Metabolites of *cis*- and *trans*-Permethrin in Lactating Goats

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cis-(1*RS*)- and *trans*-(1*RS*)-permethrins, radiolabeled with ^{14}C in either the acid or alcohol moiety, are rapidly metabolized and excreted after oral administration to lactating goats. Twenty-six metabolites of the permethrin isomers are fully or partly characterized by thin-layer chromatography and/or gas–liquid chromatography–mass spectrometry. The identified metabolites arise through hydrolysis of the ester linkage, hydroxylation at the *cis*- or *trans*-methyl of the geminal dimethyl group, and hydroxylation at the 4' position of the phenoxybenzyl moiety. Certain of these products are further oxidized and/or conjugated with glycine, glutamic acid, glucuronic acid, or other unidentified compounds before excretion. Unmetabolized permethrin and certain ester metabolites are found in feces, milk, and fat from the treated goats, but only metabolites arising from ester hydrolysis are seen in urine. GLC–mass spectral data are reported for the *cis*- and *trans*-permethrin isomers and 40 of their metabolites and analogues.

The synthetic pyrethroid permethrin [3-phenoxybenzyl *cis,trans*-(1*RS*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] is a highly efficacious insecticide that has sufficient environmental stability to permit its effective use against insects and certain other arthropod pests that attack agricultural crops as well as livestock and poultry. Several reports have dealt with the fate of permethrin in various components of the environment, including its photochemistry (Holmstead et al., 1978) and its fate in soils (Kaneko et al., 1978; Kaufman et al., 1977), plants (Gaughan and Casida, 1978; Ohkawa et al., 1977), insects (Bigley and Plapp, 1978; Shono et al., 1978), birds (Gaughan et al., 1978b), fish (Glickman et al., 1979), and mammals (Elliott et al., 1976; Gaughan et al., 1977, 1978a). The current studies were undertaken to provide additional information on the fate of permethrin in mammals, specifically the delineation of permethrin metabolites in orally treated lactating goats.

MATERIALS AND METHODS

Source of Samples. Samples of urine, feces, milk, and tissues analyzed for permethrin metabolites were obtained from lactating Nubian and Nubian–Saanen cross goats that had been treated orally with [^{14}C]permethrin isomers. Definition of the distribution and excretion rates of radiocarbon in these goats, without metabolite identification, formed the basis of a previous report (Hunt and Gilbert, 1977). In these studies, four goats were treated orally with either *cis*-(1*RS*)-permethrin (*c*-per), labeled at the carboxyl group of the acid moiety ([^{14}C-acid]-*c*-per) or at the methylene carbon of the alcohol moiety ([^{14}C-alc]-*c*-per), or with *trans*-(1*RS*)-permethrin (*t*-per), labeled at the same position in the acid ([^{14}C-acid]-*t*-per) or alcohol ([^{14}C-alc]-*t*-per) moiety. Each of the goats received ten successive daily oral doses of one of the four [^{14}C]per preparations; each dose ranged from 0.2–0.3 mg/kg of body weight per day, depending upon the ^{14}C label and isomer given. Urine, feces, and milk samples were collected periodically during the treatment period, and tissue samples were obtained when the animals were sacrificed 24 h after the last dose (Hunt and Gilbert, 1977). Samples were held frozen at Kerrville, TX, for approximately 2 months after

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termination of the study and were then transferred frozen to College Station, TX, where they were held at −70 °C until analysis. Only those fecal, urine, and milk samples collected 3, 6, and 9 d after initiation of the [^{14}C]per treatments were retained for study.

Sample Analysis. *Feces.* Samples of feces (3 g) were extracted with 30 mL of methanol by homogenization with a Willems Polytron homogenizer, the extract was decanted off after centrifugation to sediment the residue, and the residue was extracted twice again with methanol as before. Radiocarbon in the combined extracts was quantitated by liquid scintillation counting (LSC), and then the extracts were concentrated and analyzed by two-dimensional thin-layer chromatography (TLC; vide infra). Radiocarbon remaining in the extracted feces residues was quantitated by combustion in an oxygen atmosphere (Oehler and Ivie, 1980) but was not further analyzed.

Urine. Direct ether extraction of samples of whole urine from the four goats resulted in very poor partitioning of ^{14}C into the organic phase (<5% in all samples), but acidification before extraction resulted in greatly improved extractability. Thus, 10-mL samples of whole urine were adjusted to pH ∼2.0 with HCl and were then partitioned five times with equal volumes of ether. Radiocarbon in the combined ether extracts was quantitated by LSC and the extracts were then dried over anhydrous Na$_2$SO$_4$, concentrated, and subjected to two-dimensional TLC analysis. Radiocarbon in the residual aqueous phases of these samples was likewise quantitated, and in some samples the water-soluble radiocarbon was further analyzed by acid hydrolysis procedures (vide infra).

Milk. Samples of whole milk (100 mL) were adjusted to pH ∼2.0 with HCl and were partitioned five times with equal volumes of ether. Radiocarbon in the aqueous and combined organic phases was quantitated by LSC, and the organic fractions were dried and concentrated until only an oily residue remained. The oil was partitioned between 100 mL of hexane and 50 mL of acetonitrile, and the hexane phase was extracted twice again with 50-mL volumes of acetonitrile. Radiocarbon in the combined acetonitrile phases was quantitated and the samples were then concentrated and analyzed by TLC. Radiocarbon in the hexane phase was quantitated by LSC but was not further studied.

Tissues. Samples of fat (5 g) were extracted five times with 20–30-mL volumes of hexane by blending with a Polytron homogenizer, and the extracts were dried, adjusted to 100 mL, and partitioned three times with 50-mL volumes of acetonitrile. The radiocarbon content of each fraction was quantitated by LSC (or by oxygen combustion

of the small amounts of fibrous residue remaining after fat extraction), and then the acetonitrile phase was concentrated for TLC analysis.

