Some Sulfur-Containing Metabolites of Tri-*n*-butyltin Chloride in Male Rats

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In an attempt to elucidate metabolic destination of TBTO, sulfur-containing metabolites were investigated in the urine. Tri-*n*-butyltin chloride (TBTC), tri-*n*-butyltin oxide (TBTO), and their in vitro metabolites in rat liver microsomal enzyme systems, di-*n*-butyl(3-hydroxybutyl)tin chloride (T3OH), di-*n*-butyl(3-oxobutyl)tin chloride (T3CO), dibutyltin dichloride (DBTC), and monobutyltin trichloride (MBTC), were intraperitoneally administered to rats. In particular, administration of T3OH and T3CO gave higher amounts of mercapturic acid derivatives, such as *N*-acetyl-*S*-(3-oxobutyl)-L-cysteine (3CO-MA) and *N*-acetyl-*S*-(3-hydroxybutyl)-L-cysteine (3OH-MA), than TBTC or TBTO. On the other hand, DBTC and MBTC did not yield measurable amounts of 3CO-MA and/or 3OH-MA. The appearance of organotin metabolites in urine indicates that T3OH, T3CO, and hypothesized secondary metabolites, such as *n*-butyl(3-hydroxybutyl)(3-oxobutyl)tin chloride, *n*-butyl-(3-hydroxybutyl)(4-hydroxybutyl)tin chloride, etc., are subject to the action of glutathione *S*-transferase to give mercapturic acid derivatives. These sulfur-containing metabolites (3CO-MA and 3OH-MA) were also found in control rat urine.

Keywords: *TBTO; TBT; TBTC; tributyltin; mercapturic acid; glutathione conjugate; metabolism; sulfur-containing metabolite*

INTRODUCTION

Organotin compounds have been widely used as active ingredients in antifouling agents during the last three decades, and annual fuel savings due to TBT-based paints are estimated to be about $3 billion worldwide (Rouhl, 1998). However, they have also been responsible for many deleterious effects to nontarget marine and aquatic ecosystems. This situation prompted industrialized countries to introduce regulations aimed at reducing the impact of this toxic compound in the environment. It have been generally accepted that the production and uses of these chemicals have decreased in developed countries and that TBT concentrations in water, sediment, and bivalve tissues have been declining (O'Conner, 1996; Russel et al., 1996; U.S. Navy and U.S. Environmental Protection Agency, 1997; Cardwell et al., 1999; Ueno et al., 1999), but the annual production of TBT remains almost unchanged in the past 10 years on a worldwide basis (Bennett, 1996). This means the production of TBT has shifted from developed countries to developing countries.

In previous papers, we reported that dibutyltin compounds (DBTs) and tributyltin compounds (TBTs) administered to rats are metabolized to tin containing metabolites, such as hydroxylated-, oxo-compounds, carboxylic acid, and compounds having lower molecular weights than the parent molecules (Ishizaka et al., 1989; Matsuda et al., 1993). Previously (Suzuki et al., 1984b), we reported that tributyl phosphate (TBP) or bromobutane that has *n*-butyl alkyl chains is easily transferred to mercapturic acid (MA) conjugates after reaction with glutathione (GSH). They were excreted into urine as *N*-acetyl-*S*-*n*-butyl-L-cysteine (*n*-butyl-MA), *N*-acetyl-*S*-(2-hydroxybutyl)-L-cysteine (2OH-MA), *N*-acetyl-*S*-(3-hydroxybutyl)-L-cysteine (3OH-MA), *N*-acetyl-*S*-(2-oxobutyl)-L-cysteine (2CO-MA), and/or *N*-acetyl-*S*-(3-oxobutyl)-L-cysteine (3CO-MA). In the present study, the authors examined the presence of MA conjugates in the urine of rats injected intraperitoneally with TBTs and their in vitro metabolites, which have similar structures with TBP, and investigated the elimination behavior of TBTs in mammals.

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MATERIALS AND METHODS

The chemical names and their abbreviations used throughout are shown in Table 1. For the sake of brevity, each of the organotin species is referred to in the paper as if it existed only in chloride form, but this is not meant to imply the exact identities of these species in their matrices.

All organic solutions were dried over anhydrous Na_2SO_4 and concentrated in a rotary evaporator under reduced pressure.

Thin-Layer (TLC) and Column Chromatography. Silica gel (Kiesel gel 60, 70−230 mesh) and Extrelut column (100 mL) were purchased from E. Merck (Darmstadt, Germany). Bond Elut (SCX, 500 mg) was purchased from Varian Associates, Inc. (Harbor City, CA). The purity of synthesized MAs and their methyl (Me) esters was assessed by thin-layer chromatography (TLC) on silica gel HF_{254} (E. Merck, 0.25 mm thick). After development using solvent systems of chloroform ($CHCl_3$)−methanol (MeOH)−acetic acid (60:30:10) for MAs and 10% MeOH−$CHCl_3$ for Me esters of MA, spots were visualized using a chromogenic reagent: 0.002 M platinochloric acid (4 mL)/1 M KI (0.25

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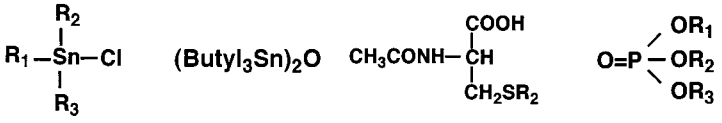
Table 1. Standards and Abbreviations



