# Molecular Species of Tri-*n*-butyltin Compounds in Marine Products

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Molecular species of organotin compounds derived from tri-*n*-butyltin compounds ($Bu_3Sn^+$) were investigated at the parts per billion level in marine products. After being divided into flesh, eggs, and liver, the samples were homogenized and extracted. After purification, the extracts were alkylated with methylmagnesium bromide, and the resulting tetrasubstituted tin compounds were identified by gas–liquid chromatography with a flame photometric detector and gas–liquid chromatography/mass spectrometry/selective ion monitoring (GC/MS/SIM) and quantified by GC/MS/SIM, using authentic specimens. Analysis of fish samples purchased in several retail stores showed the presence of 13 organotin compounds, including triphenyltin compounds. The products hydroxylated and carboxylated at a butyl moiety of $Bu_3Sn^+$ were found in liver, eggs, and flesh. The products hydroxylated at an alkyl side chain of $Bu_2Sn^{2+}$ were mainly found in liver. A carboxylated product was found in liver of only one species.

Trialkyltin and triaryltin compounds have been increasingly used since the early 1970s as biocides and antifouling paints because of their excellent ability to prevent marine organisms from becoming encrusted on ship bottoms and culturing nets. Their usage in antifouling paints, however, had caused retarded growth and abnormalities in oysters reared in the countries along the coast of the Atlantic Ocean by about 1975; they have also caused pollution of the environment and foods on a worldwide scale (Meinema et al., 1978; WHO, 1980; Mueller, 1984; Maguire and Tkacz, 1985; Takami et al., 1987; Sasaki et al., 1988a,b; Ishizaka et al., (1989a). Therefore, several countries have restricted the use of these paints; i.e., they are prohibited on ships smaller than 25 m, and The migration of these compounds from paint into seawater should be below 4 $\mu g\ cm^{-2}\ day^{-1}$ etc.

Most analyses and evaluations of toxicity originally studied only tri-*n*-butyltin (abbreviated to tributyltin, $Bu_3Sn^+$), dibutyltin ($Bu_2Sn^{2+}$), and monobutyltin ($BuSn^{3+}$). But Fish et al. (1976) and Kimmel et al. (1977) showed that $Bu_3Sn^+$ is metabolized in vitro by a rat liver microsome enzyme system to hydroxylated products at the 1-, 2-, 3-, and 4-positions of the alkyl moiety, and Ishizaka et al. (1989b) reported that dibutyltin dichloride administered intraperitoneally in the rat is metabolized to hydroxylated products at the 3- and 4-positions. The aim of present study was to clarify the molecular species of organotin compounds in marine products. The hydroxylated products at the 1- and 2-positions of the butyl moiety, however, are thought to be unstable under the conditions used in this experiment (Fish et al., 1976), and therefore only probable oxygenated derivatives at the 3- and 4-positions of alkyl moieties of $Bu_3Sn^+$ and $Bu_2Sn^{2+}$ were studied. Of the several methods that have been reported for the analysis of organotin compounds, we adopted the tetraalkylation method because it was considered the only one appropriate for this study.

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

## MATERIALS AND METHODS

**Nuclear Magnetic Resonance ($^1H$ NMR) Spectroscopy.** NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer (300 MHz) in $CDCl_3$ with tetramethylsilane as an internal standard ($\delta$ value): s, singlet; d, doublet; t, triplet; m, multiplet.

**Infrared (IR) Spectroscopy.** IR spectra were measured with a Jasco A-102 spectrometer.

**Authentic Standards.** *Tributyltin Chloride (I), Dibutyltin Dichloride (II, >97%), and Triphenyltin Chloride (III, 98%).* The chemicals were purchased from Sankyo Organic Chemicals Co., Ltd. (Tokyo), Wako Pure Chemical Industries, Ltd. (Tokyo), and Tokyo Kasei Kogyo Co., Ltd. (Tokyo), respectively.

*Diphenyltin Dichloride (IV, 96%) and Butyltin Trichloride (V, 95%).* The chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI).

*Dibutyl(3-hydroxybutyl)tin Chloride (VI), Dibutyl(3-oxobutyl)tin Chloride (VII), and Dibutyl(4-hydroxybutyl)tin Chloride (VIII).* The materials were synthesized according to the method described by Fish et al. (1976) and then purified by the method reported by Ishizaka et al. (1989b).

*Butyl(3-hydroxybutyl)tin Dichloride (IX), Butyl(3-oxobutyl)-tin Dichloride (X), and Butyl(4-hydroxybutyl)tin Dichloride (XI).* The materials were synthesized according to the method described by Ishizaka et al. (1989b).

*Dibutyl(3-carboxypropyl)tin Chloride (XII).* The chemical was synthesized as shown in eq 1. A mixture of II (760 mg, 2.5

$$Bu_2SnCl_2(II) + Bu_2SnH_2 \xrightarrow[AIBN]{CH_2CHCH_2COOCH_3} Bu_2Sn(Cl)CH_2CH_2CH_2COOCH_3\ (XIII) \xrightarrow{NaOH/CH_3CN} Bu_2Sn(Cl)CH_2CH_2CH_2COOH\ (XII) \quad (1)$$

mmol) and dibutyltin dihydride (600 mg, 2.5 mmol) was reacted with methyl vinyl acetate (500 mg, 5 mmol) at 35 °C in the presence of $\alpha,\alpha$-azobis(isobutyronitrile) (AIBN, 88 mg, 0.54 mmol) according to the method reported by Fish et al. (1976). After 15 h, the crude material obtained was purified by repeated applications of silica gel (Kieselgel 60, E. Merck) dry column chromatography using a solvent system of ethyl ether ($Et_2O$)–*n*-hexane (20:80 v/v) to yield a colorless syrupy oil, dibutyl[3-(methoxycarbonyl)propyl]tin chloride (XIII): 730 mg, 79%; IR ($CHCl_3$) 1690 $cm^{-1}$ (CO); NMR $\delta$ 0.92 (t, 6 H, $CH_2CH_3 \times 2$, $J$ = 7.2 Hz), 1.25–1.45 (m, 8 H, $CH_2CH_2CH_3 \times 2$), 1.60–1.72 (m, 6 H, $SnCH_2 \times 3$), 1.99 (5 lines, 2 H, $J$ = 6.5 Hz, $CH_2CH_2COOCH_3$), 2.42 (t, 2 H, $J$ = 6.5 Hz, $CH_2CO$), 3.71 (s, 3 H, $COOCH_3$). Anal. Calcd for $C_{13}H_{27}ClO_2Sn$: C, 42.26; H, 7.37; Cl, 9.59. Found: C, 42.46; H, 7.46; Cl, 9.52.

