking water as well as an anti fouling agent in cooling waters (Baldwin 1981; Haas et al., 1990). They are also found in municipal chlorinated waste water that has not been dechlorinated. They are formed when chlorine combines with ammonia or other nitrogenous compounds in aqueous solution (Leao and Selleck, 1983; Margerum et al., 1994). The use of inorganic chloramines in British Columbia and many parts of Canada may result in their unintentional introduction into fresh water bodies where invertebrate communities, as represented by *Daphnia magna* may be adversely affected. These organisms are a primary food source of fish and larger invertebrates. Previous studies indicate that inorganic chloramines are highly toxic to *D. magna* (Kaniewskaa-Prus, 1982; Brooks et al., 1989; Hunter/ESE 1989; Wan et al., 2000a). Inorganic chloramine LC50s medium concentration in different types of dilution water ranged from 0.011 to 0.168 mg/L (24-h), 0.017 to 0.238 mg/L (48-h), and 0.018 to 0.119 mg/L (>48-h), at pH ranging from 7 to 8.35 and temperatures varying from 10°C to 27°C. It was reported that the presence of halides, e.g., Br^-, Cl^-, I^-, and F^- affects the acute toxicity of inorganic chloramines to aquatic invertebrates (Fisher et al., 1999). Accordingly, the objective of this study was to compare the effect of the presence of small amounts of Br^- and F^- on the LT50 and LC50 values of inorganic chloramines to *D. magna* using two types of dilution water at 20°C. Specifically, the bioassays used a reconstituted water containing no Br^- and F^-, and a natural well water containing small amounts of Br^- and F^-, although it was unavoidable to have Cl^- in both water types.

MATERIALS AND METHODS

Inorganic chloramines were synthesized by adding sodium hypochlorite solution to an ammonium solution at a molar ratio of 1 to 3, respectively, (Wan et al., 2000a; Wan et al., 2000b). DPD-FAS (DPD-ferrous ammonium sulfate) and HPIC (high pressure ionic chromatography) methods were used to determine and compare the inorganic chloramine residues (Environment Canada, 1998; Wan et al., 1999).

The toxicity tests were conducted in a reconstituted water (APHA, 1995) and a well water of North Vancouver, British Columbia, Canada. The pH for both water was 8.1 (range, 8.0 - 8.2). Accordingly, it was expected that MC would be the predominant inorganic chloramine species (White, 1972; Wan et al., 2000a).

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The tests were conducted from August 11, 1999 to August 12, 1999 in accordance with the procedure outlined by Environment Canada (1990). Testing was carried out at a temperature of 20 $\pm$ 0.5°C, and with a 16-h light: 8-h dark photo period. Ten daphnia were introduced to each 200 ml inorganic chloramine test concentration. Duplicate tests per concentration plus a reference toxicity test with sodium chloride and a water control were set up. Observations of inorganic chloramine effects on daphnia were made every 3-h before teach est solution concentration was renewed. Tests were terminated when all daphnia died. Death of daphnia was defined as the cessation of all visible signs of movement or activity, including second antennae, abdominal legs, and heartbeats when viewed under a binocular (10 x magnification) microscope.

Chemical analyses were conducted to determine inorganic chloramine concentrations (with test organisms) of duplicate 250-ml test solutions at 0-h and 3-h for the 0.025 mg/L test concentration of both types of water at 20°C. The inorganic chloramine residues in test solutions were verified by using two techniques, viz., amperometric titration and high pressure ionic chromatography. In addition, samples of both types of dilution water were taken for water quality analysis. Solutions for toxicity testing and chemical analytical determinations were prepared by spiking (with Eppendorf pipettes) the reconstituted and well water with the appropriate test concentrations from a standardized (by DPD-FAS titration) stock solution of inorganic chloramines.

The cumulative daphnia mortality was recorded and, based on the observed values of LT50 (20 organisms per test concentration), the LC50 values for every 24-h were statistically calculated using the "Lethal" computer program (Stephan, 1983).

