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## Distribution of Tributyltin in Tissues of Mature Japanese Whiting, *Sillago japonica* and Their Eggs

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Tributyltin (TBT) has continued to pollute the coastal areas therein following global regulation for its use as an anti-fouling agent. The tissue dynamics of TBT in fish have been extensively documented, but few studies on maternal transfer of TBT have been performed. Previously, we reported that TBT was maternally transferred from parent fish to eggs. The present study examined the distribution of TBT in the tissues and spawned eggs of Japanese whiting, *Sillago japonica*, after dietary exposure to TBT. The percentage distribution of whole-body TBT in each tissue was estimated to be 50% in blood, 29% in muscle, 1.7% in liver, 0.4% in brain, 3.3% in ovary, and 0.47% in eggs spawned in a single day. Of the TBT in whole eggs, 28.8% was in the yolk and 13.5% in the oil droplet. However, the concentration of TBT in the oil droplet was calculated to be 43 times higher than that in the yolk. These results indicate that blood is an important tissue for the accumulation of TBT in fish and suggest that TBT is maternally transferred along with the egg components.

### INTRODUCTION

Tributyltin (TBT) has been used as a biocide in marine antifouling paints since the 1960s. Because of its high toxicity to various aquatic organisms, most developed nations have adopted policies regulating or restricting its use in antifouling paints since 1982 (Fent, 1996). However, TBT continues to persist at levels, which are capable of affecting adverse impact on ecosystem, in water, sediment and organisms in the marine environment (Bhosle *et al.*, 2004; Lee *et al.*, 2006; Inoue *et al.*, 2006). The World Health Organization reports that TBT is a pollutant that poses a risk to marine ecosystems (Damstra *et al.*, 2002). Therefore, further toxicological research is needed to verify the potential impact of TBT on marine organisms.

Tissue distribution analysis of pollutants is one of the essential approaches for elucidating ecological risk of pollutants. Previously, we showed maternal transfer of TBT from parent fish to spawned eggs and its developmental toxicity in embryo of Japanese whiting (*Sillago japonica*) (Shimasaki *et al.*, 2006) and medaka (*Oryzias latipes*) (Nakayama *et al.*, 2005). However, distribution of TBT in eggs, which might affect bioavailability of TBT, is unknown. In other chemicals, some studies have reported the affinity of vitellogenin, a yolk protein precursor, for dichloro-diphenyl-trichloroethane (DDT) and polychlorinated biphenyls

(PCBs) in Atlantic croaker (*Micropogonias undulatus*) (Ungerer and Thomas, 1996a), for cadmium in red drum (*Sciaenops ocellatus*) (Ghosh and Thomas, 1995) and for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene in channel catfish (*Ictalurus punctatus*) (Monteverdi and Di Giulio, 2000). The distribution of chemicals within eggs is expected to vary depending on its chemical property. As far as we know, however, there is no study on TBT distribution within eggs of fish in spite of marine pollution by TBT up to date.

In adult fish, on the other hands, previous studies on tissue distribution of TBT have mainly focused on whole body and muscle or metabolic organ like liver. However, a few studies pointed out the behavioral effects of TBT on fish (Triebkorn *et al.*, 1994; Nakayama *et al.*, 2004a, b). The causal mechanism of these behavioral effects of TBT is complicated and currently obscure, but the ability of TBT to permeate neural tissue may be one of the important toxic factors. However, studies on occurrence of TBT in brain of fish were limited (Martin *et al.*, 1989; Rouleau *et al.*, 1998; Harino *et al.*, 2000).

Here, we investigated the distribution of TBT in the brain, blood, muscle, liver, gonad and egg components of marine fish Japanese whiting dietarily exposed to TBT oxide. Then we also compared distribution difference of TBT between both sexes or dose groups.

### MATERIALS AND METHODS

#### Preparation of test diet

To prepare test diets containing tributyltin oxide (TBTO, >95%; Tokyo Kasei Kogyo, Japan), 500 g of commercial diets (Himezakura, Higashimaru,

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Kagoshima, Japan) for fish were wetted with 100 ml of an ethanol solution containing TBTO (1, 10, or 100 mg), and then dried with an air blower. The concentrations of TBTO in test diets were prepared at 2, 20, and 200  $\mu\text{g/g}$  of food. For the control diet, 500 g of the commercial diet was wetted with 100 ml of ethanol and dried with an air blower.