A mixture of XIII (100 mg) in acetonitrile (MeCN, 4 mL) and 4 N NaOH (4 mL) was heated at 80 °C in a water bath for 30 min. After acidification with 4 N HCl (40 mL), the mixture was

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**Table I. Mass Spectral Data of Methylated Organotin Standards**

| Me derivative of | $m/z$[a] (relative intensity) |
|---|---|
| I | 249 (34), 247 (26), 193* (100), 191 (72), 189 (44), 137 (63), 135 (66), 133 (39), 121 (29) |
| II | 249 (3), 207 (37), 205* (31), 203 (17), 151 (100), 149 (70), 147 (47), 135 (40), 133 (28) |
| III | 351* (100), 350 (45), 349 (84), 348 (42), 347 (49), 197 (69), 196 (36), 195 (53), 194 (37), 193 (40), 120 (55), 119 (29), 118 (45), 117 (22), 116 (27) |
| IV | 289* (100), 288 (51), 287 (100), 286 (47), 285 (53), 197 (62), 196 (27), 195 (49), 120 (43), 119 (29), 118 (47), 117 (23), 116 (29) |
| V | 209 (38), 165* (100), 163 (84), 161 (47), 151 (94), 149 (66), 147 (40), 135 (44), 133 (36) |
| VI | 305 (4), 265* (59), 264 (23), 263 (49), 262 (20), 261 (30), 209 (100), 208 (29), 207 (66), 206 (26), 205 (42), 135 (82) |
| VII | 279 (31), 277 (24), 275 (12), 261 (64), 260 (27), 259 (25), 258 (16), 257 (31), 209* (88), 207 (63), 193 (56), 135 (100) |
| VIII | 307 (0.2), 265* (88), 264 (29), 263 (67), 262 (26), 261 (40), 209 (22), 208 (5), 207 (16), 206 (6), 205 (11), 193 (42), 191 (33), 137 (82), 135 (100), 133 (69) |
| IX | 265 (9), 263 (5), 223* (44), 222 (15), 221 (36), 219 (20), 207 (27), 167 (100), 165 (76), 163 (50), 151 (68), 135 (59) |
| X | 279 (4.2), 261 (11), 237 (24), 235 (18), 219 (74), 218 (24), 217* (55), 216 (20), 215 (33), 207 (46), 205 (33), 167 (78), 151 (100), 135 (75) |
| XI | 265 (11), 263 (14), 223* (78), 222 (30), 221 (66), 219 (41), 207 (31), 167 (59), 165 (50), 151 (100), 135 (79) |
| XII | 293 (14), 275* (33), 274 (12), 273 (27), 272 (11), 271 (12), 249 (13), 247 (26), 245 (21), 193 (57), 191 (50), 135 (100), 133 (74) |
| XIV | 275 (6), 251 (11), 233* (46), 232 (14), 231 (32), 230 (11), 229 (18), 207 (39), 205 (56), 203 (40), 151 (100), 149 (76), 135 (65), 133 (50) |

[a] The underlined and asterisked ions were used for qualitative and quantitative analyses, respectively.

extracted three times with $Et_2O$ (50 mL), and the extract was dried over anhydrous sodium sulfate (anhydrous $Na_2SO_4$). Evaporation of the solvent gave a colorless syrupy oil, dibutyl(3-carboxypropyl)tin dichloride (XII): 83 mg, 87%. This material showed a single greenish spot on a silica gel TLC plate (Kieselgel 60) with a solvent system of dichloromethane ($CH_2Cl_2$)–$Et_2O$–AcOH (50:50:1 v/v/v), and after methylation with diazomethane ($CH_2N_2$), it gave a single greenish spot of the same $R_f$ value as XIII on TLC with the same solvent system described above, after spraying with 0.1% dithizone–$CHCl_3$; therefore, XII was used as an authentic standard without further purification.

*Butyl(3-carboxypropyl)tin Dichloride (XIV).* The chemical was synthesized as shown in eq 2. Bromine in $CHCl_3$ (10 mg/mL,

$$\text{XIII} \xrightarrow{Br_2/CHCl_3} \text{BuSn(Cl}_2\text{)CH}_2\text{CH}_2\text{CH}_2\text{COOCH}_3\ \text{(XV)} \xrightarrow{NaOH/CH_3CN} \text{BuSn(Cl}_2\text{)CH}_2\text{CH}_2\text{CH}_2\text{COOH (XIV)} \quad (2)$$

70.8 mL, 8.8 mmol) was added dropwise to XIII (1.46 g, 4 mmol) in $CHCl_3$ (50 mL) with continuous stirring over 1 h at 0 °C according to the method reported by Ishizaka et al. (1989b). The mixture was stirred overnight at room temperature, and then the solvent was evaporated in vacuo. The residue was dissolved in $Et_2O$ (100 mL), and the solution was washed with 4 N HCl (50 mL) containing sodium chloride (NaCl, 5 g). The solution was dried over anhydrous $Na_2SO_4$, followed by filtration and evaporation of the solvent. The residue was purified by silica gel (Kiesel gel 60) dry column chromatography with a solvent system of *n*-hexane–$Et_2O$–$CH_2Cl_2$–AcOH (80:10:10:0.5 v/v/v/v). After evaporation of the solvent, the residue was dissolved in $Et_2O$ (50 mL), washed with 4 N HCl (20 mL), and then dried over anhydrous $Na_2SO_4$. Evaporation of the solvent yielded a colorless oil, butyl[3-(methoxycarbonyl)propyl]tin dichloride (XV): 425 mg, 30.8%; IR ($CHCl_3$) 1685 $cm^{-1}$ (CO); NMR $\delta$ 0.96 (t, 3 H, $J$ = 7.2 Hz, $CH_2CH_3$), 1.45 (6 lines, 2 H, $J$ = 7.2 Hz, $CH_2CH_3$), 1.8–2.0 (m, 6 H, $CH_2CH_2SnCH_2CH_2CH_2CO$), 2.17 (5 lines, 2 H, $J$ = 6.5 Hz, $CH_2CH_2CO$), 2.53 (t, 2 H, $J$ = 6.5 Hz, $CH_2CO$), 3.77 (s, 3 H, $COOCH_3$). Anal. Calcd for $C_9H_{18}Cl_2O_2Sn$: C, 31.08; H, 5.22; Cl, 20.38. Found: C, 31.44; H, 5.21; Cl, 19.62.

