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Bioorganotin Chemistry. Microsomal Monooxygenase and Mammalian Metabolism of Cyclohexyltin Compounds Including the Miticide Cyhexatin

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Metabolism of cyclohexyltriphenyltin by the rat liver microsomal monooxygenase system yields *trans*-2-, *cis*-3-, *trans*-3-, and *trans*-4-hydroxycyclohexyltriphenyltin and the analogous 3- and 4-keto derivatives. The major product is *trans*-4-hydroxycyclohexyltriphenyltin. Cyclohexyldiphenyltin acetate in this microsomal system gives *trans*-2-hydroxycyclohexyldiphenyltin acetate as the principal metabolite plus the 3- and 4-hydroxy compounds. Metabolites of these ^{14}C-labeled substrates were identified by TLC cochromatography with unlabeled standards from synthesis except for *trans*-2-hydroxycyclohexyldiphenyltin acetate which was degraded to cyclohexene for determination as its oxymercuration adduct. Metabolism of tricyclohexyltin hydroxide, the important miticide cyhexatin or Plictran, yields products with the anticipated chromatographic properties for 2-, 3-, and 4-hydroxycyclohexyldicyclohexyltin derivatives. The 2-hydroxy metabolite is readily degraded to cyclohexene and dicyclohexyltin compounds. Chemical ionization mass spectrometry supports the identity of the 3- and 4-hydroxycyclohexyldicyclohexyltin derivatives. Products with chromatographic properties similar to the microsomal metabolites are present in the feces of rats, mice, guinea pigs, and rabbits orally administered [^{14}C]cyhexatin.

The miticide tricyclohexyltin hydroxide (cyhexatin or Plictran) is not readily absorbed from the gastrointestinal tract of rats, dogs, sheep, and cattle and its principal or only metabolic pathway in these species is reported to be sequential destannylation, i.e., $Cy_3SnOH \rightarrow Cy_2SnO \rightarrow CySnO_2H \rightarrow Sn^{4+}$ (Blair, 1975). Destannylation mechanisms for tetra- and tributyltin derivatives in microsomal monooxygenase systems include 1- and 2-carbon hydroxylation and subsequent degradation to 1-butanol and 1-butene, respectively, and the tri- and dibutyltin derivatives (Fish et al., 1976). Microsomal oxidation also occurs at the 3 and 4 positions of tetra- and tributyltin derivatives, yielding biologically active 3-hydroxy-, 3-keto-, and 4-hydroxybutyldibutyltin derivatives (Aldridge et al., 1977). Analogous reactions involving hydroxylation at each carbon atom might be involved with cyhexatin (Casida et al., 1971; Kimmel et al., 1977).

The present study first examines microsomal monooxygenase metabolism of [^{14}C]cyclohexyltriphenyltin and [^{14}C]cyclohexyldiphenyltin acetate for two reasons: the phenyl group does not undergo significant hydroxylation (Kimmel et al., 1977) so the cyclohexyl substituent is likely to be the dominant site for metabolism; several of the possible hydroxy and keto metabolites are available as standards from synthesis (Fish and Broline, 1978) and their TLC properties and stability are known (Fish et al., 1978). It then considers microsomal and in vivo metabolism of [^{14}C]tricyclohexyltin hydroxide.

MATERIALS AND METHODS

Thin-Layer Chromatography (TLC). Silica gel 60 chromatoplates (0.25-mm gel thickness, 20 × 20 cm, Merck) were used for one- or two-dimensional development with detection of organotin derivatives and ^{14}C compounds as previously reported (Kimmel et al., 1977). Tetraorganotin derivatives were separated with four solvent