Mono-, di – and trialkyltin chloride TBTO N-acetyl-*S*-alkyl-L-cysteine trialkylphosphate

standard	abbrev	R_1	R_2	R_3
n-butyltin trichloride	MBTC	Me	Cl	Cl
di-*n*-butyltin dichloride	DBTC	*n*-Bu	*n*-Bu	Cl
n-butyl(3-hydroxybutyl)tin dichloride	D3OH	*n*-Bu	$CH_2CH_2CH(OH)CH_3$	Cl
n-butyl(3-oxobutyl)tin dichloride	D3CO	*n*-Bu	$CH_2CH_2COCH_3$	Cl
tri-*n*-butyltin chloride	TBTC	*n*-Bu	*n*-Bu	*n*-Bu
n-butyl(3-carboxypropyl)tin dichloride	DCOOH	*n*-Bu	$CH_2CH_2CH_2COOH$	Cl
di-*n*-butyl(3-hydroxybutyl)tin chloride	T3OH	*n*-Bu	$CH_2CH_2CH(OH)CH_3$	*n*-Bu
di-*n*-butyl(3-oxobutyl)tin chloride	T3CO	*n*-Bu	$CH_2CH_2COCH_3$	*n*-Bu
tributyltin oxide	TBTO			
N-acetyl-*S*-*n*-butyl-L-cysteine	*n*-butyl-MA		*n*-Bu	
N-acetyl-*S*-(2-oxobutyl)-L-cysteine	2CO-MA		$CH_2COCH_2CH_3$	
N-acetyl-*S*-(3-oxobutyl)-L-cysteine	3CO-MA		$CH_2CH_2COCH_3$	
N-acetyl-*S*-(2-hydroxybutyl)-L-cysteine	2OH-MA		$CH_2CH(OH)CH_2CH_3$	
N-acetyl-*S*-(3-hydroxybutyl)-L-cysteine	3OH-MA		$CH_2CH_2CH(OH)CH_3$	
N-acetyl-*S*-(4-hydroxybutyl)-L-cysteine	4OH-MA		$CH_2CH_2CH_2CH_2OH$	
tributyl phosphate	TBP	*n*-Bu	*n*-Bu	*n*-Bu
dibutyl 3-hydroxylbutyl phosphate	TBP-3OH	*n*-Bu	$CH_2CH_2CH(OH)CH_3$	*n*-Bu
dibutyl 3-oxobutyl phosphate	TBP-3CO	*n*-Bu	$CH_2CH_2COCH_3$	*n*-Bu

mL)/2 N HCl (0.4 mL)/acetone (76 mL) (Toennies and Kolb, 1951).

Gas Chromatography (GC) with a Flame Photometeric Detector (FPD). The purity of synthesized Me derivatives was confirmed in the S mode of GC/FPD using a Shimadzu 15A gas chromatograph. GC column was a 1.5 m × 3 mm glass column packed with 1% OV-225 on 80−100-mesh Chromosorb W, AW. Operating conditions were as follows: injection temperature 250 °C; detector temperature 250 °C; nitrogen gas pressure 1.0 kg/cm^2; air flow 180 mL/min; hydrogen flow 180 mL/min; column temperature, 180 °C.

Gas Chromatography/Mass Spectrometry (GC/MS). Mass spectra were obtained by coupled GC/MS on a JMS-DX300 (JEOL) in the electron impact mode at an ionization voltage of 75 eV. Quantitative measurements of 3CO-MA and 3OH-MA in the urine of rats injected intraperitoneally with TBTC and related compounds and recovery tests for MAs were carried out by gas chromatography/mass spectrometry/selective ion monitoring (GC/MS/SIM) using columns described in the legend to Table 2 and *m*/*z* underlined in Table 2.

Authentic Standards. Syntheses of *n*-butyl-MA, 2CO-MA, 3CO-MA, 2OH-MA, 3OH-MA, and 4OH-MA Me esters and their physicochemical constants were already described (Suzuki et al., 1984a,b).

A general procedure for syntheses of MAs from MA Me esters is as follows. A solution of 0.2 N NaOH (2 mL) was added to MA Me ester (60 mg), mixed well, and allowed to stand for 2 h at room temperature. Then the solution was passed through Bond Elut, and the column was washed with H_2O (3 mL). The eluate were combined and evaporated to dryness under reduced pressure. Sodium sulfate (2 g) was added to the residue, and the mixture was finely powdered with the aid of spatula. Then the powder was put on a silica gel column (3 g, made from $CHCl_3$), and MAs were eluted with progressively increased rates of MeOH in an eluting solvent system ($CHCl_3$−MeOH). Fractions giving a single main spot on TLC were collected and concentrated to give gummy materials (MAs, 20−30 mg).

