Acute and Subchronic Toxicity of Tributyltin Chloride (TBTCl) to the Marine Harpacticoid Copepod *Tigriopus japonicus* Mori

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ABSTRACT
Acute and subchronic toxicity experiments of tributyltin chloride (TBTCl) were conducted with the marine harpacticoid copepod *Tigriopus japonicus*. The 48-hr LC$_{50}$ and highest non-lethal concentration (NOLC) for adult females were 0.96 and 0.14 µg/L, respectively, whereas these values for adult males were 0.58 and 0.07 µg/L, respectively. For the mean cumulative number of nauplii produced per female, the 14-day highest no observed effect concentration (NOEC), lowest observed effect concentration (LOEC) and EC$_{50}$ were 0.025, 0.05 and 0.055 µg/L, respectively. The acute-subchronic ratio, i.e. the ratio of the 48-hr LC$_{50}$ for adult females to the 14-day highest NOEC, MATC (maximum acceptable toxicant concentration) and LOEC, was 38.5, 27.2 and 19.3, respectively. These results suggest that the concentrations of current ambient TBT (tributyltin) compounds in Japanese coastal waters can be assumed as the safety range for the survival, but are unlikely to cause a reduction in the number of nauplii produced per female of *T. japonicus*. The high concentrations in seawaters, sediments and/or seawaters released from sediments that have been observed in estuarine and coastal waters in Japan may lead to a considerable reduction of survival and numbers of nauplii produced by females for *T. japonicus*.

Keywords: *Tigriopus japonicus*, toxicity tests, tributyltin chloride (TBTCl)

INTRODUCTION
Organotin compounds, especially tributyltin (TBT) and triphenyltin (TPT), dissolved principally from organotin-based anti-fouling paint on ship-bottom and fishery equipments established in estuarine and coastal waters, are one of the most hazardous marine pollutants, and are biocidal to many aquatic organisms due to their high toxicity. In addition, even extremely low concentration levels of these compounds can cause a variety of serious abnormal symptoms for aquatic invertebrates and vertebrates, e.g. impairments in morphogenesis, growth, maturity and reproduction, highly skewed sex ratio toward females or males, and endocrine disruption (e.g. Bryan and Gibbs, 1991; Koyama and Shimizu, 1992; Horiguchi and Shimizu, 1992; Fent, 1996).

