# Metabolism of Tri-*n*-butyltin Chloride in Male Rats

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Tri-*n*-butyltin chloride was orally administered to male rats, and the metabolites were investigated. After being divided into organs, blood, and urine, the samples were homogenized and extracted. After purification, the extracts were alkylated with methylmagnesium bromide and the resulting tetraalkylated tin compounds were quantified by gas–liquid chromatography with a flame photometric detector. Analytical results showed that *n*-butyl(3-carboxypropyl)tin compound in the liver and *n*-butyl(3-hydroxybutyl)tin compound in the kidney were the main products, respectively. The former metabolite showed slight accumulation in the liver and kidney and the latter in the kidney. *n*-Butyl(4-hydroxybutyl)- and *n*-butyl(3-oxobutyl)tin compounds were detected only in the urine in small amounts. Trialkyltin metabolites were not found in any organs, blood, and urine. Depending on the results of administration of possible intermediates, it was found that *n*-butyl(3-carboxypropyl)tin compound was formed not only from di-*n*-butyl(3-carboxypropyl)tin chloride and di-*n*-butyl(4-hydroxybutyl)tin chloride but also from di-*n*-butyl(3-hydroxybutyl)tin chloride. The metabolic fate of tri-*n*-butyltin chloride was postulated.

Trialkyltin and triaryltin compounds have been widely used as biocidal agents for boat paint or fishing nets. Marine products have been reported to be contaminated with those organotin compounds (Sasaki et al., 1988a,b; Ishizaka et al., 1989a), and many molecular species derived from tri-*n*-butyltin compounds (TBT) were also present, including the metabolites resulting from hydroxylation and carboxylation at the butyl group of TBT or di-*n*-butyltin compounds (DBT) (Suzuki et al., 1992).

These findings suggest that TBT can be easily metabolized in mammals. The metabolism of TBT in mammals in an in vivo system, however, has been studied only in regard to its simple dealkylated products, i.e., DBT and mono-*n*-butyltin (Iwai et al., 1979). Fish et al. (1976) and Kimmel et al. (1977) reported that TBT is metabolized in vitro by a rat liver microsome enzyme system to the products of hydroxylation at positions 1–4 of the alkyl moiety, and Ishizaka et al. (1989b) also reported that dibutyltin dichloride administered intraperitoneally to rats is metabolized to the hydroxylated products at positions 3 and 4 of a butyl group.

The aim of the present study was to examine these metabolites and determine the metabolic fate of TBT to evaluate the biological effect of TBT pollution on mammals. It is conceivable that the products of hydroxylation at positions 1 and 2 of the butyl moiety are unstable under acidic conditions and they form dibutyltin acetate and 1-butanol from the former and dibutyltin acetate and 1-butene from the latter, respectively (Fish et al., 1976). Unfortunately, this experiment was not designed to detect such metabolites, and therefore only probable oxygenated derivatives at positions 3 and 4 of the alkyl moiety of TBT and DBT were studied. DBT and its oxygenated derivatives are thought to be produced partly by biochemical degradation and partly by chemical degradation of the products formed by hydroxylation at position 1 or 2 of TBT or its oxygenated products under the acidic condition used. Because it was impossible to distinguish them, results of the analysis were expressed as the total amount obtained by both biochemical and chemical degradation. For the sake of brevity, each metabolite is referred to in this paper as if it exists only in the structure indicated, but this does not mean to imply exact identity of these compounds in rats.

Samples and authentic standards were tetraalkylated prior to analysis by a gas–liquid chromatography equipped with a flame photometric detector (GC/FPD) or by gas chromatography/mass spectrometry (GC/MS) to make analysis easier and to avoid changing their chemical characteristics.

## MATERIALS AND METHODS

**Authentic Standard.** *Tri-n-butyltin chloride* (I), *di-n-butyltin dichloride* (II, >97%), and *n-butyltin trichloride* (III, 95%) were purchased from Sankyo Organic Chemicals Co., Ltd. (Tokyo), Wako Pure Chemical Industries, Ltd. (Tokyo), and Aldrich Chemical Co. (Milwaukee, WI), respectively.

*Di-n-butyl(3-hydroxybutyl)tin chloride* (IV), *di-n-butyl(3-oxobutyl)tin chloride* (V), and *di-n-butyl(4-hydroxybutyl)tin chloride* (VI) were synthesized according to the method described by Fish et al. (1976) and then purified by the method reported by Ishizaka et al. (1989b).

*n-Butyl(3-hydroxybutyl)tin dichloride* (VII), *n-butyl(3-oxobutyl)tin dichloride* (VIII), and *n-butyl(4-hydroxybutyl)tin dichloride* (IX) were synthesized according to the method described by Ishizaka et al. (1989b).

*Di-n-butyl(3-carboxypropyl)tin chloride* (X) and *n-butyl(3-carboxypropyl)tin dichloride* (XI) were synthesized according to the method described by Suzuki et al. (1992).

The synthesized authentic standards showed a single spot after spraying of 0.1% dithizone–chloroform in TLC. The structures were also supported by the elemental analysis and NMR spectra.