XV (159 mg) was treated in the same way as described in the synthesis of XII from XIII to yield a colorless solid, butyl(3-carboxypropyl)tin dichloride (XIV): 131 mg, 86%. As this material showed a single orange spot on a silica gel TLC plate with a solvent system of $CH_2Cl_2$–$Et_2O$–AcOH (50:50:1 v/v/v) and, after methylation with $CH_2N_2$, gave a single spot of the same $R_f$ value as XV with the same solvent system described above, after spraying with 0.1% dithizone–$CHCl_3$, XIV was used as an authentic standard without further purification.

**Gas–Liquid Chromatography (GC).** A GC-15A 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 DB-5 [J&W Scientific, Folsom, CA; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)]. Operating temperatures were as follows: column oven, programmed from 50 °C (hold 4 min) at the rate of 30 °C/min to 200 °C (hold 0 min), followed by the rate of 15 °C/min to 250 °C (hold 12 min); injection port (splitless), 250 °C; detector, 280 °C. Gas flow rates were as follows: He carrier gas, 1.5 kg/cm² (head pressure); $H_2$, 150 mL/min; air, 100 mL/min. A Shimadzu C-R6A was used for data collection.

**Gas–Liquid Chromatography/Mass Spectrometry/Selective Ion Monitoring (GC/MS/SIM).** GC/MS spectra were obtained by an HP 5917A (Hewlett-Packard Co.) in the electron-impact mode at an ionization voltage of 70 eV. The column was a cross-linked methylsilicone [Hewlett-Packard; 0.2 mm (i.d.) × 12 m × 0.3 μm (film thickness)]. Operating temperatures were as follows: column oven, programmed from 35 °C (hold 2 min) at the rate of 30 °C/min to 200 °C (hold 0 min), followed by the rate of 15 °C/min to 250 °C (hold 10 min); injection port, 250 °C; radiator temperature, 185 °C; mass filter, 150 °C. The gas flow rate for He carrier gas was 20 kPa (head pressure). The mass spectrometry data of alkylated compounds are shown in Table I.

Qualitative measurement of each compound was done by SIM using the underlined mass fragment ions shown in Table I with 100 ms of dwell time for each ion after the ions were divided into two groups, i.e., 1–10 ($m/z$ 165, 193, 205, 209, 217, 223, 233, 265, 275, and 289) and 10–12 min ($m/z$ 351), according to the retention times (RTs). Quantitative measurements were carried out by SIM using the asterisked ions in Table I with 100 ms of dwell time for each ion after they were divided into six groups according to their RTs, i.e., Gr-1 (3.00–6.40 min, $m/z$ 165 and 205), Gr-2 (6.40–7.05 min, $m/z$ 217 and 223), Gr-3 (7.05–7.60 min, $m/z$ 193, 223, and 233), Gr-4 (7.60–8.18 min, $m/z$ 209 and 265), Gr-5 (8.18–8.40 min, $m/z$ 265 and 289), and Gr-6 (8.40–12.00 min, $m/z$ 275 and 351). Scanning for each ion was performed from a high mass field to a low mass field. Quantitative determination was performed by the external standards method. Detector response was linear over the ranges 0–4 ng for I, II, IV, and V and 0–2 ng for the other organotin compounds when peak area was measured. Correlation coefficients were 0.9994 (I), 0.9991 (II), 0.9977 (III), 0.9991 (IV), 0.9999 (V), 0.9992 (VI), 0.9974 (VII), 0.9987 (VIII), 0.9996 (IX), 0.9993 (X), 0.9998 (XI), 0.9897 (XII), and 0.9967 (XIV), respectively. The detection limits ($S/N$ = 2 for peak height) were 10 (I–V and X), 15 (VI–VIII), 25 (XI), 30 (IX), and 40 (XII and XIV) pg, respectively.

**Sample Preparation.** Fish flesh samples (50 g), liver (whole), and eggs (whole) were collected and frozen until samples were prepared. Then 0.9% saline (10 mL) was added to the fish sample (5 g, whole for less than 5 g) in a 50-mL centrifuge tube with a screw-cap, and the mixture was homogenized with a Biotron (Biotrona 6403, Küssnacht, SZ). HCl (36%, 12 mL) was added to the

homogenate, and the mixture was shaken vigorously and allowed to stand for 10 min. After the addition of $Et_2O$ (20 mL) and NaCl (2 g), the mixture was shaken for 10 min on a KM shaker (Iwaki Co., Ltd), followed by centrifugation at 3000 rpm for 5 min. This extraction procedure was repeated twice. The supernatant extract was dried over anhydrous $Na_2SO_4$ and evaporated in vacuo, and the residue was dissolved in *n*-hexane (20 mL) saturated with MeCN. The solution was transferred in a 50-mL centrifuge tube with a screw-cap on it, to which MeCN (20 mL) saturated with *n*-hexane was added, and the mixture was shaken vigorously and centrifuged. The underlayer (MeCN layer) was removed by pipetting with a Pasteur pipet. This extraction from the *n*-hexane layer by MeCN (20 mL) saturated with *n*-hexane was repeated once more. The remaining *n*-hexane layer was concentrated to dryness in vacuo below 35 °C, and the residue was dissolved in a small volume of $Et_2O$ and applied to a column (1-cm i.d.) prepared from a slurry of Florisil (Floridin Co., Hancock, WV; 3 g) and $Et_2O$. The column was washed with $Et_2O$ (40 mL) and then eluted with AcOH–$Et_2O$ (1:99 v/v; 40 mL), which was evaporated under reduced pressure at 35 °C. Complete removal of AcOH is facilitated by the addition of a small amount of *n*-hexane to the residue. This residue was combined with the MeCN extract described above, the mixture was concentrated in vacuo to near dryness, and the final volume of solvent was removed under an $N_2$ atmosphere. The residue was dissolved in a small volume of *n*-hexane–ethyl acetate (EtOAc) (2:1 v/v) and transferred to a 1-cm i.d. chromatographic column containing 5 g of HCl-treated silica gel (Wakogel C-100) (Hattori et al., 1984), prepared with *n*-hexane, with 1 cm of anhydrous $Na_2SO_4$ on the top. The column was eluted with a mixture of *n*-hexane–EtOAc (2:1 v/v; 50 mL). The eluate was evaporated in vacuo, and the residue was dissolved in $Et_2O$ (5 mL) and transferred into a screw-capped centrifuge tube (50 mL). Methylmagnesium bromide (MeMgBr) (Tokyo Kasei Kogyo) (ca. 3 M in $Et_2O$, 4 mL) was added carefully to the solution described above, mixed gently, screw-capped, and then allowed to stand for 1 h in a water bath at 40 °C. 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 the addition of water (1–2 mL). After gentle mixing, solid anhydrous $Na_2SO_3$ (0.2 g) and saturated $NH_4Cl$ (6 mL) were added, and the solution was shaken vigorously. This reaction mixture was extracted twice with *n*-hexane (6 mL), and the combined *n*-hexane extract was dried over anhydrous $Na_2SO_4$ and then concentrated to exactly 0.5 mL.

