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Monitoring of Tributyltin Contamination of Demersal Fish in Coastal Water in East China Sea

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To monitor the pollution of tributyltin (TBT) in East China Sea, five species of fish (*Sphoeroides pachygaster*, *Dentex tumifrons*, *Chelidonichthys kumu*, *Nippon spinosus* and *Monocentris japonica*) and two crustacean species (*Charybdis miles* and *Ibacus ciliatus*) were collected from the East China Sea on 2010. In addition, common Japanese conger (*Conger myriaster*) was collected from the East China Sea and Hakata Bay from June 2009 to September 2011. Aquaculture fish (*Paralichthys olivaceus*, *Pargus major*, and *Trachurus japonicus*) were also collected from a fish market in Fukuoka, Japan on March 10, 2010. As results, no TBT or its metabolite (dibutyltin, DBT) were detected in the blood, liver and muscle of the five species of fish, the midgut gland of the two crustaceans or the livers of conger eels collected from the East China Sea (<0.0005 µg/mL or µg/g). However, TBT (0.002–0.04 µg/g) and DBT (0.008–0.06 µg/g) were detected in the livers of common Japanese conger collected from Hakata Bay. Moreover, TBT was detected in the blood of three species of cultured fish obtained from the fish market in Fukuoka at levels ranging from no detected <0.0005 µg/mL–0.21 µg/mL. These results suggest that no TBT contamination occurred in the East China Sea, while common Japanese conger from Hakata Bay and cultured fish were still contaminated by TBT.

Key words: demersal fish, conger, pollution, tributyltin

INTRODUCTION

Tributyltin (TBT) has been widely used as an anti-fouling agent in marine environments since the early 1960s (Huggett *et al.*, 1992), having been applied to boat hulls and to marine aquaculture equipment. Because of its high toxicity of TBT to aquatic organisms (Clark *et al.*, 1988), the use of TBT has been regulated worldwide since the 1980s. The International Maritime Organization (IMO) imposed a worldwide ban on TBT in 2003 (Champ, 2000). However, TBT is still detected in coastal sediments in the world (Antizar-Ladislao, 2008).

In around northern Kyushu, Japan, the concentrations of TBT have been found to range from 0.005 to 0.094 µg/L and 0.007 to 1.1 µg/g dry weight (dw) in seawater and sediments, respectively (Inoue *et al.*, 2002), high TBT concentrations were also detected in sediments from next to the shipyard in Hakata port (0.63 µg/g wet weight), Fukuoka, Japan (Undap *et al.*, 2013a) and from 0.0014 to 0.19 µg/mL in blood from marine fish (Miki *et al.*, 2011). Contamination of sediment and aquatic organism by TBT still occurred in coastal areas around northern Kyushu. These results suggest TBT has been detected not only in water and sediments, but also in fish in coastal

areas.

Many studies have monitored TBT in coastal fish species. In the coastal area of northern Kyushu, Japan, contamination of fish such as *Pagrus major* (in blood: 0.0082 µg/mL), *Lateolabrax japonicus* (in blood: 0.190 µg/mL; liver: 0.14 µg/g), and the common Japanese conger, *Conger myriaster* (in blood: 0.0014 µg/mL; liver: 0.015 µg/g) by TBT was found (Miki *et al.*, 2011). TBT pollution of pelagic coastal fish was also reported by Kannan *et al.* (1995): <0.1–0.013 µg/g in muscle of rubberlip morwong, shovelnose ray, blue groper, long-spined snapper, sea mullet, spiny-tailed leatherjacket, Australian herring, striped sea perch, black bream, rainbow trout, Atlantic salmon, stripey, and seabass collected from Australia; and 0.0002–0.001 µg/g in muscle, and 0.0065–0.02 µg/g ww in liver of green-spotted kingfish, Indian mackerel, and paddletail snapper collected from the Solomon Islands. In pelagic fish, TBT levels of 0.0049–0.002 µg/g ww have been reported in skipjack tuna (Ueno *et al.*, 2004). A few studies have monitored TBT in marine demersal fish species; for example, in walleye pollock (*Theragra chalcogramma*) from the Bering Sea, Gulf of Alaska, and Japan Sea, the TBT concentrations ranged from 0.0011 to 0.0055 µg/g ww (de Brito *et al.*, 2002). However, pollution by TBT of demersal fish from the East China Sea is not clear. High levels of human activity and discharge of pollutants might occur in the countries surrounded by the East China Sea. Thus TBT input into the marine environment, and accumulation of TBT in demersal fish, is suspected.

