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Contamination and Accumulation Feature of Organotin Compounds in Common Cormorants (*Phalacrocorax carbo*) from Lake Biwa, Japan

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Abstract—Concentrations of organotin compounds (OTs) were determined in various body organs and tissues of adult common cormorants (*Phalacrocorax carbo*) collected from Lake Biwa, Japan. Several kinds of OTs were detected in various organs and tissues of adult common cormorants. Among the organs and tissues in which several kinds of OTs were detected, the highest concentrations of butyltin compounds (BTs), tributyltin (TBT) and its metabolites, were found in the liver (384 ng/g wet wt) and kidney (371 ng/g wet wt). Compared with BTs, phenyltin compounds (PTs), di (DPT) and triphenyltin (TPT), showed relatively uniform distribution among organs and tissues. Feathers showed significant higher concentrations of both PTs and BTs than the soft tissues and organs. This indicates possible excretion of OTs through molting feathers, similar to the trend reported for methylmercury. Lower levels of BTs and PTs were observed in the liver collected in 2007 (BTs: 8.0 ng/g, PTs: 2.0 ng/g ww) when compared with those collected in 1993 (BTs: 270 ng/g, PTs: 130 ng/g ww). This result reflects the effect of the restrictions on the usage of TBT and TPT in Japan. A significant correlation was found between the concentrations of OTs in the feathers and liver of cormorants, suggesting that cormorant feathers can be utilized for monitoring OTs contamination as a non-destructive sample.

Keywords: organotin compounds (OTs), common cormorants, Lake Biwa, non-destructive method for monitoring

INTRODUCTION

Organotin compounds (OTs) have been used as antifouling agents, biocides, wood preservative agents, PVC stabilizers and industrial catalysts (Hoch *et al.*, 2001). In Japan, usage of tributyltin (TBT) and triphenyltin (TPT) have been restricted since 1990 because of their high toxicities and bioaccumulation in the organisms. On the other hand, dibutyltin (DBT) and monobutyltin (MBT) are still in use in some industrial processes. Many studies reported the detectable levels of OTs in environmental samples such as water and sediment, and in wild anumals

such as invertebrates, fishes, birds and marine mammals (Iwata *et al*., 1995; Guruge *et al*., 1996, 1997; Stab *et al*., 1996; Strand and Jacobsen, 2005). Consequently there exists severe concern on the expansion of OTs contamination. However, to our knowledge, only few studies have analyzed OTs in birds, particularly almost no report is available on phenyltin compounds (PTs) in avian species, except for the two studies by Stab *et al*. (1996) and Strand and Jacobsen (2005).

Common cormorant (*Phalacrocorax carbo*) living in Lake Biwa, the largest lake and enclosed aquatic system in Japan, is a representative high trophic level organism. It can be presumed that common cormorants may be accumulating various OTs through food web. Guruge *et al*. (1996) has already reported BTs concentrations in common cormorants from Lake Biwa in 1993 (270 ng/g in liver, 290 ng/g in kidney, and 250 ng/g in feather, all values on a wet wt basis). Moreover Nakayama *et al*. (2006) also reported BTs concentration in liver of cormorants collected in 2001. Although they reported the contamination status of BTs in common cormorants from Lake Biwa, no data is available on other OTs such as PTs. In the present study, we analyzed OTs in various organs and tissues in common cormorants collected from Lake Biwa to elucidate their accumulation properties and body distribution. In addition, temporal trends of hepatic concentrations in cormorants collected during different sampling years were determined. Furthermore we examined correlations between feather and hepatic concentrations to establish a non-destructive (non-killing) method for monitoring OTs using the feather samples.

MATERIALS AND METHOD

Sample

The liver samples of common cormorants (*Phalacrocorax carbo*) were collected from the Lake Biwa, Japan in June 1993 (n = 13), May 2002 (n = 10), May 2007 (n = 7). Among the collected cormorants, 11 organs and tissues (kidney, brain, pectoral muscle, lung, bone, fat, skin, primary feather, dorsal feather, ventral feather and ventral down) of two birds collected in 1993 were employed for detail analysis. In addition, ventral feather of cormorants collected in 1993 were also analyzed. Sampling location is shown in Fig. 1.

