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Bioorganotin Chemistry. Metabolism of Organotin Compounds in Microsomal Monooxygenase Systems and in Mammals

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Microsomal monooxygenase metabolism of tributyltin acetate yields α-, β-, γ-, and δ-hydroxybutyldibutyltin derivatives. The major metabolites, the α- and β-hydroxy compounds, undergo destannylation reactions under acidic conditions to form dibutyltin derivatives and 1-butanol and 1-butene, respectively. The γ-hydroxy compound is further oxidized to the corresponding ketone. Tetrabutyltin yields tributyltin derivatives, via the β- and possibly also the α-hydroxybutyltributyltins. Dibutyltin diacetate undergoes monooxygenase or nonenzymatic cleavage to butyltin derivatives. Several of the metabolites formed in the monooxygenase system are also detected in the liver and/or in the feces of mice orally administered tributyltin acetate and dibutyltin diacetate. Some of the carbon-hydroxylated tributyltin metabolites retain a high level of biological activity. Other trialkyltin derivatives (ethyl, propyl, pentyl, hexyl, and cyclohexyl) appear to undergo analogous monooxygenase reactions, whereas triphenyltin acetate is more resistant to monooxygenase attack even though it undergoes destannylation in rats. Several of these relationships are also applicable to monooxygenase metabolism of organolead compounds.

Metabolism of alkyltin compounds in liver microsomal monooxygenase (MO) systems and in mammals leads to the following sequence of destannylation (carbon–tin cleavage) reactions: $R_4Sn \rightarrow R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4$ (X = anion) (Blair, 1975; Casida et al., 1971). The first step of this destannylation reaction sequence yields derivatives of increased toxicity and potency as inhibitors of mitochondrial respiration, whereas each subsequent step progressively reduces the potency and alters the type of biological activity (Luijten, 1972; Rose, 1971; Thayer, 1974). MO studies with tributyltin acetate establish that carbon hydroxylation is the major biological oxidation reaction and that destannylation occurs due to the instability of the α- and β-hydroxyalkyltin metabolites (Fish et al., 1975, 1976a,b).

The present study examines the metabolic fate in MO systems and in mammals of tributyltin acetate, dibutyltin diacetate, and triphenyltin acetate. It also provides comparative data on tetrabutyltin and other alkyltin and organolead compounds in the MO system.

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MATERIALS AND METHODS

Chemicals. The following organotin and organolead compounds were used (Et = ethyl, Pr = *n*-propyl, Bu = *n*-butyl, Pen = *n*-pentyl, Hex = *n*-hexyl, Oct = *n*-octyl, Cy = cyclohexyl, Ph = phenyl, and OAc = acetate): Et_4Sn, Bu_4Sn, Et_3SnCl, Pr_3SnCl, Bu_3SnCl, $(Bu_3Sn)_2O$, Bu_3SnOAc, Pen_3SnOAc, Hex_3SnOAc, Oct_3SnCl, Ph_3SnCl, Ph_3SnOAc, Et_2SnCl_2, Bu_2SnCl_2, Ph_2SnCl_2, $BuSnCl_3$, $PhSnCl_3$, Et_4Pb, Et_3PbOAc, Bu_3PbOAc, Ph_3PbOAc, and $Bu_2Pb(OAc)_2$ [obtained from The Institute for Organic Chemistry TNO (Utrecht, The Netherlands), M&T Chemicals Inc. (Rahway, N.J.), or G. Widmark (Institute of Analytical Chemistry, University of Stockholm, Stockholm, Sweden)] [when not of suitable purity, the compounds were purified by distillation or by thin-layer chromatography (TLC) (see below) using solvent system A for organotin compounds and solvent system F for the organoleads]; Cy_4Sn, Cy_3SnOH, $(Cy_2Sn)_2O$, and $CySnO_2H$ (provided by Dow Chemical Co., Midland, Mich.); other unlabeled $R_2Sn(OAc)_2$, $RSn(OAc)_3$, and $R_2Pb(OAc)_2$ derivatives (prepared as needed by photodecomposition of R_4Sn, R_3SnOAc, and R_4Pb compounds on silica gel chromatoplates and TLC isolation); 1-^{14}C-labeled preparations of Bu_4Sn, Bu_3SnOAc, and $Bu_2Sn(OAc)_2$ with specific activities of 12.7, 9.5, and

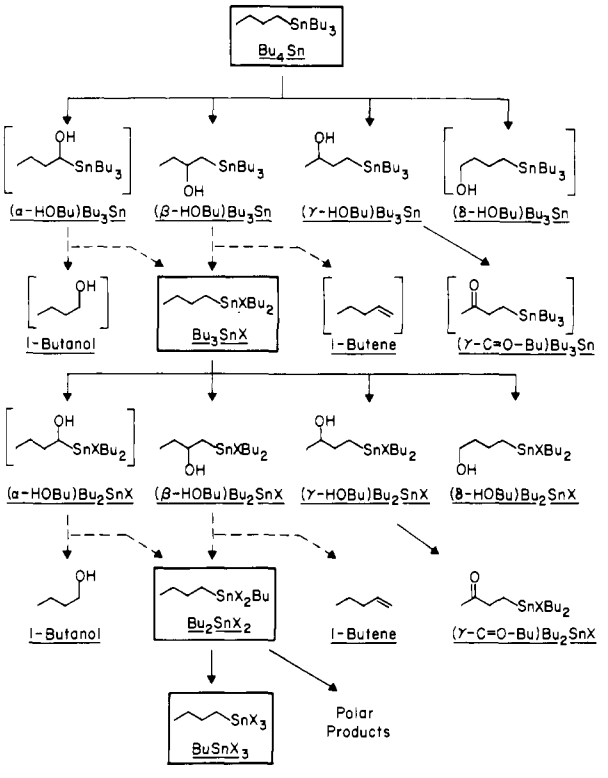

Figure 1. Metabolic pathways for butyltin derivatives in the rat liver microsome-NADPH system and in mice indicating the abbreviations used for various metabolites. Formulas in brackets designate α-hydroxy compounds that are not sufficiently stable for isolation and other possible metabolites of Bu_4Sn on analogy with the findings using Bu_3SnX. Solid arrows indicate the in vitro NADPH-dependent metabolites while broken arrows show nonenzymatic reactions that occur at physiological pH or on acidification.

6.3 mCi/mmol, respectively, and >99% radiochemical purity (Fish et al., 1976a); $Ph_3{}^{113}SnOAc$ (0.6 mCi/mmol) (provided as the hydroxide by Thompson-Hayward Chemical Co., Kansas City, Kan., and purified and converted to the acetate on TLC in solvent system A); hydroxy and keto derivatives of Bu_4Sn, Bu_3SnCl, and Bu_3SnBr as designated in Figure 1 (Fish et al., 1976a; Lahournère and Valade, 1971). The anion substituent (X) of the organometallic compounds is not stated below since, within the series examined [Bu_3SnCl, $(Bu_3Sn)_2O$, and Bu_3SnOAc], it undergoes anion exchange on chromatography in acidic solvents (Figge, 1969; Fish et al., 1976a) and probably in MO systems (Fish et al., 1976a).