**Reagents.** Silica gel (Wakogel C-100) for column chromatography was purchased from Wako Pure Chemical Industries, made 50% (v/w) with hydrochloric acid (36%), equilibrated overnight, and activated for ca. 4 h at 120 °C (Hattori et al., 1984). Methylmagnesium bromide (3 M, ether solution) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo).

**Gas Chromatography.** A GC-15A gas chromatograph (Shimadzu Co. Ltd., Kyoto) equipped with a flame photometric detector was operated under the same conditions as described in a previous paper (Suzuki et al., 1992). Quantitative measurements were performed by the external standards method, and peak heights were compared with those of authentic standards.

**Gas Chromatography/Mass Spectrometry and Gas–Liquid Chromatography/Mass Spectrometry/Selective Ion Monitoring (GC/MS/SIM).** GC/MS spectra were obtained by an HP 5971A (Hewlett-Packard Co., Avondale, PA) in the

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electron-impact mode at an ionization voltage of 70 eV. The column used was an HP-1 (cross-linked methylsilicone) [Hewlett-Packard; 0.2 mm (i.d.) × 25 m × 0.33 μm (film thickness)]. Gas flow rate: He carrier gas, 82 kPa (head pressure). Other conditions were described in the previous paper (Suzuki et al., 1992). GC/MS/SIM was performed with the column described above according to the method of the previous paper (Suzuki et al., 1992).

**High-performance liquid chromatography (HPLC)** was carried out with an LC-6A liquid chromatograph (Shimadzu). A stainless steel column (25 cm × 4.6 mm) packed with Unisil Q CN (5 μm, Gasukuro Kogyo) was used. The mobile phase was a mixture of *n*-hexane–ethyl acetate–acetic acid (80:20:5). Detection was carried out by postcolumn Morin reagent modification followed by monitoring the fluorescence (excitation 420 nm, emission 500 nm). Details are given in the previous paper (Ishizaka et al., 1989b).

**Treatment of Animals.** Wistar rats (male, 9 weeks old) were housed at 23.0 ± 2 °C and 50% relative humidity. Compound I was dissolved in soybean oil and orally administered (po) at a dose of 2 mg/kg after 12 h of fasting. The rats were housed in metabolic cages and given access to food and water ad libitum; sacrifice was by decapitation 6 h and 1, 2, 3, 4, 5, 6, and 7 days after the administration. Liver, kidney, spleen, brain, and blood were excised or collected. Urine was collected for every 24-h period.

IV–VI and X were also dissolved in soybean oil and administered intrapreritoneally (ip) at a dose of 4 mg/kg. Liver, kidney, spleen, brain, and blood were removed 1 day after administration. Urine was collected for 24 h.

The samples were frozen (−20 °C) until sample preparation and analyzed after 1–8 weeks.

**Sample Preparation.** Extraction of organotin compounds from organs and purification followed the method of Ishizaka et al. (1989b) except that organotin compounds in HCl-treated silica gel were eluted directly with a mixture of *n*-hexane and ethyl acetate (2:1 v/v) without prewashing with *n*-hexane. The eluate was evaporated to dryness under reduced pressue, and the residue was dissolved in ether (3 mL) and transferred to a screw-capped glass tube. Methylmagnesium bromide (2 mL) was added to the tube. The mixture was shaken gently and allowed to stand for 2 h at room temperature. Then water (10 mL) was added dropwise to the mixture cooled in an ice bath, and anhydrous sodium sulfite (0.2 g) and saturated ammonium chloride solution (4 mL) were added. The mixture was shaken vigorously with *n*-hexane (10 mL) and centrifuged. The organic layer was removed, and the aqueous layer was extracted with additional *n*-hexane (5 mL). The combined organic layer was dried over anhydrous sodium sulfate and carefully concentrated at 40 °C to 1–10 mL. The standard solutions for GC/FPD were prepared as described above after treatment of stock solutions of authentic standards with HCl according to the previous paper (Suzuki et al., 1992). Standard and sample solutions were stored as described previously (Ishizaka et al., 1989a). Recovery of organotin compounds added to the organs was 101–104% for I, 77.5–106% for II, 71.1–102.0% for III, 76.8–105.0% for IV, 90.6–116% for V, 72.2–93.1% for VI, 69.2–78.9% for VII, 96.2–105.0% for VIII, 66.1–78.7% for IX, 69.6–80.5% for X, and 68.1–94.3% for XI. Detection limits in the liver were 0.003 μg/g for I, 0.002 μg/g for II, 0.006 μg/g for III, 0.007 μg/g for IV, 0.006 μg/g for V, 0.005 μg/g for VI, 0.006 μg/g for VII, 0.006 μg/g for VIII, 0.005 μg/g for IX, 0.015 μg/g for X, and 0.014 μg/g for XI.

## RESULTS AND DISCUSSION

**Alkylation.** Methylmagnesium bromide was selected as an alkylating reagent for the reason given in the previous paper (Suzuki et al., 1992). The postulated reactions were also shown there.

**Identification and Determination of Metabolites in Rats Administered I.** Metabolites were identified and quantified by comparing their retention times (RTs) on the GC/FPD chromatograms after their derivatization to tetraalkyltin compounds by methylmagnesium bromide.