Organotin analysis

The details of analytical procedure for OTs were described in Murata *et al*. (2008). About 2 g of sample (wet wt) was spiked with deuterated OTs (d_9-MBT, d_{18}-DBT, d_{27}-TBT, d_5-MPT, d_{10}-DPT, d_{15}-TPT, d_{18}-MOcT, d_{34}-DOcT and d_{51}-TOcT) as internal standards. The sample was homogenized with 1M HBr/methanol-ethyl acetate (1:1). After centrifugation (3000 rpm, 15 min), OTs in the supernatant were extracted by ethylacetate/hexane (3:2) and NaBr saturated water and separated from the aqueous layer using hexane. Hexane layer was dehydrated with anhydrous Na_2SO_4 and concentrated. 1M acetate buffer, ethanol

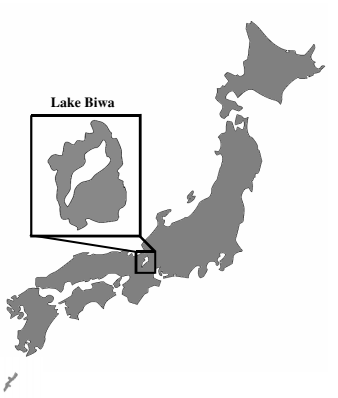

Fig. 1. Map showing sampling location.

and sodium tetraethylborate ($NaBEt_4$) were added to the solvents for ethylation. After ethylation, 1M KOH was added for saponification of lipid of in the extracts, and shaken. After that, the ethylated organotins were re-extracted by hexane. The extract was concentrated and cleaned up by SEP-PAK$^-$ florisil column and eluted with 5% diethylether/hexane. The final solution was concentrated to 1 ml and spiked with deuterated tetrabytyltin (d_{36}-TeBT) as a recovery standard.

Method for the analysis of feather was significantly different from soft tissues analysis slightly. Feathers (0.3–0.4 g wet wt) were removed from the body and washed with detergent not containing OTs and hexane washed water, dehydrated, dried over night, cut into small species and spiked with internal standards. 4M KOH was added for alkali decomposition and was sonicated while heating. The sample mixture was neutralized by 5M HCl and then the procedure for soft tissues was followed.

The quantification was made using a gas chromatograph-mass spectrometer (GC/MS) (Hewlett-Packard 6870 GC system with 5973 mass selective detector). GC/MS was equipped with a fused silica capillary column and operated in electron impact and selected ion monitoring mode (EI-SIM). The concentrations of OTs were calculated based on the internal standard method. Concentrations of MPT were not determined because the recoveries of the internal standard for MPT were less than 10% in almost all samples. Further, we did not quantify the concentration of MBT, DPT and MOcT in feather analysis because of the poor recoveries of internal standards. Concentrations of OTs in this study were reported as nanograms of corresponding cation per gram on wet weight basis.

RESULTS AND DISCUSSION

Body distribution

OTs were detected in all analyzed organs and tissues (Table 1). High

Table 1. Body distribution of organotin compounds in two common cormorants collected in 1993.

