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Simultaneous Determination of Tri-*n*-butyltin, Di-*n*-butyltin, and Triphenyltin Compounds in Marine Products

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A method for simultaneous determination of tri-*n*-butyltin (Bu_3Sn^+), di-*n*-butyltin (Bu_2Sn^{2+}), and triphenyltin species (Ph_3Sn^+) in marine products is described. The sample was homogenized with methanol, mixed with sodium chloride and hydrochloric acid, and then extracted with a mixture of ethyl ether and *n*-hexane (60:40). After fish fat was removed on a Florisil column, organotin compounds were alkylated with ethylmagnesium bromide. Extracted tetrasubstituted tin compounds were further cleaned by passage through a Sep-Pak Florisil cartridge prior to determination by gas–liquid chromatography with a flame photometric detector (detection limit: 0.2 ng for each organotin compound). Analysis of fish samples, purchased in retail stores, showed accumulations of organotin compounds, suggesting that marine pollution with these tin compounds, especially Ph_3Sn^+, is prevalent over Japan. It was also found that Bu_3Sn^+ is metabolized to Bu_2Sn^{2+} and its hydroxylated product at an alkyl side chain in fish liver.

Organotin compounds are used as stabilizers of polyvinyl chloride, catalysts, pesticides, and marine antifoulants (WHO, 1980). The biocidal properties of tri-*n*-butyltin species (Bu_3Sn^+) and triphenyltin species (Ph_3Sn^+) make them useful for the control of marine organisms such as barnacle and seaweeds, and these compounds are included in ship paints and antifoulants used in Japanese marine farms. Recently, however, pollution of the aquatic environment and marine products by these chemicals has become a major public concern.

We previously reported an analytical method for determination of Bu_3Sn^+ and di-*n*-butyltin species (Bu_2Sn^{2+}) in fish (Sasaki et al., 1988), which employs a combination of tetraalkylation with a Grignard reagent (Meinema et al., 1978) and gas–liquid chromatography with flame photometric detection (FPD–GC). In this report a concise, simultaneous analytical method for Bu_3Sn^+, Bu_2Sn^{2+}, and Ph_3Sn^+ determination, using the same principle but a different kind of cleanup method, is described. This was then applied to a survey of pollution levels in marine products.

For brevity, each of the organotin species is referred to in the paper as if it existed only in cationic form, but this formalism is not meant to imply exact identities for these species in marine products.

MATERIALS AND METHODS

Reagents. Tri-*n*-butyltin chloride, di-*n*-butyltin dichloride (>97%), triphenyltin chloride (98%), and diphenyltin dichloride (96%) were purchased from Sankyo Organic Chemicals Co., Ltd. (Tokyo), Wako Pure Chemical Industries, Ltd. (Tokyo), Tokyo Kasei Kogyo Co., Ltd. (Tokyo), and Aldrich Chemical Co. (Milwaukee, WI), respectively. Ethylmagnesium bromide [3 M in ethyl ether (Et_2O)] was purchased from Tokyo Kasei Kogyo Co., Ltd. Butyl(3-hydroxybutyl)tin dichloride and butyl(4-hydroxybutyl)tin dichloride were synthesized by the method described in the previous paper (Fish et al., 1976; Ishizaka et al., 1989). Morin reagent was purchased from Nakarai Chemicals Ltd. (Kyoto). All chemicals were analytical reagent grade, and organic solvents were HPLC grade or distilled in glass before use. Double-distilled water was used throughout. Silica gel (Wakogel C-100, Wako) was activated for ca. 4 h at 120 °C after addition of half its volume (v/w) of HCl (36%) and overnight equilibrium (Hattori et al., 1984). Florisil PR (Floridin Co., Handcock, WV) was used without further modification. Sep-Pak Florisil cartridges (Waters Associates Inc., Milford, MA) were used in conjunction with a 5-mL glass syringe.

Organotin standard solutions were prepared in ethanol solution. Working standard mixture solution was made by combining each stock solution and diluting it with *n*-hexane to 5 μg/mL for each organotin compound.

