Influence of Feeding and Organism Age on the Acute Toxicity of Sodium Bromide to *Artemia salina*

David Pillard (pillardda.tre@gmail.com)
TRE Environmental Strategies https://orcid.org/0000-0002-8227-1562
Kelly Tapp
ENSR: AECOM Ltd

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Abstract

Bromide is a common ion found in freshwater and marine systems. Although normally at relatively low concentrations, higher levels may occur in point-released wastewaters as well as nonpoint runoff from agricultural or industrial locations where bromide compounds are used as biocides and disinfectants. In this study, the potential toxicity of NaBr in a saltwater environment was studied using the brine shrimp, *Artemia salina*. The confounding factors of organism age at test initiation and pre-test feeding were included in the test design. Survival of brine shrimp nauplii in several NaBr treatments up to 11,000 mg Br^-/L (measured) was assessed after 24 h in both fed- and unfed-tests. In tests with unfed organisms, only the youngest (< 24 h old) nauplii had acceptable control survival (≥90%), while control survival for all of the tests with fed organisms (< 24 h old, < 48 h old, < 72 h old) was acceptable. There was also greater and more erratic mortality in the unfed tests. These data indicate feeding *A. salina* prior to initiating a short-term acute test improved performance. Not feeding the test organisms, especially in longer tests or when using > 24 h old organisms, may result in excessive control mortality and an invalid test. These studies show that, when healthy organisms are used in the toxicity tests, 11,000 mg/L of Br^- (~ 14,200 mg/L NaBr) is not acutely toxic to *Artemia salina*.

Introduction

Bromine (Br) compounds in the environment are typically present as bromide (Br^-) and occur as various minerals, including halides, oxides, and hydroxides (Flury and Papritz 1993). Bromide is commonly found in salt compounds combined with sodium, potassium, and other cations and, like chloride salts, is very soluble in water (WHO 2009). Bromide concentrations range from < 0.008 mg/L to 0.3 mg/L in freshwater (Woods et al. 1979; Lundström and Olin 1986) and around 65 mg/L in seawater (Sverdrup et al. 1942; Brown et al. 1995). Terminal waterbodies in arid regions can have much higher Br^- levels due to evapoconcentration. Salinity in the Dead Sea, for example, can easily exceed 200‰ (Wetzel 1983) with Br^- concentrations ranging from 4,100 to 11,000 mg/L (Livingston 1963; Sirkes et al. 1997).

Bromide, though a naturally-occurring ion, can occur at concentrations sufficiently high to have adverse ecological effects. It is generated, along with other ions, from several anthropogenic sources including oil and gas produced water, reject water from desalination operations, agricultural irrigation drain water, and metal and mine wastes (Pillard et al. 1999). Bromide is included in several biocides for different applications. The use of methyl bromide as a soil fumigant can result in the leaching of the Br^- ion and subsequent release through wastewaters (Wegman et al. 1983). Sodium bromide is used as a biocide in industrial applications and recreational swimming pools (Bartolomé and Sánchez-Fortún 2005). Although Br^- can occur in combination with several different ions and appear in a variety of compounds, sodium bromide (NaBr) is the most common form, being naturally present in freshwater, seawater, and hypersaline lakes.

Brine shrimp (*Artemia* sp.) have long been utilized as a convenient bioassay organism because of their availability as cysts, which can easily be stored and hatched out when the need arises. *Artemia* have been

used to assess the toxicity of a wide variety of materials including fungal toxins (Harwig and Scott 1971), organophosphorus insecticides (Sánchez-Fortún et al. 1996), chlorinated solvents (Sánchez-Fortún et al. 1997), agricultural antibiotics (Migliore et al. 1997), phytogenic antibiotics (Kamba and Hasan 2010), cyanobacterial extract (Jaki et al. 1999), dental materials (Pelka et al. 2000; Milhem et al. 2008), marine-derived pharmaceuticals (Carballo et al. 2002), and trace metals (Brix et al. 2003; Kokkali et al. 2011; Mohiseni et al. 2017). In this study, the influence of pre-test feeding and initial organism age on the response of *Artemia salina* to NaBr was evaluated, providing quantitative data on not only the aquatic toxicity of NaBr to a saltwater crustacean, but also for an optimal test design that can be used to minimize the impacts of factors that could confound data interpretation.