Samples of liver (5 g) were extracted by homogenization in 30 mL of a 2:1 mixture of acetonitrile acetone. The residue was separated by centrifugation, the extract decanted off, and the residue reextracted four more times as before. The combined extracts were concentrated to an oily residue, which was partitioned between 5 mL each of hexane and acetonitrile. The hexane phase, after readjustment to 5 mL, was partitioned twice again with acetonitrile. The combined acetonitrile phases were then concentrated and analyzed by TLC, and radiocarbon remaining in the hexane was quantitated but was not further studied.

The extracted liver residue was subjected to an acid hydrolysis procedure (20 mL of 3 N HCl, 100 °C, 2 h), and then the samples were partitioned three times with 15-mL volumes of ether. Radiocarbon in extracts of the liver hydrolysates was quantitated and subjected to TLC, whereas that in the residual aqueous residue slurry was quantitated (LSC) but was not studied further.

Chromatography. ^{14}C components in extracts of excreta, milk, and tissues from the [^{14}C]per-treated goats were resolved by TLC with precoated silica gel chromatoplates (0.25–2.0 mm gel thickness, 20 × 20 cm, with fluorescent indicator, Brinkmann). The plates were developed in either one or two dimensions. The resolved ^{14}C components were visualized by exposing the plates to X-ray film (Kodak No-Screen), and then they were either quantitated by direct LSC of the appropriate gel regions or were recovered from the gel for further study by extraction with an appropriate solvent (ethyl acetate, acetone, or methanol). Many of the TLC solvent systems used in this study were the same as those used previously by other workers to resolve a large number of per analogues, metabolites and their derivatives (Gaughan et al., 1977, 1978a; Unai and Casida, 1977). These systems included (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), (K) hexane–ethyl acetate (7:3), (L) carbon tetrachloride–benzene (1:1), (M) hexane–acetone (4:1), (N) acetone–ether (4:1), (O) dichloromethane–hexane–methanol (4:4:1), (P) benzene (saturated with formic acid)–ether (1:1), (Q) hexane–ethyl acetate–methanol (2:2:1), (R) hexane–acetone–methanol (10:5:1), and (S) chloroform–methanol–acetic acid (50:10:1).

Characterization of Metabolites. Metabolites were characterized by TLC and/or gas–liquid chromatography (GLC)–mass spectrometry. Direct TLC comparisons were made of radioactive components isolated from urine, feces, milk, or tissues with per analogues of known structure, sometimes after derivatization or degradation (including alkaline, acid, and enzymatic hydrolysis) of the ^{14}C metabolites by reported procedures (Gaughan et al., 1977). The ^{14}C metabolites or their derivatives were visualized by radioautography and the unlabeled per analogues were visualized by viewing the plates under shortwave ultraviolet light or by spraying with a 20% ethanolic solution of phosphomolybdic acid followed by heating the plates to 100–150 °C. The per isomers and several analogues considered as possible metabolites or metabolite derivatives were supplied for these studies by the Agricultural

Chemical Group, FMC Corp., Middleport, NY, or the Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC. We synthesized certain additional per analogues according to reported procedures (Unai and Casida, 1977). The compounds available for use in this study are indicated in Table I. The abbreviations used in Table I as structure designations for the products are generally the same as those used by earlier workers, who have also reported on the TLC behavior of most of these compounds in several solvent systems (Gaughan et al., 1977, 1978a; Unai and Casida, 1977). Structures and abbreviations of per metabolites identified in goats during the course of this study are indicated in Figure 1.

Certain per metabolites that were suspected to be glucuronide conjugates were subjected to either enzymatic or acid hydrolysis procedures to facilitate their characterization. Such products, isolated by TLC, were incubated with β-glucuronidase (type I, Sigma) in 0.1 M sodium acetate–acetic acid buffer (pH 4.5, incubation time up to 24 h), with or without D-saccharic acid, 1,4-lactone added to inhibit β-glucuronidase activity (Gaughan et al., 1977). The aglycons formed were recovered by ether extraction and were characterized by TLC comparisons (before or after derivatization with diazomethane) with compounds of known structure. Acid hydrolysis (1 N HCl, 100 °C, up to 2 h) of these conjugates was similarly used to generate aglycons that were characterized by TLC.

The availability of samples from different animals treated with the same per isomer but with the radiolabel located in different parts of the molecule (acid or alcohol moiety) permitted partial characterization of some metabolites that were not fully identified by TLC or GLC–mass spectrometry. Thus, detection of the same metabolite in goats with both [^{14}C-acid] and [^{14}C-alc] preparations of the same per isomer was considered to be sufficient evidence that the metabolite retained the ester linkage intact, whereas [^{14}C-acid] or [^{14}C-alc] metabolites that resulted from hydrolysis of the ester linkage were indicated by their occurrence in samples from one but not both of the label positions.

GLC–Mass Spectrometry. GLC–mass spectrometry was used where possible to make or confirm structural assigments of the isolated metabolites. The instrumentation used included a Varian/MAT CH 7 magnetic scan spectrometer, coupled with a Varian 2700 gas–liquid chromatograph and 620L Varian computer system. The resolutions were made on a 1.8 m × 2 mm i.d. glass column, packed with 1.5% SP-2250 + 1.95% SP-2401 on 100/120 Supelcon AW-DMCS. The column temperature varied with the compounds (Table I), and injector and detector ovens and all transfer lines were always maintained at a temperature slightly higher than the column. The flow rate of the helium carrier gas was 50 mL/min, and all spectra were recorded at 70 eV. Retention times and pertinent mass spectral data for each of the per metabolites and analogues studied are indicated in Table I.