Sample No.	Sex	Organs and tissues	Concentration (ng/g wet wt)									
			MBT	DBT	TBT	∑BTs	DPT	TPT	∑PTs	MOcT	DOcT	TOcT	∑OcTs
1	M	Liver	170	230	8.7	410	35	66	100	<7.0	<7.0	<0.5	<7.0
		Kidney	140	260	14	410	18	68	86	<7.0	<7.0	<0.5	<7.0
		Brain	29	28	6.4	63	6.7	37	43	<7.0	<7.0	<0.5	<7.0
		Pectralmuscle	25	15	9.8	49	3.0	46	49	<7.0	<7.0	<0.5	<7.0
		Lung	27	67	1.7	96	15	23	38	<7.0	<7.0	<0.5	<7.0
		Bone	38	6.0	2.4	46	14	10	24	<7.0	8.5	<0.5	8.5
		Fat	290	39	4.6	330	14	8.6	22	160	<7.0	<0.5	160
		Skin	72	64	4.8	140	15	18	33	<7.0	<7.0	<0.5	<7.0
		Primary feather	—	37	<1.9	37	N.A.	16	16	N.A.	<6.6	<2.9	<6.6
		Ventral feather	—	47	<1.9	47	N.A.	34	34	N.A.	<6.6	<2.9	<6.6
		Ventral down	—	64	<1.9	64	N.A.	86	86	N.A.	<6.6	<2.9	<6.6
		Dorsalfeather	—	65	2.6	65	N.A.	40	40	N.A.	<6.6	<2.9	<6.6
2	M	Liver	170	190	5.2	360	29	110	140	<7.0	<7.0	<0.5	<7.0
		Kidney	89	230	8.0	330	40	97	140	<7.0	<7.0	<0.5	<7.0
		Brain	33	37	4.4	74	21	67	88	<7.0	<7.0	<0.5	<7.0
		Pectralmuscle	<5.7	17	9.1	27	8.1	120	130	<7.0	<7.0	<0.5	<7.0
		Lung	12	78	1.3	91	16	28	44	<7.0	<7.0	<0.5	<7.0
		Bone	28	<0.2	<0.3	28	5.6	1.1	6.7	<7.0	<7.0	<0.5	<7.0
		Fat	150	33	2.0	190	12	0.6	13	<7.0	12	<0.5	12
		Skin	130	74	2.3	210	17	37	54	7.4	<7.0	<0.5	7.4
		Primary feather	—	5.5	<1.9	5.5	N.A.	14	14	N.A.	<6.6	<2.9	<6.6
		Ventral feather	—	180	8.5	190	N.A.	820	820	N.A.	<6.6	<2.9	<6.6
		Ventral down	—	330	7.8	340	N.A.	1500	1500	N.A.	<6.6	<2.9	<6.6
		Dorsal feather	—	290	13	300	N.A.	1300	1300	N.A.	<6.6	<2.9	<6.6

N.A.: Not analysis.

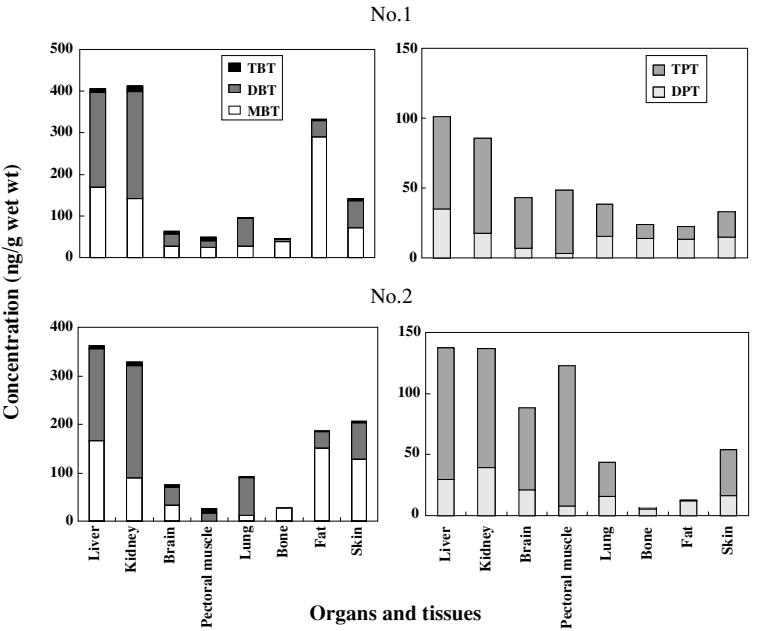

Fig. 2. Concentrations of OTs in various organs and tissues (excluding feathers) of two cormorants.

concentrations of BTs were found in liver (380 ng/g wet wt) and kidney (370 ng/g wet wt), followed by fat (260 ng/g wet wt) and skin (170 ng/g wet wt) (Fig. 2). Concentrations of BTs in kidney and fat were comparable to those in liver. These results are different from the accumulating characteristics in fish and marine mammals which accumulate BTs in liver at significantly higher concentrations than in other organs and tissues (Iwata *et al*., 1995; Kannan *et al*., 1995). Birds may have specific protein binding to BTs in their kidney, weak binding affinity of BTs to protein in liver and/or different metabolic and excretion pathways for BTs that are different from mammals. DBT and MBT accounted more than 60% in total BTs in almost all organs and tissues, while the conposition of BTs in diet fishes showed higher abundance of TBT (Guruge *et al*., 1996). This suggested that common cormorants have relatively high metabolic capacity to degrade TBT. On the other hand, common cormorants living in Lake Biwa, which is located in urban area might have been expose to DBT and MBT derived from industrial materials such as PVC and other plastic resins (Fent, 1996).