Methods And Materials

Test organisms

Artemia salina cysts were supplied by Argent Chemical Laboratories (Redmond, WA, USA), and stored in the dark at 4 °C until use. Brine shrimp nauplii were obtained by hatching the cysts at 29 °C according to USEPA procedures (USEPA 2002a). At the end of the 24 h incubation period, nauplii were harvested, separated from unhatched cysts, rinsed with USEPA Moderately Hard Water (MHW, USEPA 2002a), and transferred to a beaker containing approximately 150 ml of the brine shrimp culture water at a salinity of 29 ppt. Aliquots (~ 1 ml) were drawn and transferred to individual 300 ml crystallizing dishes containing 250 ml of culture water at 35‰. These cultures were maintained with gentle aeration at 25 °C and a 16 h light:8 h dark photoperiod until test initiation.

Comparison of fed and unfed Artemia

Both fed and unfed tests were conducted. For feeding studies, a food slurry was prepared by mixing 72 mg of yeast (Red Star®) with 25 ml of MHW. On the day of test initiation, 5 ml of the well-mixed food slurry were added to each crystallizing dish (containing the harvested brine shrimp) ~ 3 h prior to initiating the toxicity tests. The food was distributed by pipette in a spiral fashion on the water's surface and the water was gently swirled to facilitate food distribution. In tests with unfed *Artemia*, the organisms were transferred directly from the crystallizing dishes to the test chambers without any pre-feeding.

Age of Artemia salina

The time it takes for individual *Artemia* cysts to hatch varies, and the hatching rate increases as temperature rises. Sorgeloos et al. (1978) found that, at 20°C, hatching does not begin until about 25 h in seawater and 50% hatching is achieved at 30 h; at 24 °C, hatching begins at 17 h and 50% hatch is reached after 21 h. At 29 °C, it can be assumed that hatching begins somewhat earlier than 17 h and significantly exceeds 50% at the end of 24 h. In this study, therefore, the actual age of the hatched brine shrimp after 24 h of incubation was likely to vary from < 1 h to as much as 10 h. For the sake of simplicity and for easier comparison to other studies, *Artemia* test ages in this study will be referred to as < 24 h (the day nauplii are removed from the hatching funnel), < 48 h (24 h later), and < 72 h (48 h later).

Preparation of test solutions

Synthetic seawater at 35‰ (Crystal Sea® Marine Mix) was the base water (for spiking) and control water. NaBr (EM Science®) was added to the base water to produce the following nominal test concentrations (mg/L of NaBr): 84 (synthetic seawater control), 125, 250, 500, 1000, 2500, 5000, 10,000, and 15,000. The bromide concentration in each test treatment was measured using EPA Method 300, yielding the following Br^- concentrations (mg/L): 79, 97, 190, 380, 780, 1900, 3900, 7700, and 11,000 mg/L, respectively. Adding NaBr also increased the salinity of the test solutions (salinity of 47‰ in the highest concentration). Additional tests were conducted in which salinity was increased only by addition of NaCl to determine if *Artemia salina* was adversely affected by the higher salinity. No acute effects due to higher salinity (up to 47‰) were found (data not shown).

Test procedures

Artemia salina were exposed to the different NaBr concentrations for 24 h. Tests were conducted with < 24-, < 48-, and < 72-h old organisms. Two sets of tests were completed, one set that was fed the yeast slurry prior to test initiation and a second set that was unfed. All tests were conducted using four replicates of 10 organisms each per concentration. Test containers were 30-ml plastic cups containing 15 ml of test solution. The cups were randomly positioned and covered with a clean sheet of glass. The light intensity was 100 ± 50 ft-c, with a 16:8, light:dark photoperiod.