Marine pollution by TBT and TPT has been occurring globally not only in estuarine and coastal waters, but also in offshore oceanic areas (e.g. Yamada, 1999; Antizar-Ladislao, 2008). Although their concentrations in many estuarine and coastal waters in Japan have generally decreased in recent years after the regulation and prohibition of their use in the 1990s, they have been detected, occasionally in high concentrations, e.g. maximum TBT concentrations of 0.033-0.084 µg/L, 0.64-1.6 µg/g-dry weight and 0.025-0.78 µg/g-wet weight in seawaters, sea-bottom sediments and aquatic organisms in these waters in the 1990s, respectively (Harino *et al.*, 1997, 1998, 1999; Ministry of the Environment, 2007).
Many studies have been extensively performed to examine the acute and/or chronic toxic effects of TBT to marine organisms such as commercially important fishes, molluscs, shrimps and other crustaceans (e.g. Goodman et al., 1988; Kusk and Petersen, 1997; Lignot et al., 1998; Yamada, 1999; Hori et al., 2002; Ohji et al., 2002; Verslycke et al., 2003; Kwok and Leung, 2005). Most of these studies have focused on the evaluation of TBT concentration on acute and/or chronic toxicity to various marine organisms. In general, acute toxicity studies have measured the LC$_{50}$ (the lethal concentration to 50% of test organisms) at 24-96 hrs, whereas chronic toxicity studies have examined mainly the reduction of growth and reproduction in test organisms exposed to TBT. Although there is a huge amount of information available on acute toxicity of TBT, information on chronic toxicity is still relatively scarce (Antizar-Ladislao, 2008). Especially, there is yet little information on the toxic effects of TBT compounds on small marine organisms such as copepods that are important prey for various aquatic animals. For evaluating the detailed toxic effects of TBT that can be applied to the environmental risk assessment, it is necessary to examine several indexes such as the highest NOLC (no lethal concentration) in addition to the LC$_{50}$ in acute toxicity tests, the highest NOEC (no observed effect concentration), LOEC (lowest observed effect concentration), MATC (maximum acceptable toxicant concentration) and EC$_{50}$ (median effect concentration) in chronic toxicity tests and the acute-chronic ratio, which have not been simultaneously determined in most acute and chronic toxicity studies. In the present study, the toxic effects of TBTCl (tributyltin chloride) to the marine harpacticoid copepod *Tigriopus japonicus* Mori (*T. japonicus*) were examined. TBTCl is one of the 13 TBT species that have been designated as Class 2 Specified Chemical Substances in 1990 under the “Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances” in Japan. The toxicity level of TBTCl may be as high as that of tributyltin oxide (TBTO) (e.g. Ohji et al., 2002; Huang et al., 2006; Aono and Takeuchi, 2008), which has been designated a Class 1 Specified Chemical Substances under the same law. *Tigriopus japonicus* with body length of approximately 1.0 and 0.9 mm for adult female and male, respectively (Ito, 1970; Koga, 1970), is widely distributed along the coast of Japan (Ki et al., 2009), and can be an ideal marine model organisms for environmental studies such as ecotoxicity testing (Raisuddin et al., 2007). In this study, the acute and subchronic toxicities of TBTCl to *T. japonicus* were presented. The acute toxicity was expressed as the LC$_{50}$ and highest NOLC and the subchronic toxicity, which focused on the mean cumulative number of nauplii produced per female during 14 days was expressed as the highest NOEC, LOEC, MATC and EC$_{50}$.

MATERIALS AND METHODS
*Tigriopus japonicus* was collected using a hand net (100 µm in mesh opening size) at rocky tide pools located in Enoshima Island (Lat. 35°17′52″N, Long. 139°28′52″E), Fujisawa, Kanagawa, Japan. These copepods were transferred into a bottle (volume: 5 L) containing approximately 4 L of ambient surface seawater and taken to the laboratory within 1-2 hrs. Specimens were acclimatized at least for 7 days under laboratory condition (i.e. temperature: 24.0 ± 1.0°C; salinity: 34; light:dark photoperiod of 12L:12D) prior to experiment. The mono-cultured diatoms *Skeletonema costatum* or *Thalassiosira* sp. and raphidophycean flagellate *Heterosigma akashiwo* were sufficiently fed once a day prior to experiment. The seawater used for toxicity
The methods for preparing test solutions were based on Ohji et al. (2002), but an outline is briefly given as follows. Seawater (salinity: 34 psu) filtered through a glass-fiber filter (Whatman, GF/F) was used as control. Acetone solution of 0.05 mL/L, which was made by adding 0.1 mL of acetone to 2 L of filtered seawater, was used as acetone-control. The TBTCI solution was made by adding 2000 mg of tributyltin (IV) chloride (TBTCI, Wako Pure Chemical Industries, Ltd., Japan) to 1 L of filtered seawater with 0.05 mL/L acetone solution. The TBTCI solution of 500 µg/L was made by adding 0.5 mL of 2000 mg/L TBTCI solution to 2 L of filtered seawater, and the solution was stirred for 12 hrs by a magnetic stirrer. After stirring, the TBTCI solution was transferred into lidded glass bottles and stored at 4ºC. In the present study, test solutions of five TBTCI concentrations (0.1, 0.5, 1, 5 and 10 µg/L) and other five concentrations (0.01, 0.025, 0.05, 0.075 and 0.1 µg/L) were prepared by dilution of stock solution with 0.5% (v/v) acetone-filtered seawater solution. These condensed and diluted solutions were made every week, and test solutions were used for 48 hrs or renewed every 48 hrs as mentioned below. The TBTCI concentration of test solutions utilized for experiments was considered as the nominal one, although it was not determined in the present study, because Ohji et al. (2002) confirmed that the TBTCI concentrations in test solutions were almost identical to the nominal ones and the concentration levels remained the same even after 48 hrs.