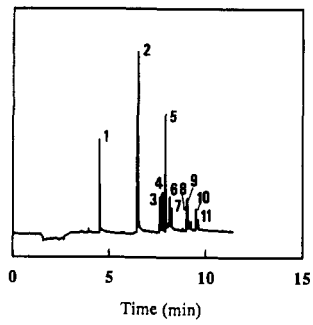


**Figure 1.** Gas chromatogram of methylated authentic standards (0.25 ng). Peaks: 1, III; 2, II; 3, VII; 4, VIII; 5, I; 6, IX; 7, XI; 8, IV; 9, V; 10, VI; 11, X.

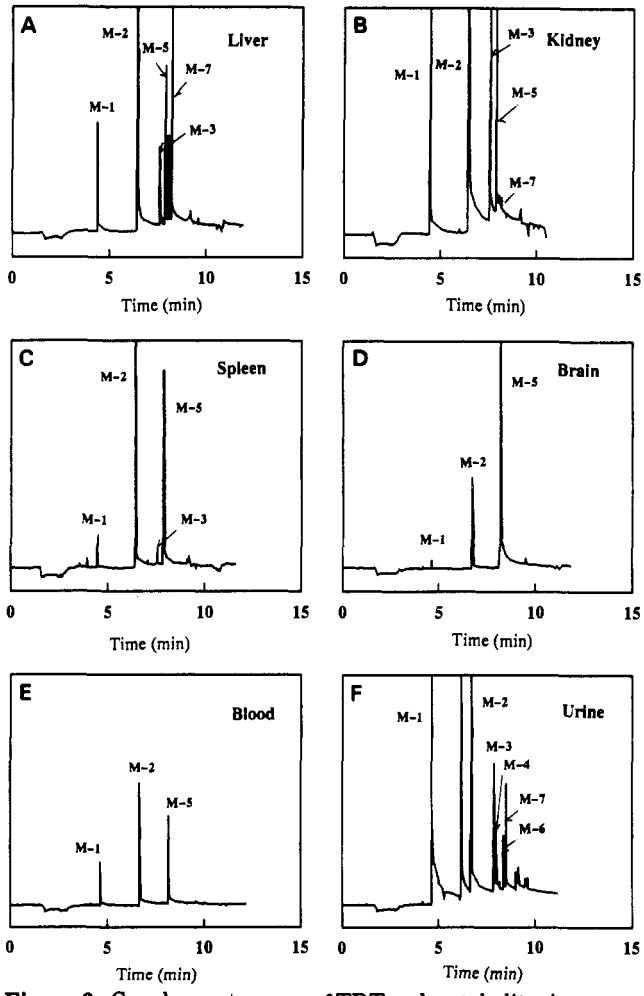


**Figure 2.** Gas chromatograms of TBT and metabolites in organs and urine extract.

The identification of metabolites by authentic standards was confirmed by GC/MS, GC/MS/SIM, or HPLC.

Figures 1 and 2 show typical GC/FPD chromatograms of methylated standard compounds and methylated metabolites extracted from rat organs and urine 1 day after administration of I. These metabolites were designated M-1, M-2, M-3, M-4, M-5, M-6, and M-7 in the order of appearance on the FPD/GC chromatograms (Figure 2). Urine extract gave a complicated chromatogram including peaks whose RTs did not agree with those of any authentic standard (Figure 2F). The chromatograms of the other organs were fairly simple, and the RTs of the peaks agreed with those of authentic standards. The RTs of metabolites and standards on the GC/FPD and GC/MS/SIM chromatograms and correspondence of metabolites to the authentic standards are listed in Table

Table I. Retention Times of Metabolites and Authentic Samples on the GC/FPD and GC/MS

| metabolite | standard, methyl derivative of | RT[a] | RT[b] |
|---|---|---|---|
| M-1 | n-butyltin trichloride (III) | 4.49 | 5.85 |
| M-2 | di-n-butyltin dichloride (II) | 6.47 | 7.69 |
| M-3 | n-butyl(3-hydroxybutyl)tin dichloride (VII) | 7.65 | 8.76 |
| M-4 | n-butyl(3-oxobutyl)tin dichloride (VIII) | 7.72 | 8.87 |
| M-5 | tri-n-butyltin chloride (I) | 7.93 | 9.16 |
| M-6 | n-butyl(4-hydroxybutyl)tin dichloride (IX) | 8.14 | 9.17 |
| M-7 | n-butyl(3-carboxypropyl)tin dichloride (XI) | 8.09 (19%)[c] 8.25 (81%) | 9.43 |
| | di-n-butyl(3-hydroxybutyl)tin chloride (IV) | 9.0 | 10.18 |
| | di-n-butyl(3-oxobutyl)tin chloride (V) | 9.06 | 10.28 |
| | di-n-butyl(4-hydroxybutyl)tin chloride (VI) | 9.49 | 10.65 |
| M-8 | di-n-butyl(3-carboxypropyl)tin chloride (X) | 9.60 | 10.88 |

[a] On GC/FPD with a capillary column coated with 5% phenylmethylsilicone (DB-5). [b] On GC/MS with a capillary column coated with methylsilicone (HP-1). [c] M-7 was split into a pair of peaks on the gas chromatograms with peak height ratio shown in parentheses.