RESULTS

Excretion Patterns and Tissue Residues. Although the patterns of radiocarbon elimination and tissue retention by these goats have been reported in detail earlier (Hunt and Gilbert, 1977), they are summarized briefly in Table II. Urine is the major route of radiocarbon excretion in goats treated with [^{14}C-acid] or [^{14}C-alc] preparations of t-per, but most of the administered radiocarbon is eliminated through the feces in c-per treated goats. Radiocarbon residues secreted into milk amounts to <1% of the total dose with each of the four [^{14}C]per-treated goats. Of the edible tissues analyzed for radiocarbon 24 h after

Table I. GLC–Mass Spectral Data for Permethrin and Certain of Its Metabolites and Analogues[a]

compound	column temp,[b] °C	retention, min	molecular ion	base peak ion	other ions
		Permethrin and Ester Derivatives			
c-per	230	6.2	390	183	255, 215, 163, 127
t-per	230	6.7	390	183	255, 215, 163, 127
2'-MeO-c-per	250	4.1	420	137	345, 237, 213, 163, 105
2'-MeO-t-per	250	4.5	420	137	345, 237, 213, 263, 105
4'-MeO-c-per	250	5.3	420	213	345, 237, 163
4'-MeO-t-per	250	5.8	420	213	345, 237, 163
t-HO-c-per	250	10.0	406	183	348, 255, 199
		Acid Moiety and Derivatives			
c-Cl$_2$CA	140	2.5	208	91	173, 163, 127
t-Cl$_2$CA	140	2.5	208	91	173, 163, 127
c-Cl$_2$CA-Me	110	3.4	222	91	187, 163, 127
t-Cl$_2$CA-Me	110	3.9	222	91	187, 163, 127
t-HO-c-Cl$_2$CA	175	3.2	224	58	206, 177, 149, 131
t-HO-c-Cl$_2$CA-Me	150	4.1	238	181	208, 207, 149, 145, 113
t-HO-t-Cl$_2$CA-Me	150	4.5	238	181	149, 145
c-HO-c-Cl$_2$CA-lactone	200	0.7	206	77	171, 149, 127, 115, 113. 91
c-HO-t-Cl$_2$CA-lactone	200	0.9	206	77	171, 149, 127, 115, 113, 91
c-Cl$_2$CA-Gly-Me	200	1.9	279	88	244, 191, 127, 116, 91
t-Cl$_2$CA-Gly-Me	200	2.2	279	88	244, 191, 127, 116, 91
t-Cl$_2$CA-Ala-Me	200	1.5	293	102	258, 234, 191, 130, 127
c-Cl$_2$CA-Glut-Me$_2$	210	5.4	365	142	202, 191, 174, 127, 114, 110
t-Cl$_2$CA-Glut-Me$_2$	210	5.8	365	142	202, 191, 174, 127, 114, 110
		Alcohol Moiety and Derivatives			
PBalc	200	1.3	200	200	181, 171, 153, 141
2'-HO-PBalc	225	1.1	216	198	197, 185, 169
4'-HO-PBalc	225	1.7	216	216	197, 187, 185, 169, 157
2'-MeO-PBalc	225	1.1	230	230	197, 185, 169, 157, 121
4'-MeO-PBalc	225	1.5	230	230	215, 171, 169, 157, 89, 77
PBacid	225	0.8	214	214	196, 169, 141
PBacid-Me	200	1.6	228	228	197, 169, 141, 115
2'-HO-PBacid	250	0.8	230	230	214, 212, 196, 184, 169, 168, 157
4'-HO-PBacid	250	1.6	230	230	214, 212, 184, 157
4'-HO-PBacid-Me	220	2.8	244	244	213, 185, 157
2'-MeO-PBacid-Me	200	3.0	258	258	227, 199, 184, 149
4'-MeO-PBacid-Me	200	3.9	258	258	243, 227, 215
PBacid-Gly-Me	250	2.3	285	197	254, 226, 169, 141
PBacid-Ala-Me	250	1.8	299	197	240, 169, 141, 115
PBacid-Glut-Me$_2$	270	3.8	371	197	339, 312, 174, 169, 141
4'-HO-PBacid-Gly-Me	270	3.0	301	213	269, 157
4'-MeO-PBacid-Gly-Me	270	2.2	315	227	199, 171, 128, 114
4'-MeO-PBacid-Gly-Me-N-Me[d]	270	2.5	329	329	241, 213, 212, 157
4'-HO-PBacid-Glut-Me$_2$	285	4.5	387	213	315, 157, 137
4'-MeO-PBacid-Glut-Me$_2$	285	3.3	401	227	369, 342, 199, 174, 171, 114
4'-MeO-PBacid-Glut-Me$_2$-N-Me[d]	285	3.6	415	241	383, 227, 213, 174, 157

[a] Trivial names for compounds as indicated in text, tables, or Figure 1, or as defined in subsequent footnotes. [b] 1.8 m × 2 mm i.d. glass column packed with 1.5% SP-2250 + 1.95% SP-2401 on 100/120 mesh Supelcon AW-DMCS. [c] 70 eV. [d] N-Methyl derivative of the indicated amino acid conjugates formed during methylation reactions (diazomethane).

Table II. Summary of Radiocarbon Elimination and Residue Retention by Lactating Goats Treated Orally for Ten Consecutive Days with [^{14}C]Alcohol- or [^{14}C]Acid-Labeled cis-(1RS)- or trans-(1RS)-Permethrin Isomers[a,b]

label and isomer	radiocarbon eliminated, cum % dose			tissue residues, ppm[c,d]		
	feces	urine	milk	liver	kidney	fat
[^{14}C-acid]-c-per	67.5	25.8	0.66	0.12	0.05	0.23
[^{14}C-alc]-c-per	51.7	36.4	0.53	0.13	0.05	0.24
[^{14}C-acid]-t-per	15.0	72.1	0.17	0.04	0.03	0.02
[^{14}C-alc]-t-per	12.3	79.4	0.24	0.01	0.03	0.02

[a] Data summarized from Hunt and Gilbert (1977). [b] Goats treated with 10 successive daily oral doses of the appropriate [^{14}C]per isomer (0.2–0.3 mg/kg per d). [c] Tissues taken 24 h after last dose. [d] Other edible tissues contained lower residues.

the final per doses, fat, kidney, and liver contain the highest residues. Radiocarbon retained by the tissues, and that secreted into the milk are appreciably higher in goats treated with c-per than with t-per (Table II, Hunt and Gilbert, 1977).

Metabolites in Feces. Methanol extraction of samples of feces collected 3, 6, or 9 d after initiation of a 10-d treatment schedule with [^{14}C]per isomers results in >90% recovery of the radiocarbon from all samples (Table III). Analysis of the extracts by two-dimensional TLC [developed once in solvent system A and then twice in solvent system B (A, B × 2)] resolves several metabolites from each sample.

