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Acute Toxicity of Chlorine-Produced Oxidants (CPO) to the Marine Invertebrates *Amphiporeia virginiana* and *Eohaustorius washingtonianus*

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Inorganic chloramines are used as a disinfectant in drinking water as well as an anti-fouling agent in cooling water (Baldwin 1981; Haas et al., 1990) and are found in chlorinated wastewater that has not been dechlorinated. They are formed when chlorine combines with ammonia or other nitrogenous organic compounds (Leao and Selleck, 1983; Margerum et al., 1994). The use of chloramine-treated water in Canada, notably, in British Columbia, Nova Scotia, and Newfoundland, may result in its discharge or unintentional introduction into marine water bodies.

When inorganic chloramine-treated water is discharged to the estuarine or marine environment, a number of possible oxidants may be formed (Fisher et al., 1994). They include both free and combined chlorine-, bromine-, iodine-, and fluorine-containing oxidants due to the presence of varying concentrations of Cl^-, Br^-, I^-, and F^- in estuarine or marine water (Jolley and Carpenter, 1983; Wilson, 1975). Collectively, these oxidants are called chlorine-produced oxidants (CPO). The current analytical methods used for measuring CPO as chlorine equivalents are incapable of differentiating these oxidants.

To date, few data are available on the acute toxicity of CPO to marine amphipods, which are one of the main food sources for fish, whales and larger invertebrates. A predictive mortality study of Margrey et al. (1981) suggests that 0.3 mg/L chloramines (total residue chlorines) produced about 20% mortality to estuarine amphipods of *Gammarus* species at 96 h in 10°C sea water. In this study we (a) determined the LT50 of CPO to the Atlantic marine amphipod *Amphiporeia virginiana* at 10°C and to its Pacific marine counterpart, *Eohaustorius washingtonianus* at 15°C, both in pH of 7.5 seawater and (b) estimated the LC50s values every 24 h for a period of 7 d.

MATERIALS AND METHODS

Inorganic chloramines were synthesized by adding sodium hypochlorite solution to ammonium solution at a molar ratio of 1 to 3. The speciation of various inorganic chloramines in fresh water at different pH is summarized in Table 1. Although it is presently not possible to distinguish CPO in sea water, Mono-CPO were found to be the predominant species in the water at pH = 7.5 *(Pasternak, unpublished data).* CPO were determined in sea water as chlorine equivalents according to the method used for inorganic chloramine determination of fresh water (Environment

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Table 1. Speciation of inorganic chloramines[a] in 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 molar ratio

Canada, 1998a). DPD-FAS [N,N-diethyl-*p*-phenylene-diamine (DPD) - ferrous ammonium sulfate (FAS)] and HPIC (high pressure ion chromatography) methods were used. 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 (bromine or iodine in the case of combined inorganic and organic chloramines) to the magenta-colored species. The red color was then titrated with a ferrous reducing agent to the colorless end point. The sea water samples were analyzed for free chlorine, combined chlorine, and total residual chlorine by the DPD-FAS titrimetric method. Inorganic MC and inorganic dichloramine (DC) were analyzed by HPIC with post-column reaction by using iodide and subsequent electrochemical 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 Burrard Inlet sea water having a pH range of 7.5 ± 0.1. A series of tests to determine the LT50s of CPO to *E. washingtonianus* were conducted from November 16 to December 5, 1998, and to *A. virginiana* from February 1 to February 8, 1999. The tests were carried out in accordance with the procedure outlined by Environment Canada (1992, 1998b). The test duration was modified to 168 h for the lower concentrations. Amphipods were not fed during the test period. Testing was carried out at two temperatures: 10°C ± 0.1°C *(A. virginiana)* and 15°C ± 0.1°C *(E. washingtonianus)* in total darkness and without sediments. Ten *E. washingtonianus* or twenty *A. virginiana* were introduced to one liter of exposure water for each CPO test concentration. Triplicate tests per concentration plus reference toxicity tests with ammonia, sodium chloride, and a sea water control were conducted.

Observations of CPO effects on amphipods were made at 5, 10, 20, and 40 min; 1, 3, 6, 12, and 12 hours thereafter until 168 h. Each test solution was renewed every 12 h. The test organisms were transferred to fresh solution, using an eye dropper. Tests were terminated when all amphipods died. Death of amphipods was defined as the cessation of all visible signs of movement or activity, including antennae, abdominal legs, and heartbeats when viewed under a binocular microscope. Based on the observed LT50 values (n = 3) a mean value was calculated using the statistical program LETHAL (Stephan, 1983).