Gas–Liquid Chromatography. A GC-9A gas chromatograph (Shimadzu Co. Ltd., Kyoto), equipped with a flame photometric detector (FPD), was operated in the tin mode (filter for 610 nm) with a fused silica capillary column CBP 10 (Shimadzu; equivalent to OV-1701; 0.53 mm (i.d.) × 12 m). Operating temperatures: column oven, programmed from 130 °C (hold 4 min) at the rate of 20 °C/min to 240 °C (hold 5 min); injection port, 240 °C; detector, 300 °C. Gas flow rates: He carrier gas, 20 mL/min; H_2, 150 mL/min; air, 100 mL/min. A Shimadzu C-R2AX was used for data collection, and the concen-

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tration of tetrasubstituted tin was determined by peak height.

Gas–Liquid Chromatography/Mass Spectrometry (GC/MS). GC/MS spectra were obtained by a JMS-DX 300 (JEOL Ltd., Tokyo) in the electron-impact mode at ionization voltage 70 eV and ionization current 300 μA. The column was the same as for FPD–GC. Operating temperatures: column oven, programmed from 130 °C (hold 4 min) at the rate of 16 °C/min to 240 °C (hold 5 min); injection port, 250 °C; separator, 240 °C; ion source, 240 °C. Gas flow rate: He carrier gas, 20 mL/min.

High-Performance Liquid Chromatography (HPLC). HPLC was performed by a postcolumn fluorescence detector system (Yu and Arakawa, 1983). Pumps used were LC-6A (Shimadzu) and Hitachi 635 (Hitachi, Ltd., Tokyo) for the mobile phase and Morin reagent, respectively. A stainless steel column (4.6 mm (i.d.) × 25 cm), packed with Unisil Q CN (5 μm; cyanopropyl-bonded phase; Gasukuro Kogyo Inc., Tokyo), coupled with a precolumn (4.6 mm (i.d.) × 5 cm) with the same packing, was employed. A mobile phase of *n*-hexane–ethyl acetate (EtOAc)–acetic acid (AcOH) (80:20:5) with flow rate of 1.2 mL/min was used. Detections were carried out by postcolumn modification with Morin reagent (0.005% Morin in ethanol at a flow rate of 0.5 mL/min), followed by fluorescence monitoring (excitation 420 nm, emission 500 nm) by a Model RF-535 (Shimadzu).

Sample Preparation. Samples of marine product were purchased on the day of analysis from retail stores in June and August 1988 and stored on ice. Reared yellowtail was purchased in October 1987 and frozen until analysis. One weight of the fish samples was homogenized with two weights of methanol (MeOH) with a Biotron (Biotrona 6403, Küssnacht, SZ). A 10-g portion of NaCl and 50 mL of 3 N HCl were added to the homogenate (15 g, equivalent to 5 g of fish flesh), which was then extracted with two portions of 100 mL of Et_2O–*n*-hexane (60:40, v/v) by shaking for 5 min. After centrifugation (5 min, 3000 rpm), the combined organic extract was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure below 35 °C. The residue was dissolved in a small volume of Et_2O and applied to a Florisil PR column (3 g, 1-cm i.d.), prepared from a slurry of Florisil and Et_2O. The column was first washed with 40 mL of Et_2O and then eluted with 40 mL of AcOH–Et_2O (1:99, v/v). Collected eluate was evaporated under reduced pressure at 35 °C until AcOH was almost removed. Removal of the final volume of AcOH is facilitated by addition of a small amount of *n*-hexane (4–5 mL) to the residue and continued evaporation. The residue was transferred to a 25-mL glass tube (2.2-cm i.d.) sealed with a ground-glass stopper with 2 mL of Et_2O. Ethylmagnesium bromide (2 mL) was carefully added and the solution mixed thoroughly and allowed to stand for 30 min. A 2-mL portion of Et_2O and 10 mL of water were then added drop by drop to the solution in an ice bath until violent bubbling ceased after addition of 1–2 mL of H_2O. After gentle mixing, 0.2 g of solid anhydrous Na_2SO_3 and 2 mL of concentrated HCl were added and the solution was vigorously shaken. This reaction mixture was extracted with two portions of 5 mL of *n*-hexane, and the *n*-hexane solution was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to near dryness. The concentrate was applied to a Sep-Pak Florisil cartridge, prewashed with a mixture of Et_2O–*n*-hexane (1:99, v/v), and eluted with the same solvent mixture. The first 8 mL of eluate was collected and concentrated to 2 mL for FPD–GC.