The acute toxicity tests were conducted in conformity with the modified OECD Test Guideline, i.e. the ecological effect testing method in the risk assessment program of the Organization for Economic Cooperation and Development (OECD) (OECD, 1998). For acute toxicity tests, five adult females or adult males of *T. japonicus* were introduced into each glass bottle containing 50 mL of test solution. The experiment, with four replicates of the control, acetone-control and test solutions (TBTCI concentration: 0.1, 0.5, 1, 5 and 10 µg/L), was run for 48 hrs in an incubator (temperature: 24.0 ± 1.0ºC; light: dark photoperiod of 12L: 12D). The copepods were not fed during the experiment. After the exposure, the degree of survival of the copepods in each bottle was checked under a microscope.

The subchronic toxicity tests were conducted in conformity with the modified OECD Test Guideline (OECD, 2008). The approximate threshold response concentrations (i.e. highest NOLC) during the acute toxicity tests were selected as the highest test concentration for subchronic toxicity tests. For subchronic toxicity tests, ten ovigerous females of *T. japonicus* were individually introduced into each glass bottle containing 50 mL of test solution. For the control, acetone-control and test solutions (TBTCI concentration: 0.01, 0.025, 0.05, 0.075 and 0.1 µg/L), triplicates were incubated for 14 days at the same condition as for acute toxicity tests. Test solutions were renewed every 48 hrs. Each time these female copepods were transferred into renewed test solutions, the number of nauplii in each bottle was counted under a microscope.

Regression analysis was conducted to determine the relationships between the survival rate of *T. japonicus* and TBTCI concentration and between the inhibition rate (the
RESULTS

Acute toxicity in adult *T. japonicus*

All adult females and males of *T. japonicus* in the control and acetone-control solutions were always alive during the experiment, whereas they died in test solutions with TBTCI concentrations of 5 and 10 µg/L (Fig. 1). In addition, all adult females were always alive in test solutions with TBTCI concentration of 0.1 µg/L. In test solutions with TBTCI concentrations of 0.1, 0.5 and 1 µg/L, the survival rates of adult females were significantly higher than those of adult males (ANOVA, *p* < 0.001 for each concentration). In test solutions with TBTCI concentrations of 0.1-5 µg/L, the survival rate of adult females and males decreased with increasing TBTCI concentration. There were significant correlations between TBTCI concentration and survival rates of adult females and males, respectively (Fig. 1). From the obtained regression equations of TBTCI concentration-survival rate relationships, the 48-hr LC₅₀ for adult females and males was calculated to be 0.96 and 0.58 µg/L, respectively, while the highest NOLC for adult females and males was calculated to be 0.14 and 0.07 µg/L, respectively.

