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Kimmel, E. C., Fish, R. H., Casida, J. E., unpublished results, 1976.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).

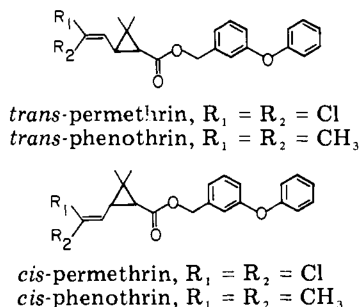
Received for review June 21, 1976. Accepted August 8, 1976. This study was supported in part by grants from the National Institutes of Health (2 P01 ES00049) and The Guggenheim Foundation.

Permethrin Metabolism in Rats

Loretta C. Gaughan, Tadaaki Unai, and John E. Casida*

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).



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.

Department of Entomological Sciences, University of California, Berkeley, California 94720.

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*-

Table I. Thin-Layer Chromatographic Properties of *trans*- and *cis*-Permethrin and of Various Metabolites and Their Derivatives

Compound	R_f values with indicated solvent systems[a]		
	A	B × 2	Others[b]
		Permethrin and Ester Metabolites	
t-Per	0.86	0.94	B (0.72), C (0.69), D (0.77), E (0.36), F (0.51)
c-Per	0.86	0.94	B (0.71), C (0.69), D (0.78), E (0.42), F (0.56)
2′-HO,*c*-per	0.86	0.89	H (0.54), I (0.73)
4′-HO,*t*-per	0.87	0.77	H (0.30), I (0.56)
4′-HO,*c*-per	0.87	0.77	H (0.35), I (0.61)
t-HO,*c*-per	0.87	0.65	H (0.22), I (0.49)
4′-HO,*t*-HO,*c*-per	0.87	0.30	H (0.06), I (0.20)
		Cl$_2$CA, Metabolites of Acid Moiety, and Related Compounds	
t-Cl$_2$CA	0.83	0.72	C (0.38) [B (0.63), C (0.67), E (0.38), F (0.44)]
c-Cl$_2$CA	0.83	0.78	C (0.44) [B (0.64), C (0.65), E (0.45), F (0.51)]
t-HO,*t*-Cl$_2$CA	0.80	0.21	G (0.15) [J (0.45), K (0.28)]
c-HO,*t*-Cl$_2$CA	0.76	0.14	G (0.11) [J (0.31), K (0.20)]
t-HO,*c*-Cl$_2$CA	0.82	0.23	G (0.18) [J (0.46), K (0.29)]
c-HO,*c*-Cl$_2$CA	0.80	0.18	G (0.13) [J (0.43), K (0.28)]
c-HO,*t*-Cl$_2$CA-lactone		0.62	G (0.55), J (0.42), K (0.36)
c-HO,*c*-Cl$_2$CA-lactone		0.63	G (0.58), J (0.49), K (0.39)
t-Cl$_2$CA-gluc[c]	0.37		
c-Cl$_2$CA-gluc[c]	0.39		
t-Cl$_2$CA-gluc-lactone[c]	0.60		
c-Cl$_2$CA-gluc-lactone[c]	0.60		
HO-*t*-Cl$_2$CA-gluc[c]	0.28		
HO-*c*-Cl$_2$CA-gluc[c]	0.31		
		PBalc, Metabolities of Alcohol Moiety, and Related Compounds	
PBalc	0.82	0.60	B (0.36), C (0.49), D (0.47)
PBacid	0.82	0.66	C (0.33), D (0.44) [B (0.63), C (0.66), E (0.30), F (0.39)]
2′-HO-PBalc		0.27	G (0.27), C × 2 (0.56), I × 2 (0.22)
2′-HO-PBacid		0.36	G (0.37) [B × 2 (0.83), G (0.67)]
4′-HO-PBalc		0.25	G (0.25)
4′-HO-PBacid		0.30	G (0.29) [B × 2 (0.85), G (0.68)]
PBacid-glycine	0.52	0.09	[B (0.21), C (0.47)]
PBacid-gluc[c]	0.37		
PBacid-gluc-lactone[c]	0.60		
2′-HO-PBacid-sulfate[c]	0.41		
4′-HO-PBacid-sulfate[c]	0.47		

[a] The TLC solvent systems are as follows: A = butanol–glacial acetic acid–water (6:1:1); B = benzene saturated with formic acid–ether (10:3); C = benzene–ethyl acetate–methanol (15:5:1); D = ether–hexane (2:1); E = hexane–ether (10:1); F = carbon tetrachloride–hexane–ether (20:2:1); G = chloroform saturated with formic acid–ether (10:3); H = carbon tetrachloride–ether (3:1); I = benzene–ethyl acetate (6:1); *J* = ether–hexane (1:1), developed twice; K = hexane–ethyl acetate (7:3). R_f values for the glucuronides, glucuronide lactones, and sulfates are below 0.05 in B × 2. [b] Brackets designate methylated derivatives. [c] R_f values determined with metabolites or products from enzymatic synthesis; in all other cases, authentic standards from synthesis were used for the determination of R_f values.

HO,*t*-Cl$_2$CA; *t*-HO,*c*-Cl$_2$CA; and *c*-HO,*c*-Cl$_2$CA. The *cis*-hydroxymethyl acids readily lactonize to form the corresponding lactones, *c*-HO,*t*-Cl$_2$CA-lactone and *c*-HO,*c*-Cl$_2$CA-lactone. Derivatives of phenoxybenzyl alcohol (PBalc) and phenoxybenzoic acid (PBacid) include those hydroxylated at the 2′ and 4′ positions (2′-HO-PBalc, 2′-HO-PBacid, 4′-HO-PBalc, and 4′-HO-PBacid). Several conjugates involving glycine, sulfate, and glucuronic acid (gluc) as the conjugating moieties are also considered, as are the lactones of the glucuronides.

Chromatography and Radiocarbon Analyses. Thin-layer chromatography (TLC) utilized precoated silica gel 60 F-254 20 × 20 cm chromatoplates with 0.25-mm layer thickness (EM Laboratories Inc., Elmsford, N.Y.). The solvent systems used are either given with the R_f values for a variety of per derivatives in Table I or they are stated in the text. Reference to solvent systems for two-dimensional development is illustrated by (A, B × 2), which indicates development in the first direction with solvent system A and in the second direction twice with solvent system B. For cochromatography of ^{14}C-labeled metabolites or their derivatives, the spots from the unlabeled standards, detected first with ultraviolet light (254 nm) and then by spraying with phosphomolybdic acid (20% in ethanol) and heating at 110–120 °C for up to 30 min, were compared as to their positions and shapes with the radioactive spots detected by radioautography.