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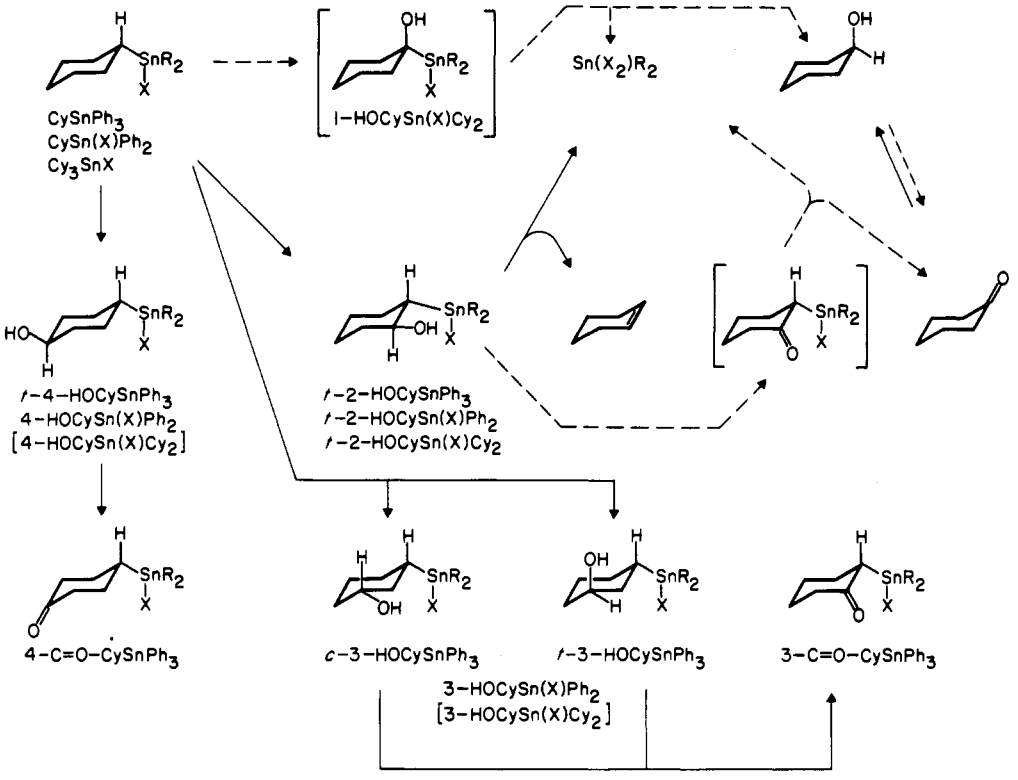

Figure 1. Partial metabolic pathways for three cyclohexyltin derivatives in the rat liver microsome-NADPH system and for tricyclohexyltin hydroxide in rats, mice, guinea pigs, and rabbits, indicating the abbreviations used for various metabolites. X is phenyl in $CySnPh_3$ or it is hydroxide, acetate, or other group with which these exchange during metabolism or analysis. Cy is cyclohexyl and Ph is phenyl. Brackets around chemical structures designate likely intermediates and around compound abbreviations indicate possible identities based only on chromatographic properties. Established reactions are designated by solid arrows and hypothetical pathways by dashed arrows.

systems: (A) diisopropyl ether–hexane (1:1), (B) benzene–ethyl acetate (19:1), (C) diisopropyl ether–hexane (17:3), and (D) benzene–ethyl acetate (7:3). Tri-, di-, and monoorganotin derivatives were resolved with three solvent systems: (E) diisopropyl ether–glacial acetic acid (49:1), two developments; (F) hexane–glacial acetic acid (9:1) two developments; and (G) carbon tetrachloride–acetylacetone–glacial acetic acid (10:1:1).

Chemicals. The chemicals are designated by abbreviations given in Figure 1.

Unlabeled Chemicals. Tricyclohexyltin hydroxide, dicyclohexyltin oxide, and cyclohexylstannic acid were provided by Dow Chemical Co. (Midland, MI). Hydroxycyclohexyltriphenyltin, ketocyclohexyltriphenyltin, and hydroxycyclohexyldiphenyltin bromides were from syntheses of Fish and Broline (1978).

[^{14}C]Cyclohexyltriphenyltin. [^{14}C]Cyclohexylmagnesium bromide was prepared under nitrogen by dropwise addition of [^{14}C]cyclohexyl bromide (526 mg, 3.2 mmol, 4 mCi, 1.24 mCi/mmol) to a stirred and heated solution of 96 mg (0.004 g-atom) magnesium chips in 5 mL of anhydrous tetrahydrofuran (THF) containing ethylene dibromide (5 drops) to entrain the Grignard reagent. The mixture was heated for 2 h after initiation of the Grignard reaction and cooled, and triphenyltin chloride (1.23 g, 3.22 mmol) was added in anhydrous THF (5 mL) at 25 °C. The reaction mixture was refluxed 4 h and treated with a saturated solution of ammonium chloride to hydrolyze excess Grignard reagent, and sodium chloride was added and the mixture was extracted with ether. The ether extract was washed thrice with water and dried over anhydrous magnesium sulfate, and the residue, following solvent evaporation, was chromatographed on Florisil (dry column technique) with hexane. Triphenyltin chloride was retained on the column and the eluate contained [^{14}C]-cyclohexyltriphenyltin [mp 132 °C, 453 mg (32.5%), 1.3 mCi] with a NMR spectrum identical with the standard compound and a radiochemical purity of >99% based on TLC with solvent systems A–D.