The common Japanese conger is a possible biomonitoring species among demersal fish. The common Japanese conger is an important commercial fish species

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in Japan, Korea, and China around the East China Sea (Kurogi *et al.*, 2012). It mainly inhabits shallow coastal waters to the edge of the continental shelf (e.g., the East China Sea; Katayama *et al.*, 2004; Ma *et al.*, 2007). In Japan, this species is distributed from Sagami Bay southward to Hakata Bay, northern Kyushu, and from western Kyushu to southern Hokkaido (Mochioka *et al.*, 1988). Common Japanese congers might therefore inhabit coastal areas including ports and shipyards, where there is abundant human activity and high levels of TBT pollution are suspected. Kurogi *et al.* (2012), found that there is a *C. myriaster* spawning area along the Kyushu–Palau Ridge, approximately 380 km, of Okinotorishima Island. The accumulation of TBT in congers prior to their return offshore for spawning is therefore a cause for concern.

In this study, we collected demersal fish, including common Japanese congers, by trawl netting from the East China Sea, and monitored pollution by TBT.

MATERIALS AND METHODS

Sample collection from East China Sea

As shown in Table 1, five fish species, i.e., *Spherooides pachygaster*, *Dentex tumifrons*, *Chelidonichthys kumu*, *Nippon spinosus*, and *Monocentris japonica*, and two crustacean species, i.e., *Ibacus ciliatus* and *Charybdis miles*, were collected by trawl netting in the East China Sea (latitude: 31°50.307' N, longitude: 127°40.679' E) during a trip on R/V Nagasaki Maru of Nagasaki University on 6 April 2010. Fish tissues (blood, muscle, and liver) and mid-gut glands of the crustaceans were individually obtained and stored at –30°C until TBT analysis.

Collection of common Japanese conger samples

Common Japanese conger samples were collected

by trawl netting in Hakata Bay and the East China Sea (latitude: 28–33° N; longitude: 125–128° E) from June 2009 to September 2011. The conger livers were individually taken and stored at –30°C until TBT analysis.

Collection of farmed fish samples

As references, samples of three farmed fish species *Paralichthys olivaceus*, *Pagrus major*, and *Trachurus japonicus* were obtained from the fish market in Fukuoka, Japan on 10 March 2010.

Reagents

Tributyltin chloride–d₂₇ (TBTCl–d₂₇) and dibutyltin chloride–d₁₈ (DBTCl–d₁₈), used as internal standards, were purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium tetraethyl borate [NaB(C₂H₅)₄] and Florisil were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All organic solvents were of pesticide residue analytical grade. Other reagents used were of analytical grade.

Analysis of organotin compounds

The concentrations of TBT and its metabolite (DBT) in the samples (muscle, liver and blood of fish; midgut gland of crustacean) were analyzed according to a method described by Undap *et al.* (2013b). Each sample was spiked with 1 µg each of TBTCl–d₂₇ and DBTCl–d₁₈ as internal standards. The sample was then determined after digesting the sample using a polytron homogenizer (Kinematica AG, Littau, Switzerland) in 10 ml of 1 M hydrobromic acid–methanol–ethyl acetate, and 10 ml of deionized water was added. The sample was shaken for 30 min, followed by centrifugation at 1,870 g for 6 min. The homogenate was extracted twice with 10 ml of ethyl acetate–hexane (3:2) solution, shaken for 10 min, and centrifuged at 1,870 g for 6 min. The combined organic

