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Accumulation of Tributyltin in Olive Flounder, *Paralichthys olivaceus*: Its Effect on Hepatic Cytochrome P450

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Abstract. Accumulation of tributyltin (TBT) was determined in liver of olive flounder exposed to TBT (3.65, 36.5, 365, 3,650, and 7,300 ng Sn/L) for 10 or 30 days, followed by 60 days depuration. Effect of TBT on hepatic cytochrome P450 content was also measured in liver of olive flounder. TBT was highly accumulated in liver of fish during the 10- to 30-day exposures, and hepatic cytochrome P450 content decreased with increasing TBT concentration. Hepatic cytochrome P450 contents were affected in olive flounder exposed to even environmentally relevant TBT concentrations, such as 3.65 ng Sn/L. In addition, the liver TBT levels demonstrated strong negative correlation to the hepatic cytochrome P450 content. The effects started to appear from 20 ng Sn/g dry weight of TBT in liver. Tributyltin concentrations and hepatic cytochrome P450 were also determined in feral fine-spotted flounder. The relationship between other organic pollutants known as cytochrome P450 inducers (*e.g.*, polychlorinated biphenyls) as well as TBT and hepatic cytochrome P450 in the feral fish implied that TBT even at ppt level could impose antagonistic effects on hepatic cytochrome P450 induction.

Since the early 1980s, tributyltin (TBT) has been one of the contaminants of environmental concerns due to its high toxicity and direct introduction into the aquatic environment mainly from antifouling paints applied to ship hulls. The high toxicities of TBT have been revealed in a number of aquatic animals, including fish (Fent 1996). Although many countries regulated the use of TBT on ships less than 25 m in length from the early 1980s, contamination persists at chronic levels. Furthermore, because there have been no regulations in most Asian countries, TBT concentrations are still high in the waters of these countries (Kannan *et al.* 1995; Kan-atireklap *et al.* 1997; Shim *et al.* 1998).

Recently, it has been known that TBT has a strong inhibitory effect *in vitro* on microsomal cytochrome P450 in marine and freshwater fish (Fent and Stegeman 1991; Fent and Bucheli 1994) and molluscs (Morcillo and Porte 1997). The hepatic microsomal monooxygenase or cytochrome P450 systems play a vital role in the metabolism of xenobiotic compounds and also in the metabolism of endogenous compounds, such as hormones. TBT was known to cause imposex (imposition of male sexual organ on female) in marine gastropods by inhibiting cytochrome P450 dependant aromatase (Bettin *et al.* 1996). Fent and Stegeman (1993) reported the interaction of TBT *in vivo* with different P450 isoforms, including those involved in the metabolism of organic pollutants and steroids. The presence of TBT in the organism can therefore have consequences both for protection against other pollutants and reproduction.

However, effects of TBT on cytochrome P450 have been demonstrated at a concentration range from 0.001 to 1 mM (Fent and Stegeman 1991; Fent and Bucheli 1994; Morcillo and Porte 1997) that is several orders higher than those found in environment. Furthermore, most studies on the interaction of environmental pollutants with hepatic microsomal cytochrome P450 have been focused on the induction of this enzyme system (for review, see Stegeman and Kloepper-Sams 1987). There have been attempts to use the P450 level and ethoxyresorufin-O-deethylase (EROD) activity in fish as a biochemical markers to determine the extent of exposure to enzyme-inducing chemicals (*e.g.*, polychlorinated biphenyls, PCBs). However, inhibition of P450 enzyme in fish was not considered while monitoring the induction of P450 in marine fish. Thus, this study aims to determine if TBT could affect cytochrome P450 levels in marine flounder exposed to environmentally relevant TBT concentrations. The effect of TBT on cytochrome P450 in experimental fish is discussed with exposure concentration and time for accumulation of TBT. This study also attempts to test the hypothesis that high TBT and low P450-inducible pollutants may suppress the induction of P450 enzyme in fish. This would complicate the use of induced P450 enzyme as a marker for other pollutants. The relationships between TBT and other organic pollutant to cytochrome P450 level were also examined in field-collected fish.