[^{14}C]Cyclohexyldiphenyltin Acetate. Hydrogen bromide (50%, 42 mg, 0.22 mmol) was added over a 5-min period to a stirred solution of [^{14}C]cyclohexyltriphenyltin (98.8 mg, 0.22 mmol, 0.284 mCi, 1.24 mCi/mmol) in 4 mL of 2-propanol and 1 mL of chloroform. The reaction mixture was stirred for 3 days, benzene was added, and the solvents were evaporated. The product was isolated by dry column chromatography with Florisil using first hexane to remove [^{14}C]cyclohexyltriphenyltin (46.6 mg) and then diisopropyl ether–acetic acid (99:1) to elute [^{14}C]cyclohexyldiphenyltin acetate (42.6 mg; 80% based on reacted starting material; identical NMR spectrum to authentic sample; >99% radiochemical purity based on two-dimensional TLC with solvent systems E–G).

[^{14}C]Tricyclohexyltin Hydroxide. [^{14}C]Tricyclohexyltin diisopropylphosphorodithioate (2 mg; 7.2 mCi/mmol; obtained from Stauffer Chemical Co., Richmond, CA) was reacted with potassium hydroxide (9.5 mg) in 1 mL of methanol–water (4:1) for 2 h at 25 °C. After addition of water (3.5 mL), the reaction mixture was extracted with chloroform (18 mL) which in turn was reextracted with water (3 mL) to remove all potassium hydroxide. TLC cochromatography revealed 97.4% tricyclohexyltin and 2.6% dicyclohexyltin derivatives, in an overall yield of 94%. Preliminary experiments with unlabeled material revealed that this procedure converted the starting material (mp 39–40 °C) to a new product with properties appropriate for cyhexatin (mp 205–210 °C, decomposition; no response of TLC to chromogenic reag-

ents specific for the P=S group).

Microsomal Monooxygenase Metabolism. Components of the reaction mixtures in 2 mL of sodium phosphate buffer (pH 7.4, 0.1 M) were as follows: male rat liver microsomal fraction (5.1 mg of protein) and 105000*g* supernatant fraction (5.5 mg of protein), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 2 μmol), and [^{14}C]cyclohexyltin compound (0.05, 0.05, and 0.01 μmol for cyclohexyltriphenyltin, cyclohexyldiphenyltin acetate, and tricyclohexyltin hydroxide, respectively) added last in ethanol (25 μL). Incubations were carried out in 25-mL Erlenmeyer flasks with shaking in air for 1 h at 37 °C.

The incubated reaction mixtures were extracted directly with chloroform (5 × 7 mL) (for cyclohexyltriphenyltin) or they were acidified to pH 1–2 with hydrochloric acid prior to the same extraction method (for cyclohexyldiphenyltin derivatives). Alternatively, reaction mixtures with tricyclohexyltin hydroxide were lyophilized to dryness, the residue was extracted with chloroform (2 × 5 mL, 2 h mild sonication) and methanol (2 × 5 mL, 2 h mild sonication), and the insoluble portion was then acidified with dilute hydrochloric acid prior to a final extraction with methanol (5 mL). ^{14}C compounds in the organosoluble fractions were analyzed by TLC and liquid scintillation counting (LSC). The remaining radiocarbon, the bound fraction, was determined by combustion and LSC.

Identification of Microsomal Monooxygenase Metabolites. TLC cochromatography of the organosoluble ^{14}C products in each of the solvent systems indicated above served as one criterion of metabolite identification.

Several metabolites were isolated by preparative TLC for spectral identification. The major metabolite of cyclohexyltriphenyltin was isolated on TLC plates (prewashed with acetone and then hexane) by developing with diisopropyl ether–hexane (17:3) and recovering the desired ^{14}C product by extraction of the silica gel with chloroform, filtration through a sintered glass filter and filter paper, and evaporation to dryness. This metabolite was dissolved in chloroform-*d* for examination by 360-MHz NMR (Bruker HXS-360 instrument of the Stanford Magnetic Resonance Laboratory) in the Fourier transform mode (13 800 acquisitions) with computer subtraction of the background. Two metabolites of tricyclohexyltin hydroxide were purified and separated by two-dimensional TLC (E × F), recovered by ethyl acetate extraction of the silica gel, and subjected to chemical ionization–mass spectrometry (CI–MS) (Finnigan Model 1015D mass spectrometer, methane reagent gas).