The cumulative amphipod mortality was also recorded, and the LC50 values at 24-h intervals were calculated using LETHAL. *E. washingtonianus* was collected locally, while *A. virginiana* was imported from eastern Canada. Prior to its importation into BC, a permit was obtained from the Canadian Federal/Provincial Transplant Committee. The amphipods were collected and shipped to North Vancouver by Harris Industrial Testing Service, Halifax, NS, Canada.

Each CPO test concentration was analyzed by DPD-FAS and HPIC technique at the beginning (0 h) and the end of each 12-h test duration. Duplicate 250-ml CPO solution samples from each test concentration series were taken for analytical determination. Test water samples (~ 85 mL) were collected from each of the test replicates, twice to fill two 250-ml amber bottles at 12 h for CPO residue analysis, which was conducted promptly thereafter. The chemical loss for the 12-h period was then determined for 0.025, 0.125, 0.625, 3.125 and 12.5 mg/L CPO solutions. Solutions for toxicity testing and chemical analytical determinations were prepared by spiking the sea water with the appropriate test concentrations of CPO. CPO bioassay solutions for the second (2000 h) 12-h water replacement of each day were adjusted for losses according to the time that was needed to prepare and store the sea water before being used. The degradation curves of each test concentration were pre-determined earlier.

Burrard Inlet sea water had the following quality (mean mg/L; n = 5): alkalinity (98), inorganic and organic carbon (18.4) metals (< 0.1) Ca (310), dissolved O_2 (9), K (320), Mg (1040), Na (8420), SO_4^- (692), total hardness (5070), pH (7.5 ± 0.1 units); filterable particles (10 mg/L).

RESULTS AND DISCUSSION

An average of about 25% (range, 11.7% - 40%) and 44% (range, 40% - 48%) of the CPO in the bioassay sea water containing test organisms disappeared at test temperatures of 10°C and 15°C respectively, during the 12-h test period in darkness (Table 2). The CPO loss for the 12.45 mg/L solution in 1 h was 0.6%. All test organisms of this concentration died within the hour; the cause of death appeared to be CPO because all test organisms in the ammonia control did not die during a 12-h exposure. It seems that CPO losses were much greater at 15°C than 10°C.

Table 3 presents the estimated LT50s of test CPO concentrations to *A.virginiana* and *E. washingtonianus.* Based on these observations, the LT50s were inversely related to CPO concentrations [R^2 = 0.9771 *(A. virginiana);* R^2 = 0.9038 *(E. washingtonianus);* Fig. I]. From Figure 1, conservative estimates of LT50s to *A. virginiana* for 0.010 mg/L and 0.005 mg/L CPO are 4000 h and 9000 h, respectively. For *E. washingtonianus,* the equivalent was about 700 h and 1000 h. However, estimates from such extrapolations should be used with caution. In reality, at very low concentrations, CPO probably is not acutely toxic to these aquatic invertebrate. It would be a challenge, nevertheless, to determine the LC50 values of very low CPO concentrations (e.g., < 0.010 mg/L) to both marine invertebrates, because this would require extending the test beyond 7 d. A feeding regime for, as well as the handling stress effects on, the organism would

Table 2. Disappearance of chlorine-produced oxidants (CPO) in test solutions of different starting concentrations at 10°C and 15°C in darkness

0 h[a] (mg/L)	12 h (mg/L)	Mean over a 12-h period (mg/L)	Loss at 12 h (%)
10°C			
0.025	0.015	0.020	40.0
0.120	0.106	0.113	11.7
0.620	0.495	0.557	20.2
3.150	2.290	2.720	27.3
12.45	12.38[b]	12.42[b]	0.6[b]
15°C			
0.025	0.013	0.019	48.0
0.120	0.064	0.092	46.7
0.620	0.371	0.496	40.0
3.150	1.899	2.525	40.7

[a] - detection limit = 0.010 mg/L of chlorine equivalents, mean (n = 2); [b] - observation at 1 h