### Fish

Adult Japanese whiting were collected from a ship by fishing off of the coast of Tsuyazaki, Fukuoka Prefecture, Japan in May 2000 at beginning of their reproductive season. Before the exposure test, about 200 captured fish were maintained in two 3-ton concrete tanks filled with about 3 m<sup>3</sup> of seawater at the Fishery Research Laboratory of Kyushu University, using a commercial diet (Higashimaru, Kagoshima, Japan) and under natural photoperiod and water temperature with flowing filtered seawater. After three weeks, 96 healthy, mature fish (60 males and 36 females) were selected and their total length and body weight were measured (mean total length, 160 mm in males, 182 mm in females; mean body weight, 38.3 g in males, 53.1 g in females). Sex was determined by observing the release of sperm after abdominal pressing. In general, Japanese whiting spawn well under a higher male-to-female ratio. Thus, the 96 fish selected were divided into twelve groups (3 females and 5 males in each group) and each group was placed in indoor 0.5-ton polyethylene tanks (test tanks) filled with flowing and aerated seawater (500 L/h) under natural photoperiod and water temperature. To minimize stress on the fish, half of the top of each test tank (aisle side) was covered with a translucent black sheet. The other half was covered with a transparent plastic sheet. These groups were fed a commercial diet three times a day for three weeks to acclimatize the fish before exposure to TBT. The daily feeding rate was 5% of the mean fish body weight.

### Exposure conditions

Fish were dietarily exposed to TBT for about two months from 26 July to 30 September in 2000. Each treatment group (3 tanks/treatment) was fed one of four test diets (0, 2, 20, or 200  $\mu\text{g/g}$  diet) three times a day. The daily feeding rate was 5% of the mean fish body weight. The diet and feces remaining in tanks was removed from tanks after 1 hour from feeding. Fish were reared under natural photoperiod and water temperature (22.5–29.6 °C) with flowing filtered seawater. Other conditions were the same as those during the acclimation period.

### Sampling of spawned eggs and tissue from parent fish

During the exposure period, the fish spawned almost daily between about 7 PM and midnight. Spawning was checked every hour from 7 PM by check in a collecting net fixed under the outlet pipe outside each test tank. Two hours after spawning, eggs were

gathered from a collecting net. During the first 30 days, reproductive toxicity test described in Shimasaki *et al.* (2006) was performed. After the first 30 days, TBT exposure was continued under same condition as first 30 days and only fecundity was checked until the time when reproductive season was almost finished (67 days from beginning of exposure). However no remark difference was observed in fecundity between control and TBT treatment groups (data not shown).

After 67 days, whole blood was withdrawn from the caudal vessel by syringe and then the gonad and liver were collected. These samples, residual fish body, and collected eggs were stored at –80 °C until TBT analysis. Brain and muscle tissue were collected from fish bodies just before TBT analysis.

### TBT analysis of tissue and whole body in parent fish

For analysis of TBT, dibutyltin (DBT) and monobutyltin (MBT), blood, brain, liver, muscle and gonad from each individual were homogenized separately with approximately 3 g of diatomaceous earth (Hyflo Super-Cel; Wako Pure Chemical, Osaka, Japan) using a mortar and pestle. Each sample was spiked with 1  $\mu\text{g}$  each of butyltin surrogates (TBT-d<sub>27</sub>, DBT-d<sub>18</sub>, MBT-d<sub>9</sub>; Hayashi Pure Chemical, Osaka, Japan).

The procedures following homogenization, including extraction and instrumental analysis, were performed by the method described in a previous study (Omura *et al.*, 2004). Briefly, homogenates were extracted with 0.1% tropolone benzene by an accelerated solvent extraction system (ASE 200; Dionex, Sunnyvale, CA, USA). The extract was concentrated by a nitrogen stream at 40 °C to 10 ml. This sample was ethylated by sodium tetraethylborate (Hayashi Pure Chemical, Osaka, Japan), then hydrolyzed with 10 ml of 1 mol/L potassium hydroxide solution. Hexane layers were cleaned up with a Florisil column (Waters, Milford, MA, USA). The resulting sample was measured with a gas chromatograph equipped with mass spectrometer (GC-MS; model 6890 gas chromatograph, model 5973 mass spectrometer; Hewlett-Packard, Palo Alto, CA, USA). The detection limit for butyltins was 10 ng/g sample.

