Bull. Environ. Contam. Toxicol. (2005) 75:356–360

DOI: 10.1007/s00128-005-0761-5

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity of Gallium and Effects of Salinity on Gallium Toxicity to Brackish and Marine Organisms

N. Onikura,* A. Nakamura, K. Kishi

Japan Pulp and Paper Research Institute, Inc., 5-13-11 Tokodai, Tsukuba, Ibaraki 300-2635, Japan

Received: 23 February 2005/Accepted: 28 April 2005

The metal gallium belongs to group IIIa of the periodic table. It often exists as a trace element in diaspore, sphalerite, germanite, bauxite, and coal, whereas some flue dusts from burning coal are known to contain as much as 1.5% gallium (Hammond, 2003). Gallium is also one of the intermetallic elements that are increasingly being used in manufacturing high-speed semiconductors such as gallium arsenide (Yang and Chen, 2003a, b). Therefore, there is a possibility that a significant amount of gallium is being discharged into the environment.

In many studies, the evaluation of metal toxicity has been simply based on the total concentrations of the metal. Metal toxicity, however, largely depends on water quality including its salinity, hardness, alkalinity, and concentrations of natural organic matter. A number of studies have revealed that the metal toxicity is strongly related to salinity (Voyer and Modica, 1990; Lin and Dunson, 1993; Blackmore and Wang, 2003; Burke et al., 2003). Although the relationships between salinity and most of the metal toxicities were reviewed by Hall and Anderson (1995), they did not consider the effect of salinity on gallium toxicity. Only a few studies have been conducted to investigate the toxic effects of gallium; furthermore, these studies are limited to the freshwater fish, common carp or tilapia (Lin and Hwang, 1998; Betoulle et al., 2002; Yang and Chen, 2003a, b). Data on the toxic effects of gallium on brackish and marine species is not available. In this study, we examined the toxic effects of gallium to three brackish and marine species, and the effect of salinity on gallium toxicity.

MATERIALS AND METHODS

Acute toxicity tests were conducted using *Americamysis bahia*, *Brachionus plicatilis*, and *Artemia salina*. These tests using *A. bahia* were performed for 96 h by standard methods (US EPA, 1993). Ten newly-hatched nauplii were transferred to 50 ml of test medium in an 80- ml vessel. Each test was performed using one control and five exposure concentrations. Four replications were used for each exposure concentration. Water salinity was found to be approximately 26 g/l, with a pH value of approximately 7.5- 8.0, and a dissolved oxygen concentration in the range 80% - 99%. Temperature was maintained at 25 ± 1°C. The mortality was observed on a daily basis, and 24-h and

* *Present address:* Fishery Research Laboratory, Kyushu University, 2506 Tsuyazaki, Fukutsu, Fukuoka 811-3304, Japan
Correspondence to: N. Onikura

96-h median lethal concentrations (LC_{50}) were calculated based on the survival rates.

Acute toxicity tests using *B. plicatilis* were conducted for 24 h. A total of 100- 200 amictic females of *B. plicatilis* were collected from the stock maintained at our laboratory; the females were reared in 100 ml of culture medium for 24 h. These amictic females were classified according to the classification proposed by Hagiwara et al. (1988). After 24 h, newly-hatched rotifers were collected from the culture medium, and five individuals were then transferred to 1 ml of test medium contained in a 2- ml cell. Each test was conducted using one control and five exposure concentrations. Three replications were used for each exposure concentration. Water salinity was found to be approximately 26 g/l, with a pH value of approximately 7.5- 8.0, and a dissolved oxygen concentration range 80% - 99%. Temperature was maintained at 25 ± 1°C. The mortality was observed after 24 h, and 24-h LC_{50} was calculated based on the survival rates.

Acute toxicity tests using *A. salina* were performed for 48 h following the method proposed by Vanhaecke et al. (1981). The standard conditions for acute toxicity tests using *A. salina* included a temperature of 25°C, salinity of 35 g/l, and dark conditions. The salinity of the normal seawater was considered to be approximately 31 g/l because we selected the EPA medium as the standard seawater medium (US EPA, 1993). The *A. salina* cysts were incubated in 1000 ml of the seawater medium at 25°C and under a light intensity of 1000 lux in a glass cylinder. The medium was aerated using a small tube placed in contact with bottom of the cylinder containing the medium. Under these conditions, the time required for the cysts to hatch was approximately 24 h. Ten hatched nauplii were subsequently transferred to 10 ml test medium in a 30- ml vessel. Each test was performed with one control and seven exposure concentrations. Two replications were used for each exposure concentration. Temperature and pH were maintained at 25 ± 1°C and 7.5- 8.0, respectively. The mortality was observed on a daily basis, and LC_{50}s for 24-h and 48-h were calculated based on the survival rates. Acute toxicity tests using *A. salina* were also performed at several levels of salinities because *A. salina* is known as a euryhaline species (Ewing et al., 1980). The synthetic seawater medium was normally used as it has been internationally standardized as the seawater medium and recommended by US EPA (1993). The low salinity mediums (with salinities of 3.3, 5.9, 10.1, and 17.7 g/l) were obtained by diluting the standard seawater medium with Milli Q water.