The standard solutions for GC/FPD and GC/MS/SIM were prepared as above except that the final volume was 2 mL. A stock solution of XII and XIV in acetone (20 μg/2 mL each) and other standard stock solutions in *n*-hexane (20 μg/2 mL each) were put into a calibrated test tube (50 mL) with a ground-glass stopper washed beforehand with HCl, and then the solvents were distilled off in vacuo. $Et_2O$ (20 mL) was added to the residue, and then the mixture was shaken vigorously; the portions of the separated $Et_2O$ layer (0–8 mL) were transferred by pipetting to a 50-mL centrifuge tube and then treated as described above. Standard and sample solutions were stored as described in the previous paper (Ishizaka et al., 1989a). Recoveries (percent, mean ± SD, $n = 3$) of organotin compounds added to mackerel flesh at a level of 0.1 μg/g of sample were 93.9 ± 12 (I), 96.8 ± 5.8 (II), 57.7 ± 2.0 (V), 112 ± 13 (XII), 99.8 ± 11 (VI), 86.5 ± 2.4 (VIII), 77.9 ± 3.6 (VII), 105 ± 8.2 (XIV), 109 ± 8.0 (IX), 80.1 ± 8.1 (XI), 92.3 ± 4.3 (X), 72.5 ± 9.7 (III), and 59.8 ± 1.8 (IV), respectively.

## RESULTS AND DISCUSSION

**Extraction and Purification.** Extraction of organotin compounds was principally performed as described in the previous paper (Ishizaka et al., 1989b), but the higher fat content in the marine fish extracts made it difficult to inject the samples directly; therefore, a further purification step, i.e., partition between *n*-hexane and MeCN, was added. After extraction with $Et_2O$ under an acidic condition, organotin compounds were partitioned between *n*-hexane and MeCN. Most of the organotin compounds were found in the MeCN layer, but I (53%) and III (11%) were partitioned into the *n*-hexane layer in a single try and, therefore, were recovered from the *n*-hexane layer throughout Florisil column chromatography, which was described in the previous paper (Ishizaka et al., 1989a). Recovered *n*-hexane-soluble organotin compounds were combined with the MeCN layer and applied to column chromatography on HCl-treated silica gel and then subjected to alkylation. Total recoveries of I and III spiked in fish were improved by this treatment from 64.3 and 70.0 to 93.9 and 72.5%, respectively.

**Alkylation.** MeMgBr was selected as an alkylating reagent for its high reactivity, high sensitivities of the products on FPD/GC (Sasaki et al., 1988b), and relatively small disturbances by matrices because of the early appearance of the products on GC/MS chromatograms. The proposed reactions of alkyltin and aryltin compounds with MeMgBr are shown in

$$BuSnCl_3\ (V) + MeMgBr \rightarrow BuSnMe_3 \quad (3)$$

$$Bu_2SnCl_2\ (II) + MeMgBr \rightarrow Bu_2SnMe_2 \quad (4)$$

$$Bu_3SnCl\ (I) + MeMgBr \rightarrow Bu_3SnMe \quad (5)$$

$$BuSnCl_2CH_2CH_2CH_2CH_2OH\ (XI) + MeMgBr \rightarrow BuSnMe_2CH_2CH_2CH_2CH_2OH \quad (6)$$

$$Bu_2SnClCH_2CH_2CH_2CH_2OH\ (VIII) + MeMgBr \rightarrow Bu_2SnMeCH_2CH_2CH_2CH_2OH \quad (7)$$

$$BuSnCl_2CH_2CH_2CH(OH)CH_3\ (IX) + MeMgBr \rightarrow BuSnMe_2CH_2CH_2CH(OH)CH_3 \quad (8)$$

$$Bu_2SnClCH_2CH_2CH(OH)CH_3\ (VI) + MeMgBr \rightarrow Bu_2SnMeCH_2CH_2CH(OH)CH_3 \quad (9)$$

$$BuSnCl_2CH_2CH_2COCH_3\ (X) + MeMgBr \rightarrow BuSnMe_2CH_2CH_2C(OH)CH_3CH_3 \quad (10)$$

$$Bu_2SnClCH_2CH_2COCH_3\ (VII) + MeMgBr \rightarrow Bu_2SnMeCH_2CH_2C(OH)CH_3CH_3 \quad (11)$$

$$BuSnCl_2CH_2CH_2CH_2COOH\ (XIV) + MeMgBr \rightarrow BuSnMe_2CH_2CH_2CH_2C(OH)CH_3CH_3 \quad (12)$$

$$Bu_2SnClCH_2CH_2CH_2COOH\ (XII) + MeMgBr \rightarrow Bu_2SnMeCH_2CH_2CH_2C(OH)CH_3CH_3 \quad (13)$$

$$Ph_2SnCl_2\ (IV) + MeMgBr \rightarrow Ph_2SnMe_2 \quad (14)$$

$$Ph_3SnCl\ (III) + MeMgBr \rightarrow Ph_3SnMe \quad (15)$$

As the carboxylic acid derivatives, especially XIV, had a tendency to be extensively adsorbed on the surface of glass without acid, the authentic samples were treated with HCl prior to alkylation.