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Materials and Methods

Experimental Design

Olive flounder (*Paralichthys olivaceus*) were purchased from an aquaculture company in Kangnung, Korea, and acclimated for 7 days in flow-through water of 15°C in the laboratory. Their mean weight and length of were 229 ± 51 g and 31 ± 2 cm, respectively. To assess accumulation and effect of TBT on hepatic cytochrome P450, olive flounder were exposed to tributyltin chloride (TBTCl) at nominal concentrations of 3.65 and 36.5 ng Sn/L for 30 days and at 365, 3,650, and 7,300 ng Sn/L for 10 days. The 30-day exposures at 3.65 and 36.5 ng Sn/L were followed by 60 days elimination in clean sea water. The fish were kept in 1000 L of five different aquaria. Control fish were separately kept in clean aquarium under the same conditions. They were fed a nutritionally complete commercial fish diet. Filtered natural sea water was renewed every day and then adjusted to the nominal TBT concentration with TBT stock solution dissolved in ethanol. During the exposure, salinity and temperature of the sea water remained at 32 ± 2‰ and 13–16°C, respectively. After 1, 2, 4, 6, 10, 20, and 30 days of exposure and following 30 and 60 days of elimination, four fish were sampled and liver was excised from the fish. A large portion of liver from each fish sample was immediately stored at −80°C for further analysis of P450, and the remainders of livers were combined and then kept in a −20°C freezer for organotin analysis.

Collection of Fish

Fine-spotted flounders (*Pleuronichthys cornutus*) were collected by gill net from four stations in Masan Bay in May 1998 (Figure 1). The feral fish samples were collected from Masan Bay, which has previously shown an apparent gradient in TBT concentrations (Shim *et al.* 1999). The number of fish caught from stations 1, 2, 3, and 4 was 4, 10, 2, and 9, respectively. Liver was removed from each fish and stored for further cytochrome P450 and TBT analyses using the method described.

Measurement of Cytochrome P450

Extraction of the microsomal fraction from the fish hepatocytes followed Collier *et al.* (1995) with minor modifications. Liver of individual fish was thawed, homogenized in 0.25 M sucrose, and then centrifuged (9,000*g*) at 4°C for 20 min. The supernatant was centrifuged (105,000*g*) again at 4°C for 1 h to obtain the pellet, called the microsomal fraction. Hepatic microsomes of individual fish were prepared by resuspending the pellet in 0.25 M sucrose solution.

Cytochrome P450 content of microsomal preparations was determined with a dithionite difference spectrum of CO-bound microsomes using an extinction coefficient of 91 $mM^{-1}\ cm^{-1}$ (450 nm to 490 nm), as described by Omura and Sato (1964). To the microsomal fraction, 0.1 M of K_2HPO_4 buffer (pH 7.5) was added to adjust protein concentration in the 0.5 to 1.0 μg/L range. One of two cuvettes containing microsome was bubbled with CO, and then $Na_2S_2O_4$ was immediately added to the cuvette. The absorbances were measured at 450 and 490 nm with a spectrophotometer. Microsomal protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Analytical Procedure for Organotins

The analytical procedure used is a modification of the method suggested by Shim *et al.* (1998). Liver pooled from four fish samples from each sampling time was homogenized with a Tekmar tissuemizer in a glass amber bottle. Tripentyltin chloride was added to the samples as a surrogate standard. The samples were digested with 10 ml of 50% (v/v) HCl for 30 min and then extracted with 20 ml dichloromethane with tropolone (0.1%) by shaking for 3 h. After 10 min of centrifugation (4,000 rpm), 2 ml of the organic extracts were transferred to 15-ml glass test tubes and concentrated under a gentle stream of nitrogen. The extracts were resuspended in 2 ml *n*-hexane and derivatized with 250 μl 2 M propylmagnesium bromide for 20 min. The remaining propylmagnesium bromide was neutralized with 4 ml 0.4 N sulfuric acid. The derivatized extracts were recovered by centrifugation and cleaned up on 12 g of activated florisil.

The cleaned liver extracts were concentrated again and spiked with tetrabutyltin as an internal standard and then analyzed by gas chromatography using a flame photometric detector. The gas chromatograph (GC) operating condition was identical to that in Shim *et al.* (1998) except for the temperature program. The GC temperature was programmed from 50°C (2 min holding time) and heated to 150°C (10 min holding time) at a rate of 10°C/min, and then heated again to final temperature of 240°C (5 min holding time) at 30°C/min.

Fresh standard stock solutions were made every 3 months. External calibration standards were run with every sample batch. The whole analytical procedure was validated by analyzing reference materials (NIES11: sea bass, *Leteolabrax japonicus*) from the National Institute for Environmental Studies of Japan. The results fell within the range of the certified value for TBT (1.3 ± 0.1 μg/g, as chloride). Recoveries of butyltin compounds from spiked in muscle samples (0.3 μg Sn/g) ranged from 71% to 98%. Relative standard deviations for three butyltins obtained with seven replicate extractions of samples were less than 7%. The method detection limits of the whole analytical procedure for tissue samples ranged from 1 to 3 ng/g. Concentrations of organotin compounds in this study are expressed as ng/g of Sn on a dry weight basis.