The standard solutions for FPD–GC were prepared as above except that the Sep-Pak Florisil cleanup procedure was omitted. Standard solutions were stored in a refrigerator in glass bottles containing a small amount of crystalline Na_2SO_3.

Liver Sample Preparation. Liver of reared yellowtail was homogenized two times with MeOH (w/w), and then 8 mL of concentrated HCl and 2 g of NaCl were added to the homogenate (15 g) in a 50-mL centrifuge tube with a ground-glass stopper. This homogenate was extracted with three portions of 20 mL of Et_2O by shaking for 5 min. The combined Et_2O layer was dried over anhydrous Na_2SO_4, evaporated under reduced pressure, and applied to HCl-treated silica gel column (5 g, 1-cm i.d.) with 1 cm of anhydrous Na_2SO_4 on top, prepared from an *n*-hexane–EtOAc (2:1, v/v) slurry. The column was eluted

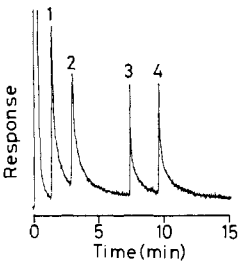

Figure 1. Typical FPD–GC chromatogram of tetrasubstituted tin compounds. Peaks 1–4 are ethyl derivatives of di-*n*-butyltin dichloride, tri-*n*-butyltin chloride, diphenyltin dichloride, and triphenyltin chloride, respectively.

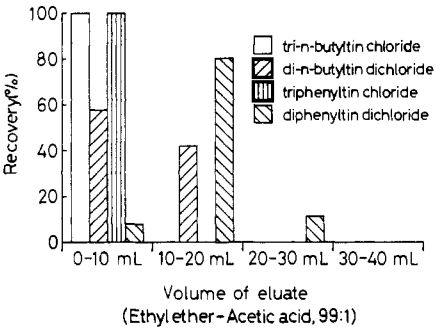

Figure 2. Elution pattern of organotins from Florisil column: 2.5 μg of each organotin was charged on the Florisil column. After the column was washed with ethyl ether (40 mL), it was eluted with ethyl ether–acetic acid (99:1).

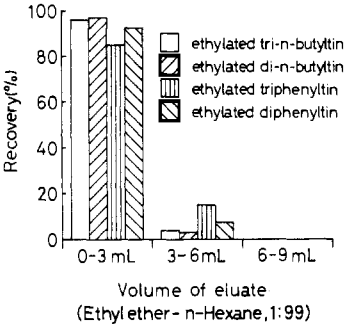

Figure 3. Elution pattern of tetrasubstituted tin derivatives from Sep-Pak Florisil cartridge: 2.5 μg of each organotin was ethylated, charged on Sep-Pak Florisil cartridge, and then eluted with ethyl ether–*n*-hexane (1:99).

with 50 mL of the same solvent mixture, the eluate was evaporated under reduced pressure, and then the residue was dissolved in 10 mL of *n*-hexane. An aliquot of this solution was applied to the HPLC, and 2 mL of the solution was mixed with 2 mL of Et_2O and alkylated as above but without Sep-Pak Florisil cleanup for GC analysis.

RESULTS AND DISCUSSION

Reactions of alkyl- and aryltin compounds with ethylmagnesium bromide are shown in eq 1–4.

$$(n\text{-Bu})_2SnCl_2 + EtMgBr \rightarrow (n\text{-Bu})_2SnEt_2 \quad (1)$$

$$(n\text{-Bu})_3SnCl + EtMgBr \rightarrow (n\text{-Bu})_3SnEt \quad (2)$$

$$Ph_2SnCl_2 + EtMgBr \rightarrow Ph_2SnEt_2 \quad (3)$$

$$Ph_3SnCl + EtMgBr \rightarrow Ph_3SnEt \quad (4)$$

A typical FPD–GC chromatogram of tetrasubstituted organotin compounds formed according to eq 1–4 is shown

Table I. Organotin Recovery under Various Extraction Conditions

HCl concn, N	extractn solvent: ethyl ether–*n*-hexane	recovery,[a] % $(n\text{-Bu})_3SnCl$[b]	$(n\text{-Bu})_2SnCl_2$[c]	Ph_3SnCl[d]	Ph_2SnCl_2[e]
3	60:40	93.2 ± 5.4[f]	108.8 ± 11.1	115.2 ± 8.2	48.5 ± 5.5
3	80:20	86.4 ± 5.3	92.8 ± 6.4	103.2 ± 7.7	48.4 ± 1.1
6	40:60	79.2 ± 3.8	96.7 ± 5.9	85.3 ± 3.4	53.0 ± 10.9
6	60:40	83.3 ± 3.0	94.4 ± 5.5	107.2 ± 7.8	69.8 ± 10.8
6	80:20	86.1 ± 5.0	86.9 ± 5.5	92.9 ± 5.4	g