**Identification and Determination of Metabolites.** Metabolites were identified by comparing their RTs with those of authentic specimens by GC/FPD and GC/MS/

**Table II. Relative Retention Times (RRT) of Metabolites and Standards**

| metabolite (peak no.[c]) | standard (Me derivative of) | RRT[a] | RRT[b] |
|---|---|---|---|
| P-1 | butyltin trichloride (V) | 0.569 | 0.579 |
| P-2 | dibutyltin dichloride (II) | 0.817 | 0.825 |
| P-3 | butyl(3-hydroxybutyl)tin dichloride (IX) | 0.964 | 0.953 |
| P-4 | butyl(3-oxobutyl)tin dichloride (X) | 0.974 | 0.967 |
| P-5 | tributyltin chloride (I) | 1.000 | 1.000 |
| P-6 | butyl(4-hydroxybutyl)tin dichloride (XI) | 1.027 | 1.003 |
| P-7 | butyl(3-carboxypropyl)tin dichloride (XIV) | 1.041 | 1.030 |
| P-8 | dibutyl(3-hydroxybutyl)tin chloride (VI) | 1.136 | 1.112 |
| P-9 | dibutyl(3-oxobutyl)tin chloride (VII) | 1.143 | 1.123 |
| P-10 | dibutyl(4-hydroxybutyl)tin chloride (VIII) | 1.198 | 1.164 |
| P-11 | dibutyl(3-carboxypropyl)tin chloride (XII) | 1.211 | 1.186 |
| | diphenyltin dichloride (IV) | 1.211 | 1.164 |
| P-12 | triphenyltin chloride (III) | 1.742 | 1.544 |

[a] RRTs on GC/FPD with a capillary column coated with 5% phenylmethylsilicone (DB-5), relative to $I$ = 8.108 min. [b] RRTs on GC/MS with a capillary column coated with methylsilicone, relative to $I$ = 7.148 min. [c] Peak numbers correspond to those in Figure 1.

SIM. The relative RTs (RRTs) of metabolites and standards are shown in Table II.

Typical FPD/GC chromatograms of tetrasubstituted organotin compounds of two marine product extracts, formed according to eqs 1–13, are shown in Figure 1. However, we could not obtain complete separation of the 13 methylated mixtures into their components on the gas chromatograms by the columns examined. For example, column DB-5, which is depicted in Figure 1 and showed the best separation, could not separate the methyl derivative of IV from that of XII, and some quenching, which was attributed to materials coexisting in the extracts, was also observed with the compounds in the latter region of the chromatograms with some fish species and organs, especially liver. Therefore, although it provided valuable information, the use of FPD/GC was restricted to an overview of the entire figure, and qualitative and quantitative measurements were mainly performed by GC/MS/SIM.

FPD/GC chromatograms of dab flesh (no. 18F) and top shell whole sample (no. 32W) are shown in Figure 1. These indicate the presence of at least 11 organotin compounds in each sample, and the RTs of peaks (P) 1–12 corresponded to those of methylated samples of V, II, IX, X, I, XI, XIV, VI, VII, VIII, XII or IV, and III, respectively.

Further identification of P-1, -2, -5, -11, and -12 with V, II, I, IV, and III, respectively, was confirmed by comparing the mass spectra with those of authentic standards (data not shown). Identification of the other peaks was carried out by employing the mass numbers underlined in Table I by comparing GC/MS/SIM with authentic standards. Among them, only six ions are depicted in Figure 2.

For example, identification of P-3 in the dab flesh extract (18F in Table III) with alkylated IX is illustrated in Figure 2. The results of SIM of standard (IX) show that the peak of $m/z$ 265 is about 6.815 min, those of $m/z$ 221, 167, and 165 are 6.795 min, and those of $m/z$ 223 and 222 are between the two. Since standard IX is a pure chemical and not a mixture, its fragment ions were expected to show the same RT, but contrary to our expectation, they did not coincide with each other. The ions with lower mass numbers showed a shorter RT. Almost the same phenomenon was observed in the dab flesh extract (18F in Figure 2). SIM electric scanning was performed from the high mass field to the low mass field, and therefore we can exclude mechanical delay. Accordingly, the time lag of 0.02 min, i.e., about 1 s, which corresponds to about one scanning cycle, should be attributable to an isotope effect. It is well-known that tin is composed of 10 stable isotopes, and it is reasonable to assume that some of them were separated from each other by the capillary column. A decrease in the dwell time from 100 to 10 ms resulted in a decreased time lag, but the phenomenon was still observed (data not shown). This means that the time lag observed in Figure 2 does not reflect the exact figure but an exaggerated one caused by the long scanning time. Changing the column from methylsilicone to cyanopropylmethylsilicone caused an increased time lag (data not shown). These results support the above explanation. One of the physical isotope effects is that the gas chromatographic retention time of the deuterated compound is shorter than that of a non-labeled compound (Bentley et al., 1965; Waller et al., 1969; Vandenheuvel et al., 1970; Miyazaki et al., 1975). We deduced that the same kind of phenomenon happened in this case.

The correspondence of P-4 and -6–11 to authentic standards X, XI, XIV, VI, VII, VIII, and XII, respectively, in several fishes is also shown in Figure 2, and a similar time lag was observed with these chemicals.

Quantitative measurements were carried out after the ions were divided into six groups according to their RTs to obtain accuracy without loss of sensitivity (Matthews and Hayes, 1976).

**Survey Study.** The Fishery Union of Japan decided to ban the use of bis(tri-*n*-butyltin) oxide for culturing nets in December 1987, as severe contamination in marine products had been observed. Complete control by law was set forth in December 1989, because of the compound's low degradability, high accumulation rate, and long-term toxicity. However, other tri-*n*-butyltin salts like chloride and fluoride and triphenyltin compounds ($Ph_3Sn^+$) were not prohibited; instead, their production and use were restricted, i.e., notice of production and import, adherence to a technical guidelines, and so on were required. Thus, an extensive survey of these substances has been carried out.

Samples of marine products of natural origin were purchased whole in several retail stores in the Tokyo metropolitan area (April–August 1991) where various marine products from Japan and other countries are available. Table III shows organotin levels in the marine products purchased. Relatively high levels of $Bu_3Sn^+$ (I) and $Ph_3Sn^+$ (III) were found in hairtail (1-3F), Japanese sea bass (22F), croaker (5F) flesh, and top-shell (32W) whole sample. The levels of $Bu_2Sn^{2+}$ (II) in liver were higher than in flesh. This tendency was also seen in the previous study on yellowtail (Sasaki et al., 1988b). Generally, the contents of $Bu_3Sn^+$ (I), $Bu_2Sn^{2+}$ (II), and $BuSn^{3+}$ (V) increased in the order flesh, eggs, and liver. The level of $BuSn^{3+}$ (V) in flesh was usually low (<8 ppb), but a relatively high value (87 ppb) was obtained in top-shell (32W) whole sample, along with increased levels of other oxygenated metabolites, although the levels of $Bu_3Sn^+$ (I, 172 ppb) and $Bu_2Sn^{2+}$ (II, 31 ppb) were relatively low.