Table 2. Mass Spectral Data of Synthesized Organotin Compounds

compounds	column 1[a] R.T. (min)	column 2[b] R.T. (min)	*m*/*z*[c]
n-butyl-MA Me ester	4.2		88, 114, 142, 174, 233
2CO-MA Me ester	10.10	2.47	88, 100, 117, 132, 176, 188, 247
3CO-MA Me ester	10.48	2.88	113, 155, 188, 229
2OH-MA Me ester	10.07		88, 100, 117, 132, 172, 191
3OH-MA Me ester	10.48	2.87	88, 113, 144, 172, 190
4OH-MA Me ester	11.93		88, 113, 140, 157, 172, 190

[a] Qualitative measurement and screening for methyl ester of each compound was done by using a cross-linked 14% cyanopropylphenyl methylsilicone [column 1: CBP10; Shimadzu Co. Kyoto; 0.2 mm (i.d.) × 25 m × 0.25 μm (film thickness)]. Operating conditions were as follows: column oven, programmed from 60 °C (hold 1 min) at the rate of 32 °C/min to 240 °C (hold 8 min). He gas flow rate: 1.5 kg/cm^2 (head pressure). Injection: splitless, 150 °C. [b] Quantitative measurement was performed using a cross-linked 14% cyanopropylphenyl methylsilicone (column 2: CBP10; Shimadzu Co. Kyoto; 0.53 mm (i.d.) × 12 m). Operating conditions were as follows: column temperature, 200 °C. He gas flow rate: 15 mL/min. Injection: on column, 220 °C. [c] The underlined ions were used for quantitative analysis.

Treatment of Animals. Wistar rats (male, 8 weeks old) were housed at 23.0 ± 2 °C and 50% relative humidity in metabolic cages, maintained on a diurnal cycle of 14 light/10 h darkness, and given access to food and water ad libitum. Each compound was dissolved in olive oil and intraperitoneally injected at doses between 2.5 and 6.3 mg ip/kg bw (Figure 3). Urine was collected for every 24-h period. Controls received only the medium without organotin compounds.

Extraction and Identification of Mercapturic Acid Derivatives from Rat Urine. Hydrochloric acid (36.5%, 2 mL) and NaCl (5 g) was added to rat urine (5 mL) under ice-cooling, and then the volume was adjusted to 20 mL with H_2O. This solution was absorbed onto Extrelut column (100 mL) and allowed to stand for 15 min until an equilibrium is reached. Then, the

column was eluted with ethyl acetate (100 mL). An ethereal solution of diazomethane (CH_2N_2) was added to the solution described above under ice-cooling until bubbling ceased [*caution*: CH_2N_2/ether (Et_2O) solution is explosive and highly toxic and therefore should be handled only under effective ventilation and with extreme caution]. After being dried over Na_2SO_4 and filtrated, the solution was concentrated just to dryness. The residue was dissolved in $CHCl_3$ and then chromatographed on silica gel (7 g) with 3 g of Na_2SO_4 on the top. The column was successively eluted with $CHCl_3$ (fraction 1: 70 mL), 1% MeOH−$CHCl_3$ (fraction 2: 70 mL), and 5% MeOH−$CHCl_3$ (fraction 3: 70 mL). Each fraction was concentrated and then subjected to GC/MS/SIM analysis; fraction 1 was examined for *n*-butyl-MA Me ester, fraction 2 was for 2CO-MA Me and 3CO-MA Me esters, and fraction 3 was for 2OH-MA, 3OH-MA, and 4OH-MA Me esters. The mass spectrum of 3CO-MA Me ester was measured without further purification after concentration of fraction 2 to 100 μL. To obtain more definitive spectra for 3OH-MA Me ester, fraction 3 was further cleaned up by a different method. Namely, fraction 3 was cleaned up by silica gel column chromatography (7 g) using a solvent system of 5% MeOH−benzene. The first 50 mL was discarded, and the following 40 mL was collected, concentrated to 50 μL, and then applied to GC/MS for measurements of 3OH-MA Me ester. For qualitative analysis, column 1 (see the legend to Table 2) was used. Control rat urine was also treated in a similar fashion.

Quantitation of Mercapturic Acid Derivatives in Rat Urine. After column chromatographic separation into fractions 1−3 as described above, each fraction was concentrated to exactly 1 mL. Quantitative measurements were performed by the external standards method using GC/MS/SIM without further purification. For quantitative analysis of 3CO-MA Me ester, authentic 2CO-MA Me ester (10 μg) was added to sample and standard solutions, respectively, for the purpose of calibrating retention time (R.T.). The time required for quantitative analysis can be reduced by using a wide bore column, column 2 (see the legend to Table 2). Control rat urine was also treated in a similar manner.

RESULTS AND DISCUSSION

Syntheses of Authentic Mercapturic Acids from Mercapturic Acid Me Esters. Purification of MA Me esters by column chromatography was considered to be much more easier than the purification of MAs themselves. Therefore, authentic MAs for recovery tests were synthesized by the hydrolysis of MA Me esters. Since MA derivatives were considered to be unstable against strong hydrolyzing conditions, a very mild condition was selected. As a result, they gave low yields of hydrolyzed products as expected. Their purity was checked by spraying the chromogenic reagent on TLC, followed by TLC and GC equipped with a FPD (S-mode) after being restored to MA Me esters by CH_2N_2. All MAs were at least 96% pure by GC. Therefore, the obtained gummy materials were used for recovery tests without further purification.