Thirteen ^{14}C components are resolved from feces extracts of the c-per-treated goats (Table III). Structures are assigned to five of these, one of which is unmetabolized c-per (TLC in B, C, E, G, H, L; GLC–mass spectrometry). Two ester metabolites of c-per are also identified in the sample extracts; one is 4'-HO-c-per [TLC of the methylated metabolite with authentic 4'-MeO-c-per (H, L, M, N); TLC of the alkaline hydrolysis product of the [^{14}C-alc] metabolite with authentic 4'-HO-PBalc (B, C, M, O)]. The other ester metabolite, which is the major ^{14}C component in most feces samples of the c-per goats, is t-HO-c-per (TLC in B, C, G, H, I, L, M; TLC of the alkaline hydrolysis product of the [^{14}C-alc] metabolite with authentic PBalc in B, C, G, M; TLC of the alkaline hydrolysis product of
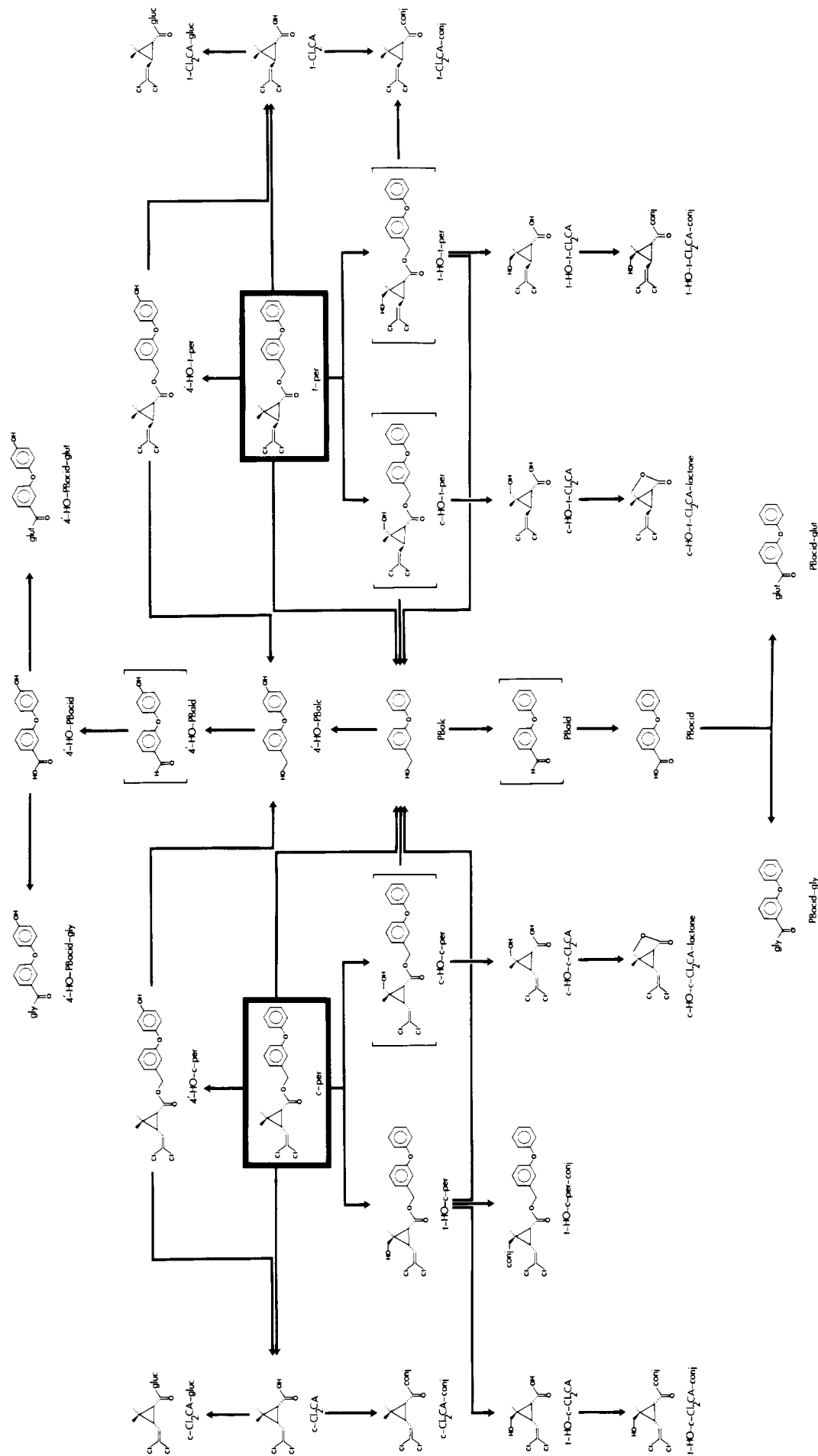
Figure 1. Metabolic pathways for *cis*- and *trans*-permethrin in lactating goats. The products represent 1*RS* isomers, although only the 1*R* isomers are shown in the figure. Compounds enclosed by brackets were not isolated, but are logical intermediates in the pathways defined. Conjugates are abbreviated as follows: gly, glycine; glut, glutamic acid; gluc, glucuronide; conj, unidentified, acid- and/or base-labile conjugate.

Metabolites of cis- and trans-Permethrin in Goats

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Table III. Metabolites in Feces of Lactating Goats Treated Orally for Ten Consecutive Days with [^{14}C]Alcohol- or [^{14}C]Acid-Labeled Preparations of cis- or trans-Permethrins[a]

metabolite[b] or fraction	% for indicated goat and sample					
	[^{14}C-alc]per			[^{14}C-acid]per		
	3[g]	6[g]	9[g]	3[g]	6[g]	9[g]
cis-Permethrin						
c-per	14	18	20	22	27	48
4-HO-c-per	13	12	10	6	5	4
t-HO-c-per	28	29	30	33	30	23
PBalc	10	12	15			
c-HO-c-Cl$_2$CA-lactone				18	18	10
unknowns: ester (8)[c,d]	28	21	17	17	15	11
unextractable[e]	7	8	8	4	5	4
trans-Permethrin						
t-per	79	41	46	73	65	75
PBalc	8	25	25			
c-HO-t-Cl$_2$CA-lactone				6	2	5
unknowns: ester (3)[c,d]	8	23	21	16	23	12
alc (2)[d,f]	3	6	4			
acid (1)[d,f]				2	4	3
unextractable[e]	2	5	4	3	6	5