Analytical Procedure for Other Organic Pollutants

Analysis of polynuclear aromatic hydrocarbon (PAH) equivalents in bile followed the method reported by Krahn *et al.* (1984). Briefly, fish bile was dissected and immediately stored at −70°C in dry ice. A 5-μl sample of bile samples were directly injected to HPLC system (Hewlett Packard 1050 series) with two fluorescence detectors. A reverse-phase column (Vydak 201TP54) was used, and column temperature was maintained at 50°C. Methanol and water with 5 μl/L acetic acid were used as mobile phase. Naphthalene and bezno-[a]pyrene equivalents were monitored at 290/335 nm (excitation/emission) and 380/430 nm (excitation/emission), respectively.

Analytical procedures for PCBs and organochlorine pesticides (OCPs) in fish muscle followed the method suggested by Sloan *et al.* (1993) with a partial modification. Briefly, a 5-g aliquot of muscle sample was dehydrated with anhydrous sodium sulfate and extracted using Soxhlet apparatus with 200 ml of dichloromethane for 16 h. Three surrogate recovery standards (DBOFB, PCB103, and PCB169) were added to samples prior to extraction. The extract was passed through a column of 20 g of silica and 10 g of alumina (5% and 1% deactivated, respectively) and eluted with 100 ml dichloromethane. The elutes were subjected to a high-performance liquid chromatography (HPLC: 250 × 22.5 mm ID of size-exclusion column packing with Phenogel 100 Å, Phenomenex Co.) for further clean-up and fractionation. A 10 ng sample of TCMX was added to the extracts as an internal standard. Finally, the extracts were analyzed with the GC equipped with electron capture detector.

The capillary column used for analyses was a DB-5 (30 m × 0.25 mm ID × 0.25 m film thickness). The oven temperature program was as follows: 100°C for 1 min, 5°C/min to 140°C (1 min holding time), 1.5°C/min to 250°C (1 min holding time), and 10°C/min to 300°C (5

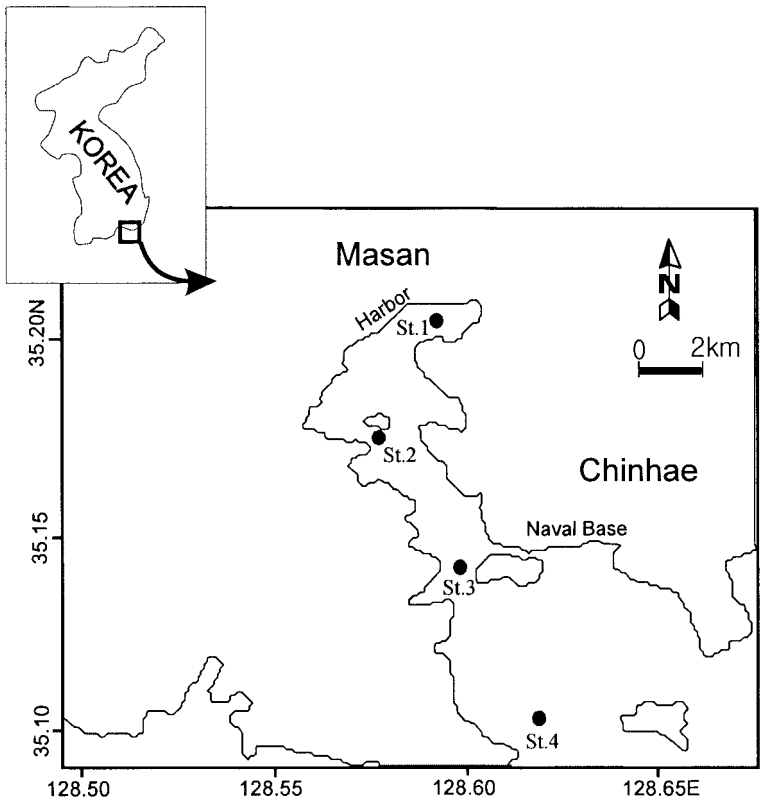

Fig. 1. Map of fine-spotted flounder sampling stations in Masan Bay, Korea

min holding time). The whole analytical procedure was validated by analysis of the certified reference material (IAEA-142).

Results

Accumulation of Organotins in Liver

The TBT concentrations in the tissues of control fish were not significantly changed for 30-day exposure plus 60-day elimination. The TBT concentrations in liver of experimental fish exposed to five TBT concentrations increased with exposure time, and the magnitude of accumulation was proportional to exposure concentration (Figure 2). After a 10-day exposure, elevated TBT concentrations (3,010 and 3,550 ng Sn/g) were found in the liver of fish exposed to the highest TBT concentrations (*i.e.*, 3650 and 7300 ng Sn/L, respectively) (Table 1).