Subchronic toxicity in the number of nauplii

All ovigerous females in the control, acetone-control and test solutions were alive during the 14-day experiments. The nauplii (N1) of *T. japonicus* appeared in the control, acetone-control and test solutions on Day 2-4, and increased with experiment time, especially in the control, acetone-control and test solutions at lower TBTCI concentrations.
concentrations (Fig. 2). All nauplii in the control, acetone-control and test solutions were alive after hatching. The mean cumulative number (± SD) of nauplii produced per female at the end of the experiment (i.e. on Day 14) was 32.2 ± 4.7 individuals/female in control, 31.0 ± 4.2 individuals/female in acetone-control, and 31.2 ± 3.6, 28.2 ± 3.7, 17.7 ± 3.6, 6.7 ± 2.2 and 4.4 ± 1.2 individuals/female in test solutions with TBTCl concentrations of 0.010, 0.025, 0.050, 0.075 and 0.1 µg/L, respectively. There was statistically no significant difference between the mean cumulative numbers of nauplii produced per female in the control, acetone-control and test solutions with TBTCl concentrations of 0.01-0.025 µg/L throughout the experiment (Fig. 2). The mean cumulative numbers of nauplii produced per female in the test solution with TBTCl concentration of 0.05 µg/L were significantly lower than those in the control on Day 6 and 12-14, whereas the values in test solutions with TBTCl concentrations of 0.075-0.10 µg/L were significantly lower than those in the control on Day 4-14 (Fig. 2). The highest NOEC and LOEC were 0.025 and 0.05 µg/L, respectively. The MATC (i.e. geometric mean of NOEC and LOEC) was calculated to be 0.035 µg/L. The acute-subchronic ratio, i.e. the ratio of the 48-hr LC50 for adult females to the 14-day highest NOEC, MATC and LOEC, was calculated to be 38.5, 27.2 and 19.3, respectively.

The inhibition rate, i.e. the proportion of inhibiting production of nauplii during 14 days, increased with increasing TBTCl concentration (Fig. 3). There was a significant correlation between TBTCl concentration and inhibition rate. From the obtained regression equations of TBTCl concentration-inhibition rate relationship, the 14-day EC50 was calculated to be 0.055 µg/L.

![Fig. 2 - The cumulative number of nauplii produced per female of *T. japonicus*. The number of nauplii is expressed as mean (●) and SD (vertical bars). Values with asterisk (*) differed significantly from the values in control](image)
DISCUSSION

In the present study, the organic solvent (i.e. acetone) used to facilitate the solubilization of TBTCl into seawater was highly water-soluble and hard to enter the organism’s body due to its low penetrability across biomembrane. In addition, the acetone concentration in the acetone-control and test solutions was very low (approximately 0.05 mL/L). The values of survival rate and cumulative number of nauplii in acetone-control were mostly identical to those in control during the two experiments (Figs. 1 and 2). Thus, it was assumed that the toxicity of acetone utilized in the present study would be very low and negligible in comparison with that of TBTCl.

Acute toxicity tests of TBTCl have been conducted for several marine invertebrates (Table 1). The 48-hr LC\textsubscript{50} values for \textit{T. japonicus} obtained in the present study are higher than that for \textit{Acartia tonsa} (48-hr LC\textsubscript{50}: 0.24 and 0.47 µg/L (Kusk and Petersen, 1997)), and lower than those for all other marine invertebrates, especially the gammarid amphipods \textit{Cerapus erae}, \textit{Eohaustorioides} sp. and \textit{Jassa slatteryi} (48-hr LC\textsubscript{50}: 17.8-23.1 µg/L (Ohji \textit{et al.}, 2002)). This indicates that \textit{T. japonicus} has higher toxic sensitivity to TBTCl and can be a bioindicator for TBTCl pollution in environmental waters as well as \textit{A. tonsa}, rather than other tested organisms. For \textit{T. japonicus}, the 48-hr LC\textsubscript{50} values of TBTCl to adult females and males obtained in the present study are 6.5-fold and 3.9-fold higher than the 96-hr LC\textsubscript{50} values, respectively (0.15 µg/L (Kwok and Leung, 2005)). This might be caused by the difference in the duration of the experiment. Hori \textit{et al.} (2002) showed that the LC\textsubscript{50} values of TBTCl to the marine decapods \textit{Heptacarpus futilirostris} and \textit{Marsupenaeus japonicus} decline considerably with lengthening exposure time (Table 1). Koyama and Shimizu (1992) reviewed the time-dependent acute toxicity (i.e. 24-96-hr LC\textsubscript{50}) of TBTCl and TBTO to some marine fishes. Similarly, Ara \textit{et al.} (2004) stated the time-dependent acute toxicity (i.e. 24-96-hr LC\textsubscript{50}, highest NOLC and lowest LC\textsubscript{100}) of Bunker C refined oil to the Japanese littleneck clam \textit{Ruditapes philippinarum}. Thus, the acute toxic effects of chemical substances to marine organisms might be time-dependent, although the time-dependent acute toxicity of TBTCl to \textit{T. japonicus} was not evaluated in the present study.