Stock solutions of gallium were prepared by dissolving $GaCl_3$ (purity: 99.9 %; Wako Chemicals, Osaka, Japan) in Milli Q water to attain the gallium concentration of 10000 mg/l (in the form of Ga ion).

The results of each acute tests conducted during the study were used to calculate the median lethal concentration (LC_{50}) at 95% confidence interval (CI) using the trimmed Spearman-Karber method or the probit method (US EPA, 1993). Data analyses using linear regressions were conducted using the StatView 5 program (SAS Institute Inc.).

RESULTS AND DISCUSSION

To date, no study has been conducted to examine the toxic effects of gallium on brackish and marine animals. This is the first report on gallium toxicity to marine species. Acute gallium toxicities in *A. bahia*, *B. plicatilis*, and *A. salina* are summarized in Table 1. The 96-h LC_{50} values ranged from 10 to 50 mg/l with the highest sensitivity of *B. plicatilis* to gallium toxicity among these species.

Table 1. The median lethal concentrations (LC_{50}) and 95 % confidence interval (95% CI) on the gallium acute toxicity tests by normal test mediums using *A. bahia*, *B. plicatilis* and *A. salina*

Species	Exposure periods	LC_{50} (mg/l)	95% CI (mg/l)
A. bahia	24 h	22.47	14.92 - 28.67
A. bahia	96 h	12.76	10.36 - 15.71
B. plicatilis	24 h	11.48	8.11 - 16.25
A. salina	24 h	54.64	51.07 - 58.46
A. salina	48 h	52.78	47.97 - 58.07

The 96-h LC_{50} was found to be 96 mg/l for adult common carp (Betoulle et al., 2002), and it was 13 and 20 mg/l for the fry and juvenile stages of common carp, respectively (Yang and Chen, 2003a, b). The 96-h LC_{50} was 36 mg/l for the 3-day-old tilapia (Lin and Hwang, 1998). These values indicate that gallium is not as acutely toxic as other well known heavy metals such as cadmium, copper, or zinc to either the freshwater species or the marine water species. Although a few reports are available on the concentration or distribution of rare earth elements in environmental waters (He et al, 2004), gallium concentrations are generally not reported. Therefore, for the toxicity evaluation of gallium, it is necessary to measure the gallium concentration in these effluents or waters.

The 24-h and 48-h LC_{50}s of gallium were similar, suggesting that the exposure periods did not affect the acute toxicity in *A. salina*. As expected, the longer the test duration, the lowest the gallium concentration needed to achieve the same toxic effect. Other researchers (Yang and Chen, 2003a) have noted that the growth rates of younger organisms (fry stage) of the common carp were more affected by gallium toxicity than the older stages. Therefore, chronic effects of gallium might be observed at much lower concentrations than its acute toxicity for the freshwater species or the marine water species. These results suggest that the chronic effects of gallium might be more significant than its acute toxicity.

The effect of salinity on the gallium toxicity was investigated for *A. salina*. Both 24-h and 48-h LC_{50} values were strongly correlated with salinities and the positive relationships were observed between the salinities and LC_{50} values (Fig. 1), indicating that higher salinity interfered with the gallium toxicity to *A. salina*.

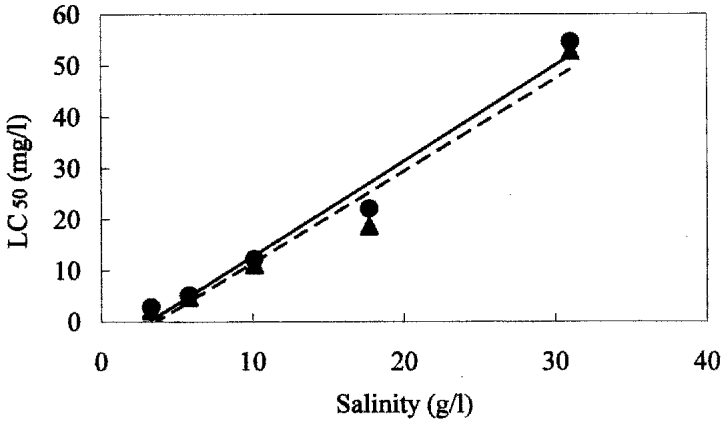

Figure 1. Gallium toxicity (24 h: Circle; 48 h: Triangle) to *Artemia salina* a function of salinity. Linear regression between LC_{50} (L) and salinity (S was fitted as follows:
24 h (Solid line): L = 1.866 S - 5.901 (r = 0.989, P = 0.0014)
48 h (Dotted line): L = 1.801 S - 6.557 (r = 0.980, P = 0.0033)