Purification of Mercapturic Acid Derivatives in Urine for GC/MS. In the previous paper (Suzuki et al., 1984b), the authors used a gas chromatograph equipped with a FPD (S-mode) for analysis of sulfur-containing metabolites in the urine of rats dosed with TBP or bromobutane. In the present study, however, more sensitive, highly specific techniques were needed because of decreased body burdens due to high toxicities of TBTC and related compounds. In this respect, GC/MS/SIM is not always highly specific but was considered to be sufficiently sensitive and specific to allow monitoring of μg/10 mL urine levels of MA. After methylated urine extracts were fractionated into three portions by silica gel column chromatography, each eluate was used for quantitative determination by GC/MS/SIM without further purification. For the purpose of identification by GC/MS, each eluate was further cleaned up by additional column chromatography using different solvent systems as described under Materials and Methods.

Identification of Sulfur-Containing Metabolites in Rat Urine. The urine samples from rats injected intraperitoneally with TBTC and its in vitro rat microsomal metabolites were tested for the presence of sulfur-containing metabolites. Mass spectra and ion chromatograms of 3CO-MA Me ester are shown in Figure 1. The detailed explanations of the figure are described in the legend to Figure 1. The overall results show the presence of 3CO-MA in both treated and control rat urine. In the previous paper (Suzuki et al., 1984b), the authors reported that 3CO-MA and 3OH-MA were found in the urine of rats dosed with TBP or bromobutane. However, the authors did not pay any special attention to the presence of these chemicals in control rat urine, because their doses were very high compared with those in the present study and therefore there was no need for sensitive methods.

Mass spectra and ion chromatograms of 3OH-MA Me ester are shown in Figure 2. The detailed explanations of the figure are described in the legend to Figure 2. The overall results show that 3OH-MA is present in both the rat urine injected with TBTC and control rat urine (indicated by the arrows in Figure 2C,E).

The rat urine samples were also tested for other sulfur-containing metabolites. Despite extensive investigation, however, the other metabolites, namely *n*-butyl-MA, 2CO-MA, 2OH-MA, and 4OH-MA, were not found in either the control rat urine or the rat urine treated with TBTC.

Quantitation of Sulfur-Containing Metabolites Excreted in the Urine After the Administration of TBTC and Its in Vitro Metabolic Intermediates to Rats. The recoveries of MA Me esters in fractions 1−3 from silica gel column were between 96 and 98%. The recoveries of MAs through the whole analytical procedure from spiked urine samples at a level of 100 μg/5 mL were relatively low due to their high polarities. The overall recoveries were 99 ± 8 (*n*-butyl-MA), 85 ± 5 (2CO-MA), 90 ± 6 (3CO-MA), 68 ± 4 (2OH-MA), 70 ± 4 (3OH-MA), and 70 ± 6 (4OH-MA). Backgrounds of 3CO-MA and 3OH-MA found in control urine were subtracted from the obtained values. Concentrations of MAs reported in the present paper were not corrected for recovery. The level of quantitation (LOQ) and the level of detection (LOD) for these analytical methods were 0.05 and 0.005 μg/mL urine for *n*-butyl-MA and 0.1 μg/mL and 0.01 μg/mL urine for other MAs.

Rats were intraperitoneally treated with TBTC, TBTO, and their in vitro metabolites at about 5 mg/kg body weight (bw), as shown in Figure 3, except for DBTC which is highly toxic in intraperitoneal administration and was lethal within a week at that dose, and therefore its dose was decreased to one-half the other chemicals.