Table 3. LT50s (mean, n = 3) and times to 100% mortality for *Amphiporeia virginiana* and *Eohaustorius washingtonianus* in different CPO concentrations

CPO* (mg/L)	Mean LT50 (h) (95% C.I.)	Time (h) to 100% mortality
***A. virginiana* @ 10°C**		
0.020	>168	>168
0.113	≤168	≤168
0.557	62.2 (60 - 72)	≤72
2.72	5.7 (3 - 24)	≥24
12.42	≤1	≤1
***E. washingtonianus* @ 15°C**		
0.019	>168	≥168
0.092	101.1 (95.5 - 107.1)	≥168
0.496	61.4 (58.8 - 63.7)	≥84
2.525	11.2 (6 - 24)	≥35

* - mean CPO concentrations (chlorine equivalents) as verified by DPD-FAS & HPIC techniques

have to be considered. These factors could complicate the results of test regimes of longer duration. Based on the cumulative mortality observations of different test concentrations, the estimated LCSOs of CPO to *A. virginiana* were 0.99 mg/L, 0.63 mg/L, and 0.134 mg/L for 24 h, 48 h, and 168 h, respectively (Fig. 2). The respective equivalent for *E. washingtonianus* was 1.85 mg/L, 0.567 mg/L, and 0.043 mg/L.

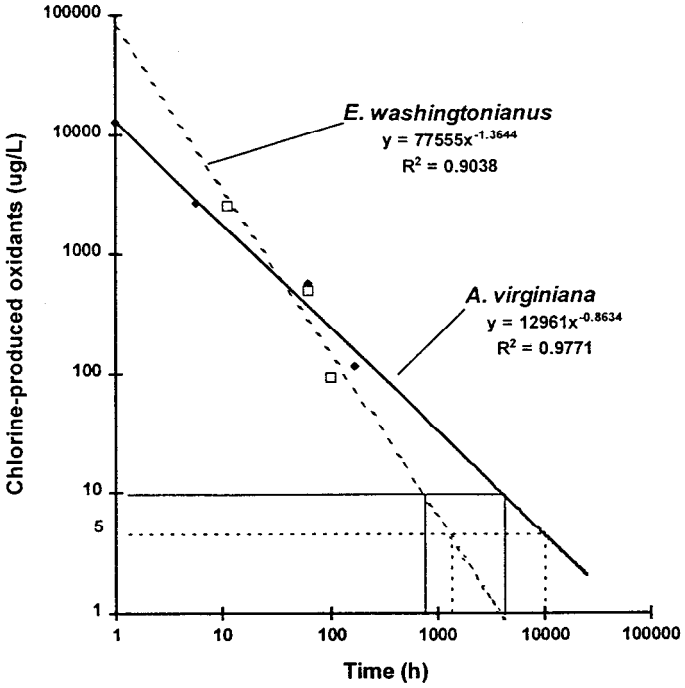

Figure 1. LT50 of chlorine-produced oxidants of *Amphiporeia virginiana* and *Eohaustorius washingtonianus*

This study has three limitations. First, the LT50 of each test concentration was an estimated (via statistical calculation) mean time of the 50% amphipod mortality and not an observed mean LT50. Second, the current method cannot differentiate MC from other inorganic amines. When sea water was spiked with small amount of MC (i.e., 0.020 to 12.5 mg/L), undetermined amounts of other inorganic amines were also likely formed, as sea water contains about 67, 19350, 0.6, and 1.3 mg/L Br^-, Cl^-, I^-, and F^-, respectively (Wilson, 1975; Jolley and Carpenter, 1983). It is known that some of these inorganic amines may have a higher reactivity than MC, e.g., bromamines, thereby having greater toxicity to living organisms (Fisher et al., 1999). Third, both LT50s and LC50s may likely be over/under estimates of toxicity, as varying amounts of CPO disappeared during the exposure period. This problem was partially addressed by using the arithmetic mean concentration of the verified initial (0 h) and final (12 h) CPO concentration of each test solution to determine toxicity. No published CPO LT50 nor LC50 toxicity data for *A. virginiana* and *E. washingtonianus* are available for comparison.