Chromatography. Silica gel 60 TLC plates (0.25 mm layer thickness, without fluorescent indicator, Merck) of 20 × 20 cm were used for resolving the metabolites of ^{14}C- and ^{113}Sn-labeled compounds alone or mixed with unlabeled standards (cochromatography) and of 10 × 10 cm for the metabolites of unlabeled substrates. The following three solvent systems were used to separate R_3SnX and R_2SnX_2 derivatives, with R_4Sn compounds at or near the front and $RSnX_3$ and SnX_4 derivatives at the origin: (A) diisopropyl ether–HOAc (49:1); (A′) two developments with diisopropyl ether–HOAc (99:1); (B) hexane–HOAc (9:1). Separation of Bu_4Sn metabolites was achieved with solvent system C, diisopropyl ether–hexane (1:1). To separate R_2SnX_2 and $RSnX_3$ derivatives, with SnX_4 at the origin and R_3SnX and R_4Sn at or near the front, the systems were: (D) carbon tetrachloride–acetylacetone–HOAc (20:1:1); (E) acetylacetone–acetone–HOAc–water (15:10:2:1). For organolead compounds, solvent system F, ethyl acetate–water–HOAc (20:1:1), separated R_4Pb (at or near the front), R_3PbX and R_2PbX_2 derivatives (intermediate R_f), and $RPbX_3$ and PbX_4 derivatives (at or near the origin).

Chromogenic reagents used in detecting organotin compounds were as follows (Casida et al., 1971): HQ—0.1% (w/v) solution of 8-hydroxy-5-quinolinesulfonic acid in 90% ethanol giving golden fluorescent spots on a purple-violet background when viewed under 365-nm uv light (Pal and Ryan, 1969); PCV—saturated solution of pyrocatechol violet in 95% ethanol yielding blue-violet spots on a yellow background (Petrowitz, 1969; Williams and Price, 1960); dithizone–0.1% (w/v) in chloroform (Petrowitz, 1969; Türler and Högl, 1961). The HQ and PCV reagents detect R_2SnX_2, $RSnX_3$, and SnX_4 derivatives but not R_4Sn and R_3SnX derivatives. The dithizone reagent does not detect R_4Sn compounds but yields yellow spots with R_3SnX derivatives and salmon spots with R_2SnX_2, $RSnX_3$, and SnX_4 derivatives. Thus, R_3SnX and R_2SnX_2 derivatives are differentiated by the presence or absence of a chromogenic response with the HQ and PCV reagents and by the color obtained with dithizone. Photodestannylation (Casida et al., 1971; Williams and Price, 1960) by irradiating the chromatoplates under a germicidal uv lamp was used in order to detect R_4Sn and R_3SnX derivatives. The following sequence proved most useful with the organotins: HQ spray, detecting R_2SnX_2 and $RSnX_3$ derivatives and SnX_4; uv irradiation; HQ spray to locate new spots due to R_4Sn and R_3SnX derivatives; PCV spray for a more permanent record. Only the dithizone reagent was employed in the studies with organoleads, the response being the same as that with the corresponding organotin derivatives. The dithizone reagent is less sensitive than the HQ and PCV reagents, the detection limits being 10–100 and 1–10 μg/spot, respectively.

^{14}C- and ^{113}Sn-labeled compounds were detected by radioautography and quantitated by scraping the appropriate TLC gel regions and liquid scintillation counting (lsc).

Gas–liquid chromatography (GLC) was used for analysis of cyclohexanol and cyclohexanone (Ullrich, 1969; the retention time of cyclohexanone is about one-half that of cyclohexanol) and of 1-butene (Fish et al., 1976a).

Microsomal Monooxygenase Reactions. The microsomal and postmicrosomal supernatant (soluble) fractions were prepared from the livers of male albino rats (150–160 g) at 20% (w/v) fresh tissue weight equivalent in sodium phosphate buffer (0.1 M, pH 7.4). Comparable preparations were also made from the livers of male rabbits (1–3 kg) and male mice (18–20 g) and from the abdomens of houseflies (*Musca domestica* L.; strains with low to moderate MO activity).

The reaction mixtures in 2 ml of phosphate buffer contained the following components added in sequence: microsomal preparation (5.1 mg of protein, 200 mg of fresh liver weight equivalent); soluble fraction (5.5 mg of protein, 80 mg of fresh liver weight equivalent); reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 2 μmol); substrate (0.003 μmol of [^{14}C]butyltin derivative; 0.002, 0.007, or 0.02 μmol of $Ph_3{}^{113}SnOAc$; 0.5 μmol of unlabeled compound) in ethanol (50 μl) injected with a syringe directly into the reaction mixture. The 25-ml Erlenmeyer flasks were incubated with shaking in air at 37 °C for 1 h.

Several extraction procedures were used depending on the substrate and the analyses to be performed. With [^{14}C]butyltin substrates, the reaction mixture was extracted

with chloroform (5 × 7 ml), the aqueous phase was treated with trichloroacetic acid (Cl_3CCOOH) (25% final concentration), and the precipitate from centrifugation was washed with acetone (1 × 3 ml) and ethanol (2 × 3 ml) to yield the acetone–ethanol extract. With $Ph_3{}^{113}SnOAc$, the reaction mixture was extracted with chloroform (2 × 5 ml) and the aqueous phase was then acidified to pH 1–2 with HCl (50 μl, 6 N) and reextracted with ether–acetylacetone (9:1) mixture (2 × 5 ml) and finally with ether–ethanol (2:1) mixture (2 × 5 ml). With unlabeled substrates, the extraction utilized the ether–acetylacetone (9:1) mixture (4 ml) which gave recoveries comparable to those using chloroform as above. Identical reaction mixtures were acidified prior to extraction, a procedure which afforded increased recoveries of organometallic compounds; this treatment also decomposed some acid-labile metabolites, providing a useful criterion in certain identifications.

Organometallic products were analyzed by TLC using the PCV (organotin) or dithizone (organolead) color intensity and size of the metabolite spots to estimate yields with unlabeled substrates and lsc to quantitate labeled products. Radiocarbon designated as bound or not extracted was analyzed by combustion with the Packard Tri-Carb Model 306 sample oxidizer. Determinations of 1-[^{14}C]butene as the oxymercuration adduct [methanol, $Hg(OAc)_2$] and 1-[^{14}C]butanol as the phenylcarbamate were made by described procedures (Fish et al., 1976a). For analysis of cyclohexanol and cyclohexanone, the reaction mixtures were acidified with HCl as above and extracted with ethyl acetate (1 × 2 ml) which was then added to 200 mg of distilled water and concentrated at ~80 °C to 190–195 mg total weight. A 5.0-μl aliquot was used for GLC with corrections for the extraction efficiencies of cyclohexanol and cyclohexanone and for variations in the aqueous volume resulting from evaporation of the ethyl acetate–water mixture.

In Vivo Metabolism. [^{14}C]Bu_3SnOAc and [^{14}C]-$Bu_2Sn(OAc)_2$ were administered in methoxytriglycol (125 μl) by stomach tube to male albino mice (20 g, Swiss-Webster strain, Horton Laboratories, Oakland, Calif.) to yield a dose of 1.2 and 1.1 mg/kg, respectively. The metabolism cages, holding conditions, and procedures for collection and quantitation of the radiocarbon content of urine, feces, and $^{14}CO_2$ were as previously described (Krishna and Casida, 1966). The urine was diluted with water and extracted with chloroform (2 × 1 vol) before and after acidification to pH 1–2 by addition of HCl. The feces were dried and pulverized for determination of total radiocarbon content (combustion) or extraction by a multistep procedure. Thus, aliquots of the feces (40–100 mg) were homogenized in chloroform (5 ml) with a Polytron homogenizer (Kinematica GmbH, Lucerne, Switzerland), and the homogenate was centrifuged and filtered to give the chloroform-soluble fraction. The precipitate and residue on the filter paper were combined and homogenized (Polytron) in methanol (5 ml) for recovery of the methanol-soluble fraction as above. Finally, the centrifugal precipitate and filter residues were moistened with a few drops of water, acidified to pH 1–2 by addition of HCl, and reextracted with methanol as above to give the acid–methanol extract and unextractable residue (analyzed by combustion). Tissue residues were analyzed by combustion on sacrifice of the animals at 138 h after treatment. In separate experiments with each labeled compound, the 1-[^{14}C]butene expired within 24 h after treatment was trapped and analyzed as previously described (Fish et al., 1976a). Also, the liver was removed 3 h after treatment, immediately frozen on dry ice, and then homogenized in water (10 ml) and the homogenate extracted with chloroform (5 × 35 ml), followed by a second chloroform extraction (5 × 35 ml) after acidification of the aqueous phase to pH 1–2 with HCl.