A carboxylic acid derivative of $Bu_3Sn^+$ (XII) and a hydroxy derivative at the 3-position of a butyl side chain of $Bu_3Sn^+$ (VI) were found at relatively high levels compared with the other two oxygenated metabolites (VII and VIII), in each organ of hairtail (1–4), yellowtail (15 and 16), dab (18–21), and barracuda (27 and 28). The carboxylic acid derivative of $Bu_2Sn^{2+}$ (XIV) was found only in dab (19–21L) liver. The hydroxylated derivative of $Bu_2Sn^{2+}$ (IX) is one of the commonly found metabolites among dibutyltin derivatives, and its presence in yellowtail liver has already been reported (Ishizaka et al., 1989a). Relatively high levels were observed in croaker (5L), yellowtail (16L), dab (19–21L), Japanese sea bass (22L), and barracuda

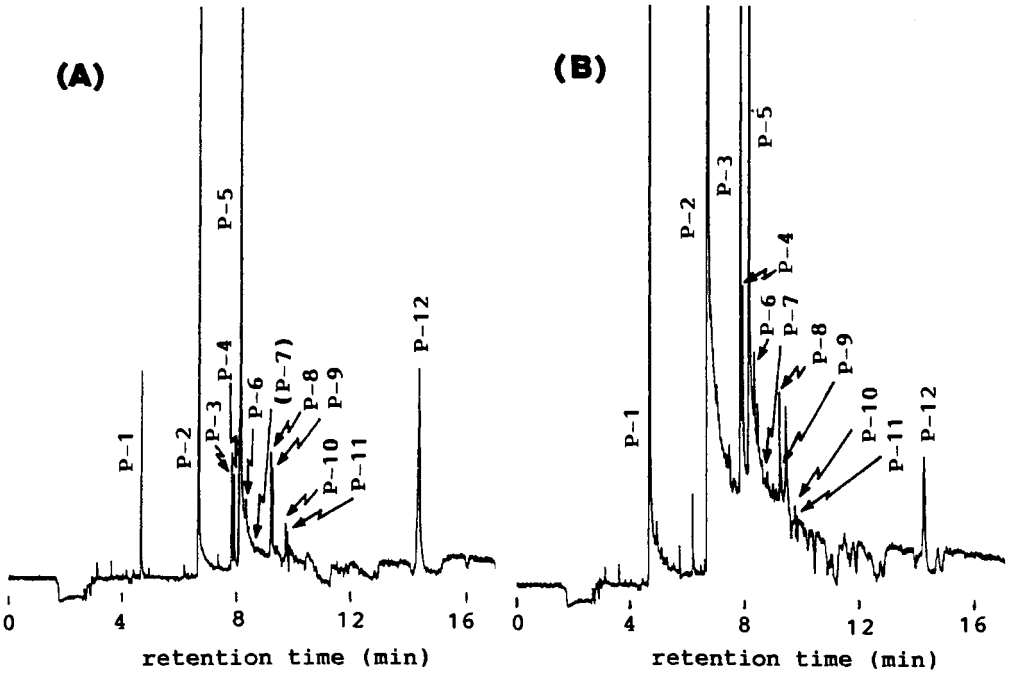


**Figure 1.** Typical FPD/GC chromatograms of marine product extract. (A) Dab flesh extract (18F). (B) Top-shell whole extract (32W). The correspondence of each peak to the authentic sample and the analytical result obtained by GC/MS/SIM are shown in Tables II and III, respectively.