I. The GC/MS spectra and the GC/MS/SIM chromatograms are shown in Figures 3 and 4, respectively.

(1) *M-1, M-2, and M-5.* The RTs of M-1, M-2, and M-5 in each figure corresponded to those of methylated products of authentic standards III, II, and I, respectively. Their identities were confirmed by comparing the mass spectra with those of tetraalkylated authentic specimens (data not shown).

(2) *M-3.* The RT of M-3, which was observed on the GC/FPD chromatograms of methylated liver, kidney, spleen, and urine extracts, coincided with that of the methylated product of the authentic standard, VII, and its identity with VII was confirmed by comparing the mass spectrum at RT = 8.76 min of the methylated kidney extract (Figure 3A) with that of the methylated authentic standard (Figure 3A′).

(3) *M-4.* The RT of M-4 on the GC/FPD chromatogram of the methylated urine extract (Figure 2F) corresponded to that of the methylated product of the authentic standard, VIII. The mass spectrum of M-4 (RT = 8.87) in the methylated urine extract and that of the standard are shown in Figure 3C,C′, respectively. Although their spectra were not superimposable because of the small quantity of M-4, the main mass fragment ions and their isotopes were identical; M-4 was confirmed as VIII on the basis of these data.

(4) *M-6.* The RT of M-6 on the GC/FPD gas chromatogram of the methylated urine extract (Figure 2F) coincided with that of methylated IX. The identity with IX was confirmed by GC/MS/SIM as shown in Figure 4A,A′.

(5) *M-7* in the methylated liver, kidney, and urine extract had the same RT as that of XI on the GC/FPD gas chromatogram (Figure 2A,B,F). The mass spectrum at RT = 9.43 of the methylated liver extract revealed fragment peaks at $m/z$ 293, 275, 251, 233, 205, 167, 151, and 135 and their isotope peaks (Figure 3B). These peaks were also observed in methylated XI (Figure 3B′). The GC/MS/SIM chromatograms (RT = 9.4) of rat urine 2 days after administration are shown in Figure 4B with the methylated authentic standard, XI (Figure 4B′). On the other hand, the HPLC chromatogram of liver extract 1 day after administration (Figure 5A) showed a peak corresponding to XI (Figure 5B) at RT = 12 min, and both peaks shifted to the same RT shorter than those of the original peaks after esterification with diazomethane (Figure 5A′,B′). For this reason, M-7 was identified as XI. A peak observed between M-5 and M-7 in Figure 2A was thought to be an artifact because the peak was also observed in the same ratio in the rat liver sample treated by the authentic standard (XI) alkylated with methylmagnesium bromide as shown in Table I (8.09 min, 19%; 8.24 min, 81%), and the peak did not disturb the determination of M-6.

(6) *Unknown peaks* were observed at RT = 6.19, 8.74, 8.89, 9.24, and 9.37 min on the gas chromatogram of urine extract, but confirmation was not carried out (Figure 2F).

**Distribution and Excretion of Tri-n-butyltin Chloride (I) and Its Metabolites.** Figure 6 shows the concentration of I and its metabolite in the organs and in the urine after administration of I. I (M-5) and its simple dealkylated metabolites M-2 and M-1 were present in every organ, blood, and urine 6 h after administration of I and decreased gradually after the maximum, 6 h–1 day, except brain. In brain, I was present at a far higher level than M-2 and M-1 and showed slight accumulation (Figure 6D). These results almost agree with the results obtained by Iwai et al. (1979). The level of I in brain reached its highest (0.85 μg/g) 2 days after treatment and was 0.1 μg/g even 7 days after treatment. The high concentration and accumulation of I in brain tissue is probably due to the high lipophylicity of I and its easy penetration through the blood–brain barrier. On the other hand, M-1 is the principal metabolite in urine. This suggests that M-1 plays a role as carrier for excretion of organotin compounds because of its high polarity.

In contrast to the observation that only the dealkylated metabolites were present in the blood and brain, M-3, a hydroxylated metabolite, was found in other organs (liver, kidney, and spleen) and in urine (Figure 6A–C,F). The metabolite had also been observed in these organs after the administration of II (Ishizaka et al., 1989b). The decrease in M-3 level in the kidney was slower than in the liver and spleen. The maximum concentration of M-3 in the kidney was 4.3 μg/g 2 days after administration of I and was 0.835 μg/g even 7 days after administration when the metabolite was not detected in other organs. The concentration of M-3 in urine also decreased gradually with the decrease in the concentration of M-3 in the kidney, and 0.4 μg of M-3 was excreted in the urine between 6 and 7 days after administration of compound I. The high concentration and slow decrease of M-3 in the kidney suggest the high affinity of the compound to kidney tissue. Ishizaka et al. (1989b) also reported slight accumulation of M-3 in the kidney after intraperitoneal administration of II.