$Ph_3{}^{113}SnOAc$ in methoxytriglycol was administered by the intraperitoneal (ip) route (60 μl carrier) or by stomach tube (210 μl carrier) to male albino rats (170 g, Horton Laboratories) to yield a dose of 1.6 mg/kg. Urine and feces collected daily for 10 days were analyzed for total radiotin content by direct counting of an aliquot of each urine sample and of each feces sample after homogenization in water. An aliquot (2 ml) of each feces homogenate sample was treated in the following manner: extraction with chloroform (2 × 6 ml); acidification of the aqueous phase to pH 1–2 with HCl, saturation with $(NH_4)_2SO_4$, and extraction with ether–acetylacetone (9:1) (2 × 6 ml); evaporation of the aqueous phase to dryness; addition of methanol (2 ml) to the residue, sonication of the suspension for 30 min, centrifugation, and recovery of the methanol-soluble products; lsc and TLC analyses of the individual chloroform, ether–acetylacetone, and methanol extracts using two-dimensional development first with solvent system A′ and then with B × 2 and, on separate plates, one-dimensional development with solvent system D. As a control, $Ph_3{}^{113}SnOAc$ was added directly to feces from untreated rats and analyzed by the same procedure.

RESULTS

Properties and Optimization of Monooxygenase System. Rat liver microsomal preparations were used with ^{14}C-labeled substrates, mouse liver microsomes with $Ph_3{}^{113}SnOAc$, and rabbit liver microsomes with unlabeled substrates, unless noted otherwise. The same metabolites (TLC pattern) of unlabeled Bu_3SnX and Cy_3SnX are obtained using rat or rabbit microsomal systems and of [^{14}C]Bu_3SnOAc with rat microsomes and the less active preparations of mouse and housefly microsomes. With both rat and rabbit preparations, a small amount of soluble fraction (optimum 40–100 mg fresh liver weight equivalent) increases the activity of the microsome–NADPH system although the soluble fraction is not active by itself or with NADPH. There is a considerable variation in the activity of different enzyme preparations from the same species, for unknown reasons. It is necessary in all cases to fortify the microsomal preparations with NADPH to obtain detectable metabolism of the organotin and organolead substrates (Casida et al., 1971). The rat liver microsome–NADPH system acting on [^{14}C]Bu_3SnOAc is totally inhibited by carbon monoxide and to a large extent by 4(5)-α-naphthylimidazole (Fish et al., 1976a). These findings suggest that a cytochrome P450 dependent monooxygenase system is responsible for metabolism of the organometallic substrates (Fish et al., 1976a,b).

Monooxygenase Metabolites of Labeled and Unlabeled Organotin Compounds. *[^{14}C]Butyltin Derivatives and Rat Liver Microsome–NADPH System.* Metabolites of [^{14}C]Bu_4Sn, [^{14}C]Bu_3SnOAc, and [^{14}C]-$Bu_2Sn(OAc)_2$ were analyzed in a series of chromatographic systems that resolve all of the authentic unlabeled compounds and therefore allow tentative metabolite identification by cochromatography (Figure 2) and quantitation by lsc (Table I).

[^{14}C]Bu_4Sn yields eight identified NADPH-dependent metabolites, i.e., (β-HOBu)Bu_3Sn, (γ-HOBu)Bu_3Sn, and all products detected with [^{14}C]Bu_3SnOAc as the substrate (see below) except (γ-C=O–Bu)Bu_2SnX. No (δ-HOBu)Bu_3Sn is detected as a metabolite of Bu_4Sn. There are two unusual features of the results: a large loss of radiocarbon, possibly attributable to volatilization of

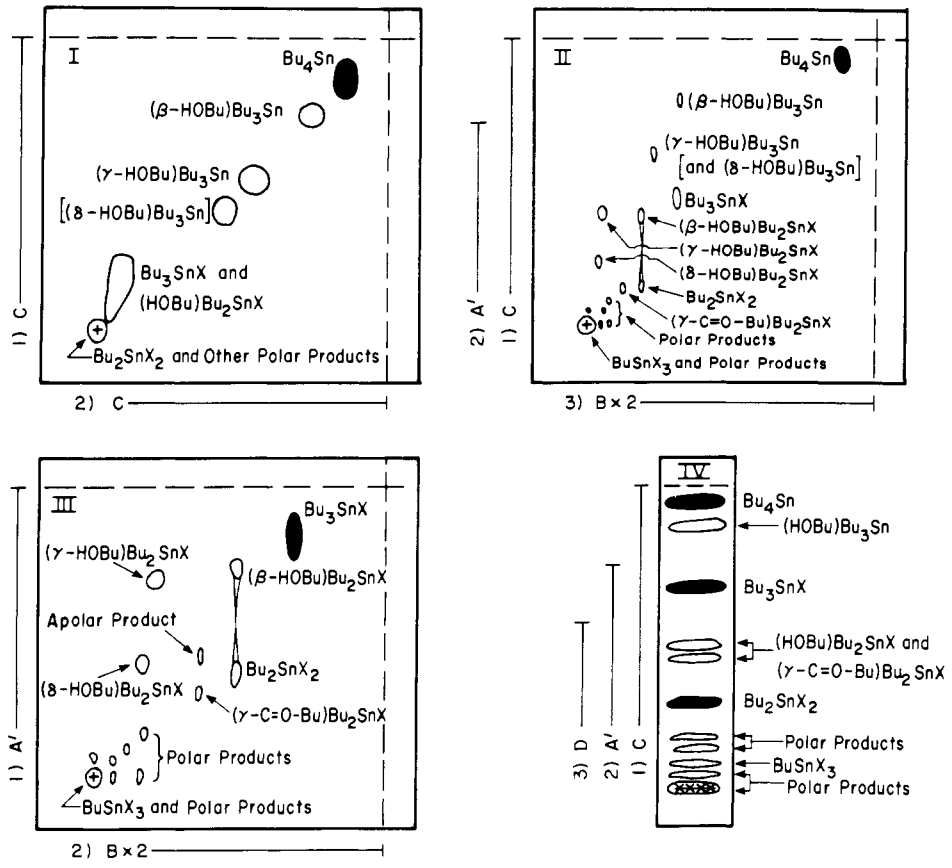

Figure 2. Metabolites of [^{14}C]Bu_4Sn, [^{14}C]Bu_3SnOAc, and [^{14}C]$Bu_2Sn(OAc)_2$ formed by the rat liver microsome-NADPH system as resolved by TLC. The substrates are indicated by dark spots and the metabolites by open spots. Compounds designated in brackets indicate that they are not detected as metabolites even though their chromatographic positions are shown. The β-hydroxybutyltin derivatives, (β-HOBu)Bu_3Sn and (β-HOBu)Bu_2SnX, decompose in acidic media so they chromatograph as Bu_3SnX and Bu_2SnX_2, respectively, in the second direction of development in systems II and III. The chromatographic systems used to analyze metabolites from different substrates were as follows: Bu_4Sn, I, II, and IV; Bu_3SnOAc, III and IV; $Bu_2Sn(OAc)_2$, IV.