[a] See footnote b, Table II. [b] As resolved by 2-dimensional TLC (A, B × 2). [c] Intact ester metabolites as indicated by the occurrence of the same metabolites in samples from both [^{14}C-alc]- and [^{14}C-acid]per-treated goats. [d] Numbers in parentheses indicate the number of unidentified metabolites resolved from the sample extracts. [e] Radiocarbon remaining in the feces residues after methanol extraction. [f] Metabolites arising through hydrolysis of the ester moiety, as indicated by their occurrence in samples from either [^{14}C-alc]- or [^{14}C-acid]per-treated goats, but not both. [g] Days.

the [^{14}C-acid] metabolite with authentic t-HO-c-Cl$_2$CA in B, C, G, M). PBalc is a metabolite in feces from the [^{14}C-alc]-c-per goat, and c-HO-c-Cl$_2$CA-lactone occurs in samples from the [^{14}C-acid]-c-per goat (TLC in B, C, G, H, L, M in each case). Eight additional unidentified metabolites are resolved from feces extracts of the c-per goats, but none of these individually comprise >6% of the total radiocarbon in any sample. All of these products are apparently intact ester derivatives of c-per because each is seen in samples from both [^{14}C-acid]- and [^{14}C-alc]-c-per treated goats (Table III). TLC studies with some of these metabolites, both before and after derivatization or degradation procedures, did not result in their tentative identification.

Characterization of ^{14}C constituents in the feces of t-per treated goats was restricted by the low levels of radiocarbon present (Table II), but samples from both [^{14}C-acid]- and [^{14}C-alc]-t-per treatments do contain unmetabolized t-per as the major radioactive component in each case, as evidenced by TLC cochromatography (B, C, E, G, H, L) with authentic t-per and by GLC–mass spectral analysis. t-per comprises 41–79% of the total radiocarbon in these samples, depending on the sample and label position (Table III). Two additional metabolites in feces of the t-per-treated goats are tentatively identified: PBalc (TLC in B, C, G, H) and c-HO-t-Cl$_2$CA-lactone (A, B, I). Of the six unidentified metabolites in feces extracts of the t-per treated goats, three are apparently intact esters, two arise from the alc moiety, and one arises from the acid moiety (Table III). The major unidentified ester metabolite comprises 5–16% of the total radiocarbon in samples from both [^{14}C-acid]- and [^{14}C-alc]-t-per-treated goats, and on the basis of its TLC behavior in comparison with published data (Unai and Casida, 1977), this metabolite may be either c-HO-t-per or its t-HO isomer. However,

Table IV. Metabolites in Urine of Lactating Goats Treated Orally for Ten Consecutive Days with [^{14}C]Alcohol-Labeled cis- or trans-Permethrin[a]

metabolite[b] or fraction	% for indicated per isomer and sample					
	[^{14}C-alc]-c-per			[^{14}C-alc]-t-per		
	3[f]	6[f]	9[f]	3[f]	6[f]	9[f]
PBalc	<1	<1	<1	0	0	0
PBacid	2	2	1	1	1	<1
4'-HO-PBalc[c]	<1	1	1	0	0	0
4'-HO—PBacid	2	2	1	<1	<1	<1
PBacid-Gly	66	70	76	89	89	89
PBacid-Glut	4	4	5	3	3	3
4'-HO-PBacid-Gly	12	9	6	4	4	4
4'-HO-PBacid-Glut	3	2	2	1	1	1
unknowns: alc (2)[d]	4	4	2	1	1	1
water soluble[e]	7	6	5	1	1	1

[a] See footnote b, Table II. [b] As resolved by 2-dimensional TLC (A, B × 2). [c] Possibly a mixture with other, unidentified metabolites. [d] See footnotes d and f, Table III. [e] Radiocarbon remaining in the aqueous phase after ether extraction of acidified whole urine. [f] Days.

authentic standards of these compounds were not available for direct comparisons, and results in degradation studies with the rather small amounts of this metabolite obtained from feces were not conclusive. The occurrence of c-HO-t-Cl$_2$CA-lactone as a metabolite of t-per in feces further suggests that c-HO-t-per may, in fact, be present in these samples.

Metabolites in Urine. Two-dimensional TLC analysis (A, B × 2) of urine extracts from the per-treated goats resolves several metabolites in each sample, and most of these are identified by TLC and/or GLC–mass spectrometry. Eight metabolites are identified in extracts of urine from goats treated with either [^{14}C-alc]-c-per or [^{14}C-alc]-t-per (Table IV). PBalc and PBacid and their 4'-HO-analogues are identified by TLC with appropriate authentic standards in solvent systems B, C, D, F, M, and O. Also, methylation of the PBacid metabolite from urine gives a product that cochromatographs on TLC with authentic PBacid-Me (B, C, D, F, M, O). Treatment of 4'-HO-PBacid from urine with diazomethane results in the expected two-step methylation, first to 4'-HO-PBacid-Me, then to 4'-MeO-PBacid-Me, both of which are confirmed by TLC cochromatography with appropriate standards (B, C, D, F, M, O). The glycine and glutamic acid conjugates of PBacid and 4'-HO-PBacid are also identified from these urine samples (Table IV). These metabolites from urine, after methylation, cochromatograph on TLC (B, C, D, Q) with the appropriate unlabeled standards (Table I), and the assigned structures are confirmed in each case by GLC–mass spectrometry. Although Gaughan et al. (1978a) reported earlier that solvent system B is not acceptable for the resolution of PBacid-Gly and PBacid-Glut, this system (B × 2) in our hands adequately separated these products. Two minor uncharacterized metabolites are seen in urine extracts of the [^{14}C]alc-per-treated goats. Radiocarbon not extracted from the urine aqueous phase accounts for only 1–7% of the total (Table IV), and this radiocarbon was not further studied.