[^{14}C]Cyclohexene, from a 1,2-deoxystannylation reaction of *trans*-2-hydroxy[^{14}C]cyclohexyltin derivatives in the presence of acid, was determined as the oxymercuration adduct formed on reaction with mercuric acetate and methanol. The incubated enzyme reaction was acidified to pH 1–2, fortified with unlabeled cyclohexene (25 μL), and placed in a 25-mL distillation flask closed at the top by a rubber septum containing a capillary for air flow. The end of the long sidearm was submerged in a trapping mixture of 30 mL of methanol, 1 mL of water, 87 mg of mercuric acetate, and 7 drops of glacial acetic acid. The distillation flask was placed in a boiling water bath for 1 h with the receiver at reduced pressure to insure continuous air flow through the flask and trapping mixture. The oxymercuration adduct was isolated by rotary evaporation of the methanol/acetic acid solution, followed by recrystallization from hexane. The white crystals (mp 63–64 °C) were weighed to determine the efficiency of the derivatization and analyzed by TLC for radiocarbon content by LSC and for purity by cochromatography with an authentic standard [diisopropyl ether–glacial acetic acid (49:1), R_f 0.09; 1-propanol–triethylamine–water (2:1:1), R_f 0.12; detected by autoradiography and the *S*-diphenylcarbazone spray reagent (0.1% in ethanol)].

To determine the possible presence of [^{14}C]cyclohexanone, the incubated enzyme reaction was extracted with 2 mL of ethyl acetate and 0.2 mL of water was added to the ethyl acetate fraction. The ethyl acetate was evaporated and enough ethanol was added to the remaining aqueous phase to make 1-mL volume. 2,4-Dinitrophenylhydrazine (5 mg) and concentrated hydrochloric acid (3 drops) were added, and the mixture was boiled for 3 min. One-tenth of the total volume was used for TLC analysis (chloroform). Studies with [^{14}C]cyclohexanone in buffer established that the labeled dinitrophenylhydrazone from this procedure cochromatographed with the authentic standard and on recrystallization from ethyl acetate it gave the expected mp of 160 °C. This derivatization procedure was 25% efficient based on ^{14}C recovery.

[^{14}C]Cyclohexanol was extracted from the acidified incubation mixture with chloroform (2 mL) to which phenyl isocyanate (1 mL) was added. After 18 h at 25 °C, the solvent was evaporated, and water (2 mL) and hexane (6 mL) were added. The phenyl carbamate of [^{14}C]cyclohexanol was extracted into hexane and analyzed by two-dimensional TLC [chloroform and hexane–acetone (9:1)]. The labeled derivative cochromatographed with an authentic sample (mp 83 °C, from hexane). Recovery studies gave an evaporation loss of 25% during enzyme incubation and a final 25% recovery of [^{14}C]cyclohexanol as the phenyl carbamate; these results were unaffected by NADPH fortification.

In Vivo Metabolism. [^{14}C]Tricyclohexyltin hydroxide was administered to male Swiss-Webster mice (~20 g), male albino Sprague-Dawley rats (~160 g), and male Hartley guinea pigs (~250 g) from Simonsen Laboratories, Inc. (Gilroy, CA) and to male rabbits (~500 g) from Western Scientific Supply Co. (West Sacramento, CA) at dosages of 1.35, 0.84, 0.64, and 0.32 mg/kg, respectively. The cyhexatin was administered in methoxytriglycol by stomach tube for mice and rats and by application to lettuce which was quickly consumed by the guinea pigs and rabbits (starved overnight prior to treatment). Animals were placed in individual metabolism cages and urine and feces were collected for 72 h. Urinary radiocarbon was determined by direct LSC of an aliquot, whereas the feces were dried, pulverized, and combusted for total ^{14}C determination. Another portion of feces was homogenzied (polytron) in chloroform and/or methanol and then further extracted with methanol after acidification with dilute hydrochloric acid. The extracts were individually analyzed by TLC (solvent systems E and F) and LSC. Unextractable fecal radiocarbon was determined by combustion.

[^{14}C]Tricyclohexyltin hydroxide and its metabolites were analyzed in the liver of mice at 1–24 h after they were treated by stomach tube. The excised livers were immediately lyophilized, and the resulting dry powder was extracted with chloroform and methanol and finally acidified with hydrochloric acid and reextracted with methanol. The residue was analyzed by combustion. The chloroform and methanol extracts were subjected to TLC (solvent systems E and F), autoradiography, and LSC.