Bu_4Sn during incubation, extraction, workup, and TLC analysis; extensive formation of polar metabolites which chromatograph in positions appropriate for Bu_3SnX derivatives with two sites of carbon hydroxylation or for Bu_2SnX_2 derivatives with one site of carbon hydroxylation.

[^{14}C]Bu_3SnOAc gives six NADPH-dependent metabolites tentatively identified by TLC cochromatography with additional structural evidence as follows (Fish et al., 1976a): [^{14}C](β-HOBu)Bu_2SnX undergoes protodestannylation to [^{14}C]Bu_2SnX_2 and 1-[^{14}C]butene; (γ-HOBu)Bu_2SnOAc recovered as a metabolite gives an appropriate chemical ionization mass spectrum; unlabeled (γ-HOBu)Bu_2SnX is metabolized in the rat liver microsome–NADPH system to (γ-C=O–Bu)Bu_2SnX.

[^{14}C]$Bu_2Sn(OAc)_2$ yields one NADPH-dependent metabolite (free, polar) in a small amount but the formation of $BuSnX_3$ does not appear to be dependent on NADPH fortification.

Unlabeled n-Alkyltin Derivatives and Rabbit Liver Microsome–NADPH System. The extent of NADPH-dependent metabolism of tri-*n*-alkyltin compounds decreases in the order Et_3SnX, Pr_3SnX, Bu_3SnX > Pen_3SnX > Hex_3SnX ≫ Oct_3SnX. Two-dimensional TLC (A × B) reveals the corresponding di-*n*-alkyltin derivative as a major product in each case; Oct_3SnX yields only trace amounts of Oct_2SnX_2 and no other detectable products. Each of the other substrates gives a major metabolite with the following characteristics: chromatographs above the di-*n*-alkyltin derivative in solvent system A and the same as the corresponding di-*n*-alkyltin derivative in solvent system B (Figure 3) suggesting that it undergoes protodestannylation in the more acidic solvent used in the second direction of development; detected on developed chromatograms with HQ and PCV reagents even without uv irradiation; decomposes to the corresponding dialkyltin derivative on treatment of the incubation mixture with HCl. When this metabolite of Bu_3SnX is separated in solvent system A, it gives the yellow dithizone color of a triorganotin derivative when sprayed before evaporation of the diisopropyl ether from the chromatoplate and the salmon dithizone response of a diorganotin derivative when the solvent is completely evaporated and the gel is again acidified with HOAc before spraying (reacidification is necessary for a good color reaction). GLC analysis reveals that 1-butene is released on acidification of the reaction mixture so this metabolite yields the alkene and diorganotin derivative upon protodestannylation (Fish et al., 1976a). Finally, this Bu_3SnX metabolite cochromatographs with authentic (β-HOBu)Bu_2SnX which also undergoes the same protodestannylation reaction. By analogy, a major metabolite of Et_3SnX, Pr_3SnX, Pen_3SnX, and Hex_3SnX is designated as the corresponding β-hydroxy compound even though authentic standards were not synthesized for direct comparisons.

The metabolites discussed above account for all of the organotin products detected with Et_3SnX and Pr_3SnX but the higher trialkyltin derivatives show additional metabolites (Figure 3). The number of metabolites depends on both the alkyl substituent and the degree of metabolism, two interrelated features. Bu_3SnX yields three

Table I. Metabolites of Tetrabutyltin, Tributyltin Acetate, and Dibutyltin Diacetate following Incubation with the Rat Liver Microsome System with and without NADPH

Compd or fraction	Recovery of initial radiocarbon, %[a]					
	Bu_4Sn		Bu_3SnOAc		$Bu_2Sn(OAc)_2$	
	−NADPH	+NADPH	−NADPH	+NADPH	−NADPH	+NADPH
Tetrabutyltin Derivatives						
Bu_4Sn	95.9	62.9				
(β-HOBu)Bu_3Sn	<0.1	0.6				
(γ-HOBu)Bu_3Sn	<0.1	0.5				
(δ-HOBu)Bu_3Sn	0.0	0.0				
Tributyltin Derivatives						
Bu_3SnX[b]	1.1	5.8	92.7	70.6		
(β-HOBu)Bu_2SnX	0.0	1.6	0.3	4.0		
(γ-HOBu)Bu_2SnX	0.0	1.4	0.2	1.3		
(γ-C=O-Bu)Bu_2SnX	0.0	0.0	0.0	0.2		
(δ-HOBu)Bu_2SnX	0.0	0.1	0.2	1.4		
Di- and Monobutyltin Derivatives						
Bu_2SnX_2[b]	0.0	2.7	2.3	8.7	88.9	91.3
$BuSnX_3$[b]	0.0	2.6	0.4	5.5	3.9	2.2
Unknowns						
Free, apolar[c]	0.0	0.5	0.0	0.1	0.0	0.0
Free, polar[d]	0.0	18.2	0.8	3.8	0.0	0.2
Bound	<0.1	0.5	0.3	1.3	0.8	0.2
Nonenzymatic products[e]	3.0	2.6	2.8	3.1	6.4	6.1
Loss correction used[f]	29.8	19.5	−2.8	7.3	12.8	8.0

[a] For compounds designated by structure the recovery values are normalized for change in specific activity on destannylation. The results are the average of two experiments with Bu_4Sn, four with Bu_3SnX, and two with Bu_2SnX_2. [b] Combined recovery of designated compounds in chloroform extracts and extracts of Cl_3CCOOH-precipitated protein. The following percentages of the total appeared in the chloroform extracts: Bu_3SnX, 67-73% from Bu_3SnX substrate; Bu_2SnX_2, 54-75% from Bu_3SnX substrate and 28-37% from Bu_2SnX_2 substrate; $BuSnX_3$, 70-100% from Bu_3SnX and Bu_2SnX_2 substrates. Extracts of the Cl_3CCOOH-precipitated protein were not analyzed with Bu_4Sn as the substrate. [c] One or more NADPH-dependent metabolites(s) chromatographing in the A′ × B×2 TLC solvent system between (δ-HOBu)Bu_2SnX and Bu_2SnX_2. [d] NADPH-dependent metabolites chromatographing at or near the origin in the A′ × B×2 TLC solvent system and of unknown number except that only one was detected slightly above the origin with Bu_2SnX_2 as the substrate. [e] Summation of all products detected with boiled microsomes + NADPH. The values for individual products are subtracted from those obtained in the fresh microsome and microsome-NADPH systems in the remaining data. [f] Losses on extraction, workup, and TLC analysis, some of which may be due to volatile substrates (e.g., Bu_4Sn) or products (e.g., 1-butene and 1-butanol). Quantitative data on the identified products are normalized values (see footnote *a*) whereas the unknowns are reported directly in relation to the initial radiocarbon; some of the unknowns probably consist of carbon fragments released on formation of other organotin metabolites and this is not taken into account in the tabulation.

additional metabolites, i.e. (γ-HOBu)Bu_2SnX and two diorganotin metabolites, one acid labile and the other acid stable. The pentyl compound gives the most complex pattern with two acid-stable triorganotin metabolites and comparable numbers of acid-labile tri-, acid-stable di-, and acid-labile diorganotin metabolites. The trihexyltin derivative yields two acid-stable triorganotin and one acid-stable diorganotin metabolites. Some of these acid-labile metabolites may contain β-hydroxyalkyl substituents.