M-7, which was present in the liver at a high concentration after oral administration of I (Figure 6A), was not detected after intraperitoneal administration of II (Ishizaka et al., 1989b). M-7 was the principal metabolite of I in the liver but it was not observed in the blood. The concentration of M-7 in the liver increased until 2 days after treatment and then decreased slowly (Figure 6A). The highest level of M-7 in liver was 11.8 μg/kg (2 days).

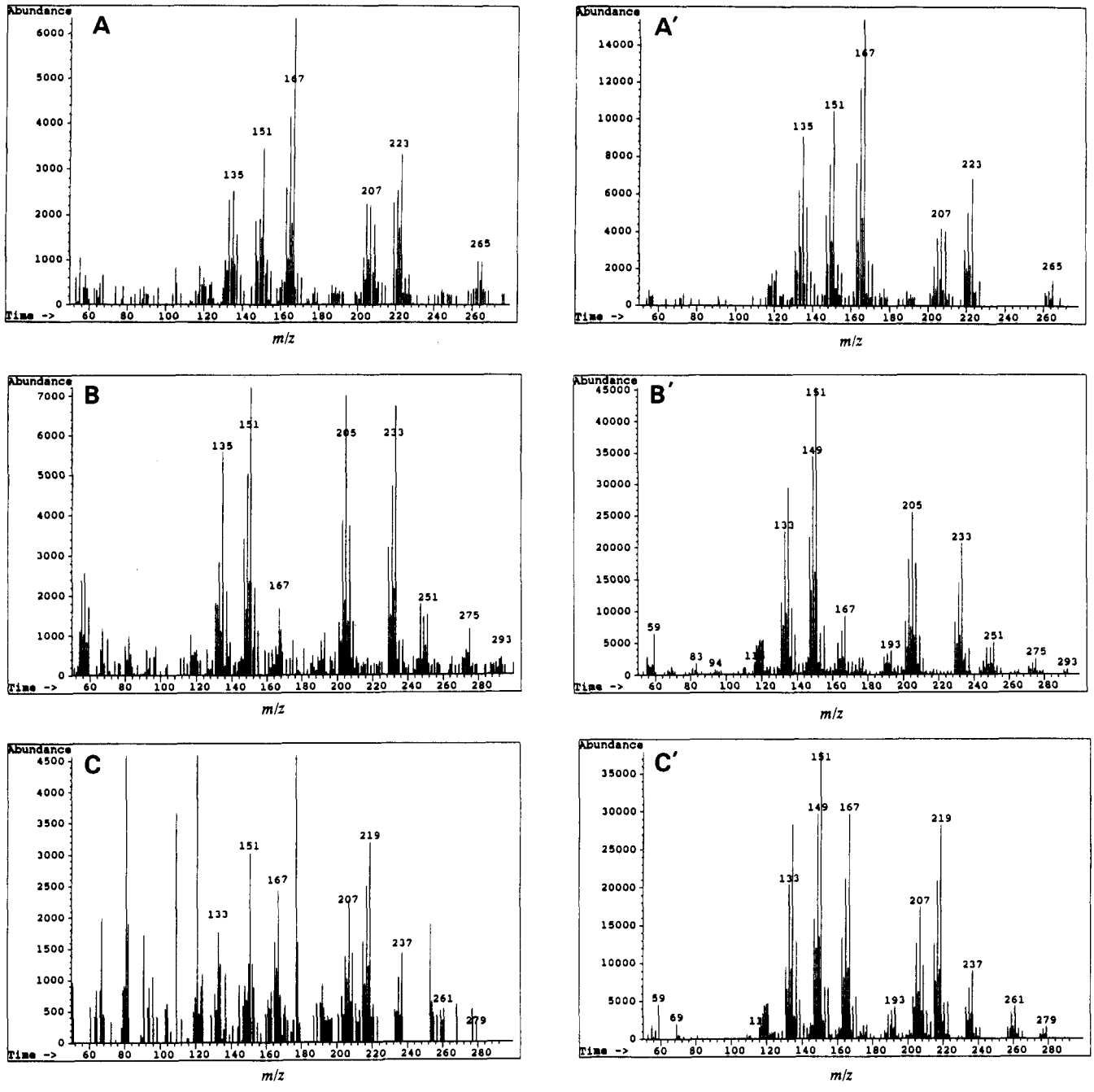


**Figure 3.** GC/MS spectra of methylated metabolites in organs and standards. A, M-3 in kidney extract; A′, authentic standard of VII; B, M-7 in liver extract; B′, authentic standard of XI; C, M-4 in urine extract; C′, authentic standard of VIII.

The decrease in M-7 was so slow that the concentration was 3.4 μg/g 7 days after administration when the concentrations of other metabolites in the liver were very low or below the limit of detection. M-7 as well as M-3 accumulated in the kidney (Figure 6B). The level of M-7 in the kidney did not vary from 2 to 7 days. The metabolite was excreted in urine until 4 days after administration, but no excretion of M-7 into urine was found 5 days after administration in spite of the presence of the metabolites in kidney tissue in fairly high concentration (Figure 6F,B).

In addition to the above-mentioned metabolites, M-4 and M-6 were excreted in urine at lower levels in the first 2 days (Figure 6F). These metabolites were not detected in the kidney or liver. These findings suggest rather low production and rapid excretion of M-4 and M-6.