We analyzed whole body samples for six females in the 20- $\mu\text{g/g}$  diet group. The body sample remaining after individual tissue analyses was homogenized in a blender (Oster, Milwaukee, WI, USA). Approximately 5 g of each homogenized sample was spiked with 1  $\mu\text{g}$  of TBTCI-d<sub>27</sub> as an internal standard. Procedures for extraction and instrumental analysis for TBT were the same as that described above for tissue samples. The whole-body TBT concentration was calculated by adding the sample weights and TBT amounts measured in blood, brain, liver, muscle, and ovary to those from the remaining whole body sample.

In present study, we didn't analyze TBT concentration of diet, therefore we use nominal concentration in results and discussion sections.

### Fractionation of eggs

We analyzed eggs from the 200- $\mu\text{g/g}$  diet group spawned 33 days after exposure because of its richness of eggs volume for fractionation of eggs. Samples were stored at  $-80^\circ\text{C}$  until analysis. Eggs were fractionated into three components (yolk, oil droplet, and chorion) by a method previously reported (Ungerer and Thomas, 1996b), with slight modification. Approximately 4.5 g (wet weight) of eggs was homogenized in 20 ml of 0.5 mol/L NaCl and centrifuged at  $14,500 \times g$  for 45 min. The resultant three fractions contain primarily the oil droplet in the surface layer, yolk in the middle layer, and chorion in the precipitate.

### TBT analysis of three egg fractions

For TBT analysis, each egg fraction was first spiked with  $1\mu\text{g}$  of TBTCl- $d_{27}$  as an internal standard, and then 10 ml of 1 mol/L hydrochloric acid-methanol solution was added. The rest of the procedure has been described previously (Inoue *et al.*, 2004). Briefly, the sample solution was extracted twice with 10 ml of 0.1% (w/w) tropolone-hexane solution. The extract was ethylated with sodium tetraethylborate solution and then hydrolyzed with 10 ml of 1 mol/L potassium hydroxide solution. Hexane layers were cleaned up with a Forisil column. The resulting sample was sub-

jected to measurement by GC-MS.

### Statistical analysis

To compare difference of butyltin concentrations between males and females, student's t-test was used after homogeneity of variance was confirmed by Levene's test. Probability of a significant difference was set at  $p < 0.05$ . The statistical package used in this study was SPSS Base 10.0J (SPSS, Tokyo, Japan).

## RESULTS

### Concentration of butyltins in tissues of parent fish

The concentrations of TBT, DBT and MBT in tissues of fish are shown in Table 1. In all treatment groups and for both sexes, the highest tissue concentrations of TBT were detected in blood, at levels 1.3–5.8 times those of brain tissue, 1.6–12.2 times those of liver, 1.7–16.3 times those of muscle, 2.5–5.7 times those of ovary, and 3.6–9.6 times those of testis. Brain tissue had the second-highest concentration of TBT in most groups. In contrast, there was no consistent order in TBT concentrations in liver, muscle, and gonad tissue.

For DBT and MBT the highest levels were observed in liver tissue. However, in almost all tissues except for liver, the concentrations of DBT and MBT were more