Some differences are evident in studies with labeled and unlabeled butyltin compounds that may result from variations in the enzyme source and substrate level (0.003 μmol of labeled substrate with rat preparations; 0.5 μmol of unlabeled substrate with rabbit preparations) and the greater sensitivity of the radioassay. (δ-HOBu)Bu_2SnX and (γ-C=O-Bu)Bu_2SnX are not detected in the standard rabbit assays with unlabeled Bu_3SnX. Separate studies with rat preparations established that the proportion of (δ-HOBu)Bu_2SnX to other hydroxybutyl derivatives decreases as the substrate level is increased and that (γ-C=O-Bu)Bu_2SnX is a detectable metabolite with this enzyme source. There is no apparent NADPH-dependent conversion of unlabeled Bu_4Sn to Bu_3SnX by rabbit microsomes whereas [^{14}C]Bu_4Sn yields [^{14}C]Bu_3SnX with rat microsomes. On the other hand, unlabeled Bu_2SnX_2 undergoes NADPH-dependent conversion to $BuSnX_3$ (TLC cochromatography in D and E) but no other products are detected with rabbit microsomes, while [^{14}C]Bu_2SnX_2 yields only one polar NADPH-dependent metabolite and no $BuSnX_3$ with rat microsomes.

As expected, Et_4Sn is extensively metabolized based on the TLC detection (A × B) of Et_3SnX and Et_2SnX_2.

Unlabeled Cyclohexyltin Derivatives and Rabbit Liver Microsome–NADPH System. The TLC chromatographic pattern of the NADPH-dependent microsomal metabolites of Cy_3SnX is similar to that obtained with the corresponding *n*-alkyltin derivatives (Figure 3) and the degree of metabolism falls between that of Pen_3SnX and Hex_3SnX. The metabolite tentatively designated as (2-HOCy)Cy_2SnX is similar to (β-HOBu)Bu_2SnX in relative chromatographic position and in the following additional features: undergoes protodestannylation to the dialkyltin derivative; yields a yellow dithizone color before decomposition and a salmon dithizone color after decomposition on TLC. Cy_2SnX_2 is a major product (TLC, A × B) and $CySnX_3$ a minor one (TLC, D and E) in the microsomal NADPH-dependent metabolism of Cy_3SnX. Three acid-stable triorganotin metabolites of Cy_3SnX (Figure 3) each undergo photodecomposition on TLC plates to yield Cy_2SnX_2 and additional di- or monoorganotin derivatives of greater polarity than Cy_2SnX_2; these metabolites are possibly hydroxylated at the 3 or 4 position of the cyclohexyl group or are formed by oxidation of these (HOCy)Cy_2SnX derivatives to their corresponding ketones. The acid-stable metabolite with the highest R_f in solvent system A is a monohydroxy derivative of Cy_3SnX based on isolation of this metabolite and observing an appropriate chemical ionization mass spectrum (Fish et al., 1976b). An additional triorganotin metabolite is sufficiently polar for a dihydroxy derivative and its acid-labile nature suggests that one of the cyclohexyl groups is hy-

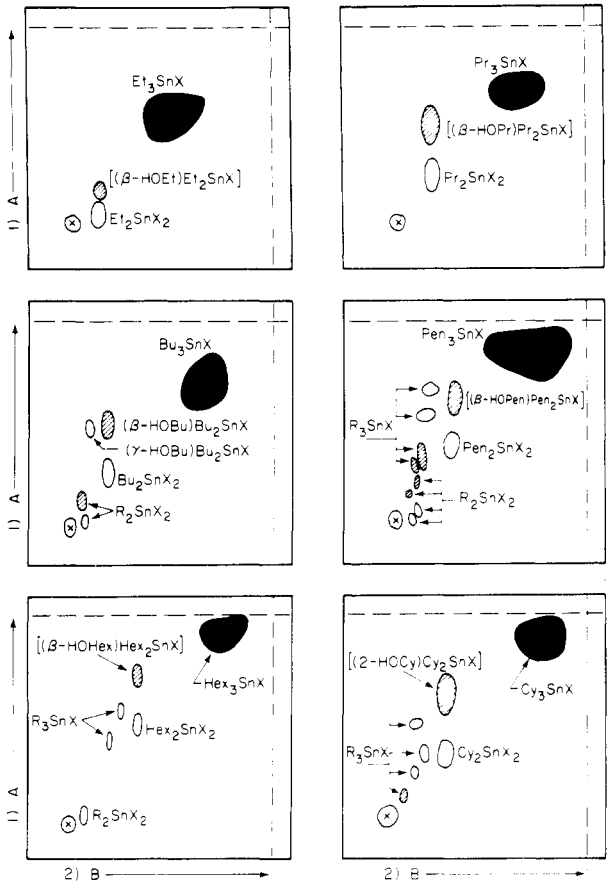

Figure 3. Metabolites of unlabeled trialkyltin derivatives formed by the rabbit liver microsome-NADPH system as resolved by TLC. The substrates are indicated by dark spots. The β-hydroxyalkyltin compounds indicated in brackets chromatograph and undergo protodestannylation as anticipated for such products although authentic standards were not synthesized. Additional metabolites are designated as triorganotins (R_3SnX) or diorganotins (R_2SnX_2) based on the response to HQ before and after uv irradiation and as acid-stable (open spots) or acid-labile (spots with diagonal lines) derivatives.

droxylated at the 2 position (Fish et al., 1976b).

Examination of the organotin metabolites of other cyclohexyltin compounds revealed that Cy_4Sn is not metabolized to detectable amounts of tri- or dicyclohexyltin derivatives, possibly due in part to its low water solubility, that Cy_2SnX_2 yields a small amount of $CySnX_3$ (TLC, D and E) in a NADPH-dependent reaction and that $CySnX_3$ does not undergo detectable metabolism.

The microsomal metabolism of cyclohexyltin compounds was compared with that of cyclohexane, cyclohexanol, and cyclohexanone using GLC analysis to determine the amount of cyclohexanol and cyclohexanone present after acidification of the reaction mixtures, extraction with ethyl acetate, and evaporation of the ethyl acetate into a small volume of water (Table II). Cy_4Sn, $CySnX_3$, and cyclohexanol undergo no detectable NADPH-dependent metabolism in the rabbit liver microsome system and, as expected (Ullrich, 1969), only cyclohexanol is evident with cyclohexane as the substrate. Cy_3SnX and Cy_2SnX_2 are metabolized to similar extents, yielding almost equivalent amounts of cyclohexanol and cyclohexanone. Maximum yields of cyclohexanol and cyclohexanone are obtained from the Cy_3SnX and Cy_2SnX_2 metabolites by treating the reaction mixtures with HCl and by holding the final aqueous solution after evaporation of the ethyl acetate for at least 1 day prior to analysis. These conditions presumably decompose the acid-labile metabolites. It appears that one or more metabolites of Cy_3SnX and Cy_2SnX_2 decompose at physiological pH or in acid solution to liberate both cyclohexanol and cyclohexanone. With each of Cy_3SnX and Cy_2SnX_2, the cyclohexanol may result from hydroxylation at the 1-carbon followed by destannylation and the cyclohexanone may be formed by hydroxylation at the 2-carbon followed by oxidation to the corresponding ketone and then destannylation. If the destannylation reaction occurred during the normal incubation at pH 7.4, the released cyclohexanol would undergo no further metabolism, but some of the cyclohexanone would be enzymatically reduced to cyclohexanol (see also Markovič et al., 1971). Further studies are in progress concerning the reactions of the hydroxycyclohexyltin derivatives.