RESULTS

Microsomal Monooxygenase Metabolism. Metabolism of the cyclohexyltin derivatives examined requires both microsomes and NADPH for product formation and

Table I. Metabolites of Cyclohexyltriphenyltin and Cyclohexyldiphenyltin Acetate following Incubation with the Rat Liver Microsomal System with and without NADPH

compound or fraction	recovery of initial radiocarbon, %, in the presence of NADPH (and in its absence)	
	$CySnPh_3$	$CySn(OAc)Ph_2$
unmetabolized	81.0 (99.7)	85.7 (99.2)
hydroxy derivatives		
trans-2	0.2 (0.0)	6.5 (0.2)[a]
trans-3	0.3 (0.0)	2.2 (<0.1)[b]
cis-3	0.6 (0.0)	
trans-4	8.1 (0.0)	1.4 (<0.1)[b]
keto derivatives		
3	0.1 (0.0)	
4	0.2 (0.0)	
unknowns		
hydroxy or keto deriv		2.1 (<0.1)
apolar	0.6 (0.0)	0.8 (0.2)
polar	6.8 (0.3)	0.7 (<0.1)
bound	2.1 (0.0)	0.6 (0.4)
loss correction used[c]	14.3 (2.8)	20.9 (17.3)

[a] This compound decomposes under the extraction and TLC conditions used but was determined in separate experiments as the oxymercuration adduct of cyclohexene following acidification of the incubation mixture. [b] Stereochemistry not assigned. [c] Loss of volatile compounds (e.g., cyclohexanol and cyclohexanone) and other losses on extraction, workup, and TLC analysis. The loss includes cyclohexene with $CySnPh_3$ but not with $CySn(OAc)Ph_2$.

gives the metabolite pattern shown in Figures 2 and 3 and the yields noted in Tables I and II.

[^{14}C]Cyclohexyltriphenyltin ($CySnPh_3$). Six of the relatively apolar metabolites resolved by two-dimensional TLC are identified as shown in Figure 2 using cochromatography in the indicated solvent systems (C and D) and also in another two-dimensional system (A and B) as the criteria. The metabolite designated *t*-2-$HOCySnPh_3$ is completely decomposed on treatment with glacial acetic acid, yielding [^{14}C]cyclohexene [and presumably $Sn(OAc)Ph_3$ although this fragment bears no label]. This indicates that only the trans 2-hydroxy derivative is formed since the cis 2-hydroxy compound would not destannylate

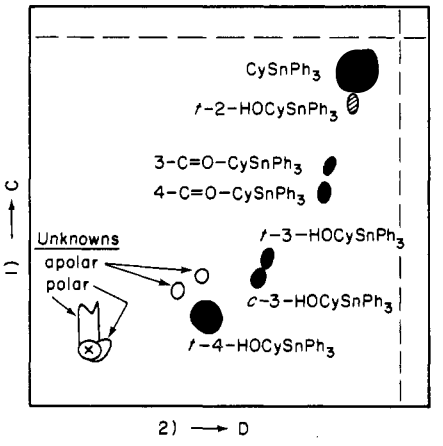

Figure 2. Metabolites of cyclohexyltriphenyltin formed by the rat liver microsome-NADPH system as resolved by TLC.

under these conditions (Fish et al., 1977). The identity of the major metabolite, cochromatographing with *t*-4-$HOCySnPh_3$, was verified by preparative TLC isolation in sufficient amount (11 μg) for identification by NMR. This metabolite gives a multiplet (nonet) at 3.58 ppm (H–C–OH), J_{ax}–J_{ax} = 11 Hz; J_{ax}–J_{eq} = 4.0 Hz, consistent with an authentic sample of *t*-4-$HOCySnPh_3$, and the absence of a multiplet at ~3.72 ppm (H–C–OH) which would be the chemical shift corresponding to the cis 4-hydroxylated compound. There is a high specificity for hydroxylation at the trans 4 position (Table I).

[^{14}C]Cyclohexyldiphenyltin Acetate [$CySn(OAc)Ph_2$]. This substrate gives three NADPH-dependent metabolites which are stable under the acidic TLC conditions used (Figure 3, Table I). Two of these metabolites cochromatograph (solvent systems E and F) with 3-HO-$CySn(Br)Ph_2$ and 4-$HOCySn(Br)Ph_2$ which are converted to their acetates during chromatography. Unfortunately, TLC does not separate the cis and trans isomers of 3-$HOCySn(X)Ph_2$ and 4-$HOCySn(X)Ph_2$ so no stereochemical assignments could be made. The corresponding ketones appear in chromatographic regions with interfering materials so TLC identifications were not possible. However, the unknown metabolite chromatographs similar to but not identical with 4-C=O-$CySn(X)Ph_2$. *t*-2-HO-