**Table 1.** Butyltin concentrations in tissues of Japanese whiting, *Sillago japonica*. (ng/g wet wt; mean  $\pm$  SD)

		Blood	Brain	Liver	Muscle	Gonad	
Control	Male	TBT	49 $\pm$ 16	31 $\pm$ 26	7 $\pm$ 4	7 $\pm$ 4	nda
		DBT	nda	nda	17 $\pm$ 4	nda	nda
		MBT	nda	6 $\pm$ 4	7 $\pm$ 4	nda	nda
	Female	TBT	28 $\pm$ 5	20 $\pm$ 24	nda	6 $\pm$ 3	nda
		DBT	8 $\pm$ 4	nda	14 $\pm$ 3	nda	nda
		MBT	nda	8 $\pm$ 5	8 $\pm$ 5	nda	nda
2 $\mu\text{g/g}$	Male	TBT	670 $\pm$ 228	115 $\pm$ 50	55 $\pm$ 15	41 $\pm$ 13	70 $\pm$ 24
		DBT	18 $\pm$ 11	6 $\pm$ 3	50 $\pm$ 21	nda	24 $\pm$ 5
		MBT	10 $\pm$ 7	7 $\pm$ 3	12 $\pm$ 8	nda	9 $\pm$ 6
	Female	TBT	589 $\pm$ 216	116 $\pm$ 26	51 $\pm$ 16	40 $\pm$ 10	110 $\pm$ 60
		DBT	23 $\pm$ 10	7 $\pm$ 6	57 $\pm$ 16	nda	26 $\pm$ 17
		MBT	13 $\pm$ 13	7 $\pm$ 3	22 $\pm$ 9	nda	7 $\pm$ 4
20 $\mu\text{g/g}$	Male	TBT	2447 $\pm$ 574	507 $\pm$ 155	339 $\pm$ 133	188 $\pm$ 44	261 $\pm$ 125
		DBT	139 $\pm$ 59	36 $\pm$ 28	759 $\pm$ 598	nda	62 $\pm$ 16
		MBT	39 $\pm$ 23	12 $\pm$ 7	99 $\pm$ 65	nda	28 $\pm$ 21
	Female	TBT	2250 $\pm$ 409	508 $\pm$ 189	428 $\pm$ 105	199 $\pm$ 43	397 $\pm$ 90
		DBT	136 $\pm$ 34	41 $\pm$ 26	824 $\pm$ 265	nda	76 $\pm$ 42
		MBT	17 $\pm$ 12	11 $\pm$ 6	134 $\pm$ 88	nda	24 $\pm$ 22
200 $\mu\text{g/g}$	Male	TBT	4069 $\pm$ 943	2193 $\pm$ 525	1615 $\pm$ 694	1370 $\pm$ 244	1141 $\pm$ 341
		DBT	394 $\pm$ 101	81 $\pm$ 74	4345 $\pm$ 2379	34 $\pm$ 32	192 $\pm$ 48
		MBT	232 $\pm$ 179	22 $\pm$ 19	723 $\pm$ 476	nda	54 $\pm$ 30
	Female	TBT	4001 $\pm$ 1524	3104 $\pm$ 1525	2430 $\pm$ 1155	2401 $\pm$ 1396	1604 $\pm$ 740
		DBT	391 $\pm$ 268	133 $\pm$ 100	3490 $\pm$ 1022	64 $\pm$ 47	191 $\pm$ 54
		MBT	209 $\pm$ 224	23 $\pm$ 11	351 $\pm$ 121	6 $\pm$ 9	28 $\pm$ 10

“nda” means not detected in any samples analyzed (detection limit  $< 10\text{ ng/g}$ ). For calculation of the mean values, undetected samples were assigned a value of one half the detection limit ( $5\text{ ng/g}$ ). Number of samples was 6, except for the female gonad at 20  $\mu\text{g/g}$  ( $n=5$ ). TBT, tributyltin; DBT, dibutyltin; MBT, monobutyltin

than an order of magnitude lower than that of TBT. In particular, the metabolites in muscle tissue were below the detection limit (10 ng/g), except in the 200- $\mu$ g/g group. The order of mean metabolite concentrations in tissues was generally liver > blood > gonad > brain > muscle.

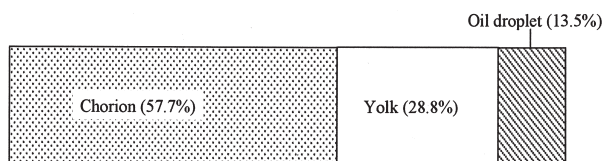
No overt differences in butyltin concentrations were observed between males and females, although there were statistic difference between both sexes only in TBT concentration in blood among control group ( $p=0.01$ ) and MBT concentration in liver among 2  $\mu$ g/g group ( $p=0.047$ ).

### Whole-body TBT concentrations

The whole-body concentration of TBT in females from the 20- $\mu$ g/g diet group was  $300 \pm 83$  ng/g (mean  $\pm$  standard deviation,  $n=6$ ). Thus, the bio-magnification factor—the ratio of the TBT concentration in the whole body to that in the diet—was estimated to be 0.015.

### Distribution of TBT in eggs

We determined the percent distribution of TBT in whole eggs among the three egg fractions (Fig. 1). Chorion, yolk, and oil droplet contained 57.5%, 28.8%, and 13.5% of the TBT, respectively.