The number of organisms surviving in each replicate was recorded after 24 h of exposure. Acceptable control survival was set at 90%, which meets the USEPA (2002b) acute requirement for whole effluent toxicity (WET) tests and Vanhaecke et al. (1981). However, No Observed Adverse Effect Concentrations (NOAECs) and 24-h median lethal concentrations (LC_{50}s) were determined for all tests even if the controls did not meet the 90% survival criterion. Hypothesis testing to determine NOAECs was conducted, where appropriate, using Toxstat Version 3.5 (WEST, Inc. and Gulley 1996). The LC_{50}s were calculated, where possible, using USEPA (1994) software.

Results

Bromide concentrations

Measured Br^- concentrations in each test treatment were very similar to nominal values (Table 1). As Na^+ is the dominant cation in seawater, the impact of additional Na^+ from the added NaBr was relatively small, particularly in the lower test treatments. To calculate the NaBr concentration in each test concentration, it was assumed that all the Br^- in the Crystal Sea® salts used to prepare the synthetic seawater was originally present as NaBr.

Table 1
Nominal and measured concentrations of bromide in the test treatments

NaBr (mg/L, Nominal)	NaBr (mg/L, based on Measured Br)	Bromide (mg/L, Nominal)	Bromide (mg/L, Measured)
84 (control)	102	65	79
125	125	97	97
250	245	194	190
500	489	388	380
1,000	1,004	777	780
2,500	2,447	1,941	1,900
5,000	5,022	3,883	3,900
10,000	9,915	7,766	7,700
15,000	14,165	11,650	11,000

Comparison of fed and unfed Artemia

There were notable differences between survival of fed and unfed brine shrimp, with differences becoming more pronounced as the animals aged. Fed animals were darker and distinctly larger than the unfed brine shrimp, which grew increasingly pale. The naupliar stage of the test organisms was not determined, but anecdotal observations suggest that fed organisms tended to develop faster into later pre-metamorphic instars. All fed organisms had acceptable (100%) control survival (Table 2). In fact, the only tests to have 100% survival in the control treatment were those that were fed. In addition to the control organisms, survival in all NaBr treatments was ≥92.5% in the tests with fed animals. In the test with < 72 h brine shrimp that had been fed, a larger proportion (relative to < 24 h and < 48 h fed organisms) of treatments showed less than 100% survival, with the lowest survival (92.5%) in the highest (11,000 mg/L) Br^- treatment. These responses suggest that, even though survival of *A. salina* was not statistically reduced at 11,000 mg/L, their sensitivity may have been elevated, relative to younger animals.

Table 2
Survival of three different ages of fed and unfed *Artemia salina* nauplii when exposed to sodium bromide (NaBr)

		Survival (%) of *A. salina* (three age groups) after 24-h exposure to NaBr					
NaBr (mg/L)	Total Br^- (mg/L)	Unfed Organisms			Fed Organisms		
		< 24 h	< 48 h	< 72 h	< 24 h	< 48 h	< 72 h
102	79 (control)	95	85	80	100	100	100
125	97	82.5	85	90	100	100	100
245	190	87.5	92.5	72.5	100	97.5	97.5
489	380	87.5	100	85	100	100	95
1,004	780	95	95	82.5	100	100	100
2,447	1,900	92.5	90	82.5	100	97.5	95
5,022	3,900	97.5	92.5	92.5	100	100	97.5
9,915	7,700	92.5	77.5	80	97.5	100	92.5
14,165	11,000	90	67.5[a]	77.5	95	100	92.5
24-h LC50 (mg/L Br^-)		> 11,000	> 11,000	> 11,000	> 11,000	> 11,000	> 11,000
NOAEC (mg/l Br^-)		11,000	7,700	11,000	11,000	11,000	11,000