Table II. Metabolites of Cyclohexyltin Derivatives, Cyclohexane, Cyclohexanol, and Cyclohexanone following Incubation with the Rabbit Liver Microsome System with and without NADPH Fortification

		Recovery, %[a]		
Substrate	NADPH	Cyclohexanol	Cyclohexanone	Total
Cy_4Sn	−	<0.2	<0.4	
	+	<0.2	<0.4	
Cy_3SnX	−	1.1	0.5	1.6
	+	7.6	8.7	16.3
Cy_2SnX_2	−	1.4	1.1	2.5
	+	5.1	6.2	11.3
$CySnX_3$	−	<0.2	<0.4	
	+	<0.2	<0.4	
Cyclohexane	−	<0.2	<0.4	
	+	1.6	<0.4	1.6
Cyclohexanol	−	55.0	<0.4	55.0
	+	55.2	<0.4	55.2
Cyclohexanone	−	1.0	24.0	25.0
	+	12.3	14.2	26.5

[a] The results are expressed on a molar equivalent basis and are averages from eight experiments with Cy_3SnX and Cy_2SnX_2 and four experiments with the other substrates.

Ph_3SnOAc and Liver Microsome–NADPH Systems. No metabolism of unlabeled Ph_3SnOAc was detected in the rabbit microsome–NADPH system, despite many experiments with enzyme preparations active on trialkyltin derivatives. The refractory nature of this substrate was confirmed with $Ph_3{}^{113}SnOAc$ in the mouse microsome–NADPH system, without added soluble fraction; no detectable $Ph_2{}^{113}SnX_2$ was formed. As with [^{14}C]Bu_3SnX, there is extensive and NADPH-independent binding of $Ph_3{}^{113}SnX$ in the microsome system; thus, the radiotin recoveries on sequential extraction with chloroform (before acidification) and ether–acetylacetone and ether–ethanol (after acidification) are 27, 44, and 8%, respectively. At the two higher substrate levels (0.007 and 0.02 μmol) but not at 0.002 μmol substrate, one apolar metabolite is detected in trace amount. This metabolite is formed only on NADPH fortification, appears mostly in the chloroform extract, and chromatographs in a region (solvent system A′) similar to the apolar metabolite noted later in the in vivo studies with rats.

Monooxygenase Metabolites of Unlabeled Organolead Compounds. In NADPH-dependent reactions, rabbit liver microsomes convert Et_4Pb to Et_3PbX and Bu_3PbX to Bu_2PbX_2 but not Ph_3PbX to Ph_2PbX_2. These findings are based on extraction with or without addition of HCl to the incubation mixtures and TLC in solvent system F which gives R_f values as follows: Et_4Pb, not detected possibly due to volatility; Et_3PbX, 0.79; Bu_3PbX, 0.90; Bu_2PbX_2, 0.37; Ph_3PbX, 0.91; Ph_2PbX_2, 0.43. 1-

Table III. Distribution of Radiocarbon at Various Times after Oral Administration of [^{14}C]Tributyltin Acetate and [^{14}C]Dibutyltin Diacetate to Mice

Compd or fraction	Recovery of initial radiocarbon, %				
	0-24 h	24-42 h	42-90 h	90-138 h	Total
	Tributyltin Acetate Administered				
Urine	10.7	3.9	1.5	0.2	16.3
Feces	25.1[a]	22.4[a]	4.5[a]	0.8	52.8
Carbon dioxide	16.1	3.5	2.4		22.0
1-Butene	1.7				1.7
Total	53.6	29.8	8.4	1.0	92.8
	Dibutyltin Diacetate Administered				
Urine	3.0	3.0	3.1	1.0	10.1
Feces	No feces	7.9[a]	52.1[a]	6.0	66.0
Carbon dioxide	2.1	2.0	3.0		7.1
1-Butene	0.1				0.1
Total	5.2	12.9	58.2	7.0	83.3

[a] Radiocarbon recoveries in different extracts are independent of the compound administered and the time after treatment. Average values for four analyses after Bu_3SnOAc treatment and three analyses after $Bu_2Sn(OAc)_2$ treatment are: 29% recovery in chloroform extract, 24% in methanol extract, 28% in acidic methanol extract, 10% not extracted, and 9% loss at an unknown step.

Table IV. Products in the Liver, Feces, and Urine after Oral Administration of [^{14}C]Tributyltin Acetate and [^{14}C]Dibutyltin Diacetate to Mice

Compd or fraction	Recovery of initial radiocarbon, %[a]				
	Tributyltin acetate admin.			Dibutyltin diacetate admin.	
	Liver, 3 h after admin.	Feces, 0-42 h after admin.	Urine, 0-24 h after admin.	Liver, 3 h after admin.	Feces, 24-90 h after admin.
	Tributyltin Derivatives				
Bu_3SnX	2.9[b,c]	14.6[b,c]	<0.1[c]		
(β-HOBu)Bu_2SnX	<0.1[c]	<0.1[c]	0.0		
(γ-HOBu)Bu_2SnX	0.3[b,c]	0.1[c]	0.0		
(γ-C=O-Bu)Bu_2SnX	0.1[b,c]	0.0	0.0		
(δ-HOBu)Bu_2SnX	0.3[b,c]	<0.1[c]	0.0		
	Di- and Monobutyltin Derivatives				
Bu_2SnX_2	<0.1[b]	6.7[b,c]	0.2[b,c]	<0.1[b,d]	41.1[b,e]
$BuSnX_3$	0.0	3.3[b,e]	1.0[b,c]	0.0	3.5[b,e]
	Unknowns				
Polar	1.3[c]	11.4[e]	2.5[d]	2.6[d]	6.4[e]
Not extracted plus loss	0.6	11.4	7.0	0.9	9.0
Total	5.5	47.5	10.7	3.5	60.0

[a] The results are the average of two experiments (liver analyses) or of analyses at different times after treatment (urine at 0-18 and 18-24 h, feces from Bu_3SnOAc at 0-18, 18-24, and 24-42 h, and feces from $Bu_2Sn(OAc)_2$ at 24-42, 42-66, and 66-90 h after administration) with no time-dependent differences in the product ratios. [b] Identity established by cochromatography. [c] Major portion recovered in initial chloroform extract. [d] Major portion recovered in chloroform extract of acidified fraction after initial chloroform extraction. [e] Major portion recovered in methanol and acidic methanol extracts.

Butene is released on acidification of the incubated Bu_3PbX–microsome–NADPH system but not when NADPH is deleted. On analogy with studies on Bu_3SnOAc (see above), it appears likely that the 1-butene, and at least a portion of the Bu_2PbX_2, originate from (β-HOBu)-Bu_2PbX.

In Vivo Metabolites of Labeled Organotin Compounds. *[^{14}C]Bu_3SnOAc and [^{14}C]$Bu_2Sn(OAc)_2$ in Mice.* These butyltin derivatives undergo extensive in vivo cleavage at the tin–carbon bond and further oxidation of the liberated carbon fragment(s) to $^{14}CO_2$, the amount being equivalent within 90 h after oral treatment to destannylation of 66% of the [^{14}C]Bu_3SnOAc dose and 14% of the [^{14}C]$Bu_2Sn(OAc)_2$ dose (Table III). In addition, expired 1-[^{14}C]butene is equivalent to 5.1 and 0.2% of the administered [^{14}C]Bu_3SnOAc and [^{14}C]$Bu_2Sn(OAc)_2$, respectively. Thus, a significant portion of the in vivo metabolism of Bu_3SnOAc involves (β-HOBu)Bu_2SnX as an intermediate.