Concentrations of TBT after a 10-day exposure at 3.65, 36.5, and 365 ng Sn/L were 30, 71, and 385 ng Sn/g, respectively. During the accumulation period (10 or 30 days), there were no signs of reaching steady-state at any of the five exposure levels. The 30-day exposure at 3.65 and 36.5 ng Sn/L of TBT was followed by a 60-day depuration period in clean sea water. TBT concentrations steeply decreased during the following 60 days of elimination at 3.65 and 36.5 ng/L.

Dibutyltin (DBT) and monobutyltin (MBT), degradation products of TBT, were also detected in liver of experimental fish. The DBT and MBT concentrations in all the tissue samples were generally lower than that of TBT except in fish exposed to the lower TBT concentration. The percent contribution of TBT to the total butyltin concentration in the liver were relatively low at the lower exposure levels (46% at 3.65 and 36.5 ng Sn/L) compared to higher exposure levels (62% at 365 ng Sn/L, 78% at 3,650 ng Sn/L, and 82% at 7,300 ng Sn/L). DBT concentrations were slightly higher than TBT during the elimination stage after exposure to 3.65 ng Sn/L.

Microsomal P450 Contents in Experimental Fish

The total content of hepatic microsomal cytochrome P450 in the control group fish ranged from 1.52 to 1.85 nM/mg protein and did not significantly change for 30-day exposure plus 60-day elimination (Figure 3). However, the hepatic cytochrome P450 content in fish exposed to five different concentrations of TBT apparently decreased comparing to that of the control group after the 10-day exposure (Table 1). The mean P450 content in the control group was 1.60 $\pm$ 0.12 nM/mg protein after 10 days exposure. The mean P450 contents of the group of fish exposed to 3.65, 36.5, and 365 ng Sn/L ranged from 0.65 to 0.93 nM/mg protein. Although they are significantly different from the control, no significant differences were found among these three exposure groups. The mean P450 contents in fish of the other groups (3,650 and 7,300 ng Sn/L) were 0.23 $\pm$ 0.01 and 0.21 $\pm$ 0.07 nM/mg protein, respectively, which were also significantly different from the control.

The change of liver cytochrome P450 content with time in the presence of TBT is shown in Figure 3. A significant