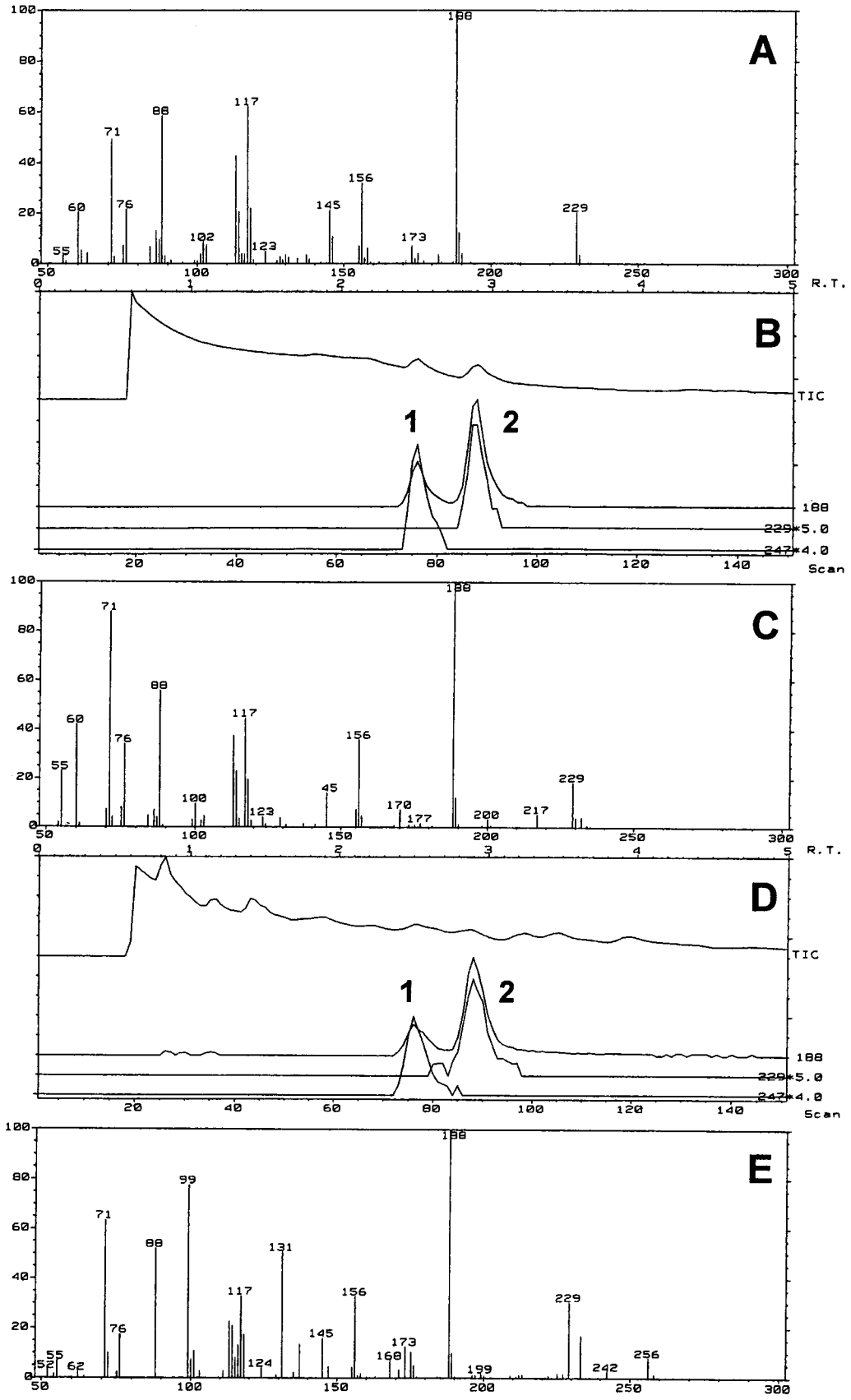


Figure 1. GC/MS spectra and ion chromatograms of 3CO-MA Me ester of standard, metabolite, and control rat urine: **A**, authentic 3CO-MA Me ester (column 1 in the legend to Table 2, R.T. = 10.48 min); **B**, ion chromatograms of authentic 3CO-MA Me ester (column 2 in the legend to Table 2; peak 1, authentic 2CO-MA Me ester, an internal standard for calibration of R.T.; peak 2, authentic 3CO-MA Me ester); **C**, 3CO-MA Me ester of the 24-h urine extract of rats injected with TBTC (column 1, R.T. = 10.48 min); **D**, ion chromatograms of 3CO-MA Me ester of the 24-h urine extract of rats injected with TBTC (column 2; peak 1, authentic 2CO-MA Me ester, an internal standard for calibration of R.T.; peak 2, 3CO-MA Me ester, metabolite); **E**, 3CO-MA Me ester of control rat urine (column 1, R.T. = 10.48 min).