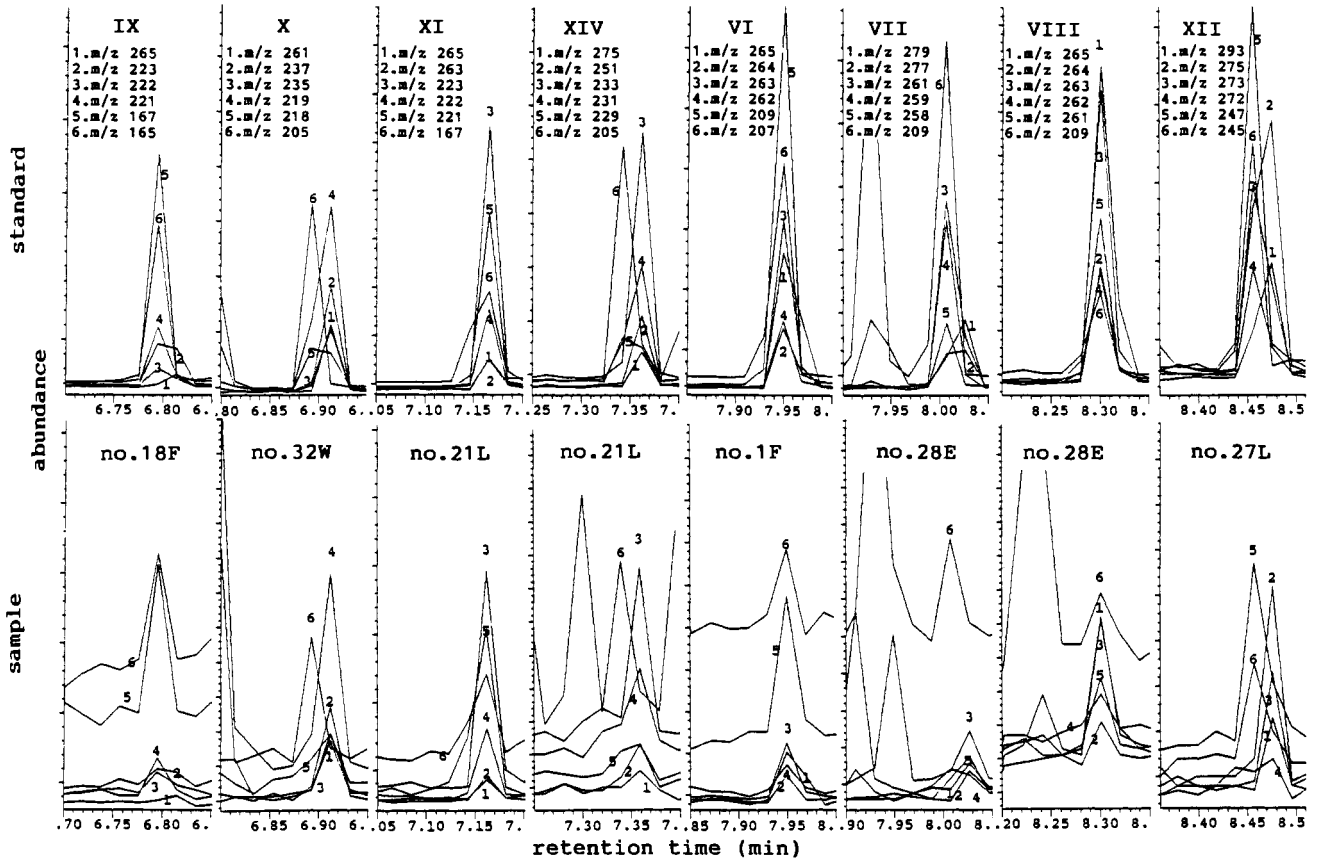


**Figure 2.** Identification of organotin compounds in marine products by GC/MS/SIM in comparison with methylated authentic standards. The authentic samples and the sample numbers and portions used correspond to those in Tables II and III, respectively.

(28L) livers and top-shell (32 and 33W) whole sample, and its content decreased in the order liver, eggs, and flesh. A 4-hydroxybutyl derivative of $Bu_2Sn^{2+}$ (XI) and a 3-oxo derivative of $Bu_2Sn^{2+}$ (X) were also found mostly in liver. It seemed that the metabolic pattern of $Bu_3Sn^+$ in eggs was between those of flesh and liver, and usually the level of each organotin compound was higher in eggs than in flesh. From the point of view of species, many metabolites were observed in dab (18–21F) and yellowtail (16F) flesh, regardless of the low levels of the mother compound, $Bu_3Sn^+$. The levels of $BuSn^{3+}$ in dab liver (19–21L, 37–49 ppb) were especially high compared with those in other fish livers. On the other hand, hairtail (1–4F) and Japanese sea bass (22F) flesh showed no metabolite or only a small amount, if any, in contrast to their high content of $Bu_3Sn^+$. It is not clear whether this reflects the differences in

