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Validation of the Acute Toxicity of Inorganic Chloramines to the Fresh Water Invertebrate *Daphnia magna*

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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 after reaching equilibrium in an aqueous solution at a designated pH (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 benthic communities, as represented by Daphnia magna may be adversely affected. Benthic organisms are a primary food source of fish and larger invertebrates. Previous studies indicate chloramines are highly toxic to *D. magna* (Kaniewskaa-Prus, 1982; Brooks et al., 1989; Hunter/ESE 1989). Inorganic chloramine LC50s medium concentration ranged from 0.011 to 0.168 mg/L (24-h), 0.033 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. However, inorganic chloramine concentrations of those studies were determined either by amperometric titration or DPD (N,N-diethyl-*p*-phenylenediamine) techniques. In this study we (a) determined the LT50 medium of inorganic chloramines to *D. magna,* and (b) validated the 24 h and 48 h LC50s values at 20°C and pH of 8, using both DPD and HPIC (high pressure ion chromatography) techniques to determine inorganic chloramine concentrations.

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. The speciation of various inorganic chloramines at different pH is summarized in Table 1.

DPD-FAS (DPD-ferrous ammonium sulfate) and HPIC methods were used to determine and compare the inorganic chloramine residues (Environment Canada, 1998). The inorganic monochloramine (MC) produced was standardized by the DPD-FAS titrimetric method in which DPD was used as an indicator. DPD was oxidized by chlorine (or iodine in the case of combined inorganic and organic chloramines) to the magenta-color species. The red color was then titrated with a ferrous reducing agent to the colorless endpoint. The water samples were

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Table 1. Speciation of inorganic chloramines[a] of fresh water

pH range	Ratio Cl_2:N[b]	Predominant chloramine species	Abbreviation
7.9 - 8.5	3:1	monochloramine	MC
6.5 - 7.9	5:1	monochloramine + dichloramine	MC + DC
4.4 - 6.5	5.1-7.6:1	dichloramine	DC
< 4.4	>7.6:1	trichloramine	TC

[a] - White 1972; [b] - chlorine to nitrogen weight ratio

analyzed for free chlorine, combined chlorine, and total residual chlorine by the DPD-FAS titrimetric method. Inorganic MC and dichloramine (DC) were analyzed by HPIC with post column reaction by using iodide and subsequent electro-chemical detection (DC Amperometry) of the liberated iodine. Standardized MC and DC solutions were injected into the HPIC Dionex system for instrument calibration. Derived organic chloramines were obtained by subtracting the free chlorine, MC and DC (of HPIC) from total residual chlorine. The toxicity tests were conducted in reconstituted water having a pH range of 8 ± 0.1. Accordingly, it was expected that MC would be the predominant inorganic chloramine species. Test series of inorganic chloramine concentrations to determine LT50s to *D. magna* were conducted from October 1997 to April 1998 in accordance with the procedure outlined by Environment Canada (1990). Testing was carried out at a temperature of 20 ± 0.5°C, and with a 16-h light: 8-h dark photoperiod. Ten daphnia were introduced to each 200 ml inorganic chloramine test concentration. Triplicate 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 at 5, 10, 20, and 40 min; 1, 2, 4, 8, 24, 32, 48, and 72 h. Each test solution was renewed every 24 h. 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 on duplicate 750-ml inorganic chloramine solutions for each test concentration. Each solution contained equal loading density of daphnia. Water samples were collected by filling a 250-ml amber bottle at 0 h, 8 h, and 24 h for inorganic chloramine residue analysis, which was conducted promptly thereafter. The decay profile of 0.035 mg/L inorganic chloramines in reconstituted water stored in darkness at 20°C was pre-determined earlier.

Solutions for toxicity testing and chemical analytical determinations were prepared by spiking the daphnia culture water with the appropriate test concentrations of inorganic chloramine. Daphnia were cultured in reconstituted water having the following water quality (mean values as mg/L, except as noted; n = 5): alkalinity (60), chloride (2.2) nitrate/nitrite nitrogen (<0.02) phosphate (<0.005), sulphate (79), inorganic and organic carbon (11.9) inorganic metals (< 0.1) calcium (14.5), dissolved oxygen (9), potassium (2.2) magnesium

(12.7), sodium (27.5), sulfur (27.4), total hardness (88.5), conductivity (286 uS/cm), pH (8 ± 0.1 units), filterable particles (10).