The nature and distribution of metabolites in urine of the goats treated with [^{14}C-acid]-labeled preparations of c-per or t-per are shown in Table V. Five metabolites are identified in extracts of urine from each of these goats, including the appropriate c or t isomers of Cl$_2$CA and c-HO-Cl$_2$CA-lactone, each of which is identified on the basis of TLC (B, C, D, F, M, O, Q) with authentic standards. The methyl esters of the Cl$_2$CA metabolites from

Table V. Metabolites in Urine of Lactating Goats Treated Orally for Ten Consecutive Days with [¹⁴C]Acid-Labeled cis- or trans-Permethrin[a]

metabolite[b] or fraction	% for indicated per isomer and sample					
	[¹⁴C-acid]-c-per			[¹⁴C-acid]-t-per		
	3[g]	6[g]	9[g]	3[g]	6[g]	9[g]
Cl_2CA	4	2	2	10	47	15
Cl_2CA-gluc	37	45	47	71	27	67
Cl_2CA-conj[c]	0	0	0	5	10	5
t-HO-Cl_2CA	2	1	1	3	3	2
c-HO-Cl_2CA	11	9	9	5	5	4
c-HO-Cl_2CA-lactone	16	12	11	1	<1	1
unknowns: acid (5,2)[d]	11	11	12	<1	1	1
water soluble:[e,f]		20	18		6	5
Cl_2CA	1			3		
t-HO-Cl_2CA	4			1		
unknowns (3,2)[d]	6			<1		
water	8			<1		

[a] See footnote b, Table II. [b] As resolved by 2-dimensional TLC (A, B × 2). [c] Conjugate of t-Cl_2CA that was hydrolyzable by acid but not β-glucuronidase. [d] See footnotes d and f, Table III. The numbers of unidentified metabolites in samples from c-per- and t-per-treated goats, respectively, are indicated in parentheses. [e] See footnote e, Table IV. [f] Acid hydrolysis of 3-d water-soluble radiocarbon gave the indicated distribution of aglycons and residual water-soluble products. [g] Days.

urine are also confirmed by TLC to be the same as authentic c- and t-Cl_2CA-Me, and the t-Cl_2CA metabolite from urine (methylated) is additionally confirmed by GLC–mass spectrometry. t-HO-c-Cl_2CA from urine of the c-per treated goat (and its methyl ester) are the same on TLC (B, C, D, F, M, O, Q) as authentic t-HO-c-Cl_2CA and t-HO-c-Cl_2CA-Me. Although t-HO-t-Cl_2CA was not available as an authentic standard, this product is a metabolite in urine of the [¹⁴C-acid]-t-per-treated goat, on the basis of similar TLC properties to authentic t-HO-c-Cl_2CA and upon GLC–mass spectral studies that indicate that this metabolite has GLC behavior and mass fragmentation patterns similar to those of authentic t-HO-c-Cl_2CA (Table I). Cis and trans isomers of c-HO-Cl_2CA were likewise not available as authentic standards, but these products are identified in urine on the basis of their rapid lactonization

to c-HO-c- or t-Cl_2CA-lactones upon attempted isolation from silica gel, as confirmed by TLC (B, C, D, F, M, O, Q) with the authentic lactones and by GLC–mass spectrometry.

The major metabolites in urine of both [¹⁴C-acid]-c-per and [¹⁴C-acid]-t-per treated goats are the glucuronide conjugates of Cl_2CA. The isolated metabolites yield either c- or t-Cl_2CA (TLC of the aglycons and their methyl esters with appropriate authentic standards in B, C, D, F, M, O, Q) upon incubation with β-glucuronidase, a reaction that is inhibited by the addition of D-saccharic acid 1,4-lactone. The conjugates are also cleaved to Cl_2CA upon heating with acid (TLC in B, C, D, F, M, O, Q). Another, unidentified conjugate of Cl_2CA is present in extracts of urine from the goat treated with t-per, but not from the goat treated with c-per. This metabolite yields t-Cl_2CA (TLC in B, C, D, F, M, O, Q) upon hydrolysis with acid but not β-glucuronidase. Results in TLC studies with the intact conjugate (after methylation) indicate that it is not the glycine, alanine, or glutamic acid conjugate of t-Cl_2CA. Five additional unidentified metabolites are resolved from urine extracts of the [¹⁴C-acid]-c-per-treated goat, but none of these comprise >3% of the radiocarbon in any sample. Urine extracts of samples from the [¹⁴C-acid]-t-per treated goat contain only trace levels of two unidentified metabolites.

About 20% and 5% of the urinary radiocarbon remains as water solubles after ether extraction of urine samples from the [¹⁴C-acid]-c-per- and -t-per-treated goats, respectively (Table V). Acid hydrolysis of the 3-d water-soluble urinary radiocarbon, followed by solvent extraction and TLC, indicates that Cl_2CA and t-HO-Cl_2CA, as well as unidentified products, are present as conjugates in these water phases (Table V).

Metabolites in Milk. Ether extraction of acidified samples of whole milk from each of the [¹⁴C]per-treated goats recovers 80–100% of the radiocarbon present, and subsequent removal of milk fats with hexane does not result in appreciable losses of radiocarbon (Table VI). TLC analyses of the milk extracts show that unmetabolized per is a major component in all samples, on the basis of TLC with the appropriate per isomer, of alkaline hydrolysis of the [¹⁴C-alc]-per in milk to PBalc, and of alkaline hydrolysis of the [¹⁴C-acid]-per in milk to c- or

Table VI. Metabolites in Milk of Goats Treated Orally for Ten Consecutive Days with [¹⁴C]Alcohol- or [¹⁴C]Acid-Labeled Preparations of cis- or trans-Permethrin[a]

label position, per isomer, and day	% indicated metabolite[b] or fraction						
	per	t-HO-c-per	PBacid-Gly	4'-HO-PBacid-Gly	origin[c]	hexane soluble[d]	water soluble[e]
[¹⁴C]alc-c-per							
3	43	5	10	3	10	16	13
6	47	3	11	1	9	18	11
9	53	4	12	1	4	19	7
[¹⁴C]acid-c-per							
3	58	7			2	23	10
6	62	8			3	11	16
9	68	5			3	17	7
[¹⁴C]alc-t-per							
3	22		71	1	1	2	2
6	21		70	4	<1	5	0
9	24		68	3	<1	3	2
[¹⁴C]acid-t-per							
3[f]							
6	41				26	13	20
9	45				24	14	17

[a] See footnote b, Table II. [b] As resolved by TLC (2 × B) of milk extracts. [c] Radiocarbon remaining at the origin after TLC. [d] Radiocarbon remaining in the hexane phase after hexane/acetonitrile partitioning of the milk extracts to remove milk fats. [e] Radiocarbon remaining in the aqueous phase after ether extraction of acidified whole milk. [f] Sample inadvertently contaminated with urine from [¹⁴C-alc]-c-per- or -t-per-treated goat, and thus metabolite distribution could not be accurately determined.