Fig. 3 - Relationship between the inhibition rate (IR) of \textit{T. japonicus} and TBTCl concentration. Inhibition rate is expressed as mean (●) and SD (vertical bars).

\[
IR = 939\times\text{TBTCl} - 2.04 \quad (r^2 = 0.966,\ p < 0.0001)
\]
**Table 1 - Acute toxicity of TBTCl to marine invertebrates**

<table>
<thead>
<tr>
<th>Test animal</th>
<th>Temp. (°C)</th>
<th>Condition (salinity)</th>
<th>T* (hrs)</th>
<th>LC50 (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Copepoda</strong></td>
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<tr>
<td>Acartia tonsa</td>
<td>17.5 ± 0.5</td>
<td>Brackish water (18)</td>
<td>48</td>
<td>0.47</td>
<td>Kusk and Petersen (1997)</td>
</tr>
<tr>
<td>A. tonsa</td>
<td>17.5 ± 0.5</td>
<td>Brackish water (28)</td>
<td>48</td>
<td>0.24</td>
<td>Kusk and Petersen (1997)</td>
</tr>
<tr>
<td>Tigrigopus japonicus (adults)</td>
<td>25.0 ± 1.0</td>
<td>Seawater (34.5 ± 0.5)</td>
<td>96</td>
<td>0.15</td>
<td>Kwok and Leung (2005)</td>
</tr>
<tr>
<td>T. japonicus (adult females)</td>
<td>24.0 ± 1.0</td>
<td>Seawater (34)</td>
<td>48</td>
<td>0.96</td>
<td>This study</td>
</tr>
<tr>
<td>T. japonicus (adult males)</td>
<td>24.0 ± 1.0</td>
<td>Seawater (34)</td>
<td>48</td>
<td>0.58</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Mysidacea</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Mysidopsis bahia (1 day-old)</td>
<td>25.0 ± 1.0</td>
<td>Brackish water (19.0-22.3)</td>
<td>96</td>
<td>1.1</td>
<td>Goodman et al. (1988)</td>
</tr>
<tr>
<td>M. bahia (3 day-old)</td>
<td>25.0 ± 1.0</td>
<td>Brackish water (19.0-22.3)</td>
<td>96</td>
<td>2.0</td>
<td>Goodman et al. (1988)</td>
</tr>
<tr>
<td>M. bahia (5 day-old)</td>
<td>25.0 ± 1.0</td>
<td>Brackish water (19.0-22.3)</td>
<td>96</td>
<td>2.2</td>
<td>Goodman et al. (1988)</td>
</tr>
<tr>
<td>Neomysis integer</td>
<td>15.0</td>
<td>Brackish water (5)</td>
<td>96</td>
<td>0.15</td>
<td>Verslycke et al. (2003)</td>
</tr>
<tr>
<td><strong>Amphipoda: Caprellidae</strong></td>
<td></td>
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<tr>
<td>Caprella danilevskii (5.7 ± 1.0 mm)</td>
<td>20.0</td>
<td>Seawater</td>
<td>48</td>
<td>5.9</td>
<td>Ohji et al. (2002)</td>
</tr>
<tr>
<td>C. equilibra (8.0 ± 2.5 mm)</td>
<td>20.0</td>
<td>Seawater</td>
<td>48</td>
<td>6.6</td>
<td>Ohji et al. (2002)</td>
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<tr>
<td>C. penantis R-type (5.5 ± 1.0 mm)</td>
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<td>Seawater</td>
<td>48</td>
<td>1.2</td>
<td>Ohji et al. (2002)</td>
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<tr>
<td>C. sabinermis (6.3 ± 1.4 mm)</td>
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<td>48</td>
<td>4.3</td>
<td>Ohji et al. (2002)</td>
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<tr>
<td>C. verrucosa (4.9 ± 1.1 mm)</td>
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<td>48</td>
<td>1.3</td>
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</tr>
<tr>
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<td>Cerapus erae (3.2 ± 0.5 mm)</td>
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<td>48</td>
<td>21.2</td>
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<td>Eohaustorioides sp. (6.2 ± 0.7 mm)</td>
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<td>Seawater</td>
<td>48</td>
<td>23.1</td>
<td>Ohji et al. (2002)</td>
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<tr>
<td>Jassa slatteryi (5.1 ± 0.7 mm)</td>
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<td>Seawater</td>
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<td>17.8</td>
<td>Ohji et al. (2002)</td>
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<td><strong>Decapoda</strong></td>
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<td>Heptacarpus fuliurostris</td>
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<td>Seawater (34)</td>
<td>24</td>
<td>7.8</td>
<td>Hori et al. (2002)</td>
</tr>
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<td>Seawater (34)</td>
<td>48</td>
<td>5.6</td>
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<td>72</td>
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<td>Seawater (34)</td>
<td>96</td>
<td>3.2</td>
<td>Hori et al. (2002)</td>
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<td>Marsupenaeus japonicus</td>
<td>25.0</td>
<td>Seawater (34)</td>
<td>24</td>
<td>85.5</td>
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<td>Seawater (34)</td>
<td>48</td>
<td>5.2-53.5</td>
<td>Hori et al. (2002)</td>
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<tr>
<td>M. japonicus</td>
<td>25.0</td>
<td>Seawater (34)</td>
<td>72</td>
<td>4.8-42.8</td>
<td>Hori et al. (2002)</td>
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<td>M. japonicus</td>
<td>25.0</td>
<td>Seawater (34)</td>
<td>96</td>
<td>3.0-42.8</td>
<td>Hori et al. (2002)</td>
</tr>
</tbody>
</table>