The initial inorganic chloramine bioassay solution was adjusted for losses before being aged in darkness at 20°C for about 16-18 h. It was then used for toxicity testing or test volume replacements. Based on observed LT50s values (n = 3), a mean value for each test concentration was calculated statistically using Stephan's program (1983). The cumulative daphnia mortality was recorded and the LC50 values for every 24 h were calculated using the "lethal" computer program developed by Stephan (1983).

RESULTS AND DISCUSSION

Approximately 30 - 35% of the inorganic chloramines (without test organisms) disappeared during the first 8 h from a 0.035 mg/L inorganic chloramine solution stored in darkness at 20°C and about 7% disappeared during the next 40 h. In test solutions, however, ≥50%, 55.9%, and 25.9% inorganic chloramine disappeared for starting concentrations of 0.020, 0.060, and 0.300 mg/L, respectively (Table 2). For 2.60 mg/L, about 3.5% disappeared after 3 h. These were additional losses, after each inorganic chloramine concentration had been aged for about 16 - 18 h and adjusted for the initial loss. This result shows that inorganic chloramines continued to disappear during the 24 h bioassay period after the test organisms were introduced, and that the rate of disappearance varied for different test concentrations. It was probably unnecessary to "stabilize" the inorganic chloramine solution by aging in darkness as long as inorganic chloramine concentrations of test solutions were verified at different times, e.g., 0 h, 8 h, and 24 h. Moreover, losses for concentrations below 0.010 mg/L cannot be validated accurately, as this was the detection limit for both the DPD-FAS and HPIC techniques.

Table 2. Inorganic chloramine disappearance of test solutions of different starting concentrations at 20°C in a 16-h light: 8-h dark photo period

0 h (mg/L)	8 h (mg/L)	24 h (mg/L)	Mean over a 24 h period (mg/L)	Loss at 24 h (%)
0.020	0.010	0.010[a]	≤0.013	≥50
0.059	0.047	0.026	0.044	55.9
0.293	0.242	0.217	0.251	25.9
2.600	2.540[b]	2.510[c]	2.550	3.5[c]

[a] - detection limit = 0.010 mg/L; [b] - analyzed at 2 h (100% mortality); [c] - at 3 h

Kaniewska-Prus and Sztrantowicz (1979) verified the concentrations of inorganic chloramines in their test solutions by means of a titration method using Mohr's salt in the presence of orthotolidine, while Brooks et al. (1989) and Hunter/ESE (1989) used the DPD method. We confirmed our inorganic chloramine residues in the test solutions using the DPD-FAS as well as the state-of-the-art HPIC