RESULTS AND DISCUSSION

Well water has a greater alkalinity and conductivity than reconstituted water (Table 1). It is slightly harder, and contains a greater amount (~ 1.5 x more) of total dissolved chemical elements/ions, particularly chlorides (~50 x more). Well water also contains small amounts of Br and F^- which were not found in reconstituted water. In addition, it contain two times more filterable residues, and a SO_4 content of about 9 times less than reconstituted water. Despite the occurrence of very different concentrations of selected ions, both water types were routinely used for culturing daphnia and toxicity tests in our laboratory without incurring any abnormal mortality in the cultures and controls. In this study, the only factor that caused daphnia mortality was the introduction of freshly prepared and standardized inorganic chloramines.

Approximately 20% of the inorganic chloramines disappeared in both reconstituted and well water during each of the 3 h test exposure duration (Table 3). This indicates that the test solution contained at least 80% of the test chemicals during each test solution renewal. A previous study with well water subjected to similar ambient conditions shows that inorganic chloramines degraded at the rate of about 10% hourly (*Farrell, unpublished data*).

Table 1. Water quality* of dilution water

Parameter analyzed	Reconstituted water	Well water
Alkalinity	30.3 ± 0.5	54.4 ± 0.5
Chemical elements/ions		
Br	< 0.05	0.15 ± 0.01
C (total)	13.5 ± 0.5	12.9 ± 0.1
Ca	14.3 ± 0.3	36.5 ± 0.5
Cl	2.1 ± 0.1	105 ± 1
F	< 0.05	0.12 ± 0.01
I	< 0.05	<0.05
K	2.5 ± 0.1	4.3 ± 2
Mg	2.8 ± 0.2	3.7 ± 0.1
Na	26.2 ± 0.5	44 ± 0.5
Si	0.06 ± 0.01	14.5 ± 0.5
SO_4	78 ± 1	8.6 ± 0.5
Total Chem. elements/ions	150 ± 5	230 ± 2
Conductivity (μmhos/cm)	291 ± 1	480 ± 1
Hardness (total)	88 ± 0.9	107 ± 0.7
Residue (filterable)	180 ± 1	320 ± 1
pH (Rel. U)	8.1 (8.0 - 8.2)	8.1 (8.0 - 8.2)

* - parameter measured in mg/L (mean ± S.E.; n = 4); detection limits: halides = 0.01 mg/L; other chemicals = 0.1 mg/L

Table 2. Disappearance[a] of inorganic chloramines and chlorine-produced oxidants (CPO) in test solutions containing test organisms at 20°C during each 3-hourly changes

Test solutions	Concentration (mg/L) at 0-h	Concentration (mg/L) at 3-h	Loss (%)
Reconstituted	0.025	0.020	20
Well	0.025	0.020	20

[a] - detection limit = 0.010 mg/L (CPO as chlorine equivalents); n = 2; confirmed by amperometric and HPIC techniques

The data in Table 4 indicates that inorganic chloramines in reconstituted water appeared to be slightly more toxic to *D. magna* than in well water, except for the trend of LT50 for the two highest test concentrations. We are unable to find a logical explanation to indicate why the dose-response of *D. magna* was different for these two test concentrations for both types of dilution water. Due care was taken to ensure that the correct amount of stock solution (the same stock solution used in the preparation of the other test concentrations) was used to produce the required test concentrations. It appeared that the LT50 was not a monotonic function of exposure concentration because it increased at the highest concentration. Notwithstanding this observation, the 48-h LC50 dose-response

Table 3. Estimated LT50* and the time to 100% mortality of *Daphnia magna* in different inorganic chloramine concentrations of reconstituted and well water

Test concentrations** (mg/L)	Observed LT50 (h)		Time (h) to 100% mortality	
	Recon***	Well	Recon***	Well
0.001	> 48	> 48	> 48	> 48
0.005	> 48	> 48	> 48	> 48
0.010	45	> 48	> 48	> 48
0.015	27	> 48	> 48	> 48
0.020	10.8	23.8	24	48
0.025	21.9	32.2	36	48

