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<https://doi.org/10.5109/12859>

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出版情報：九州大学大学院農学研究院紀要. 53 (2), pp.467-469, 2008-10-28. Faculty of Agriculture, Kyushu University

バージョン：

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## Acute Toxicity of Thallium and Indium toward Brackish-Water and Marine Organisms

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(Received June 13, 2008 and accepted July 16, 2008)

We examined the toxic effects of thallium and indium on brackish-water and marine species. Acute toxicity tests were conducted on *Americamysis bahia*, *Brachionus plicatilis*, *Artemia salina*, and *Sillago japonica*. The  $LC_{50}$  values of thallium ranged from 3.48 to 100 mg/L, and this metal exhibited the strongest toxic effects on *A. bahia*. With regard to indium toxicity, the  $LC_{50}$  values ranged from 24 to 51 mg/L, and the strongest toxic effects were noted in *B. plicatilis*. The toxicity of thallium is higher than that of other rare metals toward both freshwater and marine species. Further, we examined the effects of salinity on thallium and indium toxicities toward *A. salina*. We found that the survival rates of *A. salina* individuals following exposure to thallium or indium were strongly influenced by the salinity of the medium. The 48-h  $LC_{50}$  of these metals decreased as the salinity of the medium decreased.

### INTRODUCTION

The metals thallium and indium belong to group IIIa of the periodic table. Thallium has industrial applications as a catalyst for many organic reactions and in the production of alloys and electric devices (LeBlanc and Dean, 1984). It is also used in the manufacture of electronic devices and special glass (Zitko, 1975). In the past, thallium has been used widely as a pesticide (Ralph and Twiss, 2002). Indium is used in the manufacture of bearing alloys, germanium transistors, rectifiers, thermistors, liquid crystal displays, high-definition televisions, batteries, and photoconductors (Hammond, 2003). Indium has also been used effectively in semiconductor technology (Lin and Hwang, 1998a). Although these metals are relatively obscure and rare, they have various industrial uses. Therefore, it is possible that significant levels of thallium and indium are continuously discharged into the environment. Here, we examined the toxic effects of thallium and indium in 3 brackish-water and marine species.

### MATERIALS AND METHODS

#### Methods used for testing brackish-water and marine species

Acute toxicity tests were conducted on *Americamysis bahia*, *Brachionus plicatilis*, *Artemia salina*, and *Sillago japonica*. These tests on *A. bahia* were performed for 96 h using standard methods (US Environmental Protection Agency (EPA), 1993). Ten newly hatched nauplii were transferred to 50 mL of the

test medium in an 80-mL vessel. Each test was performed using 1 control and 5 exposure concentrations, with 4 replications per exposure concentration. The 96-h median lethal concentrations ( $LC_{50}$ ) were calculated on the basis of the survival rates.

The acute toxicity tests on *B. plicatilis* were conducted for 24 h according to a previously reported method (Onikura *et al.*, 2005). Five newly hatched rotifers were transferred to 1 mL of the test medium in a 2-mL cell. Each test was conducted using 1 control and 5 exposure concentrations, with 3 replications per exposure concentration. The 24-h  $LC_{50}$  was calculated on the basis of the survival rates.

The acute toxicity tests on *A. salina* were performed for 48 h, following the method proposed by Vanhaecke *et al.* (1981). Ten hatched nauplii were transferred to 10 mL of the test medium in a 30-mL vessel. Each test was performed using 1 control and 7 exposure concentrations, with 2 replications per exposure concentration. The 48-h  $LC_{50}$  was calculated on the basis of the survival rates. Further, the acute toxicity tests for *A. salina* were also performed at several salinity levels because this species is a euryhaline species (Ewing *et al.*, 1980). Synthetic seawater is usually used as the medium for such tests because it has been internationally standardized and is recommended by the US EPA (2002). Low-salinity media (salinity levels, 3.3, 5.9, 10.1, and 17.7 g/L) were obtained by diluting the standard seawater medium with Milli-Q water.