[a] 2.5 μg of each organotin chloride was fortified to 5 g of flatfish. [b] Tri-*n*-butyltin chloride. [c] Di-*n*-butyltin dichloride. [d] Triphenyltin chloride. [e] Diphenyltin dichloride. [f] Mean of triplicate analyses and standard deviation. [g] Not recovered.

Table II. Organotin Recovery from Various Species of Fish

species	recovery,[a] % $(n\text{-Bu})_3SnCl$	$(n\text{-Bu})_2SnCl_2$	Ph_3SnCl	Ph_2SnCl_2
flatfish	83.3 ± 3.0[b]	94.4 ± 5.5	107.2 ± 7.8	69.8 ± 10.8
flying fish	90.7 ± 4.6	85.4 ± 3.2	95.6 ± 4.5	20.6 ± 2.8
hairtail	88.9 ± 3.0	85.0 ± 2.7	88.2 ± 7.3	11.1 ± 1.2

[a] 2.5 μg of each organotin chloride was fortified to 5 g of each fish. Extraction of MeOH homogenate was practiced by using 6 N HCl and an ethyl ether–*n*-hexane (60:40) mixture. [b] Mean of triplicate analyses and standard deviation.

in Figure 1. The tailing of the peaks is characteristic of organotin compounds in FPD system, and so the peak area is not linearly related to concentration (Maguire and Huneault, 1981). Detector response was, however, linear over the range 0–5.0 ng for each organotin chloride when peak height was measured. Correlation coefficients were 0.9986, 0.9995, 0.9990, and 0.9871 for each chloride of tri-*n*-butyltin, di-*n*-butyltin, triphenyltin, and diphenyltin, respectively. The detection limit for each compound was 0.2 ng (S/N = 3).

In a previous paper (Sasaki et al., 1988), Et_2O–*n*-hexane extract was treated with Grignard reagent and purified on silica gel containing 10% H_2O. Although the cleanup method is effective for the analysis of Bu_2Sn^{2+} and Bu_3Sn^+, interfering substances did not allow the analysis of diphenyltin species (Ph_2Sn^{2+}) and Ph_3Sn^+ in some samples. This interference could be removed by repeated silica gel column chromatography, indicating that fish extracts were overloaded on the column. Ethylated tin compounds are less polar than the original tin compounds and easily eluted from silica gel column with *n*-hexane along with fat overloaded on the column. Therefore, an attempt to remove the fat prior to the Grignard reaction was tried. It is already reported that tri-*n*-butyltin chloride is held on Florisil when *n*-hexane–Et_2O (25:75) is used as an eluting system but easily eluted when 1% AcOH is added to the solvent system (Chikamoto et al., 1988); in addition, Florisil does not hold large amounts of fat.

When 40 mL of Et_2O was used as the solvent, almost all the chemicals were held on Florisil (3 g) and eluted by an AcOH–Et_2O (1:99, v/v) elution solvent system. This procedure could separate tin compounds from 95% of the original fat when mackerel was used as a test sample.

Figure 2 shows an elution pattern of organotins from Florisil PR column. In light of this, the following cleanup procedure was used in subsequent experiments. Sample extract was applied to Florisil PR column (3 g, 1-cm i.d.), and interfering compounds were washed offwith 40 mL of Et_2O. Adsorbed organotin compounds were eluted with 40 mL of an AcOH–Et_2O (1:99, v/v) mixture. Recoveries of di-*n*-butyltin dichloride, tri-*n*-butyltin chloride, diphenyltin dichloride, and triphenyltin chloride from the Florisil PR column were 91.4 ± 1.4, 96.8 ± 1.4, 79.5 ± 6.6, and 88.2 ± 7.7%, respectively.