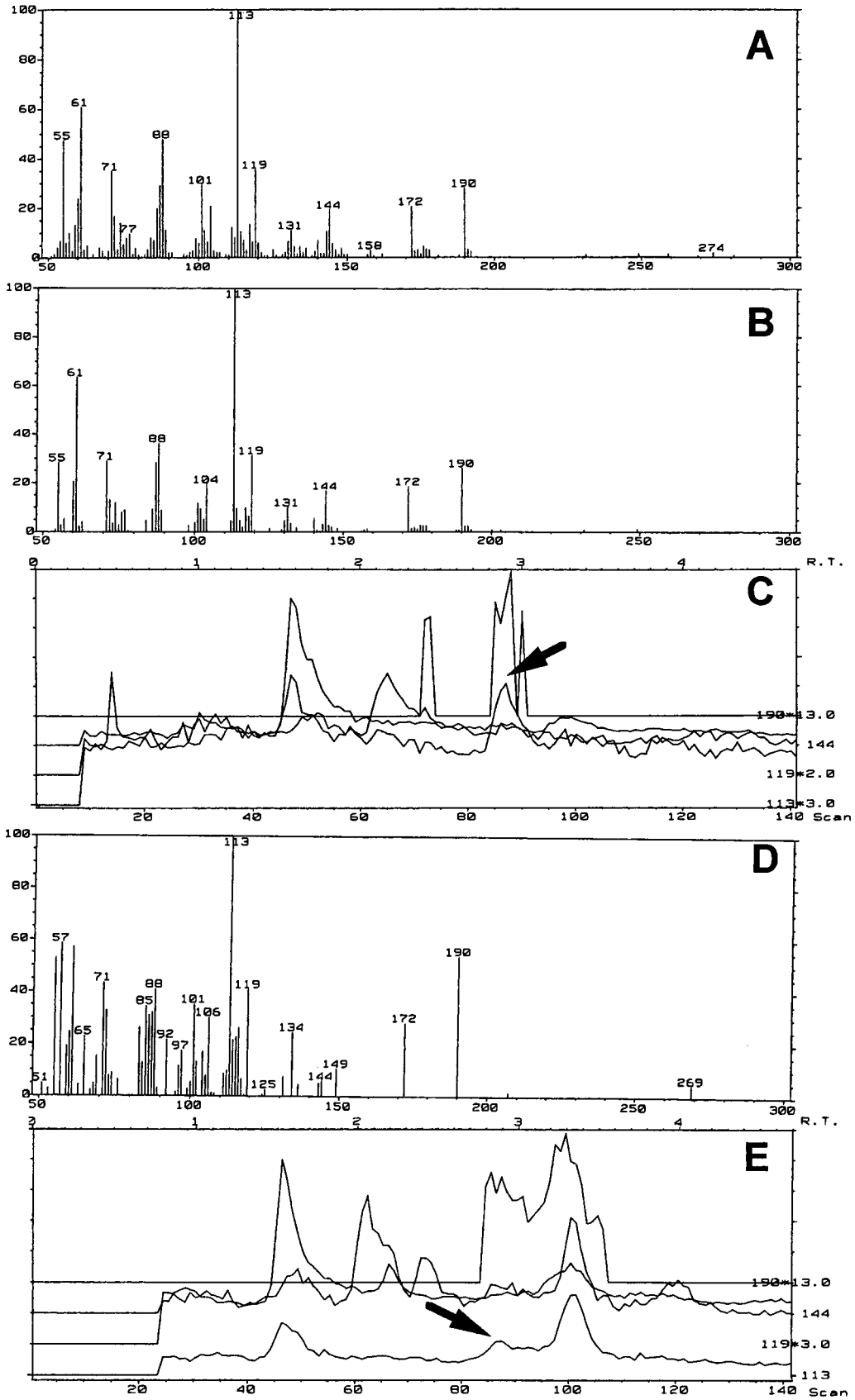


Figure 2. GC/MS spectra and ion chromatograms of 3OH-MA Me ester of standard, metabolite, and control rat urine: **A**, authentic 3OH-MA Me ester (column 1 in the legend to Table 2, R.T. = 10.48 min); **B**, 3OH-MA Me ester of the 24-h urine extract of rats injected with TBTC (column 1, R.T. = 10.46 min); **C**, ion chromatograms of 3OH-MA Me ester of the 24-h urine extract of rats injected with TBTC (column 2 in the legend to Table 2); **D**, 3OH-MA Me ester of control rat urine (column 1, R.T. = 10.46 min); **E**, ion chromatograms of 3OH-MA Me ester of the urine extract of control rats (column 2).

The excretions of 3CO-MA and 3OH-MA were extremely rapid and decreased to nearly control level within 24 h after the administration of TBTC and related compounds. In the case of TBP and related compounds, the

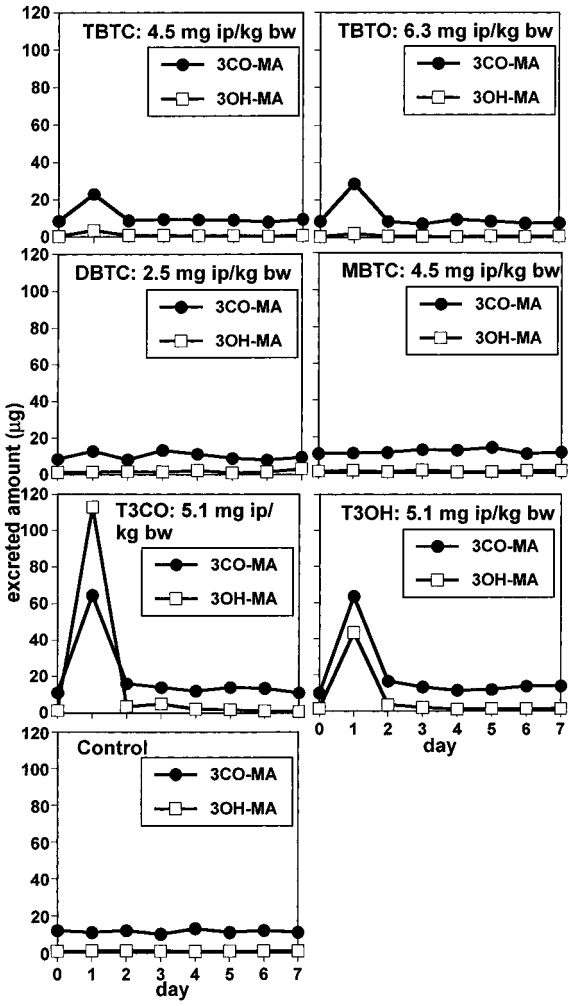


Figure 3. Daily excretion of 3CO-MA and 3OH-MA in rats after the intraperitoneal administrations of TBTC, TBTO, DBTC, MBTC, T3CO, and T3OH and in control rats; each point represents the mean of three rats.