The acute toxicity tests on *S. japonica* were performed for 24 h according to the method proposed by Onikura *et al.* (2006). Ten to twenty fertilized eggs were transferred to 2.5 mL of the test medium in a 3.0-mL vessel. Each test was performed using 1 control and 5 exposure concentrations, with 4 replications per exposure concentration. The 24-h  $EC_{50}$  was calculated on the basis of the hatching rates. In both tests, the medium conditions such as the salinity, temperature, and dissolved oxygen were controlled, as shown in Table 1.

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**Table 1.** The conditions used for acute toxicity tests on several brackish-water and marine species in this study

species	exposure period (h)	Salinity (mg/L)	Temperature (°C)	Dissolved oxygen (%)
<i>A. bahia</i>	96	26	25±1	80–99
<i>B. plicatilis</i>	24	26	25±1	80–99
<i>A. salina</i>	48	31	25±1	80–99
<i>S. japonica</i>	24	32±1	26±1	80–99

### Test chemicals and data analyses

Stock solutions of thallium and indium were prepared by dissolving  $Tl_2SO_4$  and  $InCl_3 \cdot 4H_2O$  (99.9% purity; Wako Chemicals, Osaka, Japan) in Milli-Q water to a concentration of 10,000 mg/L (in the form of the Tl and In ions).

The results of each acute toxicity test conducted during the study and the theoretical concentrations of the dissolved chemicals were used to calculate the median lethal concentrations at 95% confidence intervals (*CI*s) by using the trimmed Spearman–Karber method or the probit method (US EPA, 1993) and to calculate the median-effective concentrations (*EC*<sub>50</sub>) and standard deviation (*SD*) by using point estimation techniques (Hamilton *et al.*, 1977).

## RESULTS AND DISCUSSION

### Toxicity of thallium and indium toward brackish-water and marine species

Thus far, only a few studies have been conducted to investigate the toxic effects of thallium and indium; furthermore, these studies have been limited to freshwater species (Zitko, 1975; Zitko *et al.*, 1975; LeBlanc and Dean, 1984; Lin and Hwang, 1998a; Pickard *et al.*, 2001; Ralph and Twiss, 2002; Lin *et al.*, 2005). To date, no study has been conducted to examine their toxic effects on brackish-water and marine animals. The present report is the first to discuss the toxicity of these metals toward marine species. The results of the acute toxicity tests for thallium performed on *A. bahia*, *B. plicatilis*, *A. salina*, and *S. japonica* are summarized in Table 2. This metal was found to be toxic in the concentration range of 3.48–100 mg/L and to exert the strongest toxic effects on *A. bahia*. The results of the acute toxicity tests for indium performed on *A. bahia*, *B. plicatilis*, *A. salina*, and *S. japonica* are summarized in Table 2. This metal was found to be toxic in the range of 24–51 mg/L and to exert the strongest toxic effects on *B. plicatilis*.

The median lethal, effective, or inhibitory concentrations of gallium, thallium, and indium, as determined by toxicity tests performed on several species in previous studies, are summarized in Table 3. These values indicate that the acute toxicity of thallium (Pickard *et al.*, 2001; Lin *et al.*, 2005) is higher than those of other rare metals such as gallium (Lin and Hwang, 1998b; Betoulle *et al.*, 2000; Yang and Chen, 2003; Onikura *et al.*, 2005) and indium (Lin and Hwang, 1998a) toward both freshwater and marine species. In addition, a few reports

**Table 2.** The median lethal concentrations (*LC*<sub>50</sub>) and 95% confidence intervals (*CI*s) determined in the acute toxicity tests for thallium and indium performed on *Americamysis bahia*, *Brachionus plicatilis*, and *Artemia salina*

Species	Exposure periods	<i>LC</i> <sub>50</sub> (mg/L)	<i>CI</i> (mg/L)
Thallium			
<i>A. bahia</i>	96 h	3.48	3.34–3.62
<i>B. plicatilis</i>	24 h	100	71.4–140
<i>A. salina</i>	48 h	10.7	8.60–13.4
<i>S. japonica</i>	24 h	NE*	NE*
Indium			
<i>A. bahia</i>	96 h	30.48	21.75–42.71
<i>B. plicatilis</i>	24 h	24.42	15.39–38.77
<i>A. salina</i>	48 h	51.00	45.60–56.90
<i>S. japonica</i>	24 h	NE*	NE*

\* NE\*: No effects noted at the highest concentration (20 mg/L).