Table II. Radiolabeled Chemicals Used for Metabolism Studies

No.	Compd	mCi/mmol
1	[^{14}C-acid-1*R*,*t*]per	6.4
2	[^{14}C-alc-1*R*,*t*]per	4.6
3	[^{14}C-acid-1*R*,*c*]per	1.7
4	[^{14}C-alc-1*R*,*c*]per	4.6
5	[^{14}C-acid-1*RS*,*t*]per	58.2
6	[^{14}C-alc-1*RS*,*t*]per	55.9
7	[^{14}C-acid-1*RS*,*c*]per	58.2
8	[^{14}C-alc-1*RS*,*c*]per	55.9
9	[^{14}C-1*R*,*t*]Cl$_2$CA	6.4
10	[^{14}C]PBalc	4.6

The procedures for radioautography and for quantitation of the radiocarbon content of various materials by liquid scintillation counting (lsc) are given by Ueda et al. (1975b).

Chemicals. Procedures of synthesis or sources for the unlabeled standards referred to below or in Figure 1 are given by Unai and Casida (1976) with some exceptions noted later. The unlabeled esters or products derived from the acid moiety are 1*RS* compounds while the ^{14}C-labeled esters and ^{14}C-labeled products derived from the acid moiety are 1*RS* or 1*R* compounds as specified.

Ten radiolabeled chemicals were used as shown in Table II. Compounds **1–4**, **9**, and **10** with Cl$_2$C*=CH labeling

Figure 1. Metabolic pathways for [1*R*,*trans*]- and [1*R*,*cis*]-permethrin indicating the abbreviations used for various metabolites. These pathways are also applicable to [1*RS*,*trans*]- and [1*RS*,*cis*]-permethrin and to phenoxybenzyl alcohol and [1*R*,*trans*]-Cl_2CA. Additional pathways which are not shown yield several minor metabolites, most of these being formed from *cis*-permethrin.

in the acid moiety and uniform phenoxy labeling in the alcohol moiety are described by Elliott et al. (1976). The 1*RS* compounds, labeled in the *C=O group of the acid moiety (**5** and **7**) and the α-$^*CH_2$ position of the alcohol moiety (**6** and **8**), were obtained from preparations of [^{14}C-1*RS*,*trans*,*cis*]per (provided by FMC Corporation, Middleport, N.Y.) by TLC separation of the trans and cis isomers, using two developments with carbon tetrachloride–benzene (4:1) (R_f values, 0.54 for *t*-per and 0.68 for *c*-per). Each [^{14}C]permethrin preparation had a radiochemical purity of >99% based on TLC and radioautography, using the following solvent systems for two-dimensional development: benzene–carbon tetrachloride (1:1) in the first direction (*t*-per, R_f 0.48; *c*-per, R_f 0.58) and two developments with hexane–chloroform (3:1) in the second direction (*t*-per, R_f 0.31; *c*-per, R_f 0.37); carbon tetrachloride–benzene (4:1) in the first direction (*t*-per, R_f 0.37; *c*-per, R_f 0.45) and carbon tetrachloride–ether–hexane (8:1:1) in the second direction (*t*-per, R_f 0.52; *c*-per, R_f 0.53). The radiochemical purities of compounds **9** and **10** were also >99% (Elliott et al., 1976).

3-Phenoxybenzoylglycine (PBacid-glycine) was provided as the methyl ester by J. Miyamoto (Sumitomo Chemical Co., Takarazuka, Japan). Four labeled compounds, [^{14}C]PBacid (Elliott et al., 1976), [^{14}C]PBalc, [^{14}C-1*R*,*t*]-Cl_2CA, and [^{14}C-1*R*,*c*]Cl_2CA (recovered as a metabolite of [^{14}C-1*R*,*c*]per), were converted to their glucuronic acid conjugates in 40–60% yields by incubating the appropriate labeled material (10 nmol) with uridine diphosphoglucuronic acid (1.4 μmol) (Sigma Chemical Co., St. Louis, Mo.), magnesium chloride (100 μmol), and rat liver microsomes (4 mg of protein, washed by resuspension in buffer and recentrifugation prior to use) in 50 mM Tris–hydrochloric acid buffer, pH 7.0 (4 ml), for 30 min at 37 °C (Mehendale and Dorough, 1971). The glucuronic acid conjugates were isolated by addition of ammonium sulfate (0.5 g), extraction with ether–ethanol (3:1) mixture (3 × 1 vol), and then TLC of the organic extracts (solvent system A; R_f 0.37, 0.37, 0.37, and 0.39 for PBalc-gluc, PBacid-gluc, *t*-Cl_2CA-gluc, and *c*-Cl_2CA-gluc, respectively). These glucuronic acid derivatives were cleaved by glucuronidase and the latter three were converted to the corresponding lactones on treatment with acid as detailed later.

Treatment of Rats, Collection of Excreta, and Determination of Tissue Residues. Male, albino Sprague-Dawley rats (160–200 g, Horton Laboratories Inc., Oakland, Calif.) were individually treated by stomach tube with ^{14}C-labeled compounds **1–10** in dimethyl sulfoxide (Me_2SO) followed by a Me_2SO rinse (150 μl) of the stomach tube. With compounds **5–8**, the urine, feces, and [^{14}C]-carbon dioxide were collected for up to 12 days, while the treated rats were held in all-glass metabolism cages (rat metabolism unit Model MC 3000-1, Stanford Glassblowing Labs, Inc., Palo Alto, Calif.) and provided ground rat chow and water ad libitum with [^{14}C]carbon dioxide collection in a monoethanolamine–methyl Cellosolve (2:1) mixture. Less efficient metabolism cages were used in the earlier studies with compounds **1–4**, **9**, and **10**, resulting in lower overall recoveries than with compounds **5–8**. Tissue samples recovered on sacrifice after 4 or 12 days were analyzed for total radiocarbon by combustion (Packard Tri-Carb Sample Oxidizer Model 306, Downers Grove, Ill.) and lsc, with corrections for combustion efficiency and quench. Excreted radiocarbon is expressed as percent of the administered dose, and tissue residues are given as parts per billion equivalents of the administered ^{14}C-labeled compound based upon tissue wet weights.

Analysis of Excreta. The feces were extracted with methanol (10 ml/g of feces) by homogenization with a Virtis Homogenizer (Virtis Research Equipment, Gardner, N.Y.) (feces from administration of compounds **1–4**, **9**, and **10**) or with a Polytron homogenizer (Kinematica, GmbH, Lucerne, Switzerland) (feces from administration of compounds **5–8**). The methanol-soluble radiocarbon was determined by lsc and the unextractable portion by combustion and then lsc. Aliquots of the urine were analyzed directly by lsc.

For separation and quantitation of the excreted metabolites, the urine (40–100 μl) or the methanol extract of feces (equivalent to 40–90 mg of feces) was subjected to two-dimensional TLC (A, B × 2), radioautography, and lsc. In addition, the relatively apolar feces metabolites were partially purified so that an aliquot representing a larger amount of feces could be analyzed by TLC (B × 2). This purification was accomplished by evaporating the methanol, recovering the ether-soluble portion, and drying the ether (sodium sulfate) prior to spotting.