**Fig. 1.** Percentage distribution of total TBT in eggs of Japanese whiting, *Sillago japonica*.

### Ratio of TBT concentrations in yolk and oil droplet

The yolk globule and oil globule of Japanese whiting have almost spherical forms with radii of 0.34 mm and 0.075 mm, respectively (Kumai and Nakamura, 1977). Thus, the volumes of the yolk and oil droplet, calculated by using the formula for the volume of a sphere ( $V=4/3\pi r^3$ ), were 0.163 mm<sup>3</sup> (equal to 0.163  $\mu$ l) and 0.00177 mm<sup>3</sup> (equal to 0.00177  $\mu$ l). Therefore, for example, our distribution ratios indicated that if one egg contained 1 pg of TBT then the yolk would contain 0.288 pg and the oil droplet would contain 0.135 pg. The resulting TBT concentrations in yolk and oil droplet were calculated at 1.77 pg/ $\mu$ l and 76.4 pg/ $\mu$ l, respectively. Therefore, the TBT concentration in the oil droplet was estimated to be 43 times higher than that in the yolk.

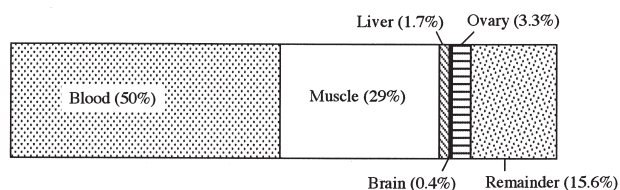
## DISCUSSION

As a result of dietary exposure of Japanese whiting to TBT for about 2 months, high concentrations of TBT accumulated in the blood of the test fish, at concentrations 1.7–16.3 times higher than in the muscle. High

concentrations of TBT in blood have been reported in some fish species. Oshima *et al.* (1997) reported that the mean concentrations of TBT in the plasma of cultured or wild fish were higher than concentrations in muscle by a factor of 17.1–23.5 in Japanese flounder (*Paralichthys olivaceus*), 20.1 in flatfish (*Limanda yokohamae*), 6.6 in red sea bream (*Pagrus major*), and 2.5 in yellow tail (*Seriola quinqueradiata*). On the basis of tissue dry weights, Shim *et al.* (2002) reported that the concentration of TBT in serum was 200 times higher than that in the muscle of fine-spotted flounder (*Pleuronichthys cornutus*). Concentrations of TBT in the blood of mummichog (*Fundulus heteroclitus*) were 1.7–2.4 times higher than in muscle following 56 days of waterborne exposure (Hori *et al.*, 2004), and in rainbow trout (*Salmo gairdneri*), blood concentrations were 1.3–2.1 times higher than muscle concentrations after 15 days of waterborne exposure (Martin *et al.*, 1989). In contrast, relatively low concentrations of TBT were detected in the blood of the finless porpoise, a marine mammal, at levels 0.007–0.2 times that in muscle (Iwata *et al.*, 1995). Furthermore, a previous study in mammals indicated that TBT levels in the blood of rats rapidly decreased after injection of TBT (Matsuda *et al.*, 1993) and the concentration of TBT in blood was 0.09 times that in the muscle of mice exposed to TBTO for 30 days via drinking water (Evans *et al.*, 1979). From these data, accumulation of TBT in blood may be a phenomenon that occurs mainly in fish. The accumulation of TBT in the blood of fish may result from binding of TBT to a TBT-binding protein, which has been identified in the blood of Japanese flounder (Shimasaki *et al.*, 2002; Oba *et al.*, 2007), as well as from lower metabolic activity in the livers of fish than that of mammals.

Table 1 shows that TBT accumulates in the brain of fish at levels intermediate to those in blood and other tissues analyzed. Several studies report the occurrence of TBT in the brains of rainbow trout (Martin *et al.*, 1989), Japanese sea perch (*Lateolabrax japonicus*), white croaker (*Pennahia argentatus*) and yellowtail (Harino *et al.*, 2000). Rouleau *et al.* (1998) also indicated uptake of <sup>113</sup>Sn in the brains of rainbow trout fed [<sup>113</sup>Sn]-TBT. TBT is known to have neurotoxicity in organisms (Fent, 1996). Some studies have pointed out the behavioral effects of TBT on fish. Triebkorn *et al.* (1994) reported that TBTO-treated fish exhibited abnormal swimming pattern. Recently, Nakayama *et al.* (2004a, b) revealed that TBT affected the general and sexual behavior of male medaka. The causal mechanism of these adverse effects of TBT on behavior of fish is complicated and currently obscure, but the ability of TBT to permeate neural tissue may be one of the important toxic factors.