Table 1. TBT and DBT concentrations in fish and crustacean samples

Fish species	Sampling site	Blood*			Liver		Muscle	
		N	DBT	TBT	DBT	TBT	DBT	TBT
Fish								
Blunthead puffer (<i>Spherooides pachygaster</i>)	East China Sea	5	ND	ND	ND	ND	ND	ND
Yellowback seabream (<i>Dentex tumifrons</i>)	East China Sea	5	ND	ND	ND	ND	ND	ND
Black scraper (<i>Thamnaconus modestus</i>)	East China Sea	6	ND	ND	ND	ND	ND	ND
Bluefin gurnard (<i>Chelidonichthys kumu</i>)	East China Sea	6	ND	ND	ND	ND	ND	ND
Ara (<i>Nippon spinosus</i>)	East China Sea	4	ND	ND	ND	ND	ND	ND
Common Japanese conger (<i>Conger myriaster</i>)	East China Sea	11			ND	ND		
Common Japanese conger (<i>Conger myriaster</i>)	Hakata Bay	18			0.008–0.06	0.002–0.04		
Japanese flounder (<i>Paralichthys olivaceus</i>)	Fish market	1	ND	0.03	ND	0.03	ND	ND
Japanese jack mackerel (<i>Trachurus japonicus</i>)	Fish market	1	ND	0.11	ND	0.07	ND	ND
Red sea bream (<i>Pagrus major</i>)	Fish market	1	ND	0.21	ND	0.16	ND	ND
Crustacean								
Mid-gut gland								
Japanese fan crab (<i>Ibacus ciliatus</i>)	East China Sea	5	ND	ND				
Swimming crab (<i>Charybdis miles</i>)	East China Sea	5	ND	ND				

* Whole blood; ND: <0.0005 µg/mL for blood, <0.0005 µg/g for liver, muscle, and mid-gut gland.

extract was then concentrated to 1 ml by using a Turbo Vap II (Zymark, Hopkinton, MA, USA) with N₂ gas flushing at 35°C and brought to a volume of 10 ml with hexane. To ethylate the sample, 0.5 ml of a 5% sodium tetrathylborate solution was added, and then 10 ml of deionized water was added. The mixture was shaken for 30 min and hydrolyzed with 10 ml of 1 N potassium hydroxide solution by shaking for 60 min. After centrifugation at 1,870 g for 6 min, the supernatant was passed through a Florisil-packed chromatography column, and the organotin was eluted with hexane. The elute was concentrated again to 1 ml and analyzed by a gas chromatograph (GC; model 6890, Agilent, Avondale, PA, USA) equipped with an Agilent 5973 mass spectrometer (MS) operated in EI + mode. The GC-MS analysis was performed with a 30 m×0.25 mm i.d. and 0.25 μm film thickness capillary column (HP-5MS, Agilent) in splitless mode, and the carrier gas was helium. Ions of DBT, DBTCl-d₁₈, TBT, and TBTCl-d₂₇ were monitored at m/z of 261, 279, 263, and 318, respectively.

RESULTS

As shown in Table 1, no TBT was detected (<0.0005 μg/g) in the tissues of fish, including the common Japanese conger, and crustaceans collected from the East China Sea.

In contrast, TBT (0.002–0.04 μg/g) and DBT (0.008–0.06 μg/g) were detected in the livers of common Japanese congers collected from Hakata Bay. The DBT concentrations were higher than the TBT concentrations. In addition, TBT was detected in the blood, liver, and muscle of three species of farmed fish obtained from the fish market at Fukuoka, in the range from not detected (ND, <0.0005 μg/mL or <0.0005 μg/g) to 0.21 μg/g tissue. The highest concentration of TBT was found in the blood of *P. major* (0.21 μg/mL).

DISCUSSION

No TBT pollution was detected in demersal fish collected from the East China Sea. In contrast, Lee *et al.* (2005) found high concentrations of TBT in demersal fish (Japanese sea bream, *P. major*, 0.508 μg/g dw) collected from Taiwan coastal areas. The TBT concentrations in demersal walleye pollock (*T. chalcogramma*) from the Bering Sea, Gulf of Alaska, and the Japan Sea ranged from 0.0011 to 0.0055 μg/g ww (de Brito *et al.*, 2002). In pelagic fish, TBT pollution (0.0049–0.200 μg/g ww) was detected in the liver of skipjack tuna (*Katsuwonus pelamis*) collected from Asian offshore waters (off Japan, the Japan Sea; off Taiwan, the East China Sea, the South China Sea; off the Philippines; off Indonesia; the Bay of Bengal), off the Seychelles, off Brazil, and in the open sea (the North Pacific) (Ueno *et al.*, 2004). Contamination of TBT in pelagic fish suggests considerable input of TBT from shipping activities. No or low contamination of TBT in demersal fish in the East China Sea suggests a low input of TBT in the bottom area of the East China Sea. TBT data on bottom water or sedi-

ments of the East China Sea are not available, so further study is needed to monitor TBT in water and sediments of the East China Sea.