The metabolites caused by hydroxylation at a butyl moiety of I, which Kimmel et al. (1977) identified in their in vitro experiment, were not detected in any organ. These results show that I is excreted into urine mainly in the form of M-1, M-3, and M-7 but rather a large amount of tin is present in liver and kidney in the form of M-3 and M-7 after elimination of tin compounds in the blood.

**Administration of Expected Intermediates and Their Metabolism.** That M-7 was not present after administration of II indicates that metabolite is not necessarily formed by simple carboxylation of M-2. It is probable that the metabolite is produced by dealkylation of oxidized metabolites of I. Therefore, the possible intermediates, hydroxylated, carbonylated, and carboxylated products of I, that is IV, VI, V, and X, were intraperitoneally administered to rats to confirm this presumption. Figures 7–9 show the level of each metabolite in liver, kidney, and urine 1 day after administration. Structures of administered compounds (Roman numerals) and the metabolites (Arabic numerals) are shown in Figure 10. In any organ the concentrations of M-1 and M-2 were

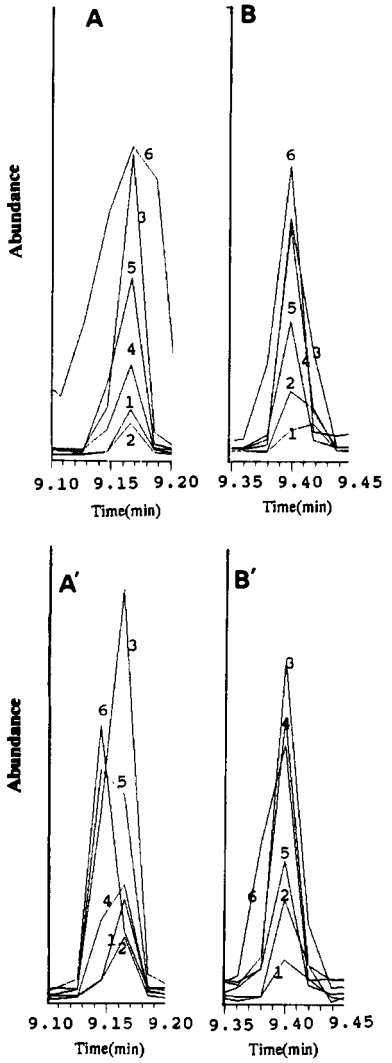


**Figure 4.** GC/MS/SIM of methylated metabolites in urine extract and the standards. A, M-6 in urine extract; A′, authentic standard of IX; B, M-7 in urine extract; B′, authentic standard of XI. Ion: A and A′, 1, *m/z* 265; 2, *m/z* 263; 3, *m/z* 233; 4, *m/z* 222; 5, *m/z* 221; 6, *m/z* 167. B and B′, 1, *m/z* 275; 2, *m/z* 251; 3, *m/z* 233; 4, *m/z* 231; 5, *m/z* 229; 6, *m/z* 205.

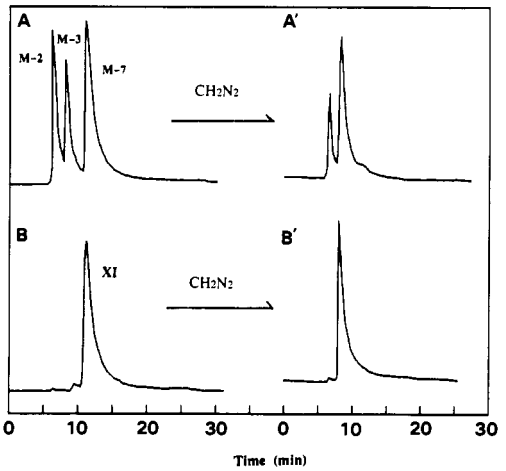


**Figure 5.** High-performance liquid chromatograms of liver extract and authentic standard XI. A, Liver extract; A′, liver extract treated with diazomethane; B, authentic standard of XI; B′, authentic standard of XI treated with diazomethane.

very low in comparison with the levels after administration of I, although the routes and doses were different (I, 2 mg/kg, po; others, 4 mg/kg, ip), and the debutylated