*T*: exposure duration

On the basis of the LC50 and highest NOLC values for *T. japonicus* obtained in the present study, the toxic susceptibility of adult males to TBTCl was 1.6 to 2-fold higher than adult females. This can be explained by the copepod susceptibility to external stress that males are less tolerant to environmental stress than females (Davis, 1984).

The acute-subchronic ratio obtained for *T. japonicus* in the present study (19.3-38.5) was similar to the acute-chronic ratio obtained for the marine copepod *Eurytemora affinis* (15.2 to >25.0 (Hall et al., 1987, 1988; US EPA, 2003)). These values are within the range of the 50-90th percentile for each of the three trophic levels (i.e. algae, daphnids and fish) (Ahlers et al., 2006), although chemical substance, test organism (i.e. species, life stage), endpoint for acute and chronic toxicity tests and obtained acute and chronic values differed depending on the study. The present study showed that the 14-day EC50 was 11 to 17-fold lower than the 48-hr LC50, and that the 14-day highest NOEC was 2.8 to 5.5-fold lower than the 48-hr highest NOLC (Figs. 1 and 2). Egg, larval and early life stages of marine organisms are generally more sensitive and much less tolerant to environmental stress than adults and the later stages. The mean cumulative numbers of nauplii produced per female in the control, acetone-control and test solutions with TBTC concentrations of 0.01-0.025 µg/L during 14 days were similar to the mean brood size (15-35 eggs produced by a female copepod per brood) of *T. japonicus* (Koga, 1970; Lee and Hu, 1981; Hagiwara et al., 1995; Takaku et al., 2009). In fact, during the 14-day experiments, all ovigerous females of *T. japonicus*.
produced another brood, and nauplii hatched from these females two times, i.e. from the first brood on Day 2-4 and from the second one on Day 10-14, in the control, acetone-control and test solutions (Fig. 2). In these cases, the spawning interval would be 2-4 days, which is similar to that of 1-6 days (mean: 2-3 days) at a temperature of 24°C in laboratory culture experiments for *T. japonicus* (Takaku et al., 2009). In addition, there was no significant difference between the numbers of nauplii hatched from the first brood (previously produced before experiment) and second one (produced during experiment) in the control, acetone-control and test solutions, respectively. This implies that TBTCI would induce the failure of hatching success, but probably not the decrease in brood size, although individual brood size and hatching success of *T. japonicus* were not evaluated in the present study.