Table II. Metabolites of Tricyclohexyltin Hydroxide following Incubation with the Rat Liver Microsomal System with and without NADPH and in the Feces of Orally Treated Rats, Mice, Guinea Pigs, and Rabbits

compound or fraction	recovery of initial radiocarbon, %				
	rat liver microsome + NADPH (and – NADPH)	in vivo, portion in 0–24 h feces			
		rat	mouse	guinea pig	rabbit
unmetabolized	63.8 (90.1)	58.4	59.5	73.4	52.1
hydroxy derivatives					
t-2-$HOCySn(X)Cy_2$	4.7 (0.6)[a,b]				
[3-$HOCySn(X)Cy_2$]	2.1 (0.2)	0.9	0.3	0.6	2.4
[4-$HOCySn(X)Cy_2$]	1.3 (0.1)	0.6	4.1	0.3	0.6
destannylation product, $Sn(X_2)Cy_2$	3.6 (3.0)[b]	1.1	4.8	3.8	7.2
unknowns					
major apolar	1.7 (0.2)	0.5	0.0	0.3	0.8
other apolars	1.5 (0.3)	4.2	0.0	2.1	0.6
polar	17.3 (2.6)[c]	24.8	24.1	10.9	24.9
bound	4.0 (2.9)	9.5	7.2	8.6	11.4
loss correction used	16.0 (12.4)[d]	19.7[e]	33.3[e]	66.1[e]	50.1[e]

[a] This compound decomposes under the extraction and TLC conditions used but was determined in separate experiments as the oxymercuration adduct of cyclohexene following acidification of the incubation mixture. [b] Recovery values normalized for change in specific activity on destannylation. [c] Includes 0.8% (– NADPH) and 2.0% (+ NADPH) of monocyclohexyltin derivatives. [d] Loss of volatile compounds (e.g., cyclohexanol and cyclohexanone) and other losses on extraction, workup, and TLC analysis. [e] Portion of the dose not present in the 0–24 h feces. Radiocarbon recovery in the urine at 0–24, 24–48, and 48–72 h and in the feces at 0–24, 24–48, and 48–72 h and the 0–72 h total for excretion, respectively, are as follows: rat, 1.4, 0.3, 0.1, 80.3, 3.0, 0.4, and 85.5; mouse, 2.3, 1.0, 0.3, 66.7, 12.8, 5.5, and 88.6; guinea pig, 2.0, 0.7, 0.7, 33.9, 34.2, 12.0, and 83.5; rabbit, 1.8, 1.3, 0.1, 49.9, 40.8, 9.8, and 103.7.

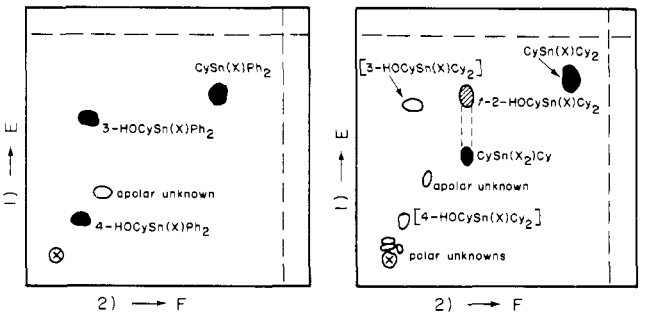

Figure 3. Metabolites of cyclohexyldiphenyltin acetate and tricyclohexyltin hydroxide formed by the rate liver microsome-NADPH system as resolved by TLC.

$CySn(X)Ph_2$ would not be detected on TLC since the labeled moiety would be lost as [^{14}C]cyclohexene due to the acidic solvent systems. The [^{14}C]cyclohexene liberated after acidification was identified as the oxymercuration adduct which cochromatographed with the standard compound.

[^{14}C]Tricyclohexyltin Hydroxide [$CySn(OH)Cy_2$]. There is a definite similarity in the metabolite TLC patterns obtained with $CySn(OH)Cy_2$ and $CySn(OAc)Ph_2$ (Figure 3) and metabolism in each case requires the cofactor NADPH. This is not surprising since the metabolism probably takes place entirely on the cyclohexyl groups rather than the phenyl substituents (Kimmel et al., 1977) and the hydroxy and acetate groups are readily exchangeable. No authentic standards were available for 3- and 4-$HOCySn(X)Cy_2$ which are anticipated metabolites from their TLC characteristics. However, there is further evidence for the structures of the metabolites designated, on the basis of chromatographic properties, as [3-$HOCySn(X)Cy_2$] and [4-$HOCySn(X)Cy_2$] since their acetate derivatives recovered from TLC gave CI–MS spectra with ions at m/e 385 (100 and 77%, respectively) for [$HOCySnCy_2$]$^+$ from loss of the acetate moiety. t-2-$HOCySn(X)Cy_2$ is identified as with t-2-$HOCySn(X)Ph_2$ by its instability in acid, the liberation of cyclohexene identified as the oxymercuration adduct, and the formation of Cy_2SnX_2 on destannylation.