Metabolite Characterization. An initial search for the presence of metabolites retaining the ester linkage was made by comparing the ^{14}C-labeled products obtained after administration of [^{14}C-acid]- and [^{14}C-alc]per and with those obtained after administration of [^{14}C-1*R*,*t*]Cl_2CA and [^{14}C]PBalc. Individual metabolites were separated by one-dimensional (B × 2) or two-dimensional TLC development (A, B × 2 and H, I) and then extracted from the appropriate gel region with methanol. These metabolites were used for direct cochromatography with authentic standards from synthesis. The structures of metabolites identified in this way were usually confirmed by appropriate derivatization and degradation procedures.

Methylation by treatment with diazomethane (Ueda et al., 1975a) converted carboxylic acids and phenols to the corresponding methyl esters and ethers for cochromatography with the appropriate unlabeled standards.

Individual products retaining the ester linkage were hydrolyzed with 1 N methanolic sodium hydroxide (25 °C, 18 h), and the degradation products were recovered (Ueda et al., 1975b) and cochromatographed with the appropriate standards both before and after methylation.

Conjugates were subjected to cleavage by enzyme, acid, and base, and then to chromatographic examination of the cleavage products with and without methylation. Three conjugate-cleaving enzymes were individually utilized in sodium acetate–acetic acid buffer, pH 4.5 (0.6 ml): β-glucuronidase/aryl sulfatase (20 000 Fishman units of β-glucuronidase and 2000 Whitehead units of aryl sulfatase; Calbiochem, San Diego, Calif.); β-glucuronidase (1.0 mg, type I bacterial powder, Sigma); and aryl sulfatase (1.0 mg, type III from limpets, Sigma). D-Saccharic acid 1,-4-lactone (Sigma) was sometimes added to inhibit the β-glucuronidase activity (Capel et al., 1974) and thereby verify, by comparing with appropriate controls lacking this inhibitor, that the cleavage resulted from β-glucuronidase action. The samples were incubated 6 h at 37 °C, in the presence and absence of the enzyme, and then extracted with an ether–ethanol (3:1) mixture (2 × 3 vol) prior to TLC. The conjugates were also subjected to hydrolysis with weak base (0.2 N sodium hydroxide, 37 °C, 24 h), strong base (1 N sodium hydroxide, 100 °C, 6 h), and strong acid (3 N hydrochloric acid, 100 °C, 30 min). The alkaline hydrolysis mixtures were acidified with hydrochloric acid and then extracted and analyzed as above; no decomposition of the dichlorovinyl group was observed with either of these alkaline hydrolysis procedures. The acid hydrolysis mixtures were evaporated to dryness, and the methanol-soluble portion was used for TLC.

Lactonization was a useful criterion in metabolite identification, but it was also a potential source of artifacts in TLC separations with acidic solvents; thus, the lactones sometimes form as the result of the high concentrations of acetic and formic acids when solvents are evaporated from the chromatoplates. The glucuronides were partially converted to lactone derivatives (Dutton, 1966) (such as PBacid-gluc-lactone, *t*-Cl_2CA-gluc-lactone, and *c*-Cl_2CA-gluc-lactone) when dilute methanolic hydrochloric acid solutions were evaporated to dryness at 25 °C. Partial lactonization also occurred in the process of recovering the glucuronides, following TLC in solvent system A, by evaporation of the chromatographic solvent, extraction of the gel with methanol, and evaporation of the methanol. The *c*-HO-Cl_2CA derivatives lactonize even more readily, particularly *c*-HO,*c*-Cl_2CA which is quantitatively converted to *c*-HO,*c*-Cl_2CA-lactone under the methanolic acid and TLC conditions given above and partially lactonized during solvent evaporation following TLC in solvent system B × 2; under comparable conditions, *c*-HO,*t*-Cl_2CA also lactonizes, but in lower yields.

Bioassays. The mouse intraperitoneal (ip) LD_{50} values were determined according to Ueda et al. (1974).

RESULTS

Distribution of Radiocarbon in Excreta and Tissues. On oral administration to rats, the hydrolysis products of [^{14}C-acid-1*R*,*t*]per and [^{14}C-alc-1*R*,*t*]per (i.e., [^{14}C-1*R*,*t*]Cl_2CA and [^{14}C]PBalc) are rapidly eliminated in the urine with very little of the radiocarbon appearing in the feces or expired gases or being retained in the tissues after 4 days (Table III). A similar radiocarbon distribution is to be expected from the [^{14}C]per isomers if they undergo rapid hydrolysis. This proved to be the case with [1*R*,*t*]- and [1*RS*,*t*]per but not with [1*R*,*c*]- and [1*RS*,*c*]per (Table III).

Twelve days after oral administration of either acid- or alcohol-labeled [^{14}C-1*RS*,*t*]- or [^{14}C-1*RS*,*c*]per, 97–100% of the radiocarbon is recovered in the excreta and essentially none is expired as [^{14}C]carbon dioxide or remains in the body, except for low levels in the fat with [1*RS*,-*c*]-per. Similar studies at 4 days after treatment with [^{14}C-1*R*,*t*]- and [^{14}C-1*R*,*c*]per preparations gave 76–87% excretion (these are probably low values due to the inefficient metabolism cages, see Materials and Methods), essentially no [^{14}C]carbon dioxide, and similar patterns of tissue residues to those obtained at 12 days with the [1*RS*]per preparations. With the [^{14}C-acid]per samples the fat and liver retained the highest radiocarbon levels, and with [^{14}C-alc]per preparations the fat, liver, and kidney retained the most radiocarbon. The fragments from *c*-per tend to persist longer than those from *t*-per and those from the alcohol moiety longer than those from the acid moiety; this latter observation is consistent with the higher proportionate levels of radiocarbon retained in fat with PBalc than with [1*R*,*t*]-Cl_2CA. The most striking result is the finding that only 45–54% of the excreted radiocarbon from *c*-per appears in the urine whereas 81–90% of that from *t*-per is excreted in the urine. This suggests that the ester group of *c*-per may be less rapidly or completely cleaved than that of *t*-per.