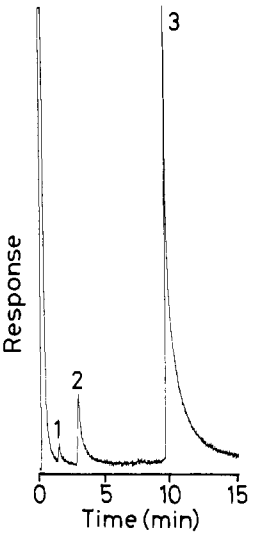

Figure 4. FPD–GC chromatogram obtained from mackerel extract (mackerel no. 1 in Table III). Retention times of peaks 1–3 correspond to ethyl derivatives of authentic di-*n*-butyltin dichloride, tri-*n*-butyltin chloride, and triphenyltin chloride, respectively.

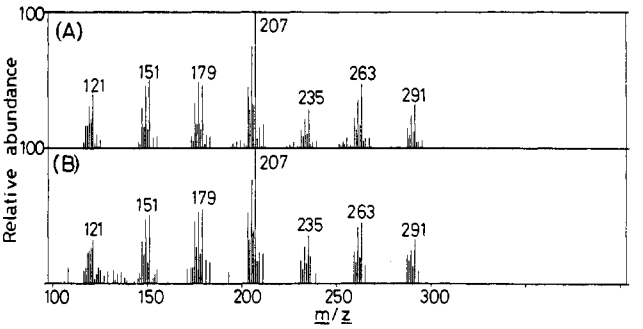

Figure 5. GC/MS spectra of tetrasubstituted tin derivatives: (A) ethyl derivative of authentic tri-*n*-butyltin chloride (RT 2′18″); (B) ethyl derivative of mackerel no. 1 extract.

The above procedure removed almost all interfering substances on the gas chromatogram. However, some samples required further cleanup on a Sep-Pak Florisil cartridge using Et_2O–*n*-hexane (1:99, v/v) as an elution solvent mixture. An elution pattern of tetrasubstituted tin compounds from a Sep-Pak Florisil cartridge is shown in Figure 3. On the basis of these results, the first 8 mL of eluate was collected after application of the samples to the Sep-Pak Florisil cartridge. The recoveries of di- and tri-*n*-butyltin and di- and triphenyltin derivatives from this cartridge were 84.3 ± 5.3, 88.6 ± 6.3, 107.0 ± 7.1, and 107.2 ± 6.4%, respectively.

Extraction of organotin chloride from MeOH homogenate of flatfish was examined using various ratios of Et_2O to *n*-hexane and 3 or 6 N HCl (Table I). Ethyl

Table III. Organotin Analysis in Marine Products

sample	concentration,[a] ppb $(n\text{-Bu})_3SnCl$	$(n\text{-Bu})_2SnCl_2$	Ph_3SnCl
atka mackerel	nd[b]	nd	176
jack mackerel			
no. 1	tr[c]	nd	345
no. 2	tr	nd	111
no. 3	nd	nd	294
grunt	nd	nd	238
butterfly bream	nd	nd	nd
wart perch			
no. 1	55	22	661
no. 2	nd	nd	nd
sardine			
no. 1	186	nd	438
no. 2	206	tr	455
no. 3	125	nd	337
northpacific rockfish	nd	nd	nd
bonito	tr	nd	57
barracuda	319	44	399
flatfish			
no. 1	nd	nd	nd
no. 2	tr	tr	442
black scraper	157	nd	472
sand borer	nd	nd	455
red bream			
no. 1	nd	nd	nd
no. 2	nd	nd	560
salmon	93	tr	50
mackerel			
no. 1	272	47	1530
no. 2	135	31	231
no. 3	209	32	519
spanish mackerel	283	32	1450
pacific saury			
no. 1	nd	nd	nd
no. 2	nd	nd	nd
no. 3	nd	nd	nd
tongue sole	nd	nd	nd
hairtail			
no. 1	278	nd	1090
no. 2	nd	nd	68
no. 3	nd	nd	nd
cod	nd	nd	238
flying fish	nd	nd	nd
pacific herring	213	28	160
bastard halibut	71	nd	103
yellowtail			
no. 1	78	nd	613
no. 2	75	nd	468
no. 3	75	tr	765
no. 4	75	nd	648
no. 5	101	16	177
no. 6 (raised)	1490	56	nd
sea bream	nd	nd	nd
tuna	nd	nd	nd
silver pomfret	nd	nd	nd
short-necked clam	30	tr	166
	tr	tr	118
	nd	21	132
top-shell	tr	tr	nd
japanese fresh water clam	tr	nd	45
	nd	nd	nd
whelk	nd	nd	30
scallop	211	tr	33