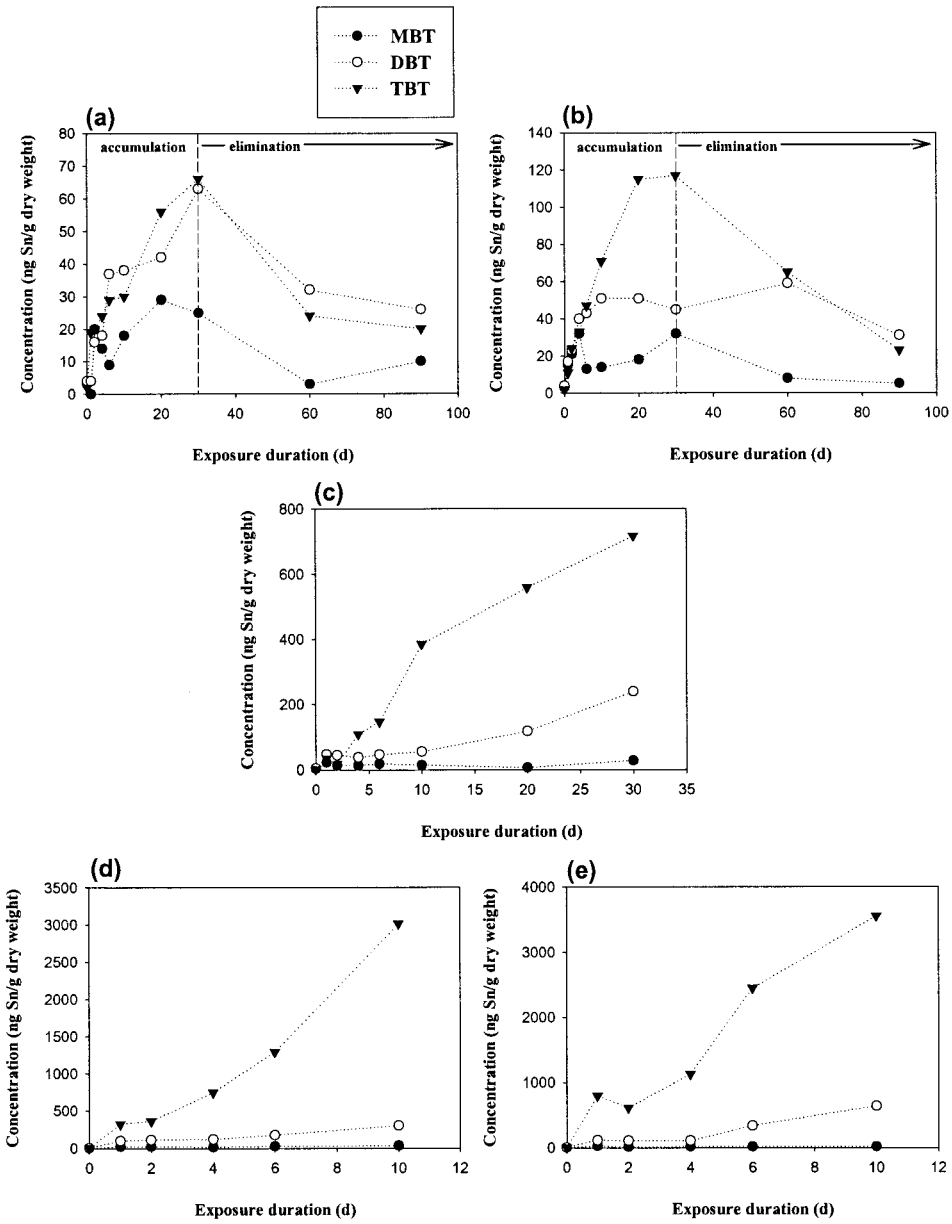

Fig. 2. Accumulation of tributyltin in liver of olive flounder exposed to tributyltin chloride at five different concentrations: (a) 3.65, (b) 36.5, (c) 365, (d) 3,650, and (e) 7,300 ng Sn/L

(Student *t*-test, $p < 0.01$) decrease of spectrally measured P450 content took place rapidly after exposure to all the TBT exposure concentrations except 3.65 ng Sn/L, at which the rate of decrease was slower. After a 24-h exposure to TBT concentrations of 36.5, 365, 3,650, and 7,300 ng Sn/L, the liver P450 content decreased to less than half in that in the control group. At the exposure level of 3.65 ng Sn/L, it took 6 days to reach to less than 50% of the control. However, the mean hepatic P450 content in fish exposed to 3.65 ng Sn/L of TBT was a little lower than those exposed to 36.5 and 365 ng Sn/L on the 6th and the 10th day of exposure. The exposure to 3.65, 3,650, and 7,300 ng Sn/L of TBT led to a decrease of P450 content with time during the first days of exposure. In contrast, no significant ($p > 0.05$) change was detected after the first day of exposure at concentrations of at 36.5 and 365 ng Sn/L (Fig. 3).

A 30-day exposure to TBT concentrations of 3.65 and 36.5 ng Sn/L was followed by a 60-day depuration period in clean sea water. Although the mean hepatic P450 contents of fish exposed to 3.65 and 36.5 ng Sn/L of TBT were significantly different from the control, they did not show any time-dependent decrease. The mean hepatic P450 content did not change during the first 30 days of depuration, and then tended to slightly increase toward the end of the depuration period.

Relationship Between Accumulation of TBT and Hepatic P450 Content

The relationship between TBT concentrations and cytochrome P450 content in the liver of olive flounder is shown in Figure