excretion of MAs persisted for a much longer time (3 days; Suzuki et al., 1984b). Although there is no substantial proof, this may be due to the difference of dose; i.e., extremely high body burdens of TBP and related compounds compared with those of TBTC and related compounds in the present study. Rats injected with TBTC or TBTO gave 28−30 μg of 3CO-MA in the urine compared with about 10 μg in the control. On the other hand, the urinary excretion of 3OH-MA was slightly higher than the control level (∼1 μg). The intraperitoneal administration of DBTC or MBTC contributes little to excretion levels of 3CO-MA and 3OH-MA (Figure 3). This may be due to the fact that they are not as actively metabolized as TBTC or TBTO and are polar enough for excretion through kidneys without MAs formation. The administration of T3CO or T3OH, which are known in vitro metabolites of P-450-dependent microsomal enzyme systems of rats (Fish et al., 1976), caused increased urinary excretion of both 3CO-MA and 3OH-MA. T3CO increased the amount of 3OH-MA excreted into the 24 h urine up to 110 μg and that of 3CO-MA up to 54 μg. The administration of T3OH increased the amount of 3CO-MA up to about 64 μg and that of 3OH-MA up to 42 μg. It is interesting to note that the administration of the 3-oxo compound, T3CO, gave much more 3OH-MA than 3CO-MA and that the administration of the 3-hydroxy compound, T3OH, showed inversely a higher excretion of 3CO-MA than 3OH-MA. In the previous paper (Suzuki et al., 1984b), the authors reported that the intraperitoneal administrations of possible metabolic intermediates of TBP, namely dibuty 3-hydroxylbutyl phosphate (TBP-3OH), dibutyl 3-oxobutyl phosphate (TBP-3CO), or butyl bis-(3-hydroxylbutyl) phosphate, to rats gave very similar ratios of 3CO-MA/3OH-MA; namely, 1.89, 2.03, and 1.69. However, when bromobutane was administered, the ratio obtained was 3.4. Anyway, the concentration of 3CO-MA predominated over that of 3OH-MA in both cases. The values obtained in the present experiment were different from those in TBP and related compounds, and the reason the ratios of 3CO-MA/3OH-MA were different among the rats injected with T3CO, T3OH, or TBTC remains unclear.

Mechanistic Consideration for Sulfur-Containing Metabolites. Fish and co-workers (1976) and Kimmel and co-workers (1977) reported that tributyltin acetate is oxidized to DBT, T3CO, T3OH, and T4OH in in vitro rat microsomal monooxygenase systems, but they did not detect any GSH or MA conjugates. On the other hand, Matsuda et al. (1993) reported that TBTC orally administered to rats was oxidized to DCOOH, DBTC, D3OH, and D3CO in the liver and that T3CO, T3OH, and T4OH were not found. The authors also reported that intraperitoneal injection of T3CO or T3OH gave predominantly DCOOH and lesser amounts of MBTC, DBTC, and D3OH in the liver and predominantly D3OH and D3CO and lesser amounts of MBTC, DBTC, D4OH, and DCOOH in the urine. The present in vivo experiment proved the presence of MA conjugates in the rat urine. This difference may be attributed to the difference between in vitro and in vivo experimental systems. That is, in vitro microsomal enzyme systems cannot give MA conjugate because of the lack of cytosolic GSH. TBTC can be primarily transformed to di-*n*-butyl(1-hydroxybutyl)tin dichloride or di-*n*-butyl-(2-hydroxybutyl)tin dichloride by 1- or 2-hydroxylation. However, these hydroxylated compounds at the 1- or 2-position of a butyl moiety are too unstable to be isolated and immediately degraded to DBTC and butanol or DBTC and 1-butene (Fish et al., 1976). On the other hand, the products formed via oxidation at the 3- or 4-position of TBTC (T3OH, T4OH, and T3CO) are stable and can be isolated in an in vitro system (Fish et al., 1976). In an in vivo system, however, thus produced T3OH, T3CO, and T4OH can afford D3OH, D3CO, and D4OH, respectively, by further 1- or 2-hydroxylation at an intact butyl moiety on them, followed by immediate elimination of 1- or 2-hydroxylated butyl moieties as butanol or 1-butene. T3OH, T4OH, and T3CO can also give DBTC by 1- or 2-hydroxylation at the oxygenated butyl moieties on them. Furthermore, T3OH, T3CO, and T4OH will be subject to oxidation at the C-3 or C-4 positions of an intact butyl moiety on them to give hypothesized secondary metabolites such as T3CO−OH [example: *n*-butyl(4-hydroxybutyl)(3-oxobutyl)tin chloride], T3OH−CO [example: *n*-butyl(3-hydroxybutyl)(3-oxobutyl)tin chloride], T3CO(T3OH)−COOH [example: *n*-butyl(3-carboxypropyl)(3-oxobutyl)tin chloride], and T3OH−OH [example: *n*-butyl bis(3-hydroxybutyl)tin chloride] as illustrated in Figure 4. However, these secondary metabolites as well as T3CO and T3OH may be highly reactive and immediately transformed to GSH conjugates in the presence of GSH, resulting in the

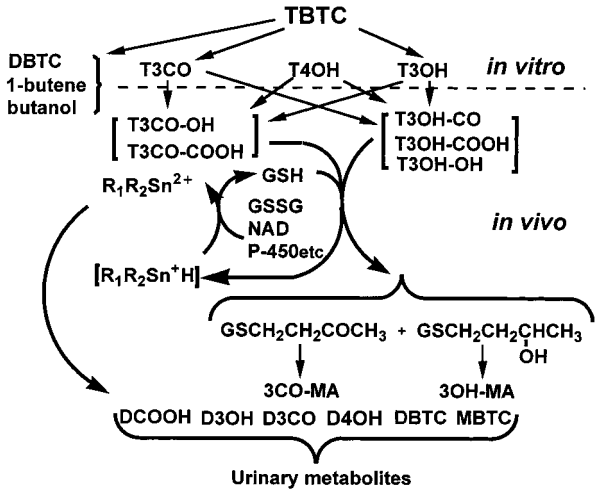


Figure 4. Proposed metabolic fates of alkyl moieties of TBTC, T3CO, and T3OH in rats.