Identification of Excreted Metabolites. Figure 2 shows the TLC chromatographic patterns of the urinary and fecal products as detected with each of the ^{14}C-labeled compounds examined. Excreted esters, differentiated by their identical chromatographic positions from the corresponding ^{14}C-acid- and ^{14}C-alc-labeled preparations, appear in the feces but not in the urine. Unmetabolized *t*-per and *c*-per were identified by cochromatography with unlabeled standards in (B × 2, C) and in (E, F). The four ester metabolites (2′-HO,*c*-per; 4′-HO,*c*-per; *t*-HO,*c*-per; and 4′-HO,*t*-HO,*c*-per) from *c*-per and the one ester metabolite (4′-HO,*t*-per) from *t*-per were obtained from the partially purified feces extract by TLC with neutral solvent systems (H, I). Each individual metabolite was extracted from the silica gel with methanol and the appropriate authentic standard was added before concentration under nitrogen; this procedure minimized de-

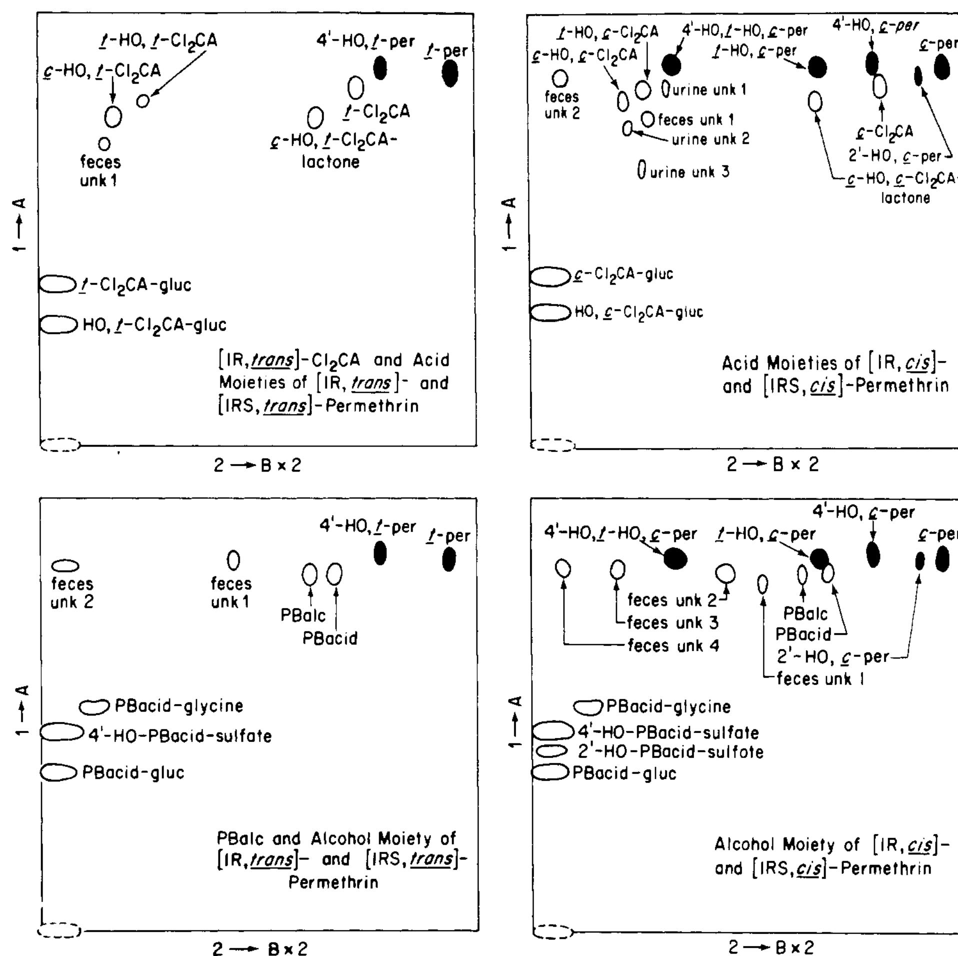


Figure 2. ^{14}C-Labeled compounds in the urine and in the methanol extract of feces of rats after oral administration of [^{14}C-1*R*,*trans*]Cl_2CA, [^{14}C]phenoxybenzyl alcohol, and various ^{14}C-acid- and ^{14}C-alcohol-labeled preparations of *trans*- and *cis*-permethrin as resolved by two-dimensional TLC. The solvent fronts are the appropriate outlines of the figures. The origin, which contained no ^{14}C-labeled compounds, is indicated at the lower left of each figure. Permethrin and ester metabolites detected with both ^{14}C-acid- and ^{14}C-alcohol-labeled preparations are indicated by solid circles. Open circles indicate the metabolites detected with either the ^{14}C-acid- or ^{14}C-alcohol-labeled preparation, as appropriate, but not with both. Metabolites designated as "unk" are unidentified. Figure 1 gives the structures of these compounds and Tables IV–VI give the quantitative data for the individual ^{14}C-labeled metabolites.

composition of the ester metabolites. These individual esters were identified by direct cochromatography (H, I) and their structures were confirmed by cochromatography of the hydrolysis products as follows: from hydrolysis of 2′-HO,*c*-per—2′-HO-PBalc in (C × 2, I × 2) and *c*-Cl_2CA in (B × 2, C); from hydrolysis of 4′-HO,*c*-per—4′-HO-PBalc in (B × 2, G) and *c*-Cl_2CA in (B × 2, C); from hydrolysis of *t*-HO,*c*-per—PBalc in (B, C) and *t*-HO,*c*-Cl_2CA in (B × 2, G); from hydrolysis of 4′-HO,*t*-HO,*c*-per—4′-HO-PBalc in (B × 2, G) and *t*-HO,*c*-Cl_2CA in (B × 2, G).

The acids (*t*-Cl_2CA and *c*-Cl_2CA) which result from cleavage of *t*-per and *c*-per, and some hydroxylated derivatives of these acids, are excreted in part without conjugation. The unmetabolized acid, *t*-Cl_2CA or *c*-Cl_2CA, cochromatographed with the appropriate unlabeled standard in (B × 2, C) before methylation and in (B, C) and (E, F) after methylation. The four hydroxy acids (*t*-HO,*t*-Cl_2CA; *c*-HO,*t*-Cl_2CA; *t*-HO,*c*-Cl_2CA; *c*-HO,*c*-Cl_2CA) cochromatographed with their respective unlabeled standards in (B × 2, G) before methylation and in (J, K) after methylation, with the exception that a portion of the *c*-HO,*t*-Cl_2CA and all of the *c*-HO,*c*-Cl_2CA were converted to the corresponding lactones under the methylation conditions. The structures of the 2-*cis*-hydroxy acids were confirmed by converting these metabolites, on treatment with acid, to products cochromatographing in (B × 2, G) and (J, K) with the corresponding unlabeled lactones, *c*-HO,*t*-Cl_2CA-lactone and *c*-HO,*c*-Cl_2CA-lactone. The original trans or cis configuration of the dichlorovinyl acid side chain, relative to the carboxyl group, was retained in each of these metabolites, i.e., no isomerization occurred in any case.

The nonconjugated metabolites of the alcohol moiety were identified by two-dimensional cochromatography as PBalc in (B, C) and (C, D) and as PBacid in (B × 2, C) before methylation and in (B, C) and (E, F) after methylation.