We estimated the tissue and organ distribution ratios of TBT in female Japanese whiting (Fig. 2). Since we have no information on the total proportional weight of muscle and circulating blood in Japanese whiting, we used the data for carp (42 g muscle and 6.5 g circulating blood per 100 g of whole body) (Itazawa



**Fig. 2.** Percentage distribution of total whole-body TBT in Japanese whiting, *Sillago japonica*. Values were estimated by using the total weights of muscle (42 g/100 g of whole body) and circulating blood (6.5 g/100 g of whole body) in carp. "Remainder" includes all tissues and organs not analyzed individually, such as skin, bone, gill, kidney, and viscera.

and Oikawa, 1983; Itazawa *et al.*, 1983). We estimated that half of the total TBT in the fish body was in the circulating blood. In addition, 80% of the total TBT was estimated to exist in blood and muscle combined. The liver, a metabolic organ, was estimated to have only 1.7% of the whole-body TBT. These estimates indicate that blood is a very important tissue for the accumulation and distribution of TBT in fish.

Present study, the TBT biomagnification factor for Japanese whiting was 0.015. The lower concentration of TBT in fish body than that in diet was also observed in previous reports. The biomagnification factor of TBT for red sea bream and goldfish was 0.26–0.38 (Yamada *et al.*, 1994) and 0.04 (Tsuda *et al.*, 1991), respectively. In present study, higher concentration of metabolites than that of TBT were detected in liver of Japanese whiting. These phenomena indicate the existence of metabolic and elimination activity of TBT in fish.

Our distribution analysis of TBT in the eggs of Japanese whiting revealed that more than 40% of the total TBT in eggs was in the oil droplet and yolk fractions. Lee (1993) suggested binding of TBT to lipovitellin, which is a main yolk protein in crab oocytes. Thus TBT may be transported from blood to eggs with the yolk protein precursor, vitellogenin, or an unknown carrier protein in fish. The affinity of pollutants to egg components in fish egg has also been observed for DDT, PCBs (Ungerer and Thomas, 1996a), TCDD, benzo[a]pyrene (Monteverdi and Di Giulio, 2000), and cadmium (Ghosh and Thomas, 1995). Nirmala *et al.* (1999) and Nakayama *et al.* (2005) reported that TBT and PCBs in eggs, which was transferred from parent fish, synergistically impaired the development of the next generation in medaka. Thus, the affinity of yolk components for various pollutants may involve severe transgenerational and mixture toxicity.

Furthermore, we estimated the amount of TBT in eggs spawned at one time. Previous reports (Shimasaki *et al.*, 2006) showed that Japanese whiting fed a 20 µg TBT/g diet produced 38,300 eggs/100 g of female fish, equal to approximately 11.3 g of eggs, per day. Since the mean TBT concentration in the spawned eggs was 12.5 ng/g between 5 and 30 days of exposure, the total amount of TBT eliminated with the eggs at a one-time spawning was estimated to be 141 ng (11.3 g × 12.5 ng/g). From our present results, the TBT concentration in fish

fed the 20 µg TBT/g-diet was 300 ng/g; therefore, the total amount of TBT in the whole body was 30,000 ng/100 g of female fish. The same individuals were used in the above previous study (Shimasaki *et al.*, 2006) and in our present study. Thus from these data (141 ng of TBT in spawned eggs, 30,000 ng of TBT in the whole body), we estimate that about 0.47% of TBT in the whole body was eliminated with the eggs of the female Japanese whiting in a day.

Maternal transfer of TBT is critical in ecotoxicology. Nakayama *et al.* (2005) reported that hatchability of medaka was affected by maternally-transferred TBT at more than 123 ng/g eggs. The viable hatching rate of embryos was also decreased by maternal TBT at around 160 ng/g eggs in Japanese whiting (Shimasaki *et al.*, 2006). Hano *et al.* (2007) found abnormal development of medaka embryos directly nanoinjected with TBT at 160 ng/g eggs. In fish collected from marine environment, TBT was detected in the eggs of hairtail (*Trichiurus lepturus*) at 431 ng/g, barracuda at 311 ng/g (Suzuki *et al.*, 1992), herring (*Clupea harengus*) at 290 ng/g, and ruff (*Acerina cernua*) at 150 ng/g (Senthilkumar *et al.*, 1999). These TBT levels in fish eggs were comparable to, or higher than, the lowest effective TBT level determined in laboratory tests using Japanese whiting and medaka.

Many studies have suggested that TBT pollution will continue without dramatic depletion in the marine environment (Stewart and Demora, 1990; Sarradin *et al.*, 1995; Viglino *et al.*, 2004). Thus, continuing study is needed for ongoing risk evaluation of TBT to the reproduction of marine fish.

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