Three hours after oral administration of [^{14}C]Bu_3SnOAc to mice, the liver contains a large amount of Bu_3SnX and detectable levels of (β-HOBu)Bu_2SnX, (γ-HOBu)Bu_2SnX, (γ-C=O-Bu)Bu_2SnX, (δ-HOBu)Bu_2SnX, and Bu_2SnX_2 (Table IV). Most of these products are also detected in the feces along with $BuSnX_3$. Although insignificant levels of triorganotin derivatives appear in the urine, it contains some Bu_2SnX_2 and $BuSnX_3$. On administration of [^{14}C]$Bu_2Sn(OAc)_2$, the feces contains a large amount of unmetabolized compound and some [^{14}C]$BuSnX_3$. Large amounts of polar and unextractable metabolites appear in the feces in each case. A portion of the urinary radiocarbon may consist of conjugates that no longer retain a tin–carbon bond. Thus, it is clear that the butyltin compounds are extensively absorbed and metabolized by pathways that, in at least their initial steps, are similar to or identical with those for the MO reactions.

The tissue residue results for [^{14}C]Bu_3SnOAc equivalents at 138 h show the highest level in brain (0.053 ppm equivalents), intermediate in bone, fat, heart, kidney, liver, lung, muscle, spleen, and testes (0.022–0.050 ppm), and the lowest in the stomach and intestine (0.012–0.015 ppm). Comparable values for [^{14}C]$Bu_2Sn(OAc)_2$ equivalents in tissues give the highest level in the brain (0.13 ppm equivalents), intermediate in heart, kidney, liver, and lung

Table V. Products in the Feces and Urine after Oral and Intraperitoneal Administration of [^{113}Sn]Triphenyltin Acetate to Rats

Compd or fraction[a]	TLC system, R_f A′	TLC system, R_f B × 2	TLC system, R_f D	Recovery of initial radiotin, % Oral admin.	Recovery of initial radiotin, % Ip admin.
Feces, 0-72 h Oral and 0-96 h Ip					
Ph_3SnX	0.83	0.70	0.84	28.0	1.8
Apolar unknown	0.70	0.18		0.6	0.4
Ph_2SnX_2	0.34	0.28	0.45	6.4	8.8
$PhSnX_3$	0.00	0.00	0.14	21.4[b]	14.2[b]
SnX_4 and polar unknowns	0.00	0.00	0.00		
Bound				21.2	24.1
Other Excreta and 0-240 h Total[c]					
Feces				7.1	13.4
Urine				1.2	1.8
Total				85.9	64.5

[a] Ph_3SnX, the apolar unknown, and Ph_2SnX_2 appear to the extent of 19-45, 55-71, and 0-10% in the chloroform, ether-acetylacetone, and methanol extracts, respectively. $PhSnX_3$, SnX_4, and polar unknowns appear to the extent of 9, 26, and 65% in the chloroform, ether-acetylacetone, and methanol extracts, respectively. [b] About half of the indicated amount is $PhSnX_3$ while the remainder is unidentified products, probably including SnX_4, remaining at the origin in solvent system D. A portion of the SnX_4 may result from decomposition of $PhSnX_3$ during analysis. [c] Based on total radiotin of 72-240 and 96-240 h feces following oral and ip administration, respectively, 0-48 h urine in each case (probably contaminated by radiotin from the feces), and of all 0-240 h excreta.

(0.058–0.082 ppm), and the lowest in the other tissues examined (0.008–0.053 ppm). These results do not differentiate [^{14}C]butyltin derivatives retained in the tissues from metabolites formed by cleavage of the tin–carbon bond and reincorporation of the carbon fragments into tissue components.

$Ph_3{}^{113}SnOAc$ in Rats. After oral or ip administration of $Ph_3{}^{113}SnOAc$ to rats, most of the radiotin appears in the feces within 10 days (Table V). The excretion of radiotin is relatively slow, particularly after the ip dose where 35% of the radiotin remains in the body or is not accounted for in the excreta after the 10-day period. Although some of the administered $Ph_3{}^{113}SnX$ is excreted without metabolism, a significant portion is excreted as Ph_2SnX_2, $PhSnX_3$, and other polar products. The minor "apolar unknown" has similar chromatographic properties to the microsomal "apolar unknown" but was not present in sufficient amount for direct cochromatography with the microsomal metabolite. The destannylation products noted in the feces are formed within the animal rather than as artifacts of analysis since: (1) less Ph_3SnX appears in the feces on ip than on oral administration; (2) the proportion of Ph_3SnX is highest in early feces samples whereas that of $PhSnX_3$ and other polar metabolites is highest in the later feces samples; and (3) analysis of comparable control feces directly fortified with $Ph_3{}^{113}SnOAc$ results in recovery (TLC) of only the original compound. A large amount of "bound" radiotin is not recovered from particulate material by the extensive extraction method used.

DISCUSSION

Figure 1 gives a tentative metabolic pathway for butyltin compounds in the rat liver microsomal MO system. Many aspects of this pathway are also applicable to the MO systems of rabbit and mouse liver and housefly abdomens and to the metabolism of Bu_3SnX and Bu_2SnX_2 in mice.

Bu_4Sn is hydroxylated in the MO system to yield (β-HOBu)Bu_3Sn and (γ-HOBu)Bu_3Sn. Although not detected in these studies, trace levels of (δ-HOBu)Bu_3Sn and (γ-C=O-Bu)Bu_3Sn might also form. On analogy with the Bu_3SnOAc metabolites, Bu_4Sn is also likely to yield significant amounts of (α-HOBu)Bu_3Sn which undergoes a destannylation reaction in the buffer system to Bu_3SnX and 1-butanol. Bu_3SnX may also be formed by destannylation of (β-HOBu)Bu_3Sn, with the liberation of 1-butene.

MO metabolism of Bu_3SnOAc involves hydroxylation at the α-, β-, γ-, and δ-carbons, with preference for the α and β positions (Fish et al., 1975, 1976a,b). The (γ-HOBu)Bu_2SnX is further oxidized to (γ-C=O-Bu)-Bu_2SnX. Both (α-HOBu)Bu_2SnX and (β-HOBu)Bu_2SnX decompose to Bu_2SnX_2 liberating 1-butanol and 1-butene, respectively, the first of these destannylation reactions occurring at physiological pH and the second on acidification. Finally, metabolism of $Bu_2Sn(OAc)_2$ yields $BuSnX_3$, perhaps both by nonenzymatic destannylation and by α- and β-carbon hydroxylation and decomposition of the hydroxy derivatives. The unidentified polar metabolites are probably formed by two or more sites of hydroxylation at different butyl groups. Bu_3SnX and Bu_2SnX_2 bind extensively in some tissue fractions, providing an inconvenience in analysis and a possible explanation for the relatively low metabolite yields.

Studies with a variety of unlabeled trialkyltin compounds and with Et_4Pb, Bu_3PbX, and Et_4Sn suggest that portions of this general metabolic pathway are also applicable to related compounds. The major products of the biological oxidation reactions are unstable at pH 7.4 or in acidic solution, undergoing destannylation reactions to the lower alkyl–metal derivatives; the intermediates in each case are probably the α- and β-carbon hydroxylated derivatives. The number of additional metabolites depends on the number of methylene groups susceptible to attack and the overall extent of metabolism, reaching a maximum with Bu_3SnX, Pen_3SnX, and Cy_3SnX.