**Table 3.** The median lethal concentrations (*LC*<sub>50</sub>) of gallium, thallium, and indium, as determined by performing acute toxicity tests on several freshwater species

Metals	Species	<i>LC</i> or <i>EC</i> <sub>50</sub> (mg/L)	Reference No.
Gallium	<i>Cyprinus carpio</i>	96	16
	<i>Cyprinus carpio</i>	13–20	17
	<i>Oreochromis mossambicus</i>	36	18
	<i>Brachionus plicatilis</i>	11.48	7
	<i>Americamysis bahia</i>	12.76–22.47	7
	<i>Artemia salina</i>	52.78–54.64	7
Thallium	<i>Daphnia magna</i>	2.01	14
	<i>Daphnia magna</i>	1.45–1.86	15
	<i>Ceriodaphnia dubia</i>	0.646–0.672	15
	<i>Oncorhynchus mykiss</i>	4.27	14
Indium	<i>Oreochromis mossambicus</i>	37	5

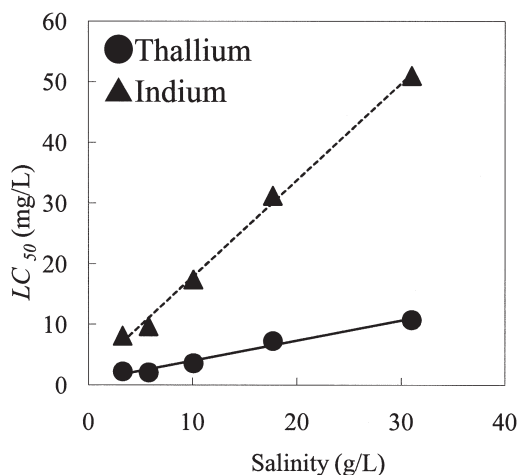
The reference numbers shown correspond to those in the Reference list of this article.

have described the concentration or distribution of rare earth elements in industrial effluents or environmental waters (He *et al.*, 2004; Chen, 2006). Indium and gallium are reported to be released as metal pollutants in groundwater through semiconductor industrial effluents in Taiwan (Chen, 2006); therefore, for evaluating the toxicity of these rare metals, it is necessary to measure their concentrations in a large number of effluent or water samples.

### Effects of salinity on the toxicity of thallium and indium

We investigated the effects of salinity on the toxicity of thallium or indium toward *A. salina*. A strong positive correlation was found to exist between the 48-h *LC*<sub>50</sub> values and the salinity of the medium (Fig. 1); this indicated that high salinity levels interfered with the toxicity of thallium and indium toward *A. salina*.

The toxicity of most of the metals, including cadmium, chromium, copper, mercury, nickel, vanadium, zinc, and gallium, increase as the salinity in the environment decreases (Voyer and Modica, 1990; Lin and Dunson,



**Fig. 1.** Toxicity of thallium (circles) and indium (triangles) toward *Artemia salina* as a function of salinity. The linear regressions between salinity (S) and  $LC_{50}$  (L) were fitted as follows:

$$\text{Thallium: } L = 0.332 S + 0.646 \quad (r = 0.989, p = 0.0014)$$

$$\text{Indium: } L = 1.599 S + 1.770 \quad (r = 0.998, p < 0.0001)$$

1993; Anderson *et al.*, 1995; Hall and Anderson, 1995; Ringelband, 2001; Craig *et al.*, 2003; Verslycke *et al.*, 2003; Onikura *et al.*, 2005). The results obtained in the tests conducted on *A. salina* in this study suggest that the effects of salinity on the toxicity on thallium or indium are similar to those on the toxicity of other metals. Therefore, further research investigating the accumulation and uptake of thallium and indium under varying environmental salinity conditions are necessary to elucidate the toxic effects of these metals in 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.

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