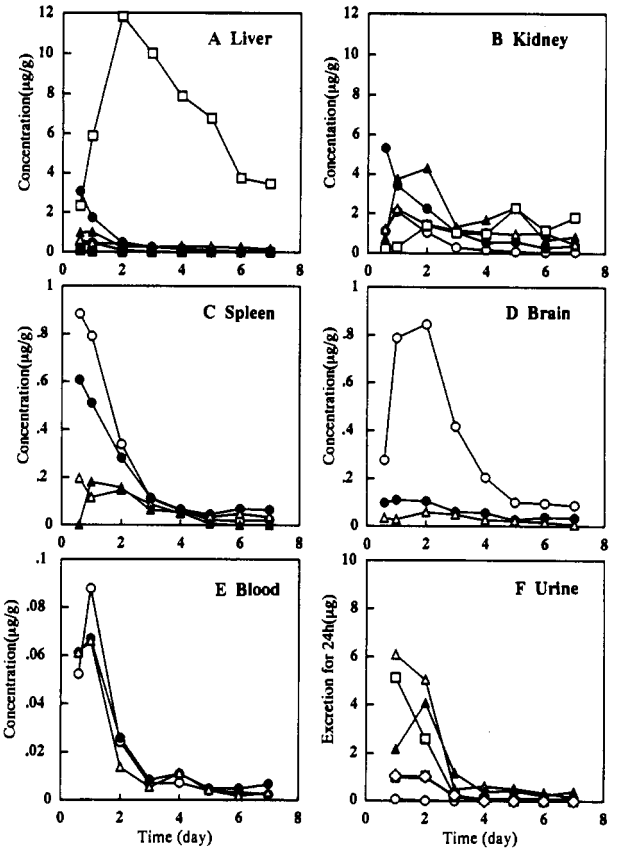


**Figure 6.** Concentrations in organs and excretion of TBT and its metabolites. Results are averages of five experiments: A, liver; B, kidney; C, spleen; D, brain; E, blood; F, urine. Symbols: (○) M-5; (●) M-2; (△) M-1; (▲) M-3; (□) M-7; (■) M-4; (◇) M-6.

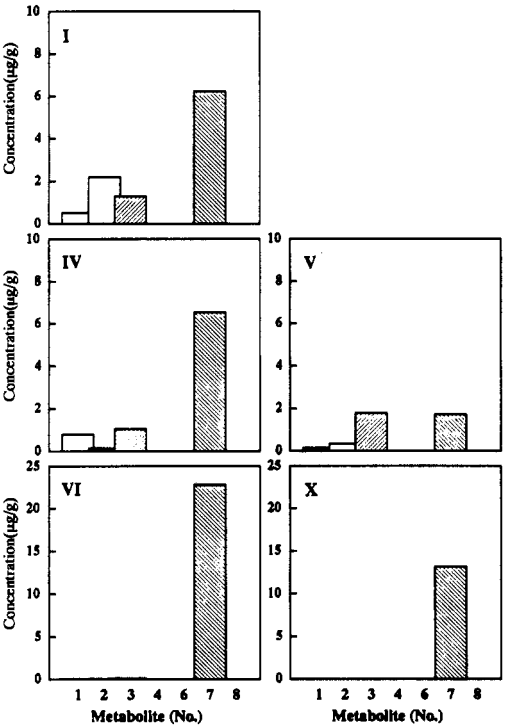


**Figure 7.** Concentration of TBT metabolites in liver after administration of I, IV–VI, and X.

product of each compound was excreted principally in the urine. Probably this means that the positions which are subject to first oxidation have an important effect on determination of final metabolites and that M-1 and M-2

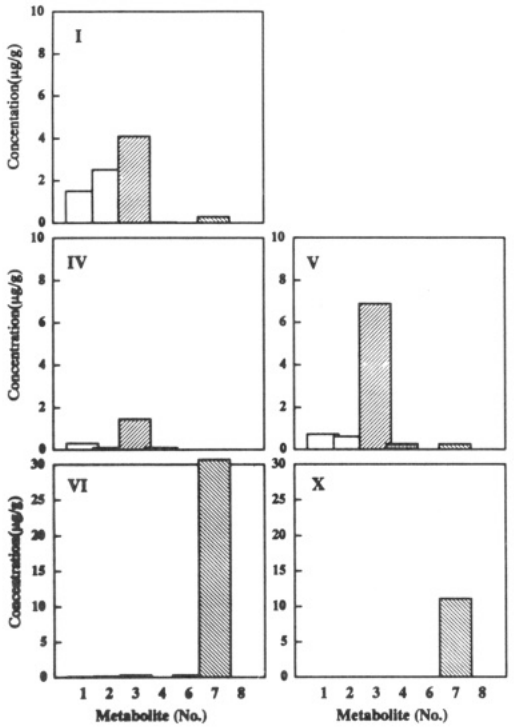


**Figure 8.** Concentration of TBT metabolites in kidney after administration of I, IV–VI, and X.

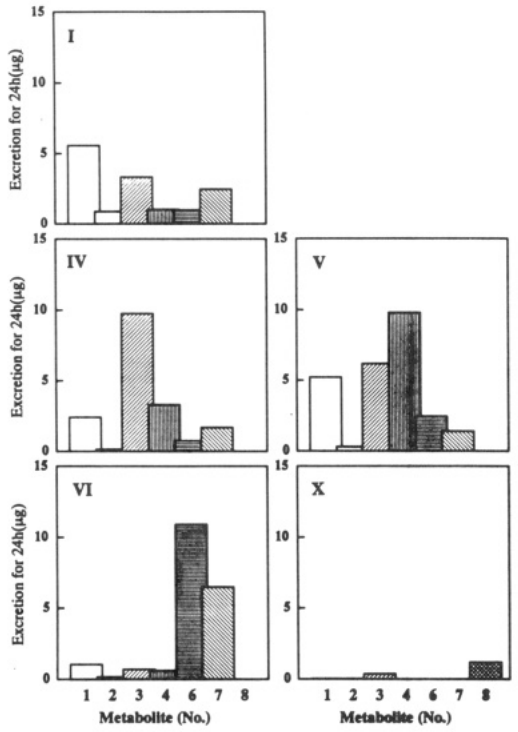


**Figure 9.** Excretion of TBT metabolites in urine after administration of I, IV–VI, and X.

were transformed via 1- or 2-oxidation at alkyl moieties of I or DBT derivatives.