**Table III. Organotin Analysis in Marine Products**

| no. | sample | | concn,[a] ppb | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | I | II | V | XII | VI | VIII | VII | XIV | IX | XI | X | III | IV |
| 1 | hairtail | F[b] | 174 | 64 | 6 | 16 | 12 | 2 | 4 | 9 | 6 | nd[c] | nd | 229 | 13 |
| 2 | hairtail | F | 501 | 30 | 7 | 12 | 11 | nd | 4 | nd | 7 | nd | nd | 223 | 18 |
| | | E[d] | 431 | 36 | 6 | 17 | 23 | nd | 9 | nd | nd | nd | nd | 131 | 17 |
| 3 | hairtail | F | 214 | 14 | 6 | nd | 5 | nd | nd | nd | nd | nd | nd | 92 | 33 |
| | | E | 243 | 24 | 3 | 19 | 16 | 2 | nd | nd | nd | nd | nd | 59 | 42 |
| 4 | hairtail | F | 92 | 18 | 4 | 10 | 5 | nd | nd | nd | nd | nd | nd | 42 | 31 |
| | | E | 97 | 27 | 3 | 12 | 19 | 2 | 7 | nd | 4 | 3 | nd | 24 | 9 |
| | | L[e] | 133 | 18 | 3 | 11 | 7 | nd | nd | nd | 10 | nd | nd | 66 | 42 |
| 5 | croaker | F | 170 | 23 | 6 | 26 | nd | nd | nd | nd | nd | nd | 6 | 36 | 2 |
| | | E | 128 | 35 | nd | 50 | nd | nd | nd | nd | 12 | nd | nd | 12 | nd |
| | | L | 345 | 96 | 16 | nd | nd | nd | nd | nd | 25 | nd | 6 | 86 | 29 |
| 6 | mackerel | F | 51 | 17 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 80 | 14 |
| | | L | 108 | 34 | 5 | nd | nd | nd | 8 | nd | nd | nd | nd | 65 | 36 |
| 7 | mackerel | F | 30 | 11 | 2 | nd | 5 | nd | nd | nd | nd | nd | nd | 88 | 2 |
| | | L | 33 | 15 | nd | nd | nd | nd | 14 | nd | nd | nd | nd | 146 | 13 |
| 8 | mackerel | F | 38 | 7 | nd | nd | nd | nd | 4 | nd | nd | nd | nd | 37 | 5 |
| | | L | 58 | 30 | 1 | nd | 4 | nd | 9 | nd | nd | nd | nd | 94 | 30 |
| 9 | horse mackerel | F | 16 | 5 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 20 | nd |
| | | L | 45 | 38 | 3 | nd | nd | nd | nd | nd | 17 | nd | nd | 60 | nd |
| 10 | horse mackerel | F | 11 | 5 | nd | nd | nd | nd | nd | nd | nd | nd | nd | 23 | 1 |
| | | L | 25 | 22 | 6 | nd | nd | nd | nd | nd | nd | nd | nd | 19 | 2 |
| 11 | filefish | F | 121 | 17 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 3 | 2 |
| 12 | squid | F | 36 | 5 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 1 | nd |
| 13 | flatfish | F | 28 | 6 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 28 | 6 |
| | | E | 26 | 5 | 2 | 10 | 3 | nd | nd | nd | nd | nd | nd | 12 | 6 |
| 14 | red sea bream | F | 20 | 5 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 29 | 1 |
| 15 | yellowtail | F | 80 | 11 | 4 | 25 | 5 | nd | nd | nd | 8 | nd | nd | 138 | 6 |
| 16 | yellowtail | F | 100 | 14 | 3 | 19 | 3 | 7 | 8 | nd | 8 | nd | 2 | 72 | 2 |
| | | L | 370 | 246 | 7 | 12 | 5 | 3 | 10 | nd | 63 | 8 | 7 | 200 | 28 |
| 17 | scorpion fish | F | 17 | 10 | 8 | nd | nd | nd | nd | nd | nd | nd | nd | 12 | 2 |
| | | E | 14 | 8 | 4 | nd | nd | nd | nd | nd | nd | nd | nd | 78 | 100 |
| | | L | 26 | 12 | 5 | nd | nd | nd | nd | nd | 4 | nd | nd | 130 | 43 |
| 18 | dab | F | 119 | 38 | 6 | 14 | 14 | 9 | 9 | nd | 14 | 9 | 8 | 83 | 3 |
| 19 | dab | F | 37 | 17 | 8 | 7 | 5 | 2 | 6 | nd | 6 | 5 | nd | 74 | 7 |
| | | E | 64 | 35 | 17 | nd | 9 | 8 | 7 | nd | 19 | 9 | nd | 118 | 32 |
| | | L | 54 | 259 | 43 | 8 | 9 | 8 | 8 | 6 | 39 | 21 | 13 | 332 | 95 |
| 20 | dab | F | 33 | 14 | 3 | nd | nd | nd | nd | nd | 6 | 2 | nd | 58 | 2 |
| | | L | 72 | 179 | 37 | 16 | 9 | 20 | 6 | 16 | 28 | 2 | 6 | 313 | 43 |
| 21 | dab | F | 21 | 24 | 5 | 16 | nd | nd | nd | nd | 10 | 2 | 3 | 66 | 4 |
| | | E | 38 | 48 | 18 | 32 | nd | nd | nd | nd | 14 | 8 | nd | 262 | 244 |
| | | L | 25 | 40 | 49 | 26 | nd | nd | nd | 22 | 27 | 31 | 5 | 594 | 66 |
| 22 | Japanese sea bass | F | 284 | 22 | 4 | 4 | 2 | nd | nd | nd | nd | nd | nd | 274 | 15 |
| | | L | 535 | 769 | 35 | 30 | 5 | 8 | 7 | nd | 54 | 5 | 5 | 627 | 226 |
| 23 | rock trout | F | 19 | 9 | 1 | nd | nd | nd | nd | nd | nd | nd | nd | 162 | 25 |
| | | E | 23 | 25 | 4 | 33 | nd | nd | nd | nd | nd | nd | nd | 76 | 18 |
| | | L | 18 | 69 | 7 | 25 | nd | 2 | nd | nd | 10 | 5 | 6 | 106 | 164 |
| 24 | gurnard | F | 67 | 7 | 3 | 12 | nd | nd | nd | nd | nd | nd | nd | 19 | 2 |
| | | L | 169 | 21 | 2 | 11 | nd | nd | nd | nd | nd | nd | nd | 67 | 107 |
| 25 | Pacific saury | F | 20 | 5 | 3 | nd | nd | nd | nd | nd | nd | nd | nd | 7 | nd |
| 26 | rockfish | F | 10 | 5 | 1 | nd | nd | nd | nd | nd | nd | nd | nd | 18 | 1 |
| | | L | 14 | 14 | 2 | nd | nd | nd | nd | nd | nd | nd | nd | 34 | 49 |
| 27 | barracuda | F | 97 | 11 | 7 | 38 | 6 | 3 | nd | nd | nd | nd | nd | 98 | 29 |
| | | E | 185 | 25 | 7 | 67 | 21 | 10 | 5 | nd | 4 | 4 | nd | 59 | 42 |
| | | L | 306 | 100 | 8 | 100 | 27 | 16 | 7 | nd | 8 | 2 | nd | 145 | 170 |
| 28 | barracuda | F | 83 | 12 | 4 | nd | 3 | 3 | nd | nd | nd | nd | nd | nd | nd |
| | | E | 311 | 34 | 4 | 37 | 18 | 26 | 3 | nd | 7 | 3 | 3 | 197 | 78 |
| | | L | 734 | 1190 | 30 | 97 | 15 | 45 | 15 | 16 | 95 | 19 | 9 | 1505 | 199 |
| 29 | clam | W | 11 | 6 | 3 | nd | nd | nd | nd | nd | nd | nd | nd | 19 | 2 |
| 30 | whelk | W | 9 | 6 | 4 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 31 | short-neck clam | W | 47 | 20 | 4 | 8 | 3 | nd | nd | nd | nd | nd | nd | 17 | 4 |
| 32 | top-shell | W | 172 | 31 | 87 | 9 | 16 | nd | 6 | nd | 95 | 6 | 14 | 36 | 10 |
| 33 | top-shell | W | 28 | 63 | 10 | nd | nd | nd | nd | nd | 31 | nd | nd | nd | nd |
| 34 | blue mussel | W | 31 | 42 | 4 | nd | nd | nd | nd | nd | nd | nd | nd | 25 | 3 |
| 35 | surf clam | W | 42 | 35 | 3 | nd | nd | nd | nd | nd | nd | nd | nd | 10 | 5 |

[a] Each organotin was analyzed as chloride. [b] F, flesh. [c] Not detected (<1 ppb for I–V and X; <2 ppb for VII and VIII; <4 ppb for XII and XIV). [d] E, eggs. [e] L, liver.

metabolism of the two fish groups or differences in feeding, which are functions of the environment in which they live.

**Conclusion.** In addition to the mother compounds $Bu_3Sn^+$ and $Ph_3Sn^+$ and their simply dealkylated compounds $BuSn^{3+}$, $Bu_2Sn^{2+}$, and $Ph_2Sn^{2+}$, which have been the subject of previous analyses, the other organotin compounds, which were oxygenated at the 3- or 4-position of an alkyl moiety of $Bu_3Sn^+$ and $Bu_2Sn^{2+}$, were also found in fish flesh, eggs, and liver. The presence of some of these metabolites has already been reported in rat liver microsomal monooxygenase systems in vitro (Kimmel et al., 1977) and in the urine of rats administered $Bu_2SnCl_2$ (Ishizaka et al., 1989b). This indicates that the same kinds of metabolic systems are present in fish and shellfish.

$Bu_3Sn^+$ and its simply dealkylated products $Bu_2Sn^{2+}$ and $BuSn^{3+}$ generally increased in the order flesh, eggs, and liver except in a few species. The oxygenated products of $Bu_3Sn^+$ also tended to increase in the order flesh, eggs, and liver, although their levels were low, while those of $Bu_2Sn^{2+}$ were found mainly in liver. Although levels of the oxygenated products were low in the edible portions of fish, the presence of metabolites in the internal organs should be taken into consideration in evaluating toxicity in shellfish.

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**Registry No.** II, 683-18-1; XII, 142237-40-9; XIII, 117747-99-6; XIV, 142237-42-1; XV, 142237-41-0; dibutyltin dihydride, 1002-53-5; methyl vinyl acetate, 3724-55-8.