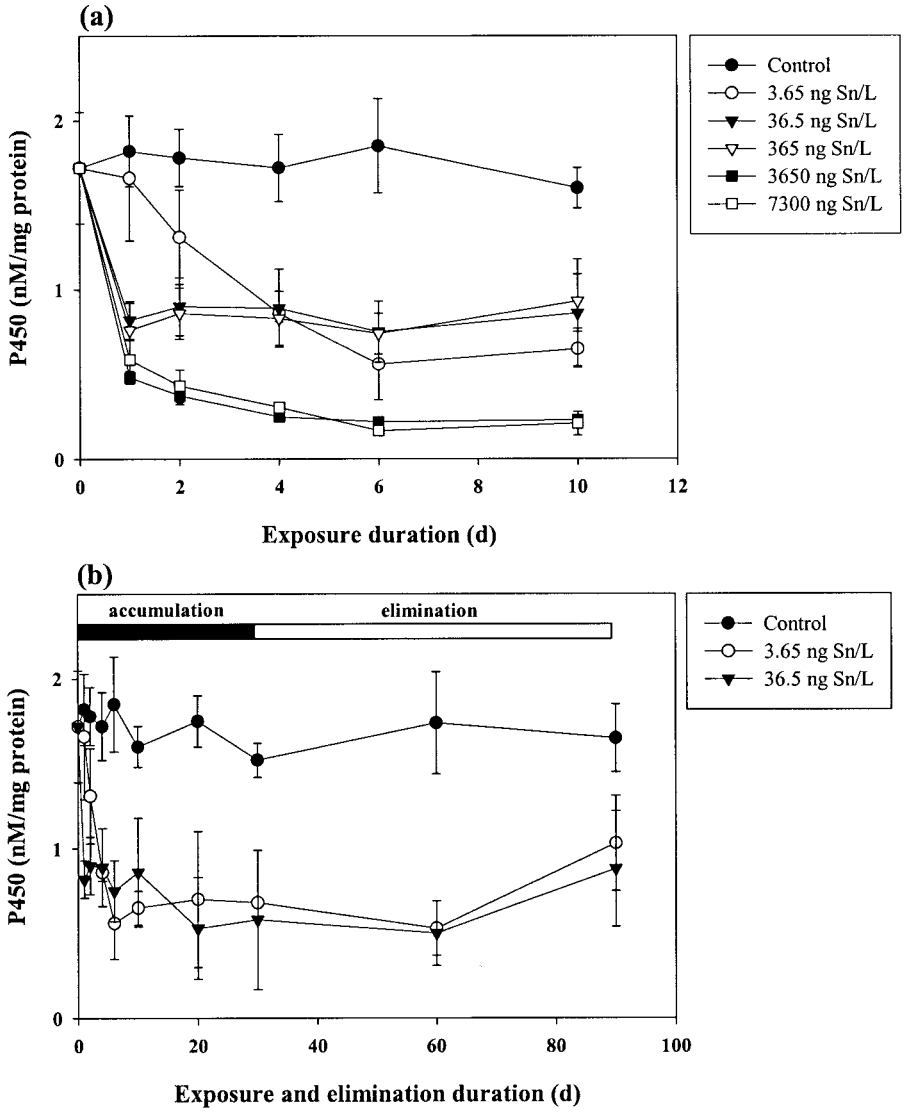

Fig. 3. Change of specific content of cytochrome P450 content in liver of olive flounder exposed to five different tributyltin (TBT) chloride concentrations for (a) after 10-day exposure and (b) after 30-day exposure followed by 60-day elimination at 3.65 and 36.5 ng/L

Table 1. The tributyltin (TBT) concentrations and specific content of hepatic cytochrome P450 in liver of olive flounder exposed to five different TBT concentrations after 10-day exposure

	Exposure Concentration of TBT (ng Sn/L)					
	Control	3.65	36.5	365	3,650	7,300
TBT concentration in liver (ng Sn/g DW)	2	30	71	385	3,010	3,550
Cytochrome P450 (nM/mg protein)	1.60 ± 0.12[a]	0.65 ± 0.10	0.86 ± 0.32	0.93 ± 0.16	0.23 ± 0.01	0.21 ± 0.07

[a] Mean ± SE ($n = 4$).

4. The hepatic cytochrome P450 contents in liver ($r^2 = 0.84$; $p < 0.001$) demonstrated a strong negative correlation with TBT concentrations. The first 6 days of exposure to TBT concentration of 3.65 ng Sn/L led to a decreasing concentration of hepatic cytochrome P450 with increasing TBT concentration ($r^2 = 0.88$, $p < 0.001$) (Figure 5). The significant effect (Student *t*-test; $p < 0.01$) started to appear from the second day of exposure and TBT concentration of 20 ng/g in liver, which was also similar to the concentration of TBT in liver at 36.5 ng Sn/L on the first day of exposure.

Although TBT was obviously accumulated in liver of olive flounder at 36.5 and 365 ng Sn/L of TBT, the hepatic cytochrome P450 content was not significantly ($p > 0.05$) changed with liver burden after 1 day of exposure (Figure 3). However, significant negative correlation ($p < 0.05$) was obtained between P450 content and TBT concentration in liver at higher

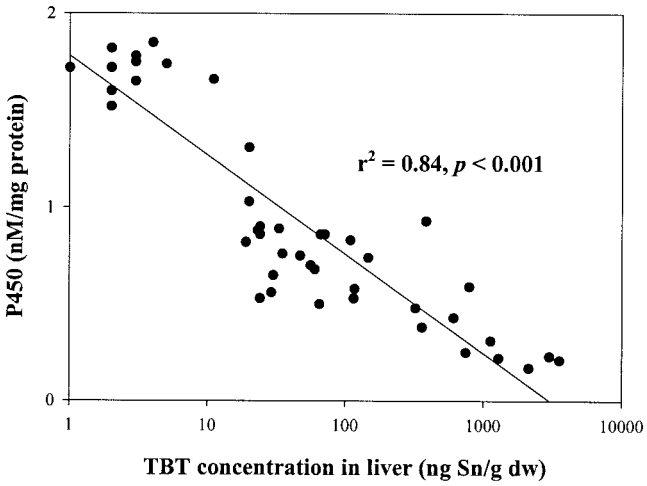

Fig. 4. Relationship between hepatic cytochrome P450 content and tributyltin (TBT) concentrations in liver of olive flounder exposed to TBT chloride, including control and five different exposure groups at all the sampling periods