Several glucuronide conjugates were found in the urine, but none appeared in the feces. Three were obtained following administration of both [^{14}C-1*R*,*t*]Cl_2CA and [^{14}C-acid-1*R*- or -1*RS*-*t*]per and two or three following administration of [^{14}C-acid-1*R*- or -1*RS*-*c*]per. One was obtained after administration of [^{14}C]PBalc, and from alcohol-labeled *t*-per and *c*-per. They are designated as glucuronides on the basis of enzymatic cleavage experiments as follows: the metabolites from the acid moiety are competely cleaved by glucuronidase or glucuronidase/aryl sulfatase, but not by glucuronidase in the presence of saccharic acid 1,4-lactone, nor by aryl sulfatase alone; the metabolite of the alcohol moiety is extensively cleaved by glucuronidase and glucuronidase/aryl sulfatase, a cleavage reaction that is completely inhibited by sac-

Table III. Radiocarbon in the Urine, Feces, Carbon Dioxide, and Tissues of Rats 4 or 12 Days after Oral Administration of ^{14}C-Acid- and ^{14}C-Alcohol-Labeled Preparations of [1*R*,*trans*]-, [1*RS*,*trans*]-, [1*R*,*cis*]-, and [1*RS*,*cis*]-Permethrin and of the Hydrolysis Products of [1*R*,*trans*]-Permethrin

	^{14}C-Acid label					^{14}C-Alcohol label				
	t-Cl_2CA	*trans*-Permethrin		*cis*-Permethrin			*trans*-Permethrin		*cis*-Permethrin	
Sample analyzed	1*R*, 4 days	1*R*, 4 days	1*RS*, 12 days	1*R*, 4 days	1*RS*, 12 days	PBalc, 4 days	1*R*, 4 days	1*RS*, 12 days	1*R*, 4 days	1*RS*, 12 days
	Administered Dose, mg/kg									
	0.5	2.0	4.8	2.9	4.8	1.4	2.1	4.4	1.6	4.4
	% of Administered Radiocarbon									
Urine										
0–1 day	76	66	57	35	34	85	70	74	35	44
1–4 or 12 days	6	4	25	4	20	1	1	5	2	8
Feces										
Methanol extract										
0–1 day	4	10	9	31	27	7	6	12	33	26
1–4 or 12 days	3	2	5	11	15	1	1	2	4	18
Unextractable	1	1	2	6	3	1	1	4	2	3
$^{14}CO_2$, 0–4 days	0.1	0.1	0.5	0.3	0.5		0.0	0.0	0.1	0.0
Total, 0–4 or 12 days	90.1	83.1	98.5	87.3	99.5	95.0	79.0	97.0	76.1	99.0
	Tissue Residue, ppb of Permethrin Equiv									
Blood	<5	6	<25	<5	69	6	7	86	16	115
Bone	<5	<5	<25	<5	<25	<5	5	43	21	<25
Brain	<5	<5	<25	<5	<25	<5	<5	<25	<5	<25
Fat	<5	7	<25	28	458	120	140	86	401	618
Heart	<5	<5	<25	<5	<25	<5	<5	<25	<5	<25
Kidney	<5	<5	<25	<5	<25	12	24	<25	40	<25
Liver	9	28	<25	11	<25	13	9	<25	55	<25
Lung	<5	<5	<25	8	<25	5	22	<25	21	<25
Muscle	<5	5	<25	<5	<25	<5	<5	<25	6	46
Spleen	<5	5	<25	<5	<25	<5	<5	<25	6	<25
Testes	<5	5	<25	<5	<25	<5	8	<25	21	<25

charic acid 1,4-lactone. Three of the conjugates are identified as *t*-Cl_2CA-gluc, *c*-Cl_2CA-gluc, and PBacid-gluc based on cochromatography in (A) with the corresponding ^{14}C-labeled product formed enzymatically. On treatment with methanolic hydrochloric acid, each of these glucuronides from both enzymatic synthesis and urine yielded three products in (A): the unreacted glucuronide, the corresponding glucuronide lactone, and the cleavage product, *t*-Cl_2CA, *c*-Cl_2CA, and PBacid, respectively. Attempts to form the glucuronide methyl esters by methylation of the metabolites with diazomethane were unsuccessful; the products recovered were instead the methyl esters of the corresponding cleavage products, and then only in low yield. All of the glucuronides are completely cleaved by strong acid, and, in the three cases examined (*t*-Cl_2CA-gluc, *c*-Cl_2CA-gluc, and PBacid-gluc), they are also completely cleaved by strong base. The products from enzymatic, strong acid, and strong base (except HO-*t*-Cl_2CA-gluc and HO-*c*-Cl_2CA-gluc) cleavage were identified by two-dimensional cochromatography as follows: from hydrolysis of *t*-Cl_2CA-gluc and *c*-Cl_2CA-gluc—*t*-Cl_2CA and *c*-Cl_2CA, respectively, chromatographed as above; from hydrolysis of HO-*t*-Cl_2CA-gluc—*t*-HO,*t*-Cl_2CA and *c*-HO,*t*-Cl_2CA-lactone in (B × 2, G); from hydrolysis of HO-*c*-Cl_2CA-gluc—*t*-HO,*c*-Cl_2CA and a product, in almost equal amount, of much lower R_f value than *c*-HO,*c*-Cl_2CA in both directions of development (B × 2, G); from PBacid-gluc—PBacid, chromatographed as above. The unidentified product released on cleavage of the HO-*c*-Cl_2CA-gluc fraction may be an oxidation or decomposition product of *t*-HO,*c*-Cl_2CA or, more likely, of *c*-HO,*c*-Cl_2CA.

The glycine conjugate of PBacid appears in the urine but not in the feces. This metabolite was not hydrolyzed by weak base or by glucuronidase/aryl sulfatase, but it was completely cleaved on treatment with strong acid to yield a product cochromatographing as above with PBacid before and after methylation. In confirmation, treatment of this metabolite with diazomethane yielded a labeled product cochromatographing in (B, C) with the authentic methyl ester of PBacid-glycine.

Two sulfate conjugates (4′-HO-PBacid-sulfate and 2′-HO-PBacid-sulfate) appear in the urine but not in the feces, the former compound originating from all of the ^{14}C-alc-labeled preparations and the latter only from [^{14}C-alc-*c*]per. These metabolites are designated as sulfates since they are readily and completely cleaved by sulfatase, partially cleaved by glucuronidase/aryl sulfatase, and suffer no cleavage by glucuronidase alone. They are partially hydrolyzed by weak base and completely by strong base and strong acid. The cleavage product of the major sulfate conjugate was identified in each case as 4′-HO-PBacid, based on cochromatography in (B × 2, G) before methylation and (B × 2, G) after methylation. The minor sulfate conjugate was more difficult to identify, even on a tentative basis. This metabolite labeled in either the [^{14}C]phenoxy or α-$^{14}CH_2$ position was cleaved as above to one or more products, none of which cochromatographed with 2′-, 3′-, 4′-, 4-, 5-, or 6-HO-PBalc in (B × 2, C) or (I) or with the corresponding HO-PBacids in (B × 2, G). The sulfate group was originally present on the phenoxy ring since the cleavage product(s) from the α-$^{14}CH_2$ preparation slowly decomposed on TLC plates ultimately yielding 3-hydroxy[^{14}C]benzoic acid (TLC cochromatography in B × 2 and G giving R_f values of 0.29 and 0.28, respectively). A comparison of the stability of the unlabeled HO-PBacids indicated above showed that the 2′-hydroxy compound was most unstable and that it yielded 3-hydroxybenzoic acid and several other products which are probably cyclohexadienone intermediates; these intermediates varied in their chemical nature and ratio depending on the condition of oxidation, e.g. thallium(III) nitrate in methanol or in sodium acetate in a mixture of acetic acid and ethyl acetate (McKillop et al., 1976; Unai and Casida, 1976). These results suggest that this minor metabolite is likely to be 2′-HO-PBacid-sulfate which gives unstable cyclohexadienones on sulfatase or chemical cleavage. The finding of 2′-HO,*c*-per as a fecal metabolite of *c*-per with no