TBT compound (expressed as TBTO) concentrations in seawater, estuarine and coastal waters in Japan have been 0.00044-0.00076 µg/L in 2005 (Ministry of the Environment, 2007), which are 770 to 2189-fold and 91 to 311-fold lower than the 48-hr LC50 and highest NOLC, respectively. These concentrations can be assumed as the “safety range” for the survival of adult females and males of *T. japonicus*, because of the concentration being 10 to 1000-fold lower than its LC50 values (e.g. Howarth, 1989). These concentrations are 72 to 125-fold and 33 to 57-fold lower than the 14-day EC50 and highest NOEC, respectively. This implies that these concentrations are unlikely to cause a reduction in the number of nauplii of *T. japonicus*, because of the concentration being lower than the safety (uncertainty) factor of 100. On the other hand, relatively high concentrations (max. 0.027-0.043 µg/L) of TBT compound in seawater have been observed in the innermost areas of estuaries and coastal waters, such as harbors, marinas, fishery and trade ports, in Japan (Wang et al., 2004; Ohji et al., 2007; Onduka et al., 2008; Suzuki et al., 2008). These concentrations are 14 to 36-fold, 1.6 to 5.1-fold, 1.3 to 2-fold and 0.6 to 0.9-fold higher than the 48-hr LC50, the highest NOLC, 14-day EC50, and the highest NOEC, respectively. In these cases, *T. japonicus* are very much unlikely to maintain their population, especially due to the reducing numbers of nauplii produced by females. Similarly, relatively high TBT compound concentrations in sea-bottom sediments have remained up to the present in estuarine and coastal waters in Japan, e.g. 0.000085-0.590 µg/g-dry-weight in 2005 (Ministry of the Environment, 2007) and 0.0004-0.0019 µg/g-dry-weight in 2009 (Japan Coast Guard, 2010). These high TBTCI concentrations in sediments and/or seawaters released from sediments can considerably reduce the survival, reproduction and hatching success of marine organisms (e.g. *T. japonicus*) which inhabit in seawater-sediment environments, although the toxicity of TBTCI in sediment to *T. japonicus* was not evaluated. The present study showed that TBTCI concentration can be one of the important factors affecting the survival, reproduction and population dynamics of *T. japonicus* in natural environments. Consequently, for evaluating the toxic effects of TBTCI to *T. japonicus*, this suggests the necessity of chronic toxicity tests using naupliar and copepodite stages in addition to the acute and subchronic toxicity tests conducted in the present study.

**CONCLUSIONS**

Acute and subchronic toxicity of TBTCI to the marine harpacticoid copepod *T. japonicus* was studied, and the following results were obtained.

1. The 48 hr LC50 for adult females and males was 0.96 and 0.58 µg/L, respectively.
(2) The 48-hr highest NOLC for adult females and males was 0.14 and 0.07 µg/L, respectively.

(3) The maximum acceptable concentration (i.e. highest NOEC) on the number of nauplii produced per female during 14 days was 0.025 µg/L.

(4) The 14-day LOEC was 0.05 µg/L.

(5) The 14-day EC50 was 0.055 µg/L.

(6) The acute-subchronic ratio, i.e. the ratio of the 48-hr LC50 for adult females to the 14-day highest NOEC, MATC and LOEC, was 38.5, 27.2 and 19.3, respectively.

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