Additional metabolites identified by cochromatography with authentic standards are $CySn(X_2)Cy$ (solvent systems E and F) and $CySnX_3$ (solvent system G). The free polar metabolites are in large amounts from $CySn(OH)Cy_2$ (17%) (Table II) but not from $CySn(OAc)Ph_2$ (0.7%) (Table I), indicating that they are di and mono derivatives which are not detected with [^{14}C]$CySn(OAc)Ph_2$ because of loss of the ^{14}C label.

Attempts to detect two potential metabolites, cyclohexanone and cyclohexanol, were not successful. Cyclohexanone was found to be rapidly converted to cyclohexanol in the microsome-NADPH system, so any ketone liberated might be quickly reduced and escape detection. Cyclohexanol, on the other hand, was quite resistant to metabolism in the microsome-NADPH system. Unfortunately, the phenyl carbamate derivative used for analysis appeared in a TLC region with interfering labeled compounds derived from [^{14}C]$CySn(OH)Cy_2$.

In Vivo Metabolism of [^{14}C]$CySn(OH)Cy_2$. Most of the radiocarbon from orally administered [^{14}C]$CySn(OH)Cy_2$ appears in the feces within 3 days with more rapid excretion by mice and rats than by rabbits and guinea pigs (Table II). From 52 to 73% of the administered dose is excreted unmetabolized in the 0–24-h feces. The TLC patterns of the 24–48-h feces extracts from rabbits and guinea pigs are essentially the same as those of the 0–24-h extracts but this comparison was not made with mouse and rat feces due to low fecal radioactivity during the 24–48-h period. The neutral feces extract contained no detectable t-2-$HOCySn(X)Cy_2$. $Sn(X)_2Cy_2$ is a major fecal product and presumably arises from in vivo 1,2-deoxystannylation of t-2-$HOCySn(X)Cy_2$ (Fish and Broline, 1978). No attempt was made to analyze for cyclohexene, cyclohexanol and cyclohexanone because under in vivo conditions they are likely to be transformed into a great variety of products. Two of the polar in vivo metabolites cochromatograph with microsomal metabolites designated 3-$HOCySn(X)Cy_2$ and 4-$HOCySn(X)Cy_2$ and a third cochromatographs with the same apolar unknown oxidized $CySn(OH)Cy_2$ derivative detected in the enzyme studies. Of these, 4-$HOCySn(X)Cy_2$ is the major metabolite in mouse feces and 3-$HOCySn(X)Cy_2$ in the other species. Unknown polar metabolites, prominent in all species although to a lesser extent in guinea pigs, are possibly oxidized at more than one cyclohexyl group.

The urine of rats and mice, analyzed by direct spotting and TLC, contains no $CySn(X)Cy_2$ or $Sn(X_2)Cy_2$ but instead it consists only of products remaining at or near the origin in solvent system E. These are presumably conjugates of cleavage products but were not further analyzed because of low overall radioactivity.

The liver of mice orally administered [^{14}C]$CySn(OH)Cy_2$ contains the following percent of the administered ^{14}C at various times after treatment: 0.9% at 1, 3, and 6 h; 0.7% at 10 h; 0.3% at 24 h. TLC analysis of liver extracts revealed $CySn(X)Cy_2$ and $Sn(X_2)Cy_2$, along with trace levels of 3-$HOCySn(X)Cy_2$ and 4-$HOCySn(X)Cy_2$ and the apolar unknown. Apparently $CySn(OH)Cy_2$ is absorbed from the gastrointestinal tract to a certain extent and metabolized in the liver.

DISCUSSION

Figure 1 gives some of the metabolic reactions of cyclohexyltin compounds based primarily on studies with $CySnPh_3$ and supported by preliminary findings on $CySn(OAc)Ph_2$ and $CySn(OH)Cy_2$. The pathways involved are considerably more complex than the simple destannylation sequence proposed by Blair (1975). Four sites of hydroxylation are established, i.e., trans-2, cis-3, trans-3, and trans-4. No evidence is available for hydroxylation at the 1 or cis-4 position. The hydroxylated derivatives are susceptible to further metabolism or degradation. The trans 2-hydroxy compound formed in vitro with each substrate was found to undergo a 1,2-deoxystannylation reaction in the presence of acid, and deoxystannylation probably also occurs in vivo to liberate cyclohexene. On analogy with chemical oxidation studies (Fish and Broline, 1978), the t-2-HOCy compounds may be oxidized to their 2-keto derivatives which would then yield cyclohexanone on destannylation. Both the t-4-HOCy and isomeric 3-HOCy derivatives in the $CySnPh_3$ series are in part further oxidized to the corresponding ketones.