excretion of MA conjugates in urine. This is the reason compounds such as T3OH or T3CO could not be found in the liver of rats dosed with TBTC, T3CO, or T3OH (Matsuda et al., 1993). On the other hand, counter molecules that contain tin, namely, $R_1R_2Sn^+H$, will be essentially produced by the nucleophilic reaction with GSH, but these compounds may be very unstable in living organisms and will be easily oxidized with the aid of enzyme, coenzyme, or oxidantlike substances, such as glutathione disulfide (GSSG), NAD, or P-450-dependent monooxygenase enzyme systems, to $R_1R_2Sn^+$-Cl ($R_1R_2SnCl_2$). As a result, these compounds will be recognized in urine as DCOOH, D3OH, D3CO, D4OH, DBTC, and MBTC as shown in the previous paper (Matsuda et al., 1993) and Figure 4. In the present experiment, the administration of D3OH or D3CO was not performed, but it is expected that these compounds also follow the same trends observed for T3CO and T3OH. Therefore, the formation of oxygenated metabolites, such as T3CO or T3OH, should be regarded as detoxification routes. On the other hand, we reported in the previous paper (Suzuki et al., 1998) that high amounts of T3CO and T3OH are accumulated in marine mussels. This indicates that mussels do not have a detoxification route in a manner analogous to mammals, or even if they have, the potency must be weak. Triphenyltin hydroxide, which had been used as fungicides on potatoes, sugar beets, etc., also react with GSH to form phenyl GSH conjugate in plants (FAO, 1991). Taken together, these observations appear to provide the information that GSH play a role in cleavage of tin−carbon bond.

About 10 μg of 3CO-MA and 1 μg of 3OH-MA/day/ rat were excreted as background levels in the control rat urine, and the ratio of 3CO-MA/3OH-MA was about 10. This ratio is relatively high and is in agreement with those obtained from the administration of TBTC but different from those obtained from the administration of metabolic intermediates of TBTC or TBP. The presence of two MA conjugates, namely 3CO-MA and 3OH-MA in the control rat urine, suggests the sources of these compounds is a compound or are compounds that have a *n*-butyl group or groups in the molecule. With our current knowledge, however, the sources that give *n*-butyl group in living organisms are unlikely.

CONCLUSION

Mercapturic acid derivatives were found in the urine of rats intraperitoneally injected with tri-*n*-butyltin chloride. The administration of two metabolites of TBTC, di-*n*-butyl(3-hydroxybutyl)tin chloride or di-*n*-butyl(3-oxobutyl)tin chloride, gave higher amounts of mercapturic acid derivatives than TBTC. The presence of hydroxylated- or oxo- and carboxylic acid derivatives of dibutyltin chloride in the rat urine suggests rats first metabolize tri-*n*-butyltin chloride to di-*n*-butyl(3-hydroxybutyl)tin chloride and di-*n*-butyl(3-oxobutyl)tin chloride, by the oxidation of the 3-position of an alkyl moiety of TBTC. These compounds will readily react with glutathione to give $GS-CH_2CH_2COCH_3$, $GS-CH_2-CH_2CH(OH)CH_3$, and dibutytin dichloride. These glutathione conjugates were excreted into urine as their mercapturic acids. Di-*n*-butyl(3-hydroxybutyl)tin chloride or di-*n*-butyl(3-oxobutyl)tin chloride could be further oxidized at an intact butyl moiety and give hypothesized secondary metabolic intermediates, such as *n*-butyl(3-hydroxybutyl)(3-oxobutyl)tin chloride, *n*-butyl-(3-hydroxybutyl)(4-hydroxybutyl)tin chloride, etc. These active metabolites would react with glutathione and result in the formation of mercapturic acid conjugates and dibutyltin derivatives in the urine. Therefore, the formation of mercapturic acid derivatives is another dealkylation pathway for oxygenated products at the 3- or 4-position of the *n*-butyl moiety. These mercapturic acids were also found in control rat urine at lower levels.

ABBREVIATIONS USED

TBTs, tributyltin compounds; DBTs, dibutyltin compounds; TLC, thin-layer chromatography; GC/MS, gas chromatography/mass spectrometry; GC/MS/SIM, gas chromatography/mass spectrometry/selective ion monitoring; FPD, flame photometric detector; Et_2O, diethyl ether; MeOH, methanol; $CHCl_3$, chloroform; GSH, glutathione; GSSG, glutathione disulfide; CH_2N_2, diazomethane; NAD, nicotineamide adenine dinucleotide; MA, mercapturic acid; T3OH−OH, *n*-butyl bis(3-hydroxybutyl)tin chloride; T3OH−CO, *n*-butyl(3-hydroxybutyl)(3-oxobutyl)tin chloride or *n*-butyl(3-hydroxybutyl)-(2-oxobutyl)tin chloride; T3CO−OH, *n*-butyl(4-hydroxybutyl)(3-oxobutyl)tin chloride or *n*-butyl(3-hydroxylbutyl)(3-oxobutyl)tin chloride; T3CO(T3OH)COOH, *n*-butyl(3-carboxypropyl)(3-oxobutyl)tin chloride or *n*-butyl(3-carboxypropyl)(3-hydroxybutyl)tin chloride. Other abbreviations are given in Table 1.

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