[a] Each organotin was analyzed as chloride. [b] nd = not detected. [c] tr = detected but below the detection limit.

ether content greater than 60% (v/v) gave better than 83.3% recoveries of tri-*n*-butyltin chloride, di-*n*-butyltin dichloride, and triphenyltin chloride. Diphenyltin dichloride, a possible degradation or metabolic product of Ph_3Sn^+, was not as efficiently recovered however (0–69.8%). The recovery of diphenyltin dichloride was also greatly influenced by the species of fish (Table II). In spite of exhaustive efforts, a more efficient extraction solvent for diphenyltin dichloride could not be found. The

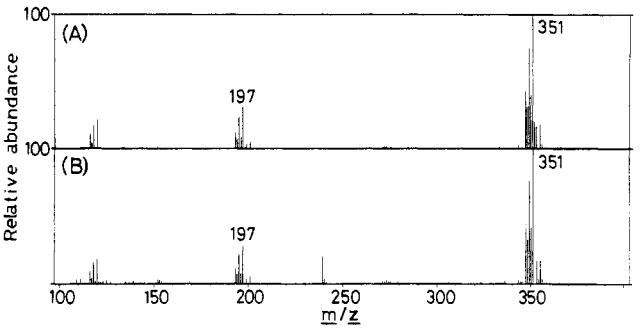

Figure 6. GC/MS spectra of tetrasubstituted tin derivatives: (A) ethyl derivative of authentic triphenyltin chloride (RT 10'10"); (B) ethyl derivative of mackerel no. 1 extract.

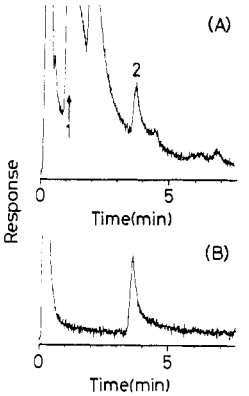

Figure 7. FPD–GC chromatograms [column temperature programmed from 140 °C (hold 4 min) at the rate of 40 °C/min to 240 °C (hold 1 min)]: (A) yellowtail liver extract, peak 1 is ethyl derivative of M – 1, peak 2 is ethyl derivative of M – 2; (B) ethyl derivative of authentic butyl(3-hydroxybutyl)tin dichloride.

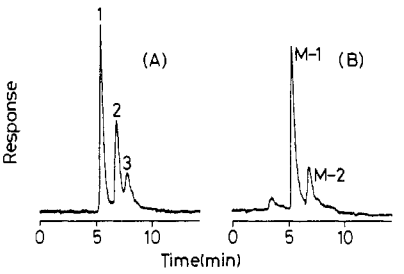

Figure 8. HPLC chromatograms: (A) authentic standards, peak 1 is di-*n*-butyltin dichloride, peak 2 is butyl(3-hydroxybutyl)-tin dichloride, peak 3 is butyl(4-hydroxybutyl)tin dichloride; (B) yellowtail liver extract.

low recovery may be attributed to strong interactions between the diphenyltin group and some components in fish, possibly protein. For example, it is reported that the amino groups of amino acid are easily coordinated to tin in alkyltin compounds (Hall and Zuckerman, 1977) and that one of two binding sites of alkyltin compounds involves histidine in cat and rat hemoglobin (Elliott and Aldridge, 1977). We also previously reported that the recovery of tri-*n*-butyltin chloride differed among fish and the recovery from animal feed was low when a solvent system without MeOH was used as an extraction solvent mixture and that this might be due to binding of the tri-*n*-butyltin group to protein (Sasaki et al., 1988). As the Sn–X bond (X = halogen) in organotin compounds has ionic character and a tendency to be subject to nucleophilic attack by other reagents, it is considered that the chemical forms of organotin compounds change by pH, ion strength, coexisting materials, etc. Accordingly, the use of HCl, NaCl, and MeOH for extraction

seems to be effective to free the bound residue of alkyl- and aryltin species from matrices and to unite the chemical forms of organotin species in fish to chloride, but this could not be applicable to diphenyltin dichloride. In view of this, the study has focused on Bu_2Sn^{2+}, Bu_3Sn^+, and Ph_3Sn^+, and therefore, a combination of 3 N HCl and Et_2O–*n*-hexane (60:40, v/v) mixture was used for extraction.