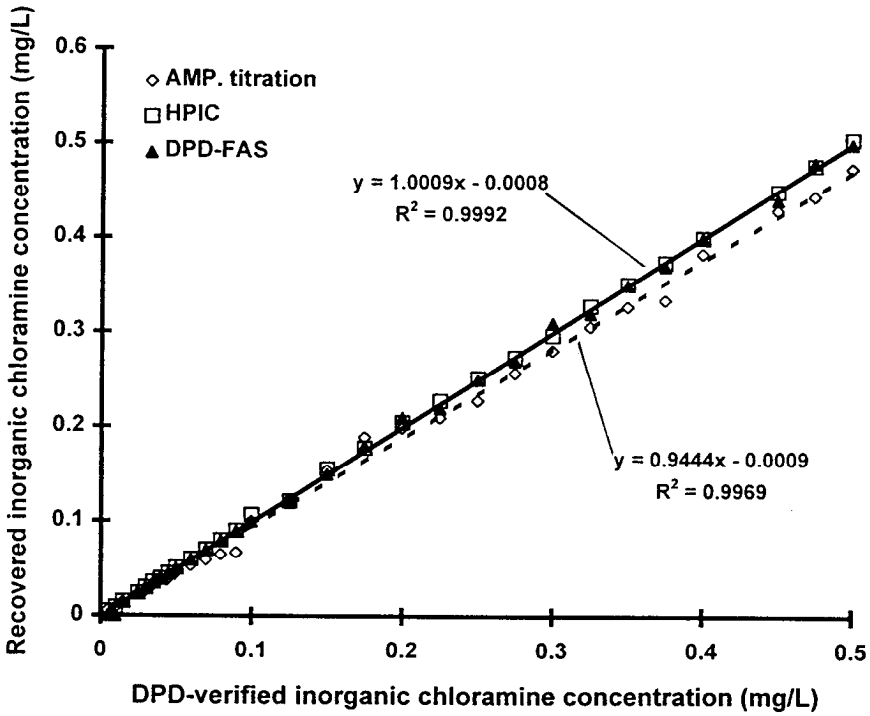

Figure 1. Inorganic chloramine residue recovery in reconstituted bioassay water

methods. Between 0.01 mg/L to 0.1 mg/L, both HPIC (R^2= 0.9992) and DPD-FAS (R^2= 0.9992) as well as amperometric techniques of inorganic chloramine residue recovery were compatible (Fig. 1). However, for concentrations at ≥0.5 mg/L, the amperometric (R^2= 0.9969) method produced about 20 - 25% lower recovery results (Fig. 1). It is not possible to compare the titration results of Mohr's salt, as this method is no longer used due to the toxic nature of ortholidine (APHA, 1995). Because of their transient characteristics, inorganic chloramine standards are not commercially available. They must be synthesized and standardized at the time of each test or bioassay run. This process may vary from laboratory to laboratory. Accordingly, it is difficult to compare the accuracy of inorganic chloramine residue recovery of different laboratories. The comparison of the three methods of recovery here was based on the assumption that the DPD method is accurate.

This study, nevertheless, confirms the results of the DPD method in verifying inorganic chloramine residues in reconstituted bioassay water. For an accurate estimation of inorganic chloramine residues, DPD-FAS and HPIC methods should be used for inorganic chloramine residue confirmation. Environment Canada is validating a technique to detect inorganic chloramine residues at concentrations below 0.010 mg/L, using the amperometric method. However, the challenge is to find an alternative method to verify this limit of detection. To date, there is no other reliable method available to verify inorganic chloramine residues below 0.01 mg/L.

Table 3. Estimated LC50, LT50 (n = 3) and the time to 100% mortality of *Daphnia* magna in different inorganic chloramine concentrations

Chloramine concentrations* (mg/L)	Estimated mean LT50 (h) (95% C.I.)	Time (h) to 100% mortality	LC50 (mg/L)
0.013	23.1 (20.6 - 25.7)	≤48	0.019 (24 h)
0.044	12.5 (8 - 24)	≤24	
0.251	6 (4 - 8)	≤24	0.017 (48 h)
2.550	2.3 (2 - 3)	≤3	

* - arithmetic mean of MC concentrations from residue recovery of water samples taken at 0 h, 8 h, and 24 h of each test solution, except the highest concentration (see Table 2), as verified by DPD-FAS and HPIC techniques

Table 3 presents the estimated LT50 of test inorganic chloramine concentrations to *D. magna.* Based on these observations, it is possible to characterize the inverse relationship (R^2 = 0.9960) of the estimated times of LT50 of this indicator organism versus actual inorganic chloramine test concentrations (Fig. 2) under the present test conditions. From this figure, it could be extrapolated that a conservative estimate of the LT50 values of inorganic chloramines at 0.010 mg/L and 0.005 mg/L to *D. magna* is probably greater than 33.3 h and 66.6 h, respectively. However, in reality, the LC50 of very low inorganic chloramine concentrations would eventually level off. Caution should therefore be taken about using such data to represent field situations. As well, this study had one limitation. The LT50 values of each actual test concentration was an estimated (via statistical calculation) mean time of the 50% daphnia mortality and not the actual observed mean LT50.

Based on the mortality observations of different inorganic chloramine test concentrations, the estimated 24h and 48h LC50 values to *D. magna* were 0.019 mg/L and 0.017 mg/L, respectively (Table 3). Both LT50s and LC50s were a mean estimate of MC toxicity.

At a pH range of 7.8 to 8.2 and under similar test conditions, the 48 h LC50s of inorganic chloramine to *D. magna* averaged 0.043 mg/L (range, 0.022 - 0.079 mg/L; n = 5) (Brooks et al. 1989; Hunter/ESE, 1989) whereas our 48-h LC50 value of inorganic chloramines to *D. magna* was about 0.017 mg/L. Our data compares favorably with the results reported by Brooks et al. (1989) and Hunter/ESE (1989) when experimental variations were taken into consideration, e.g., quality of sodium hypochlorite and ammonia solution used for inorganic chloramine synthesis, inorganic chloramine standard preparation, titration technique, etc. Both scientists used the DPD method to verify the inorganic chloramine residues in water. They also used the initial inorganic chloramine concentration of test solutions for LC50 calculation. Accordingly, theLT50s and LC50s data produced would likely be an over estimation of the true values (i.e., somewhat less toxic), as stated earlier. We felt that it was desirable to determine