The influence of salinity on metal toxicity has been reported in several studies. The toxicities of most of the metals, such as cadmium and zinc, increase with decreasing salinity (Lin and Dunson, 1993; Voyer and Modica, 1990; Hall and Anderson, 1995). The salinity is known to influence the accumulation and uptake of metals (Blackmore and Wang, 2003; Burke et al., 2003). Based on the results for *A. salina* in this study, it is suggested that the effect of salinity on the toxicity of gallium is similar to that of other metals. In addition, gallium is known to interfere with calcium uptake. Warrell (1991) applied gallium nitrate to inhibit the process of bone resorption in patients suffering with hypercalcemia. Therefore, further research involving gallium accumulation and inhibition of calcium under differing salinities is needed to elucidate the toxic mechanism of gallium in the aquatic animals.

Acknowledgements We are grateful to Ms. K. Sakai, Ms. M. Shindo, Ms. A. Iijima, and Ms. F. Hatakeyama for their technical assistance with the acute tests.

REFERENCES

Betoulle S, Etirnne JC, Vernet G (2002) Acute immunotoxicity of gallium to carp (*Cyprinus carpio* L.). Bull Environ Contam Toxicol 68: 817-823

Blackmore G, Wang WX (2003) Inter-population differences in Cd, Cr, Se, and Zn accumu,lation by the green mussel *Perna viridis* acclimated at different salinities. Aquat Toxicol 62: 205-218

Burke J, Handy RD, Roast SD (2003) Effect of low salinity on cadmium accumulation

and calcium homeostasis in the shore crab (*Carcinus meanas*) at fixed free Cd^{2+} concentrations. Environ Toxicol Chem 22: 2761-2767

Ewing RD, Conte FP, Peterson GL (1980) Regulation of nucleic acid synthesis in *Artemia salina* nauplii by environmental salinity. American J Physiol 238: R91-96.

Hagiwara A, Hino A, Hirano R (1988) Effects of temperature and chlorinity on resting egg formation in the rotifer *Brachionus plicatilis*. Nippon Suisan Gakkaishi 54: 569-575

Hall LW, Anderson RD (1995) The influence of salinity on the toxicity of various classes of chemicals to aquatic biota. Crit Rev Toxicol 25: 281-346

Hammond CR (2003) THE ELEMENT. Handbook of chemistry and physics (Editor in chief: Lide DR), 84th edit., Sec. 4, 1-36 p.

He J, Mi N, Kuwang YC, Fan QY, Wang X, Guan W, Li GH, Li CS, Wang XW (2004) Speciation and distribution characters of rare earth elements in the Baotou Section of the Yellow River. Huan Jing Ke Xue 25: 61-66

Lin HC, Dunson WA (1993) The effect of salinity on the acute toxicity of cadmium to the tropical, estuarine, hermaphroditic fish, *Rivulus marmoratus*: a comparison of Cd, Cu, and Zn tolerance with *Fundulus heteroclitus*. Arch Environ Contam Toxicol 25: 41-47

Lin HC, Hwang PP (1998) Acute and chronic effects of gallium chloride (GaCl3) on tilapia (*Oreochromis mossambicus*) larvae. Bull Environ Contam Toxicol 60: 931-935

US EPA (1993) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms (Fourth Edition). EPA 600/4-90/027F. Environmental Protection Agency, Washington, DC.

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

Voyer RA, Modica G (1990) Influence of salinity and temperature on acute toxicity of cadmium to *Mysidopsis bahia* molennock. Arch Environ Contam Toxicol 19: 124-131

Warrell RP (1991) Clinical trials of gallium nitrate in patient with cancer-related hypercalcemia. Semin Oncol 18: 26-36

Yang JL, Chen HC (2003a) Browth retardation and histopathology of common carp (*Cyprinus carpio*) exposed to gallium. Bull Environ Contam Toxicol 71: 240-247

Yang JL, Chen HC (2003b) Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere 53: 877-882