[a] Significantly (α = 0.05) reduced survival relative to the control

When using unfed *Artemia*, only the < 24 h organisms had acceptable (95%) control survival; tests using < 48 h and < 72 h unfed organisms yielded 85% and 80% control survival, respectively (Table 2). Survival of unfed *Artemia* at elevated Br^- concentrations was more variable. In 11,000 mg/L Br^-, survival was statistically reduced for the < 48 h unfed *Artemia*, but not for the < 24 h or < 72 h organisms, although survival of the latter in 11,000 mg/L Br^- was notably lower. In all tests, however, the 24-h LC_{50}s were greater than 11,000 mg/L Br^-, indicating that NaBr is not acutely toxic to *Artemia salina* at concentrations up to, and including, 14,165 mg/L.

Discussion

Relatively few studies have evaluated the toxicity of NaBr to marine organisms. Douglas and Horne (1997) reported no toxicity to *Americamysis* (formerly *Mysidopsis*) *bahia* at up to 480 mg/L Br^-. Pillard et al. (2000) studied the toxicity of several saltwater ions to *A. bahia*, inland silverside minnow (*Menidia beryllina*), and sheepshead minnow (*Cyprinodon variegatus*), and reported that NaBr toxicity to any of these three species was very low. The salinity-toxicity relationship models developed during this research

predicted Br^- LC_{50}s of 7,990 mg/L for mysid shrimp and 18,300 mg/L for the inland silverside minnow (Pillard et al. 1998). The low NaBr toxicity to these invertebrate and vertebrate species aligns with the current results for *A. salina*. Bartolomé and Sánchez-Fortún (2005) studied the acute and sublethal toxicity of NaBr and other biocides to *A. salina* and reported 24-h LC_{50}s of > 1,200 mg/L, 531.06 mg/L, and 42.42 mg/L as NaBr for 24 h, 48 h, and 72 h nauplii, respectively. These lower LC_{50} values (relative to the current investigation) may be due to unknown factors associated with their particular study design, but also may be at least partially associated with rapidly decreasing fitness in the older nauplii due to the lack of supplemental nutrition.

Many of the acute studies with *A. salina* described in the literature appear to have been conducted without feeding of the organism, although feeding details are often not explicitly stated. Vanhaecke et al. (1981) proposed a standardized short-term toxicity test method using *Artemia*, which could be utilized as a quick, uniform method of assessing chemical toxicants. No feeding was included in that 24-h test procedure. Since the 1980s, several standardized and promulgated bioassays have been developed that are now commonly used for the assessment of chemicals (e.g., OPPTS, OECD methods), as well as regulatory monitoring of wastewater discharges under the Clean Water Act (USEPA 2002a; 2002b; 2002c). While the use of *Artemia* as a bioassay organism has been criticized on several counts, including its presumed lack of sensitivity and absence from much of the marine ecosystem (Persoone and Wells 1987), there are reasons to consider it as a useful ecotoxicity test model (Nunes et al. 2006), particularly for hypersaline waterbodies, such as the Great Salt Lake, Utah, where it is the only filter-feeding representative, and thus a realistic sentinel for protection of a unique ecosystem.

The data presented here show that unfed *Artemia* nauplii can yield acceptable control survival in a 24-h test, although earlier range-finding studies indicated that acceptable 24 h control survival of unfed nauplii was neither guaranteed nor consistent (data not shown). Pre-test feeding of *Artemia* always resulted in acceptable control performance in 24-h tests. *Artemia* nauplii rapidly consume the ambient nutrition available in the egg, making supplemental pre-test food a necessity after 24 h to achieve acceptable, consistent control performance and results that accurately reflect the toxicity of a chemical or wastewater.

Declarations

Conflict of interest/Competing interests: The authors declare that they have no conflict of interest.

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Code availability: Not applicable

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