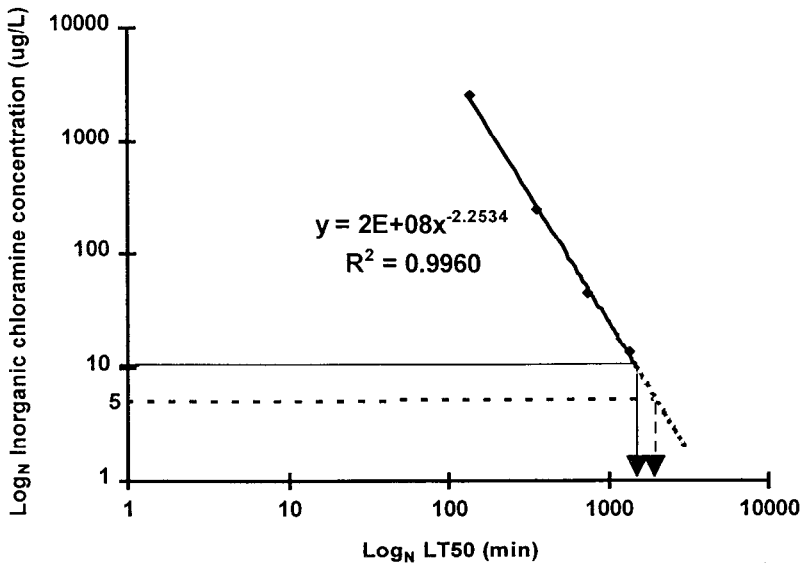

Figure 2. Inorganic chloramine LT50 *(Daphnia magna)*

the inorganic chloramine residues in the bioassay water at the beginning (0 h), between water replacement (8 h), and at 24 h. The arithmetic mean of these three determinations would probably better represent the prevailing inorganic chloramine concentration in the test solution during the 24 h period.

Inorganic chloramines could be released to the aquatic environment in three ways: (a) a sudden input of large volumes of inorganic chloramine treated water, as in the case of breaks in the water mains, (b) a steady and/or (c) intermittent release of waste treatment effluents from urban centers. Most water treatment plants would inject a concentration resulting in a residue of about 0.5 - 2 mg/L inorganic chloramine in the treated water (Haas et al. 1990; Leao and Sedeck, *1983; Pasternak, unpublished data).* Some water-main breaks have had a disastrous impact on aquatic organisms. For example, a pipe break of a fire hydrant (containing 2.53 mg/L total chlorine, 0.86 mg/L total ammonia; pH = 8.06) near Little Campbell River, Surrey, BC caused the death of about 2,000 juvenile salmonids (Nikl and Nikl, 1992).

The impact of steady or intermittent inorganic chloramine releases from effluents on aquatic invertebrates is likely a function of the resulting bio-active concentrations of these chemicals prevailing in the aquatic environment. This test demonstrated that inorganic chloramine concentrations above 0.020 mg/L in reconstituted water (pH = 8) would have an adverse acute impact on the aquatic invertebrate. It is speculated that this impact would likely occur in natural waters having a similar inorganic chloramine concentration and quality as the

reconstituted water used for the bioassay. Many water bodies in Canada have comparable pH and water quality, e.g., Norrish Creek, BC; North Saskatchewan River, SK; and Grand River, ON (Pasternak, 1998). Whether or not concentrations below 0.020 mg/L inorganic chloramines would have sub-lethal effects to *D. magna* is presently not known.

In summary, at least two recovery techniques, viz., the amperometric, DPD-FAS, or HPIC method, should be used for inorganic chloramine determination and verification. The LT50s of inorganic chloramines to *D. magna* was inversely related to the concentrations of the chemicals in water. A conservative estimate of the LT50 of this indicator organism to 0.010 mg/L and 0.005 mg/L inorganic chloramines was about 33.3 h and 66.7 h, respectively. With an estimated 24 h and 48 h LC50 value of 0.019 mg/L and 0.017 mg/L, respectively, inorganic chloramines are highly toxic (Zucker, 1985) to *D. magna* in 20°C water of pH = 8.

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