Table III shows organotin levels in purchased marine products and that Bu_2Sn^{2+}, Bu_3Sn^+, and Ph_3Sn^+ were found at levels of 0.02–0.05, 0.02–1.49, and 0.03–1.53 ppm, respectively. This clearly indicates that tri-*n*-butyltin and triphenyltin compounds are marine pollutants. The reared yellowtail was contaminated with high concentration of Bu_3Sn^+. This may be a consequence of the use of bis(tri-*n*-butyltin) oxide as an antifoulant for the raising net.

A typical FPD–GC gas chromatogram obtained from a mackerel extract (no. 1, Table III) is shown in Figure 4. The peaks at retention times 1′30″ (peak 1), 3′11″ (peak 2), and 9′56″ (peak 3) correspond to ethyl derivatives of di-*n*-butyltin dichloride, tri-*n*-butyltin chloride, and triphenyltin chloride, respectively. The identities of the latter two were further confirmed by GC/MS (Figures 5 and 6). The mass spectra of the ethyl derivative of authentic tri-*n*-butyltin chloride (Figure 5A; RT 2′18″) and that from mackerel (Figure 5B) were almost identical: a base peak at m/z 207 $[Sn(C_4H_9)(C_2H_5)]^+$ and fragment peaks at m/z 121, 151, 179, 235, and 263 and their isotope peaks. Mass spectra of the ethyl derivative of authentic triphenyltin chloride (Figure 6A; RT 10′10″) and an ethyl derivative of mackerel extract at the same retention time (Figure 6B) were also similar. A base peak and fragment peak were present at m/z 351 $[Sn(C_6H_5)_3]^+$ and at m/z 197 $[Sn(C_6H_5)]^+$, respectively, with typical tin isotope peaks.

Reared yellowtail contained very high concentrations of Bu_3Sn^+, e.g. 1.49 ppm in flesh (Table III, yellowtail no. 6), and this led us to investigate the presence of metabolites in yellowtail liver because hydroxylated metabolites had been previously noted in rats both in vivo (Ishizaka et al., 1989) and in vitro (Fish et al., 1976; Kimmel et al., 1977). Metabolites in yellowtail liver were shown by FPD–GC (Figure 7), and 1.64 ppm tri-*n*-butyltin chloride and 3.93 ppm di-*n*-butyltin dichloride (M − 1) were found. The high concentration of the latter compared to 56 ppb in flesh could be due to metabolism of tri-*n*-butyltin species or bis(tri-*n*-butyltin) oxide as it was in the rat.

Another small peak (ethyl derivative of M − 2) was observed at RT 3′4″ (Figure 7A, peak 2), which coincides with that of an ethylated product of authentic butyl(3-hydroxybutyl)tin dichloride (Figure 7B) and was supposed to be formed by eq 5. The possibility that the

$$n\text{-Bu}(CH_3CH(OH)CH_2CH_2)SnCl_2 + EtMgBr \rightarrow n\text{-Bu}(CH_3CH(OH)CH_2CH_2)SnEt_2 \quad (5)$$

small peak (2.4 ppm in fish liver) corresponded to butyl(3-hydroxybutyl)tin dichloride was further supported by comparing the retention time in HPLC with that of an authentic standard (Figure 8). The retention time of M − 2 in the liver extract was identical with that of the authentic standard (peak 2).

CONCLUSION

Although the number of fish studied was limited, the presence of *n*-butyltin and phenyltin species in most samples suggests widespread marine pollution. The especially high levels in the popular fish, mackerel, sardine, and jack mackerel, are of concern since these fish are eatables in Japan. It is recommended that polluted areas be specified and the cause of pollution be eliminated. This study also shows that tri-*n*-butyltin species are metabolized in fish to di-*n*-butyltin species and to hydroxylated metabolite at an alkyl side chain.

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Registry No. $(n\text{-Bu})_3SnCl$, 1461-22-9; $(n\text{-Bu})_2SnCl_2$, 683-18-1; Ph_3SnCl, 639-58-7; Ph_2SnCl_2, 1135-99-5; $n\text{-Bu}(CH_3CH(OH)CH_2CH_2)SnCl_2$, 123168-42-3.

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