Metabolites of *cis*- and *trans*-Permethrin in Goats

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Table VII. Nature of Radiocarbon in Fat and Liver of Goats Treated Orally for Ten Consecutive Days with [^{14}C]Acid- or [^{14}C]Alcohol-Labeled Preparations of *cis*- or *trans*-Permethrin[a]

| | % for indicated per isomer and label position | | | |
| | c-per | | t-per | |
tissue and metabolite[b] or fraction	[^{14}C-acid]	[^{14}C-alc]	[^{14}C-acid]	[^{14}C-alc]
fat				
per	59	38	75	80
t-HO-per	8	7	0	0
t-HO-per-conj	11	16	0	0
hexane soluble[c]	22	39	15	17
residue	<1	<1	10	3
liver extract				
acetonitrile	14	18	20	29
hexane soluble[c]	7	23	11	12
acid-hydrolyzed residue				
ether extract	39	48	36	59
residue	40	11	33	0

[a] See footnote *b*, Table II. [b] As resolved by TLC (B × 1 or F × 1) of tissue extracts. [c] Radiocarbon remaining in the hexane phase after hexane/acetonitrile partitioning of the tissue extracts to remove lipids.

t-Cl$_2$CA. The identifications are confirmed in each case by TLC with appropriate standards in at least four of the following solvent systems: A, B, C, D, G, M, N, O, Q, S. *t*-HO-*c*-per occurs in small quantities in the milk of the *c*-per-treated goats (TLC with authentic *t*-HO-*c*-per, TLC of the alkaline hydrolysis product of the [^{14}C-alc] metabolite with authentic PBalc, TLC of the alkaline hydrolysis product of the [^{14}C-acid] metabolite with *t*-HO-*c*-Cl$_2$CA, and each product confirmed in solvent systems B, C, D, F, M, O, Q).

The glycine conjugates of PBacid and 4'-HO-PBacid appear in trace to major amounts in milk of the [^{14}C-alc]-per-treated goats, on the basis of TLC of the milk metabolites with the products of known identity from urine (TLC in 3–6 of solvent systems B, C, G, N, Q, S). In addition, short-term methylation of these milk metabolites gives products corresponding to authentic PBacid-gly-Me and 4'-HO-PBacid-gly-Me (TLC in B, C, D, G, O, Q, S). The only other radioactive components that are seen in any of the milk extracts consist of trace to moderate percentages of ^{14}C that remain at the origin on TLC. No attempts were made to define the chemical nature of these products.

Permethrin partitions about equally between hexane and acetonitrile, and results in studies with authentic [^{14}C]per indicate that the cleanup procedure used with milk (100 mL of hexane partitioned three times with 50-mL volumes of acetonitrile) results in 10% and 6% of the *c*- and *t*-per, respectively, residing with the final hexane phase. Thus, almost certainly, part (but probably not all) of the ^{14}C milk residues defined as hexane soluble (Table VI) is, in fact, unmetabolized per.

Metabolites in Tissues. Hexane extraction of samples of fat from each of the [^{14}C]per-treated goats recovers 90–100% of the radiocarbon present, and most of the extracted ^{14}C partitions into acetonitrile during the cleanup procedure (Table VII). TLC analysis (B × 1) resolves only a single ^{14}C component from fat extracts of *t*-per-treated goats, and this product is identified as unmetabolized *t*-per (TLC in B, C, D, F, M, O, Q).

The fact that extracts of fat from goats treated with [^{14}C-acid]- or [^{14}C-alc]-*c*-per contain the same three ^{14}C components indicates that each retains the ester linkage intact. The major of these products is *c*-per (TLC with

authentic *c*-per, TLC of the alkaline hydrolysis product of [^{14}C-alc]-*c*-per from fat with PBalc, TLC of the alkaline hydrolysis product of [^{14}C-acid]-*c*-per from fat with *c*-Cl$_2$CA, and cochromatography in B, C, D, F, M, O, Q in each case). *t*-HO-*c*-per also is present in fat of the *c*-per-treated goats on the basis of TLC with authentic *t*-HO-*c*-per and on the basis of TLC of the alkaline hydrolysis products of the [^{14}C-alc] and [^{14}C-acid] metabolites with authentic PBalc and *t*-HO-*c*-Cl$_2$CA, respectively (TLC in B, C, D, F, M, O, Q). The third metabolite in fat of the *c*-per-treated goats is a relatively nonpolar ester on the basis of its similar TLC behavior to *c*-per in several solvent systems. Alkaline hydrolysis of the [^{14}C-alc] metabolites gives PBalc, and similar hydrolysis of the [^{14}C-acid] metabolites surprisingly gives *t*-HO-*c*-Cl$_2$CA (confirmed by TLC in B, C, D, F, M, O, Q and by similar TLC of the methyl ester of the hydrolyzed [^{14}C-acid] metabolite with authentic *t*-HO-*c*-Cl$_2$CA-Me). Thus, it is apparent that this metabolite results from the conjugation of *t*-HO-*c*-per with a relatively nonpolar moiety of unknown structure which is itself labile under alkaline hydrolysis conditions. Because of the small amounts of fat available, we were not successful in isolating sufficient quantities of the metabolite for spectral studies.