TBT was detected in common Japanese congers in Hakata Bay, where sediments were contaminated by TBT. The maximum TBT concentration in common Japanese congers from Hakata Bay was 0.04 μg/g ww. High TBT pollution in the sediments of Hakata Bay has been reported (0.018–1.101 mg/kg, Ohtsubo *et al.*, 2009; 0.630 μg/g ww, Undap *et al.*, 2013a), suggesting an input of TBT into the environment. Harino *et al.* (1998) reported contamination by TBT in *Physiculus maximowiczii* (0.01–0.02 mg/kg ww), seawater (0.008–0.0074 μg/L), and sediments (0.01–0.64 mg/kg dw) collected from coastal areas of Otsuchi Bay, Japan. TBT accumulations were reported in brown trout (*Salmo trutta*), 0.056 μg/g ww, and roach (*Rutilus rutilus*), 2.700 μg/g ww, and in sediments (0.024 μg/g dw) collected from Puck Bay on the Polish coast of the Baltic Sea (Senthikumar *et al.*, 1999). Sudaryanto *et al.* (2005) reported that TBT concentrations in several fish (0.0097–0.052 μg/g dw) and sediments (0.010 μg/g dw) were detected in Jakarta Bay, where the biggest port in Indonesia is located. Also, in Bohai Bay, North China, TBT concentrations in fish (0.0013–0.0146 μg/g ww) and in sediments (0.0406 μg/g dw) taken from the port were reported by Hu *et al.* (2006). These findings support the suggestion that the TBT concentrations detected in common Japanese conger samples from Hakata Bay might be attributed to TBT in sediments.

TBT was detected in farmed fish (blood: 0.03–0.210 μg/mL; liver: 0.03–0.160 μg/g ww). The TBT concentrations in fish analyzed in the present study were comparable to those found in the blood of three farmed fish species, i.e., Japanese flounder (blood: 0.609±0.210; liver: 0.049±0.019 μg/g), red sea bream (plasma: 1.351±0.706; muscle 0.206±0.045 μg/g), and yellowtail (plasma: 0.511±0.260; muscle: 0.201±0.067 μg/g), obtained from the fish market in Fukuoka, Japan (Oshima *et al.*, 1997). These results suggest that TBT contamination of farmed fish still occurs.

The high levels of TBT still detected in farmed fish might be caused by TBT contamination of food or accumulation of TBT from bottom sediments in the aquaculture system. Davies and Mckie (1987) reported that farmed Atlantic salmon (*Salmo salar*) of commercial size from farms using TBT-treated nets normally contain around 0.5–1.0 μg/kg TBT in muscle tissue. Wu (1995) reported that problems caused by high organic levels in aquaculture systems are attributable to other uses of coastal areas, and the use of chemicals such as therapeutants and antifoulants (TBT). This implies that if there are recent inputs of TBT, possibly from antifouling agents from fishing boats and aquaculture nets in these locations, sample location may also be a factor since the samples were probably collected closer to potential sources of TBT than those fish e.g. wild fish.

The high concentrations of TBT found in the blood of the three farmed fish species could be attributed to TBT-binding proteins (TBT-bps) in blood (Table 1).

Oshima *et al.* (1997; 0.015–0.609 $\mu\text{g/g}$) and Miki *et al.* (2011; 0.0014–0.19 $\mu\text{g/mL}$) detected higher TBT concentrations in blood than in other fish tissues. Shim *et al.* (2002) also reported high accumulations of TBT (0.247 $\mu\text{g/g}$) in flounder blood (*Pleuronichthys cornutus*). High levels of TBT might accumulate in the blood of farmed fish as a result of binding of TBT to TBT-bps, which have been isolated from the blood of the Japanese flounder *Paralichthys olivaceus* and identified (Shimasaki *et al.*, 2002; Oba *et al.*, 2007; Satone *et al.*, 2008; Shimasaki *et al.*, 2008). These data suggest that TBT accumulation in blood may be a phenomenon that occurs mainly in fish (Shimasaki *et al.*, 2008). We were therefore able to demonstrate that the blood of farmed fish might be a sensitive tissue for TBT monitoring.

CONCLUSIONS

In conclusion, no TBT contamination was found in the East China Sea, but common Japanese congers from Hakata Bay and farmed fish from the fish market were still contaminated by TBT, suggesting recent inputs of TBT in the coastal waters of Japan, despite regulation of its use in certain countries.

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