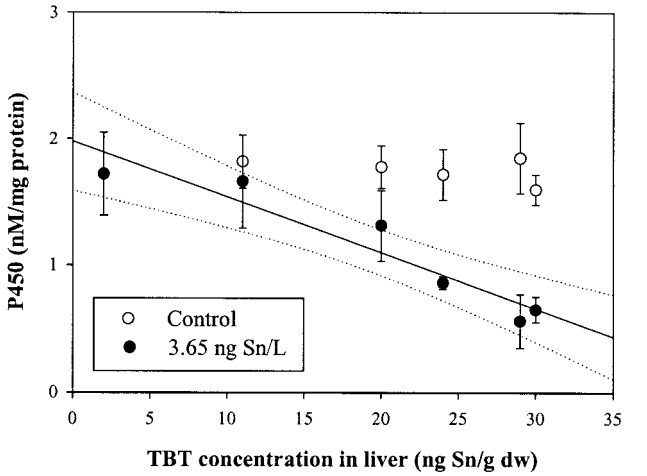

Fig. 5. Relationship between hepatic cytochrome P450 content and tributyltin (TBT) concentrations in liver of olive flounder exposed to 3.65 ng/L of TBT chloride during 6-day exposure (solid circle). Note: Hepatic P450 contents of the control group (open circle) appear together with corresponding liver TBT concentrations of fish at 3.65 ng Sn/L

exposure levels (3,650 and 7,300 ng Sn/L) after 10 days of exposure.

Concentration of Organic Pollutants and Hepatic P450 Content of Collected Fish

The total content of hepatic microsomal cytochrome P450 was measured in fine-spotted flounder collected from Masan Bay. The hepatic P450 content in fish from four stations ranged from 0.16 to 0.33 nM/mg protein (Table 2). The highest hepatic P450 was found at st. 4, the outmost station in Masan Bay. The mean hepatic P450 content showed a negative gradient from st. 1 to st. 3 (Figure 1).

All the fine-spotted flounder samples from Masan Bay contained detectable butyltins (Table 2) in liver. TBT concentration in liver ranged from 37 to 77 ng/g, respectively. The highest total butyltin concentrations were found in liver samples from st. 1. PCBs and OCPs were also detected from muscle of the fine-spotted flounder in Masan Bay (Table 2). The concentration of total PCB and OCP ranged from 147 to 509 ng/g and from 68 to 180 ng/g, respectively. Similarity to butyltins, both total PCB and OCP concentration at st. 1 were also the highest among the four stations. Relatively, high concentrations of PAHs were detected from bile of the fine-spotted flounder in Masan Bay (Table 2). The fish bile from st. 1 showed naphthalene equivalents concentration up to 256,480 ng/g, followed by st. 2 and 4 and then by st. 3. The highest concentration (3,886 ng/g) of benzo(a)pyrene equivalents was also found in fish bile from st. 1, which was followed by that in st. 4, 3, and 2.

Discussion

Effects on Hepatic P450 Content

In a previous study, Fent and Stegeman (1993) reported the significant effect of TBT on the total cytochrome P450 and EROD activity in intraperitoneally injected scup (*Stenotomus chrysops*) with a dose of 16.3 mg/kg body weight. In *in vitro* studies, cytochrome P450 and EROD activity of scup liver were affected by TBT at over 0.05 mM of exposure concentration (Fent and Stegeman 1991). Hepatic microsomal EROD activity of fresh water fish, rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*), and bullhead (*Cottus gobio*) were also apparently reduced at over 0.1 mM of TBT and triphenyltin (Fent and Bucheli 1994). However, in this study, significant effect of TBT on total cytochrome P450 of olive founder was measured at 3.65 ng Sn/L (0.031 pM) and higher exposure concentrations. TBT concentration in liver of olive flounders that showed significant reduction of cytochrome P450 content was about 20 ng/g and higher. These exposure concentrations were often reported in TBT polluted marine environment (Fent 1996). The 3.65 ng Sn/L (10 ng TBT/L) of seawater concentration is close to the water quality criteria of 2 ng TBT/L in U.K.