Table IV. ^{14}C-Labeled Compounds in the Urine and in the Methanol Extract of Feces of Rats at 1 Day after Oral Administration of [^{14}C-1*R*,*trans*]Cl_2CA and of ^{14}C-Acid-Labeled Preparations of [1*R*,*trans*]-, [1*RS*,*trans*]-, [1*R*,*cis*]-, and [1*RS*,*cis*]-Permethrin

	^{14}C-Acid label, % of administered radiocarbon								
	t-Cl_2CA, urine	*trans*-Permethrin				*cis*-Permethrin			
		Urine		Feces		Urine		Feces	
^{14}C-labeled compd	1*R*	1*R*	1*RS*	1*R*	1*RS*	1*R*	1*RS*	1*R*	1*RS*
		Permethrin and Ester Metabolites							
Permethrin		0.0	0.0	2.1	2.8	0.0	0.0	5.3	6.7
2′-HO-per		0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.5
4′-HO-per		0.0	0.0	0.0	0.0	0.0	0.0	3.1	2.7
t-HO-per		0.0	0.0	0.0	0.0	0.0	0.0	1.6	2.5
4′-HO,*t*-HO-per		0.0	0.0	0.0	0.0	0.0	0.0	3.4	3.9
		Metabolites from Acid Moiety							
Cl_2CA									
Free	5.4	2.6	5.6	4.3	2.7	1.2	0.7	2.2	0.5
Gluc	67.2	56.1	41.9	0.0	0.0	18.5	13.8	0.0	0.0
HO-Cl_2CA									
t-HO	1.4	1.4	0.3	0.4	0.8	4.7	3.3	2.5	1.5
c-HO									
Free	1.5	4.8	1.7	0.4	0.8	1.6	3.5	1.9	1.2
Lactone	0.0	1.4	0.0	0.0	0.0	1.9	3.0	0.0	1.1
Gluc	1.4	2.0	0.7	0.0	0.0	2.3	2.0	0.0	0.0
Unknowns[a]									
1				0.9	0.5	0.7	0.6	0.9	0.0
2						0.6	0.0	2.2[b]	1.7[b]
3						0.8	0.6		
		Permethrin and Metabolites							
Loss[c]	−0.9	−2.3	6.8	1.9	1.4	2.7	6.5	7.0	4.7
Total	76.0	66.0	57.0	10.0	9.0	35.0	34.0	31.0	27.0

[a] For chromatographic properties of the unknowns, see Figure 2. There are six distinct unknown metabolites from the acid moiety, i.e., one in the feces from *t*-per and three in the urine and two in the feces from *c*-per. [b] This metabolite may be an ester, the same as alcohol-labeled feces metabolite 4 from *c*-per. [c] Difference between initial radiocarbon level and that recovered as well-resolved products on TLC analysis.

comparable metabolite from *t*-per lends further support to the proposal that the minor sulfate conjugate is 2′-HO-PBacid-sulfate.

Amounts of Excreted Metabolites. The permethrin isomers are rapidly and extensively metabolized so that only a small portion is excreted without metabolic modification, this portion appearing in the feces and accounting for about 3 and 6% of the administered dose with *t*- and *c*-per, respectively (Tables IV and V). No ester metabolites of *t*-per are excreted in amounts of >0.1% of the administered radiocarbon. However, following cleanup and concentration of the fecal extracts, 4′-HO-*t*-per is detected as a very minor fecal metabolite of [1*R*,*t*]- and [1*RS*,*t*]per. The ester linkage of [1*R*,*c*]- and [1*RS*,*c*]per is more stable in vivo than that of the corresponding trans isomers since four ester metabolites of *c*-per appear in the feces, collectively accounting for 8–10% of the administered dose. The three minor ester metabolites involve single sites of hydroxylation, the phenoxy group in 2′-HO,*c*-per and 4′-HO,*c*-per and a methyl group in *t*-HO,*c*-per. The major ester metabolite, 4′-HO,*t*-HO,*c*-per, results from hydroxylation at both the phenoxy and methyl groups. Two fecal metabolites of [1*R*,*c*]- and [1*RS*,*c*]per, designated as unknowns (unknown 2 from [^{14}C-acid]per and unknown 4 from [^{14}C-alc]per), chromatograph such that they might be the same compound, i.e., an ester, but this point is not firmly established.

The major metabolite of [1*R*,*t*]Cl_2CA and of the acid moiety of [1*R*,*t*]-, [1*RS*,*t*]-, [1*R*,*c*]- and [1*RS*,*c*]per is the glucuronide conjugate of the acid moiety, Cl_2CA-gluc, which appears entirely in the urine (Table IV). In each case, 8–16% of the total amount of Cl_2CA is excreted without conjugation, appearing almost equally in the urine and in the feces. A portion of the acid moiety from each compound is excreted as the hydroxy derivatives, HO-Cl_2CA, which appear in small amounts as the glucuronides in urine. The major portion of the HO-Cl_2CA derivatives is excreted without conjugation, and to a greater extent in the urine than in the feces. A portion of the *c*-HO-Cl_2CA is detected as the corresponding lactone, but this is possibly an artifact as discussed elsewhere. There are several unknown metabolites of the acid moiety, more from *c*- than from *t*-per. A portion of these unidentified metabolites may be further oxidation products of HO-Cl_2CA.

On considering both the metabolites retaining the ester linkage and those derived from the acid moiety only, it appears that the cis methyl group is the preferred hydroxylation site with [1*R*,*t*]per and the trans methyl with [1*R*,*c*]per; however, the [1*S*]per isomers (in the unresolved [1*RS*]per) may not follow this specificity pattern. With this possible exception, the same metabolites and in almost the same amounts are detected with the 1*R* and 1*RS* preparations.