Ph_3SnX and Ph_3PbX are quite resistant to MO metabolism. However, Ph_3SnX undergoes extensive metabolism to Ph_2SnX_2 and $PhSnX_3$ in rats by a destannylation mechanism that remains to be defined.

Three of the Bu_3SnX metabolites [(γ-HOBu)Bu_2SnX, (γ-C=O-Bu)Bu_2SnX, and (δ-HOBu)Bu_2SnX] have been assayed elsewhere for potency in disturbing rat liver mitochondrial functions (effects on mitochondrial swelling and on the synthesis and hydrolysis of adenosine 5′-triphosphate). These three compounds have approximately the same potency range as Bu_3SnX in all of the mitochondrial systems assayed (Aldridge and Street, 1976) and in ip toxicity to mice (Kimmel et al., 1976). Thus, some of the metabolites may contribute to the biological activity of triorganotin compounds.

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LITERATURE CITED

Aldridge, W. N., Street, B. W., Medical Research Council Laboratories, Carshalton, Surrey, England, personal communication, 1976.

Blair, E. H., *Environ. Qual. Saf., Suppl.* **3**, 406 (1975).
Casida, J. E., Kimmel, E. C., Holm, B., Widmark, G., *Acta Chem. Scand.* **25**, 1497 (1971), and references cited therein.
Figge, K., *J. Chromatogr.* **39**, 84 (1969).
Fish, R. H., Kimmel, E. C., Casida, J. E., *J. Organomet. Chem.* **93**, C1 (1975).
Fish, R. H., Kimmel, E. C., Casida, J. E., *J. Organomet. Chem.* **118**, 41 (1976a).
Fish, R. H., Kimmel, E. C., Casida, J. E., *Adv. Chem. Ser.*, in press (1976b).
Kimmel, E. C., Fish, R. H., Casida, J. E., unpublished results, 1976.
Krishna, J. G., Casida, J. E., *J. Agric. Food Chem.* **14**, 98 (1966).
Lahournère, J.-C., Valade, J., *J. Organomet. Chem.* **33**, C4 (1971).
Luijten, J. G. A., in "Organotin Compounds", Sawyer, A. K., Ed., Vol. 3, Marcel Dekker, New York, N.Y., 1972, p 931.
Markovič, O., Theorell, H., Rao, S., *Acta Chem. Scand.* **25**, 195 (1971).
Pal, B. K., Ryan, D. E., *Anal. Chim. Acta* **48**, 227 (1969).
Petrowitz, H.-J., in "Thin-Layer Chromatography", Stahl, E., Ed., 2d ed, Springer-Verlag, New York, N.Y., 1969, p 664.
Rose, M. S., in "Pesticide Terminal Residues", Tahori, A. S., Ed., Butterworths, London, 1971, p 281.
Thayer, J. S., *J. Organomet. Chem.* **76**, 265 (1974).
Türler, M., Högl, O., *Mitt. Lebensmittelunters.-Hyg.* **52**, 123 (1961).
Ullrich, V., *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 357 (1969).
Williams, D. J., Price, J. W., *Analyst* **85**, 579 (1960).

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Permethrin Metabolism in Rats

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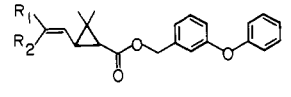
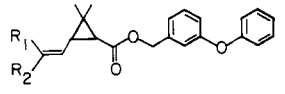
When administered orally to male rats at 1.6 to 4.8 mg/kg, the [1*R*,trans], [1*RS*,trans], [1*R*,cis], and [1*RS*,cis] isomers of the potent pyrethroid insecticide permethrin [3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] are rapidly metabolized, and the acid and alcohol fragments are almost completely eliminated from the body within a few days. *cis*-Permethrin is more stable than *trans*-permethrin and the cis compound yields four fecal ester metabolites which result from hydroxylation at the 2′-phenoxy, 4′-phenoxy, or 2-*trans*-methyl position or at both of the latter two sites. Other significant metabolites are 3-phenoxybenzoic acid (free and glucuronide and glycine conjugates), the sulfate conjugate of 4′-hydroxy-3-phenoxybenzoic acid, the sulfate conjugate of 2′-hydroxy-3-phenoxybenzoic acid (from *cis*-permethrin only), the *trans*- and *cis*-dichlorovinyldimethylcyclopropanecarboxylic acids (free and glucuronide conjugates), and the 2-*trans*- and 2-*cis*-hydroxymethyl derivatives of each of the aforementioned trans and cis acids (free and glucuronide conjugates).

Two highly insecticidal esters of 3-phenoxybenzyl alcohol, permethrin (Elliott et al., 1973a) and phenothrin (Fujimoto et al., 1973), differ in the following respects: permethrin contains a dichlorovinyl group and phenothrin an isobutenyl group in the acid moiety; the permethrin isomers are more potent and longer acting than the corresponding phenothrin isomers (Elliott et al., 1973b, 1974; Burt et al., 1974).



trans-permethrin, $R_1 = R_2 = Cl$
trans-phenothrin, $R_1 = R_2 = CH_3$



cis-permethrin, $R_1 = R_2 = Cl$
cis-phenothrin, $R_1 = R_2 = CH_3$

The alcohol moiety of [1*R*,*trans*]- and [1*R*,*cis*]-phenothrin is rapidly metabolized and eliminated from rats treated orally with these compounds, the major excreted metabolite being 4′-hydroxy-3-phenoxybenzoic acid in free and conjugated form (Miyamoto et al., 1974; Miyamoto, 1976). Three minor fecal metabolites of [1*R*,*cis*]-phenothrin retain the ester linkage but involve oxidation at other sites, i.e., in one metabolite the 4′ position of the phenoxy group is hydroxylated, in another the trans methyl of the isobutenyl group is oxidized ($R_1 = CH_3$; $R_2 = COOH$), and in the third both of these modifications are involved plus oxidation at one of the geminal dimethyl groups (isomer unspecified) (Miyamoto, 1976). Preliminary studies with rats indicate that the metabolic fate of the acid and alcohol moieties of [1*R*,*trans*]- and [1*R*,*cis*]-permethrin is very similar to that for the corresponding moieties of phenothrin, except that in permethrin the dichlorovinyl side chain is not metabolically altered (Elliott et al., 1976).

The present study considers the residence time in the body and the metabolic fate of both the acid and alcohol moieties of [1*R*,*trans*]-, [1*RS*,*trans*]-, [1*R*,*cis*]-, and [1*RS*,*cis*]-permethrin, when these esters are administered orally to rats at dosages ranging from 1.6 to 4.8 mg/kg.

MATERIALS AND METHODS

Structures and Abbreviations for Chemicals. Figure 1 gives the structures and abbreviations used for the various chemicals discussed. Permethrin is a mixture of [1*RS*,trans] and [1*RS*,cis] isomers, designated as *t*-per and *c*-per, respectively. The system used to designate the hydroxylated per isomers is illustrated for example by 4′-HO,*t*-HO,*c*-per, which represents the *c*-per derivative hydroxylated at the 4′ position of the alcohol moiety and at the methyl group of the geminal dimethyl moiety which is trans to the carboxyl group. The hydrolysis products from the acid moieties of *t*- and *c*-per are *t*-Cl_2CA and *c*-Cl_2CA, respectively. The Cl_2CA isomers hydroxylated at the geminal dimethyl position are: *t*-HO,*t*-Cl_2CA; *c*-

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