The highest concentrations of PTs were founded in the liver (120 ng/g wet wt) and the kidney (110 ng/g wet wt), followed by pectoral muscle (90 ng/g wet wt) and brain (70 ng/g wet wt) (Fig. 2). Compared with BTs, PTs did not accumulate in certain organs and tissues but rather had relatively uniform distribution expect in bone and subcutaneous fat which were contained low PTs levels. Although Harino *et al*. (2007) found that cetaceans accumulate high levels

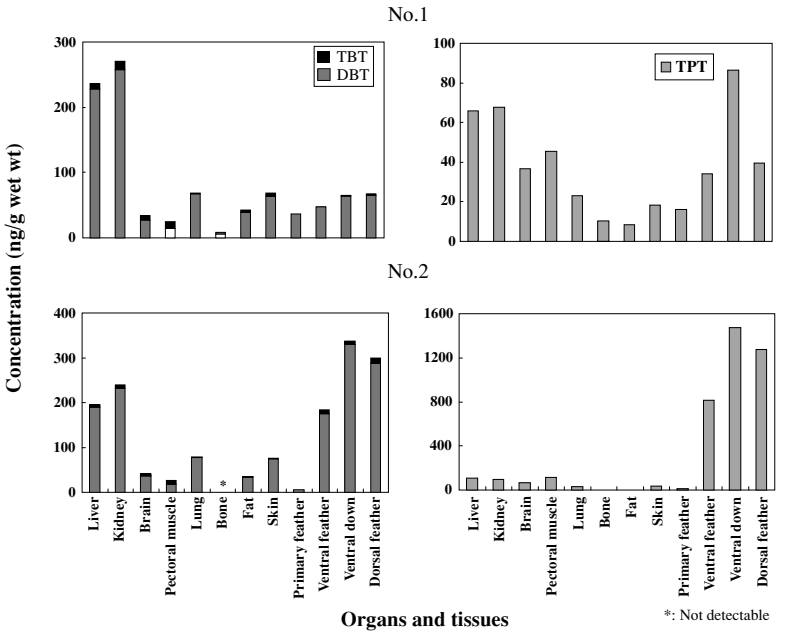

Fig. 3. Concentrations of OTs in organs and tissues including feathers of two cormorants.

of TPT in fat, in the present study, low levels of OTs were observed in fat of birds (Stab *et al*., 1996). These results on TPT in animal fat may indicate its species-specific accumulation and tissue distribution. Among PTs, TPT was the dominant compound in most of the organs and tissues and TPT concentrations were higher than TBT. Accordingly it seems that TPT is not easily biodegradable than TBT, and thus, readily retained in the body.

Concentrations of octyltin compounds (OcTs) were below detection limit in almost all the organs and tissues analyzed except bone, fat and skin (Table 1). OcTs are used as PVC stabilizers and industrial catalysts for producing polyurethane foams and silicones (Fent, 1996; Fromme *et al*., 2005), thus unlike other OTs, OcTs are land-based contaminants. Nonetheless OcTs were less in common cormorants living in inland lake. Further the molecular size of OcTs is lager than other OTs leading to reduced bioaccumulation. This result implies a lesser environmental input of OcTs around Lake Biwa or they may be less bioaccumulative.

Feathers had higher concentrations of both TPT and BTs than the soft tissues and organs (Fig. 3). The accumulation of OTs in primary feather was lower than body feathers (ventral feather, ventral down and dorsal feather). This might be due to the possible molting of primary feathers jut before sampling. Previous reports identified that pinnipeds and birds have considerable concentrations of BTs in hair and feathers as shown by Guruge *et al*. (1997), Kim, G. B. *et al*. (1996)

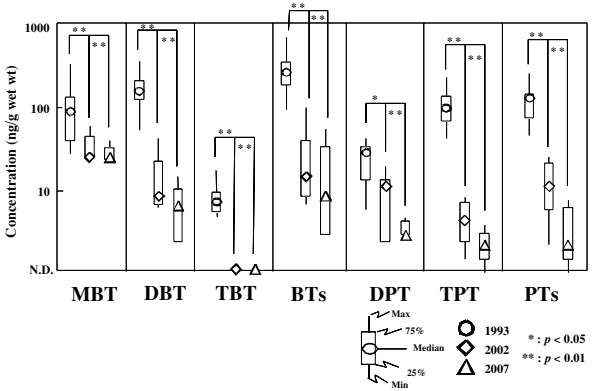

Fig. 4. Comparison of organotin concentrations in the liver of cormorants collected during 1993 to 2007.