The ^{14}C-alc-labeled metabolites of [^{14}C-1*R*,*t*]- and [^{14}C-1*RS*,*t*]per are the same as those of [^{14}C]PBalc, and they appear in almost equal proportions with this series of compounds (Table V). Thus, minor amounts of PBalc and PBacid appear in the feces, the major metabolite is 4′-HO-PBacid-sulfate in the urine, and the remainder is PBacid which is excreted, in decreasing amounts, as the glucuronide, free acid, and glycine conjugate. Furthermore, in each case the same two unidentified metabolites appear in the feces. A considerably different picture emerges with the alcohol moiety of [1*R*,*c*]- and [1*RS*,*c*]per. There is no PBalc and relatively little of PBacid and its conjugates. The proportion of HO-PBacid-sulfate conjugates is increased so that they exceed the nonhydroxylated PBacid derivatives by threefold. A new derivative is found, 2′-HO-PBacid-sulfate, and there are also two to four unknowns which are detected with *c*- but not with *t*-per. Perhaps the greater stability of the ester linkage in *c*-per permits the additional sites or degrees of oxidation prior

Table V. ^{14}C-Labeled Compounds in the Urine and in the Methanol Extract of Feces of Rats at 1 Day after Oral Administration of [^{14}C]Phenoxybenzyl Alcohol and of ^{14}C-Alcohol-Labeled Preparations of [1*R*,*trans*]-, [1*RS*,*trans*]-, [1*R*,*cis*]-, and [1*RS*,*cis*]-Permethrin

	^{14}C-Alcohol label, % of administered radiocarbon									
			trans-Permethrin				*cis*-Permethrin			
	PBalc		Urine		Feces		Urine		Feces	
^{14}C-Labeled compd	Urine	Feces	1*R*	1*RS*	1*R*	1*RS*	1*R*	1*RS*	1*R*	1*RS*
Permethrin and Ester Metabolites										
Permethrin			0.0	0.0	1.3	5.3	0.0	0.0	4.6	7.3
2′-HO-per			0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.9
4′-HO-per			0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.4
t-HO-per			0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.4
4′-HO,*t*-HO-per			0.0	0.0	0.0	0.0	0.5	0.0	5.0	3.8
Metabolites from Alcohol Moiety										
PBalc	0.0	1.3	0.0	0.0	1.3	1.7	0.0	0.0	0.0	0.0
PBacid										
Free	7.0	1.3	7.2	10.0	1.0	1.5	2.7	1.1	0.0	0.0
Gluc	23.0	0.0	14.1	14.9	0.0	0.0	1.5	7.0	0.0	0.0
Glycine	5.2	0.0	2.9	4.4	0.0	0.0	1.5	2.0	0.0	0.0
HO-PBacid-sulfate										
2′-HO	0.0	0.0	0.0	0.0	0.0	0.0	3.4	2.9	0.0	0.0
4′-HO	38.1	0.0	30.7	42.8	0.0	0.0	19.5	29.3	0.0	0.0
Unknowns[a]										
1		0.7			0.4	0.7			1.0	1.8
2		1.7			0.6	0.0			1.3	1.1
3									1.3	2.0
4									2.3[b]	2.0[b]
Permethrin and Metabolites										
Loss[c]	11.7	2.0	15.1	1.9	1.4	2.8	5.9	1.7	14.2	3.3
Total	85.0	7.0	70.0	74.0	6.0	12.0	35.0	44.0	33.0	26.0

[a] For chromatographic properties of the unknowns, see Figure 2. There are six distinct unknown metabolites from the alcohol moiety, i.e., two in the feces from both PBalc and *t*-per and four in the feces from *c*-per. [b] This metabolite may be an ester, the same as acid-labeled feces metabolite 2 from *c*-per. [c] Difference between initial radiocarbon level and that recovered as well-resolved products on TLC analysis.

to excretion, an explanation consistent with the larger proportion of ester metabolites with *c*- than with *t*-per.

The metabolites discussed above are excreted within the first 24 h following treatment. However, the 24–48- and 48–72-h samples of excreta gave TLC patterns and proportions of metabolites very similar to those obtained with the 0–24-h samples. Thus, the 0–24-h data in Tables IV and V provide a good picture of the overall metabolic pathways and products. The products entered as "loss" are likely to consist of the same materials as those already identified since much of this loss occurs from partial but not complete resolution of metabolites of similar R_f values and small degrees of decomposition during analysis. Correcting for this "loss", the identified metabolites in the excreta (urine and methanol extract of the feces) account for 80–89% of the administered radiocarbon (Table VI), and the remainder consists of unidentified metabolites, including both those in the unextractable portion of the feces and metabolites excreted between 3 and 12 days after treatment. The radiocarbon levels were insufficient for quantitation of individual metabolites at these later intervals. The unidentified metabolites at 3 days after treatment account for the following percentages of the products analyzed by TLC: 1% with [^{14}C-acid-1*RS*,*t*]per and [^{14}C-alc-1*RS*,*t*]per; 5% with [^{14}C-acid-1*RS*,*c*]per; 10% with [^{14}C-alc-1*RS*,*c*]per. The only unidentified metabolites that are resolved by TLC and account for more than 1% of the administered radiocarbon are five minor fecal metabolites of *c*-per.

Toxicity of Permethrin Derivatives. The 48-h mouse ip LD_{50} values for [1*RS*,*t*]per, [1*RS*,*c*]per, PBalc, PBacid, [1*R*,*t*]Cl_2CA, and [1*R*,*c*]Cl_2CA are >1000, 925, 575, 350, 210, and 370 mg/kg, respectively.

DISCUSSION

The residence time of *t*- and *c*-per, and that of all of their metabolites, is relatively brief in the body. About 3 and 6% of the *t*- and *c*-per doses, respectively, are excreted in the feces without metabolism, due either to incomplete absorption from the gastrointestinal tract or to enterohepatic circulation. Metabolites retaining the ester linkage account for <0.1 and 9% of the *t*- and *c*-per doses, respectively, further establishing the greater in vivo lability of the ester linkage of *t*- than of *c*-per. Since there was no significant production of [^{14}C]carbon dioxide following administration of any of the labeled compounds, the pathways of per metabolism in rats do not include extensive fragmentation of the acid and alcohol moieties.

Table VI. ^{14}C-Labeled Compounds as Identified and Unidentified Metabolites in the Urine and in the Methanol Extract of Feces of Rats at 3 Days after Oral Administration of ^{14}C-Acid- and ^{14}C-Alcohol-Labeled Preparations of [1*RS*,*trans*]- and [1*RS*,*cis*]-Permethrin

	^{14}C-Acid or ^{14}C-alcohol label, % of administered radiocarbon			
	[1*RS*,*trans*]-Permethrin		[1*RS*,*cis*]-Permethrin	
Measurement	^{14}C-Acid	^{14}C-Alc	^{14}C-Acid	^{14}C-Alc
Total excreta	90.0	89.6	86.9	89.3
Individual metabolites[a]				
Not corrected for loss				
Identified	77.6	83.6	68.4	72.8
Unidentified	0.6	0.8	4.2	8.7
Corrected for loss[b]				
Identified	89.3	88.8	81.9	79.8
Unidentified	0.7	0.8	5.0	9.5

[a] The metabolites appear in the same proportions as given in Tables IV and V. [b] The percent values for the metabolites were multiplied by the factor: initial radiocarbon level in the urine plus that in the methanol extract of feces/total radiocarbon recovered as well-resolved products on TLC analysis.

