Influence of feeding and organism age on the acute toxicity of sodium bromide to *Artemia salina*

David A. Pillard 1 · Kelly L. Tapp 2

Accepted: 12 April 2021 / Published online: 2 May 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

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*.

Keywords Bromide · Toxicity · *Artemia* · Culture · Test method

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 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‰ (Hutchinson 1957) with Br⁻ concentrations ranging from 4100 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. Methyl bromide (MeBr), though phased out to a large degree in many countries, remains in limited use as a soil fumigant to control arthropods and other crop pests (FAO and UNEP 2001). Application of MeBr 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 insec-
ticides (Sánchez-Fortún et al. 1996), chlorinated solvents
(Sánchez-Fortún et al. 1997), agricultural antibiotics
(Migliore et al. 1997), phytogenic antibiotics (Kamba and
Hassan 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
(Crystal Sea® Marine Mix) at a salinity of 29‰. Aliquots (~1 ml)
drew 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 stu-
dies, a food slurry was prepared by mixing 72 mg of yeast
(Red Star®) with 25 ml of MHW. On the day of test initia-
tion, 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 signif-
ically 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 fol-
lowering 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, 770, 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 con-
centrations 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 con-
tainers were 30-ml plastic cups (polyethylene) containing 15 ml
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. Immobile but intact (no
apparent decomposition) organisms were prodded gently
with a blunt probe to determine if there was any appendage
movement; if not they were assumed to be dead. Acceptable
control survival was set at 90%, which meets the USEPA (2002a) acute requirement for whole effluent toxicity (WET) tests and vanhaeke et al. (1981). However, No Observed Adverse Effect Concentrations (NOAECs) and 24-h median lethal concentrations (LC50 values) 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 LC50 values 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.

#### 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 premetamorphic 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 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.

When using unfed Artemia, only the <24 h organisms had acceptable (95%) control survival; tests using <48 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 LC50 values 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 Americanmysis (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 sheephead 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⁻ LC50 values of 7990 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 LC50 values of >1200, 531.06, and 42.42 mg/L, as NaBr for 24, 48, and 72 h nauplii, respectively. These lower LC50 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, as there was no indication that Artemia were fed.

Artemia salina appears to be more tolerant of NaBr than certain freshwater zooplanktonic invertebrates, although some freshwater species also exhibit high bromide tolerance, at least from an acute standpoint. Canton et al. (1983)
calculated a 48-h LC50 value of 11,000 mg Br\(^{-}\)/L for the cladoceran *Daphnia magna*, which is very similar to the 48-h LC50 of 10,500 mg/L reported by Hermens et al. (1984). *Ceriodaphnia dubia*, a smaller member of Daphnidae, was found to be more sensitive than *D. magna*, with 48-h Br\(^{-}\)LC50 values of 3470 mg/L and 7980 mg/L, respectively (A. Cohen and W. Stubblefield, personal communication).

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.

### Data availability

Study data summaries are available; raw data availability may be limited by retrieval labor and overhead costs.

### Compliance with ethical standards

**Conflict of interest** The author declares no competing interests.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

### References

Bartolomé MC, Sánchez-Fortún S (2005) Effects of selected biocides used in the disinfection of cooling towers on toxicity and bioaccumulation in *Artemia* larvae. Environ Toxicol Chem 24 (12):3137–3142

Brix KV, Cardwell RD, Adams WJ (2003) Chronic toxicity of arsenic to the Great Salt Lake brine shrimp, *Artemia franciscana*. Ecotoxicol Environ Saf 54:169–175

Brown E, Colling A, Park D, Phillips J, Rothery D, Wright J (1995) Seawater: its composition, properties and behavior. The Open University, Milton Keynes, England

Canton JH, Wester PW, Mathijsse-Spiekman EAM (1983) Study on the toxicity of sodium bromide to different freshwater organisms. Food Chem Toxicol 21(4):369–431
Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD (2002) A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnol 2:17. https://doi.org/10.1186/1472-6750-2-17

Douglas WS, Horne MT (1997) The interactive effects of essential ions and salinity on the survival of *Mysidopsis bahia* in 96-h acute toxicity tests of effluents discharged to marine and estuarine receiving waters. Environ Toxicol Chem 16:1996–2001

FAO and UNEP (2001) Global report on validated alternatives to the use of methyl bromide for soil fumigation. In Labrada R, Forsnari L (eds) Food and agricultural organization of the United Nations and United Nations Environment Programme. Nairobi, Kenya.

Flury M, Papritz A (1993) Bromide in the natural environment: occurrence and toxicity. J Environ Qual 22(4):747–758

Harwig J, Scott PM (1971) Brine shrimp (*Artemia salina*) larvae as a screening system for fungal toxins. Appl Microbiol 21(6):1011–1016

Hermens J, Canton H, Steyger N, Wegman R (1984) Joint effects of a mixture of 14 chemicals on mortality and inhibition of reproduction in *Daphnia magna*. Aquat Toxicol 5(4):315–322

Hutchinson GE (1957) A treatise on limnology. Volume I geography, physics, and chemistry. John Wiley & Sons, New York, NY

Jaki B, Orjala J, Bürgi H-R, Sticher O (1999) Biological screening of cyanobacteria for antimicrobial and molluscicidal activity, brine shrimp lethality, and cytotoxicity. Pharmaceut Biol 37(2):138–143