The administration of V resulted in a high level of M-4 in urine that was present at a lower level after administration of I (Figure 9V). In addition, a relatively high level of M-3 was present in urine after administration of V, and conversely M-4 was present after administration of IV (Figure 9IV). This indicates the possibility that IV and V or VII and VIII are transformed into each other. M-3 was also found in urine after administration of II (Ishizaka et al., 1989b). Therefore, M-3 will be formed by

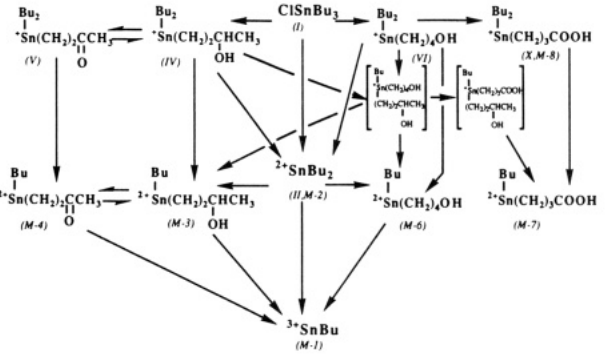


**Figure 10.** Speculated pathway of tri-*n*-butyltin chloride metabolism.

dealkylation of IV as well as hydroxylation of II. Probably most of the M-3 or M-4 which was obtained by metabolism of I could come from dealkylation depending on biochemical and/or chemical degradation of the hydroxylated products at position 1 or 2 of IV or V. This point of view can also apply to other metabolites. In kidney, M-3 is dominant after administration of IV or V (Figure 8IV,V). This agrees with the speculation that M-4 is excreted faster than M-3, which shows high affinity to kidney tissue and stays in the kidney.

M-7 was not found in urine in spite of the presence of M-6 after administration of II (Ishizaka et al., 1989b). On the other hand, administration of VI gave M-7 at much higher yield in liver (Figure 7VI), kidney (Figure 8VI), and urine (Figure 9VI), and also M-6 in urine (Figure 9VI), than that of I, IV, or V, indicating that formation of M-7 is extensively correlated with VI. Administration of IV and V also gave a relatively high amount of M-7 in the liver (Figure 7IV,V) and M-6 as well as M-7 in the urine (Figure 9IV,V). This finding suggests the possibility that M-7 could be produced via a diol, the product formed by hydroxylation at positions 3 and 4′ of two different butyl groups of I, followed by oxidation to a 3-hydroxy-3′-carboxylic acid and removal of a 3-hydroxy group resulting from hydroxylation of position 1 or 2. On the other hand, M-7 is the only metabolite in liver and kidney caused by administration of X (Figures 7X and 8X), and M-8 (X) was also excreted in urine as a main product, although the amount is not very great (Figure 9X). M-6 and M-7 were not excreted. These observations show that M-7 is produced via VI, M-8(X), and/or IV and not via M-6. It is very strange that M-7 was absent from urine after administration of X in spite of its presence in the liver and kidney and relatively low excretion into urine as total organotin compounds. It may have another excretion route such as conjugation.

A proposed metabolic fate of I in the rat is as follows (see Figure 10). I may be partially debutylated through M-2 to M-1, and the resulting M-1 may be rapidly excreted into the urine. The hydroxylation at position 3 of a butyl group of I, followed by dealkylation depending on 1- or 2-hydroxylation, will give M-3 or M-4, and 3- and 4-hydroxylation on different butyl groups of I, oxidation of $CH_2OH$ to COOH, and removal of the 3-hydroxybutyl group resulting from 1- or 2-hydroxylation will give M-7. The hydroxylation at position 4 probably gives principally M-7, partly via M-8 and mainly via 3,4-diol, a hypothetical intermediate shown in brackets in Figure 10. On the other hand, removal of the 4-hydroxybutyl group from 3,4-diol will yield M-3. These metabolites are excreted in the urine, and the excretion rate of M-3 and M-7 is slow in comparison with those of M-4 and M-6; a large part of M-3 is present in the kidney and M-7 is present in the liver and kidney

for a long time. Therefore, toxicity of M-3 and M-7 should be determined to assess the effect of TBT compound pollution.

**Conclusion.** Tri-*n*-butyltin chloride is metabolized in male rats to many oxidized forms as well as the simply dealkylated products. The principal metabolites in blood and brain are simply dealkylated species, di-*n*-butyltin and mono-*n*-butyltin compounds, but in liver and in kidney *n*-butyl(3-carboxypropyl)tin and *n*-butyl(3-hydroxybutyl)-tin compounds are dominant. These metabolites are not detected in blood and brain but are present in high concentration in liver and kidney for a long time in spite of the fairly rapid decrease in di-*n*-butyltin and mono-*n*-butyltin compound levels in blood.

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