Although there are limited sources of inorganic chloramine releases to the marine environment in British Columbia, chloraminated water could potentially be discharged to the marine aquatic environment in three ways: (a) a sudden input of large volumes of chloramine-treated water, as in the case of breaks in municipal water mains, (b) a steady and/or(c) intermittent release of waste treatment

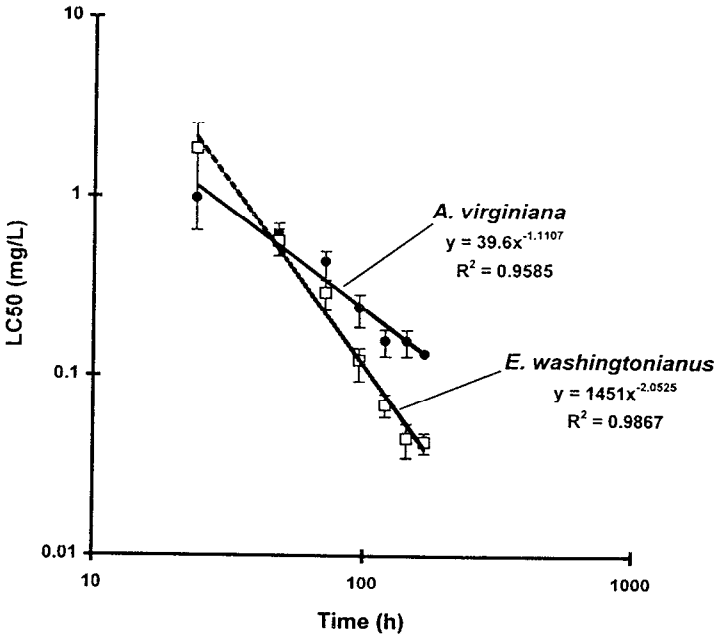

Figure 2. LC50 (mg/L) versus Time (h) of *Amphiporiea virginiana* and *Eohaustorius washingtonianus;* error bars = 95% C.I. (n = 3)

effluents from industrial complexes or urban centers. Most water treatment plants would inject a concentration equivalent to a residue of about 0.5 - 2 mg/L chloramine in the treated water (Leao and Selleck, 1983; Haas et al. 1990; Pasternak, 1998). Some water-main breaks have had a disastrous impact on fresh water 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 2000 juvenile salmonids (Nikl and Nikl, 1992). The impact of steady or intermittent chloramine releases from effluents on aquatic invertebrates in sea water is likely a function of the resulting bio-active concentrations of these chemicals prevailing in the marine aquatic environment.

It is speculated that chloramine concentrations of 0.5 -1 mg/L resulting from breaks or an unusual effluent discharge would produce CPO that may have an adverse acute impact on this aquatic invertebrate in sea water, e.g., Halifax, Nova Scotia, within 24 to 48 h. Discharges from leaks generating lower CPO concentrations, e.g., 0.02 mg/L, would probably have little or no adverse lethality impact on *A. virginiana* nor *E. washingtonianus.* This study suggests that when exposed to this low CPO concentration and under the same environmental conditions, the deleterious effect is much less severe to the Atlantic *A. virginiana* (48 h LC50 = 0.626 mg/L) and the Pacific species *E. washingtonianus* (48 h LC50 = 0.567 mg/L) when compared with *Daphnia magna* (48 h LC50 = 0.017 mg/L) *(Wan, unpublished* data). Over a 7 day (168 h) exposure period, however, *A.*

virginiana (168 h LC50 = 0.134 mg/L) appears to be more hardy than *E. washingtonianus* (168 h LC50 = 0.043 mg/L). The lower test water temperature for A. *virginiana* (10°C) when compared with *E. washingtonianus (15°C)* may have affected the sensitivity of the test organism. Whether concentrations below 0.02 mg/L CPO would have sub-lethal effects to both marine invertebrates is presently not known.

The LT50 values of CPO to *A. virginiana* and *E. washingtonianus* seem to be inversely related to the concentration of the chemicals in seawater. The projected LT50 of A. virginiana and *E. washingtonianus* to 0.010 mg/L CPO was ≥4032 h (≥168 h) and ≥700 h, respectively. With an estimated 48 h LC50 values ranging from 0.567 mg/L to 0.626 mg/L, and 168 h LC50 values varying from 0.043 mg/L to 0.134 mg/L, CPO are highly toxic (Rieder,1985) to both marine invertebrates in 10°C and 15°C sea water of pH = 7.5 relative units.

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