Kamba AS, Hassan LG (2010) Antibacterial screening and brine shrimp (*Artemia salina*) toxicity of *Securidae longepedunculata* (Polygalaceae) root bark. African J Pharma Sciences Pharm 1(1):85–95

Kokkali V, Katramados I, Newman JD (2011) Monitoring the effect of metal ions on the mobility of *Artemia salina* nauplii. Biosensors 1:36–45. https://doi.org/10.3390/bios1020036

Livingston DA (1963) Chemical composition of rivers and lakes. In: Hutchinson GE (ed) The oceans, their physical, and chemical. Oxford Univ Press, New York, NY, p 104

Lundström U, Olin A (1986) Bromide concentrations in Swedish precipitation, surface and ground waters. Water Res 6:751–756

Migliore L, Civitareale C, Brambilla G, Dojmi di Delupis G (1997) Bromide in the natural environment: factors affecting its occurrence and toxicity. J Environ Qual 26(4):747–756

Mohseni M, Farhangi M, Agh N, Mirvaghefi A, Talebi K (2017) Toxicity and bioconcentration of cadmium and copper in *Artemia urmiana* nauplii. Iranian. J Toxicol 11(1):33–41. https://doi.org/10.29252/arakmu.11.1.33

Nunes BS, Carvalho FD, Guilhermino LM, Van Stappen G (2006) Use of the genus *Artemia* in ecotoxicity testing. Environ Pollut 144:453–462

Pelka M, Danzl C, Distler W, Petschelt A (2000) A new screening test for toxicity testing of dental materials. J Dentistry 28:341–345

Persoone G, Wells PG (1987) *Artemia* in aquatic toxicology: a review. In: Sorgellos P, Bengtson DA, Deceiller DA, Jaspers E (eds) Artemia research and its applications. Morphology, genetics, strain characterization, toxicology, Vol. I. Universa Press, Wetereen, Belgium, p 259–275

Pillard DA, DuFresne DL, Tietje JE, Caudle DD (1998) Development of salinity toxicity relationships for produced water discharges to the marine environment. GRI-97/0168. Final Report. Gas Research Institute, Des Plains, Illinois

Pillard DA, DuFresne DL, Caudle DD, Tietje JE, Evans JM (2000) Predicting the toxicity of major ions in seawater to mysid shrimp (*Mysidopsis bahia*), sheephead minnow (*Cyprinodon variegatus*), and inland silverside minnow (*Menidia beryllina*). Environ Toxicol Chem 19(1):183–191

Pillard DA, Hockett JR, DiBona DR (1999) The toxicity of common ions to freshwater and marine organisms. API Publication Number 4666. American Petroleum Institute, Washington, DC

Sánchez-Fortún S, Sanz F, Barahona MV (1996) Acute toxicity of several organophosphorous insecticides and protection by cholinergic antagonists and 2-PAM on *Artemia salina* larvae. Arch Environ Contam Toxicol 31:391–398

Sánchez-Fortún S, Sanz F, Santa-María A, Ros JM, De Vicente ML, Encinas MT, Vinagre E, Barahona MV (1997) Acute sensitivity of three age classes of *Artemia salina* larvae to seven chlorinated solvents. Bull. Environ Contam Toxicol 59:445–451

Sirkes Z, Schirmer F, Essen HH, Gurgel KW (1997) Surface currents and seiches in the Dead Sea. In: Niemi T, Ben-Avraham Z, Gat J (eds) The Dead Sea: The lake and its setting. Vol 36-Geol Geophys. Oxford Monographs. Oxford Univ Press, New York, New York, NY, p 104–113

Sorgeloos P, Remiche-Van Der Wielens C, Persoone G (1978) The use of *Artemia* nauplii for toxicity tests – a critical analysis. Ecotoxicol Environ Saf 2:249–255

Sverdrup H, Johnson M, Fleming R (1942) The oceans, their physics, chemistry and general biology. Prentice-hall, Englewood Cliffs, New Jersey

USEPA (1994) USEPA toxicity data analysis software. Version 1.5. United States Environmental Protection Agency, Cincinnati, Ohio

USEPA (2002a) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth Edition, EPA-821-R-02-012. United States Environmental Protection Agency, Washington, DC

USEPA (2002b) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Fourth Edition, EPA-821-R-02-013. United States Environmental Protection Agency, Washington, DC

USEPA (2002c) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition, EPA-821-R-02-014. United States Environmental Protection Agency, Washington, DC

Vanhaecke P, Persoone G, Claus C, Sorgeloos P (1981) Proposal for a short-term toxicity test with *Artemia* nauplii. Ecotoxicol Environ Saf 5:382–387

Wegman RCC, Hamaker P, de Heer H (1983) Bromide-ion balance of a Polder district with large-scale use of methyl bromide for soil fumigation. Food Chem Toxicol 21(4):361–367

WEST, Inc., Gulley DD (1996) Toxstat Version 3.5. Western Ecot systems Technology, Inc., Cheyenne, Wyoming

WHO (2009) Bromide in drinking water. WHO/HSE/WSH/09.01/6. World Health Organization, Geneva, Switzerland

Woods AE, Carlton RF, Casto ME, Gleason GI (1979) Environmental bromine in freshwater and freshwater organisms: factors affecting bioaccumulation. Bull Environ Contam Toxicol 23:179–185