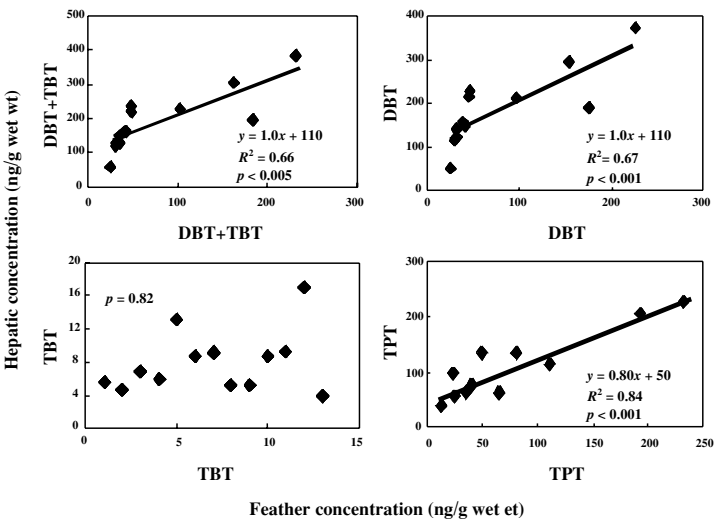

Fig. 5. Relationship between concentration of organotin compounds in feature and liver of common cormorants.

and Takahashi *et al*. (2000). The authors suggested that molting process through replacement of hair and feather is an important excretion route for BTs, as in the case of methylmercury (Honda *et al*., 1986; Birgit, 1987; Kim, E. Y. *et al*., 1996; Saeki *et al*., 2000; Nam *et al*., 2005), which birds strongly to the -SH groups of cystein in the feather after being carried by the bloodstream into the growing feather (Crewther *et al*., 1965; Smith *et al*., 1979; Guruge *et al*., 1996; Wolfe *et al*., 1998; Takahashi *et al*., 2000). This is the first report that analyzed TPT in bird

feathers. It is clear that not only BTs but also TPT accumulates in feathers, which might be due to strong binding affinity of the protein in the feather for TPT.

Temporal trend

OTs were detected in all the liver samples collected in 1993, 2002 and 2007. OcTs were below the detection limit in all samples expecting one sample collected in 2007. Lower levels of BTs and PTs levels were observed in the liver collected during 2007 (BTs: 8.0 ng/g, PTs: 2.0 ng/g ww) when compared to 1993 (BTs: 270 ng/g, PTs: 130 ng/g ww) (Fig. 4). This shows that BTs and PTs contaminations from antifouling agents are decreasing in Lake Biwa. This result reflects the positive effects of the restrictions on the usage of TBT and TPT in Japan.

Possibility of using feather as a non-destructive indicator for OTs monitoring

We investigated the possibility of the use of ventral feather samples as a non-destructive indicator for OTs monitoring. The body feathers comprised more than 70% of the total feathers in cormorant (Guruge *et al*., 1996), in addition the body feathers are not the feathers necessary for flying. So, they can be used as non-destructive indicator for OTs monitoring. A significant correlation was found between the concentrations of DBT, DBT + TBT and TPT in the ventral feathers and liver of cormorants (Fig. 5). It was found that the hepatic levels of OTs in cormorants can be estimated by measuring the concentration of OTs in the feathers using following equations.

DBT: Hepatic concentration = $1.0 \times$ Ventral feather concentration + 110,

DBT + TBT: Hepatic concentration = $1.0 \times$ Ventral feather concentration + 110,

TPT: Hepatic concentration = $0.80 \times$ Ventral feather concentration + 50.

This result suggests that cormorant feathers can be utilized for monitoring OTs contamination as a non-destructive sample.

The present study found a decreasing trend of OTs contamination in common cormorants from Lake Biwa. International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001 (AFS convention) from International Maritime Organization (IMO) has entered into force since September 17, 2008, to prohibit the usage of OTs in antifouling paints. However this convention is yet to come into force in some Asian developing and some European countries. Further studies are needed to monitor the OTs contamination in wildlife continuously by non-destructive method so as to conform AFS convention effect.

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