Comparison Between Effects on Hepatic P450 Content and Liver TBT Residue

TBT is likely to be concentrated in organisms as expected from its log K_{OW} of 4.76 (Arnold *et al.* 1997). Many supportive results have been reported in the accumulation of TBT in various tissues of fish and the toxicity of TBT on organisms has also been demonstrated from bacteria to mammalian species (Fent 1996). However, the relationship between toxicity and accumulated concentration of TBT in target organs was seldom reported with *in vivo* exposure experiments. This study showed a significant negative relationship between concentrations of TBT and specific contents of cytochrome P450 in liver of olive

Table 2. The specific content of hepatic cytochrome P450 and concentrations of butyltins, PCBs, OCPs, and bilary PAHs in fine-spotted flounder collected from Masan Bay in May 1998

Concentration	St. 1 ($n = 4$)[a]	St. 2 ($n = 10$)	St. 3 ($n = 2$)	St. 4 ($n = 9$)
Cytochrome P450[b] (nM/mg protein)	0.26 ± 0.05	0.24 ± 0.02	0.16 ± 0.02	0.33 ± 0.08
TBT[c]	76	37	77	50
ΣBTs (ng Sn/g DW)	231	148	183	96
PCBs[d] (ng/g DW)	509	440	NA[e]	147
OCPs[f] (ng/g DW)	180	154	NA	68
PAHs[g] (Naphthalene eq., ng/g wet weight)	256,480	87,620	27,200	78,926
PAHs[h] (BaP eq., ng/g wet weight)	3,886	1,699	2,029	3,219

[a] Number of fish.
[b] Mean ± SE of individual fish data.
[c] TBT in liver; ΣBTs = sum of TBT, DBT, and MBT in liver.
[d] Sum of PCB in muscle.
[e] Not analyzed.
[f] Organochlorine pesticides; sum of DDT, DDD, DDE, HCHs, chlordane, aldrin, and dieldrin.
[g] Naphthalene equivalent of bilary PAHs.
[h] Benzo[a]pyrene equivalent of bilary PAHs.

flounder. The TBT concentration in liver showing significant ($p < 0.001$) reduction of cytochrome P450 content was approximately 20 ng/g and higher. TBT concentration exceeded 20 ng/g in 32 out 45 fish liver samples collected along the coast or purchased at local markets in Korea (Shim 2000). The effects of hepatic cytochrome P450 content in fish caused by TBT may result in delay of oxidative process of other organic pollutants because cytochrome P450 is a major component of mixed function oxygenase system (Stegeman and Kloepper-Sams 1987). Further study is needed to evaluate an effect of TBT on function of hepatic cytochrome of P450 and its enzymatic activity for activation and deactivation of other organic pollutants.

Complication of Hepatic P450 for Monitoring Organic Pollutant

Most studies on the interaction of environmental pollutants with hepatic microsomal cytochrome P450 have been focused on the induction of this component as a biomarker for assessing the duration and intensity of exposure of fish to xenobiotics (Goksøyr and Förlin 1992). Exposure of fish to certain xenobiotics (*e.g.*, PCBs) led to increase of hepatic cytochrome P450 content (Collier *et al.* 1995) and EROD activity (Gallagher *et al.* 1995). In this study, exposure of olive flounder to TBT, however, reduced the hepatic cytochrome P450 content. The results imply that TBT could be an antagonist in inducing mixed-function oxygenase in fish. In feral fish samples from Masan Bay, the hepatic cytochrome P450 content was in order of st. 4 > st. 1 > st. 2 > st. 3. It is difficult to explain the P450 content in fish with only PCBs, OCPs, and PAH_{bap} concentrations. The P450 content at st. 1 could be the result from interaction between relatively high agonist (PCBs, OCPs, and PAH_{bap}) and antagonist (TBT) concentrations, because the TBT (76 ng/g) and total butyltins (231 ng/g) concentration in liver are high enough to affect hepatic P450 content as shown in the laboratory experiments. Relatively high P450 content at st. 4 could be explained with low TBT and high PAH_{bap} concentrations. The lowest P450 content at st. 3 may reflect the relatively high TBT and low PAH_{bap} concentrations. Although other xenobiotics (*e.g.*, dioxin) that have higher induction potency of P450—sex of fish, ambient water temperature, and susceptibility of fish species (which may affect on hepatic cytochrome P450 contents in feral fish)—were not measured during this study, these preliminary results indicate that only agonist exposure level could not explain distribution of hepatic P450 content in field samples. It is important to be careful when interpreting specific P450 content as a biomarker where TBT concentrations are high. Further study is obviously necessary to evaluate the potential interaction between agonist xenobiotics and TBT to induce cytochrome P450.

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