The metabolic pathways for *t*-per and *c*-per after oral administration to rats are shown in Figure 1. This figure illustrates the products originating from the [1*R*]-esters, but the same products, in nearly the same proportions, result from administration of the [1*RS*]-esters. The rate of mouse microsomal metabolism of *t*-per and *c*-per by esterase, oxidase, and combined esterase and oxidase systems is quite similar for the [1*R*]-, [1*S*]-, and [1*RS*]-esters (Soderlund and Casida, 1976); thus, it is likely that [1*S*,*t*]per and [1*S*,*c*]per are also metabolized by the pathways shown in Figure 1.

The five principal sites of metabolic attack on the per isomers are ester cleavage, oxidation at the trans or cis methyl group of the geminal dimethyl moiety, and oxidation at the 2′ or 4′ position of the phenoxy group. All of the major metabolites are accounted for by these sites of attack, individually or in sequence, plus conjugation of the phenolic hydroxy group and of the carboxylic acids including those formed on oxidation of the substituted benzyl alcohols.

Four ester metabolites of *c*-per (2′-HO,*c*-per; 4′-HO,*c*-per; *t*-HO,*c*-per; and 4′-HO,*t*-HO,*c*-per) and a very minor ester metabolite of *t*-per (4′-HO,*t*-per) are found in the feces. Those of *c*-per result from hydroxylation at the 2′-phenoxy, 4′-phenoxy, or trans methyl position or at both of the latter two sites. The other hydroxymethyl esters (*t*-HO,*t*-per; *c*-HO,*t*-per; *c*-HO,*c*-per) are probably intermediates since these compounds are detected along with *t*-HO,*c*-per in mouse liver microsomal oxidase systems when their esterase activity is inhibited by an organophosphorus compound (Unai and Casida, 1976). Conjugates of the ester metabolites are minor or are absent.

Metabolites of the acid moiety are formed by hydroxylation at the geminal dimethyl group, more extensively with *c*-per than with *t*-per, suggesting that at least some of these metabolites originate from hydroxylation before ester cleavage. The nonhydroxylated acids (*t*-Cl_2CA and *c*-Cl_2CA) are excreted in the most part as glucuronides, while the hydroxylated acids (HO-*t*-Cl_2CA and HO-*c*-Cl_2CA) are mostly excreted without conjugation. The lactones might be formed as artifacts during analysis (see Materials and Methods) or by cyclization of appropriate precursors in the body prior to excretion; candidate precursors are *c*-HO,*t*-Cl_2CA, *c*-HO,*c*-Cl_2CA, *c*-HO,*t*-per, and *c*-HO,*c*-per, the latter two compounds readily undergoing cyclization under relatively mild conditions (Unai and Casida, 1976).

All major metabolites of the alcohol moiety arise from ester cleavage of *t*-per and *c*-per, or of their ester metabolites, and oxidation of the released benzyl alcohols to the corresponding benzoic acids. PBacid is excreted either without conjugation or as glucuronide and glycine conjugates. The metabolite present in largest amount, 4′-HO-PBacid-sulfate, is probably formed by hydroxylation of the phenyl group and ester cleavage, in an undetermined sequence, followed by oxidation to the benzoic acid and sulfate conjugation. Although the amount of HO-PBacid-sulfate slightly exceeds that of the PBacid and its conjugates following *t*-per administration, this differential is much greater following *c*-per administration. This suggests that ring hydroxylation occurs most readily with the compounds that retain their apolar nature in the body for the longest period. This is supported by the finding of 2′-HO-PBacid-sulfate with *c*-per but not with *t*-per.

Permethrin combines very high insecticidal activity with low mammalian toxicity and rapid biodegradability in mammals (Elliott et al., 1973a, 1976). These are appropriate properties for a candidate insect control agent.

ACKNOWLEDGMENT

The authors thank Michael Elliott, Kenzo Ueda, David Soderlund, Toshio Shono, and Judith Engel, former or current colleagues in this laboratory, for assistance and helpful discussions.

LITERATURE CITED

Burt, P. E., Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pulman, D. A., *Pestic. Sci.* **5**, 791 (1974).

Capel, I. D., Millburn, P., Williams, R. T., *Xenobiotica* **4**, 601 (1974).

Dutton, G. J., Ed., "Glucuronic Acid Free and Combined", Academic Press, New York, N.Y., 1966.

Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pulman, D. A., Stevenson, J. H., *Nature (London)* **246**, 169 (1973a).

Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pulman, D. A., Stevenson, J. H., *Proc. Br. Insectic. Fungic. Conf., 7th*, 721 (1973b).

Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., Pulman, D. A., *ACS Symp. Ser.* **No. 2**, 80 (1974).

Elliott, M., Janes, N. F., Pulman, D. A., Gaughan, L. C., Unai, T., Casida, J. E., *J. Agric. Food Chem.* **24**, 270 (1976).

Fujimoto, K., Itaya, N., Okuno, Y., Kadota, T., Yamaguchi, T., *Agric. Biol. Chem.* **37**, 2681 (1973).

McKillop, A., Perry, D. H., Edwards, M., Antus, S., Farkas, L., Nógrádi, M., Taylor, E. C., *J. Org. Chem.* **41**, 282 (1976).

Mehendale, H. M., Dorough, H. W., *Pestic. Biochem. Physiol.* **1**, 307 (1971).

Miyamoto, J., *Environ. Health Persp.*, in press (1976).

Miyamoto, J., Suzuki, T., Nakae, C., *Pestic. Biochem. Physiol.* **4**, 438 (1974).

Soderlund, D. M., Casida, J. E., unpublished results, 1976.

Ueda, K., Gaughan, L. C., Casida, J. E., *J. Agric. Food Chem.* **22**, 212 (1974).

Ueda, K., Gaughan, L. C., Casida, J. E., *J. Agric. Food Chem.* **23**, 106 (1975a).

Ueda, K., Gaughan, L. C., Casida, J. E., *Pestic. Biochem. Physiol.* **5**, 280 (1975b).

Unai, T., Casida, J. E., manuscript in preparation, 1976.

Received for review May 17, 1976. Accepted September 7, 1976. This study was supported in part by grants from the National Institutes of Health (2 P01 ES00049); The Rockefeller Foundation; Agricultural Chemical Division, FMC Corp., Middleport, N.Y.; Agricultural Chemicals Division, ICI United States Inc., Goldsboro, N.C.; Sumitomo Chemical Co., Osaka, Japan; Roussel-Uclaf-Procida, Paris, France; Mitchell Cotts & Co. Ltd., London, England; Wellcome Foundation Ltd., London, England; National Research Development Corp., London, England.