* - based on 20 organisms per test concentration; ** - MC concentrations prepared by spiking a standardized (verified) stock solution concentration to dilution water; *** - recon = reconstituted water

Table 4. Estimated LC50* of inorganic chloramines to *Daphnia magna* at different exposure times in reconstituted and well water

Exposure time (h)	LC50 (mg/L; 95% C.I.)	
	Recon**	Well
3	> 0.025	> 0.025
6	> 0.025	> 0.025
12	> 0.025	> 0.025
24	0.015 (0.010 - 0.020)	0.021 (0.019 - 0.024)
36	0.012 (0.011 - 0.014)	0.019 (0.016 - 0.022)
48	0.010 (0.008 - 0.012)	0.016 (0.015 - 0.020)

* - calculation based on 20 organisms per test concentration; ** - recon = reconstituted water

trend for all test concentration is within the realm of expectation (Table 4).

Based on 95% C.I.of the 48-h LC50 values, inorganic chloramines in reconstituted water was significantly more toxic than in well water with increasing test concentrations. A 3-h test solution renewal regime indicated that inorganic chloramines are significantly more toxic to *D. magna* in the same type of test solution than a 24-h renewal regime, i.e., 48-h LC50 of 0.010 mg/L (0.008 mg/L - 0.012 mg/L:95% C.I.) versus 0.017 mg/L (0.015 mg/L - 0.020 mg/L: 95% C.I.), respectively, (Wan et al., 2000a).

This study, however, has two limitations. First, the estimated LC50 via statistical calculation was based on n = 2 (10 animals/test concentration/replicate), instead of the norm n = 3. Nevertheless, this study design was purposely chosen due to considerations relating to cost-effectiveness, personnel safety, time constraint,

logistics, and the labor-intensive nature of the tests involved. Second, a reconstituted water having selected chemical ions similar to that of the natural well water should have been considered for the test comparison.

Notwithstanding the above limitations, this study suggests that inorganic chloramines in reconstituted water seems to be more toxic to *D. magna* than the natural well water. Both reconstituted and well water did not cause daphnia mortality in the routine cultures of the organism nor the controls of the toxicity tests, despite the presence of larger amounts chemical elements/ions and filterable residues, higher conductivity, and greater hardness. The introduction of inorganic chloramines caused daphnia mortality in both water types. Of importance here, however, was the presence of about 0.15 mg/L bromide (Br^-) in well water and how it affected the acute toxicity to daphnia of inorgaic chloramines when compared with reconstituted water in which Br^- was not present. When inorganic chloramines are introduced to water containing bromide, inorganic bromamines may also be formed (Bousher et al., 1989; Bousher et al., 1990). Inorganic bromamines are more toxic to aquatic organisms than inorganic chloramines (Fisher et al., 1999). In addition to Br^-, well water contained a large amount of Cl^- and trace amounts of F^-, both of which were not found in reconstituted water (Table 1). It was possible that these halides collectively reacted with inorganic chloramines to form various chlorine-produced oxidants (CPO) in the test solutions. Accordingly, this study indicates that CPO play a significant role in determining the outcome of the acute toxicity test results in tests involving very low inorganic chloramine concentrations. To date, the general belief is that the presence of small amounts of Br^- increases the toxicity of inorganic chloramines to invertebrates (Fisher et al., 1999).

In summary, the 48-h LC50 of inorganic chloramines to *D. magna* in reconstituted water and a natural well water was 0.010 mg/L (95% C.I., 0.008 - 0.012) and 0.016 mg/L (95% C.I, 0.015 - 0.020), respectively. This study suggests that, in contrast to the general belief, the presence of small amounts of Br^-, Cl^-, and F^- in well water may decrease the acute toxicity of inorganic chloramines to *D. magna* when compared with a reconstituted water containing low Cl^- but no Br^- and F^-. This hypothesis needs further investigations.

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