Destannylation of the mixture of cyclohexyltin metabolites involves not only cyclohexene liberation, as established in this study, but also the generation of cyclohexanone, some of which is reduced to cyclohexanol as shown earlier (Casida et al., 1971; Kimmel et al., 1977). The previous investigations used cyhexatin levels 50-fold higher than the present study, a factor which may contribute to the failure to detect [^{14}C]cyclohexanol or [^{14}C]cyclohexanone from the labeled substrates. Also the earlier gas–liquid chromatography method was more reliable than the ^{14}C-TLC method with regard to eliminating interfering materials.

Cyhexatin is relatively resistant to in vitro and in vivo metabolism and is largely recovered intact under the

metabolic conditions examined. Two features probably contribute to this refractory property. First, a portion of the dose may bind to tissue components so it is isolated from the microsomal monooxygenase site. Second, it appears that only a small amount of the compound is absorbed following ingestion. These are important factors in toxicological evaluations of cyhexatin and other triorganotin derivatives.

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Plant Experiments on the Bioavailability of Unextracted [*carbonyl*-^{14}C]Methabenzthiazuron Residues from Soil

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The extractability of methabenzthiazuron residues from soil decreases with time after application. In this study the bioavailability of this soil bound or adsorbed fraction was investigated using maize plants. A sandy soil and [*carbonyl*-^{14}C]methabenzthiazuron (10 ppm) were incubated at 22 °C and 65% of the water holding capacity for 111 days. After extractions with H_2O/acetone/ethyl acetate/chloroform, unextracted, bound residues remaining in this soil amounted to 41% applied. Plant uptake studies were conducted in special experimental pots. After 29 days the maize shoots contained 0.7% of the radioactivity originally applied to the soil before incubation in comparison to 2.2% from the incubated soil not extracted. When the [*carbonyl*-^{14}C]methabenzthiazuron was added to the soil immediately before the plant experiment, about 4% of the radioactivity was found in the shoots. The plant availability of the unextracted radiocarbon, as compared with the extractable portion, has decreased to about one-third and in comparison with the availability after direct methabenzthiazuron application to about one-sixth. Desorption studies with water showed similar ratios.

In experiments with certain ^{14}C-labeled compounds, unextractable radioactivity in the soil which represents the bound residue fraction, increases with time (Führ and Mittelstaedt, 1976; Lichtenstein et al., 1977) and especially with increasing soil temperatures (Führ and Mittelstaedt, 1979; Katan et al., 1976). Thus, in the soil, pesticides appear to behave like other organic residues (i.e., straw, green manure, or roots) due to turnover processes and buildup of organic matter components (Stevenson, 1976). The organic fraction of a soil has the potential for forming strong chemical linkages with pesticides or residues arising from their partial degradation by microorganisms. Besides chemical reactions, adsorption by organic matter has been shown to be a key factor in inactivation of many pesticides in the soil (Hayes, 1970).

So far no analytical methods in soil organic matter chemistry have been developed to provide a precise separation and identification of the different compounds which contribute to this bound or conjugated pesticide residue fraction in the soil (Kaufman, 1976). Therefore it is of interest to know if and to what extent soil-bound pesticide residues or metabolites will become bioavailable to plants and especially to what extent they are taken up by the roots of untreated rotational crops following the treated crop.

Since outdoor lysimeter experiments with ^{14}C-labeled pesticides are not only time-consuming but also highly expensive, a plant experiment was designed to study the root uptake of unextracted pesticide residues and their turnover in the soil under standard climatic conditions. These studies were proposed at the Symposium on Bound and Conjugated Pesticide Residues at Vail, Colorado (Führ, 1976). The results from the plant experiment are discussed in relation to data from lysimeter experiments under outdoor conditions. Methabenzthiazuron [1,3-dimethyl-3-(benzthiazol-2-yl)urea, active ingredient in Tribunil (trade name, Bayer AG, Leverkusen, Germany)], a broad spectrum herbicide for weed control in cereal crops, was chosen since it was shown to be a stable compound in a lysimeter experiment with spring wheat (Führ and Mittelstaedt, 1976).

MATERIALS AND METHODS

Chemicals. [*carbonyl*-^{14}C]Methabenzthiazuron was provided by the Bayer AG, Leverkusen (Ecker, 1975).

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