In samples of liver, only 21–41% of the radiocarbon is recovered by extraction with acetonitrile–acetone, but acid hydrolysis of the extracted liver residue, followed by ether extraction, recovers an additional 36–59% (Table VII). Radiocarbon not extracted from the liver residue or hydrolyzate is minimal (0–11%) in samples from the [^{14}C-alc]per-treated goats, but comprises as much as 39% of the total radiocarbon in liver from the [^{14}C-acid]per-treated goats (Table VII).

TLC studies with the liver extracts obtained both before and after acid hydrolysis do not result in tentative characterizations of any of the ^{14}C present. TLC (B × 1) of the acetonitrile/acetone extracts after hexane cleanup shows the presence of only trace amounts of radiocarbon that remains at the origin after TLC. Extracts of the acid-hydrolyzed liver appear to contain as many as five or more ^{14}C components (depending upon the sample) that do migrate above the origin on TLC (B × 1); however, none of these products are present in more than trace amounts, and none are successfully characterized. On the basis of these data, the very low residues in liver of per-treated goats are largely, perhaps totally, in the form of conjugates or other polar metabolites or are "bound" residues.

GLC–Mass Spectrometry of Per Metabolites and Analogues. Table I shows GLC–mass spectral data for *c*- and *t*-per and each of their metabolites and analogues considered. Given the large number of per analogues studied, GLC parameters could not likely have been developed to adequately resolve each product from all others, and we did not attempt to do so here. Rather, the parameters reported in Table I were selected simply to facilitate metabolite characterization and to show the applicability of GLC–mass spectrometry to the study of a wide variety of per degradation products and analogues.

DISCUSSION

The metabolism of *c*- and *t*-per in goats as defined in the current study is summarized in Figure 1. Twenty-six metabolites are fully or partly characterized, and several others (enclosed by brackets in Figure 1), although not isolated, are logical intermediates in the pathways defined. In the goat, per is subjected to rapid and extensive degradation through hydrolytic, oxidative, and conjugative reactions. PBalc and its 4'-HO analogue, once generated within the animal, are almost quantitatively oxidized to

PBacid or 4'-HO-PBacid, which are excreted in urine primarily as conjugates with glycine or glutamic acid (Table IV). The acid moiety is likewise extensively excreted in urine, primarily as free Cl_2CA or its glucuronide conjugate, but also as hydroxlyated Cl_2CA derivatives, both free and conjugated (Table V).

That part of the dose eliminated through the feces consists primarily of unmetabolized per or hydroxylation products of intact per esters, although cleavage products (PBalc and c-HO-Cl_2CA-lactones) also appear in feces in appreciable quantity (Table III). We do not know whether the fecal metabolites are generated within the digestive tract or occur as the result of biliary or intestinal secretion.

Levels of radiocarbon secreted into milk and retained by the tissues are quite low and consist in large part of unmetabolized per in most samples, with the exception of liver. Glycine conjugates of PBacid and 4'-HO-Pbacid are significant to major metabolites in milk (Table VI), an observation that is somewhat surprising in view of their high polarity. It is most unlikely that these conjugates appear in milk through contamination by urine because they are present at consistent levels in all [^{14}C-alc]per milk samples analyzed. Large quantities of these glycine conjugates are generated within the animals (Table IV), and their absolute quantities in milk are exceedingly low; thus, they likely occur as true milk metabolites through passage in trace quantities across the blood–milk barrier.

The distribution of metabolites in feces, urine, and milk extracts is highly consistent among the 3-, 6-, and 9-d samples analyzed (Table III–VI), and the data obtained from these samples likely are representative of all milk and excreta generated throughout the 10-d treatment period. Thus, our studies result in the characterization of large percentages of the radiocarbon administered to these goats. An average of 70, 81, 74, and 75% of the total ^{14}C in feces is identified in samples from goats treated with [^{14}C-alc]-c-per, [^{14}C-acid]-c-per, [^{14}C-alc]-t-per, and [^{14}C-acid]-t-per, respectively. Identified ^{14}C comprises 89, 70, 98, and 87% of the total radiocarbon in urine and 64, 69, 95, and 43% in milk.

With reference to previously published studies on the fate of [^{14}C]per in mammals, specifically rats (Elliot et al., 1976; Gaughan et al., 1977) and lactating cattle (Gaughan et al., 1978a), the following similarities and differences in metabolism between goats and these mammals are noted. (1) In each of the three species, a greater percentage of an administered c-per dose is eliminated in the feces than is a t-per dose, a pattern that appears to be most pronounced in goats and least in cattle. It may be that t-per is absorbed more rapidly than c-per from the gastrointestinal tract; alternatively, isomer differences in the rates of biliary excretion of per and/or its metabolites may account for these observations. (2) Although retention of per by tissues and its secretion into milk of mammals is minimal, c-per and its metabolites in each of these three species are retained by tissues to a more significant extent than is t-per. In goats, a greater percentage of administered [^{14}C]-c-per is secreted into milk than is t-per, but these differences are not apparent in cattle. (3) Primary metabolism of per by rats involves attack at five major sites, including ester cleavage, hydroxlyation at the *cis*- or *trans*-methyl of the geminal dimethyl moiety, and hydroxlylation at the 2' or 4' position of the phenoxybenzyl moiety. Cattle and goats metabolize per similarly, except that 2'-hydroxylation apparently does not occur in these ruminants. (4) Ester

metabolites of per are eliminated primarily through the feces of rats, cattle, and goats. Cattle eliminate large quantities of ester metabolites of both c-per and t-per in feces, but rats and goats eliminate considerably more c-per esters than t-per esters in feces. (5) Conjugation of per metabolites before urinary excretion is extensive in each species. Rats, cattle, and goats eliminate the acid moiety in urine primarily as conjugates with glucuronic acid. The alcohol moiety is excreted mostly as PBacid-Gluc or as 4'-HO-PBacid-sulfate in rats, but amino acid conjugates of PBacid comprise most of the excreted products in cattle and goats. Conjugation of PBacid with glycine is preferred in goats, but conjugation with glutamic acid is favored in cattle.

From the standpoint of minimizing toxicological hazards to man and animals, pesticides should be highly biodegradable and should show minimal tendency toward retention or accumulation of residues by animal tissues or toward secretion into edible animal byproducts. Results in studies with permethrin in mammals, including two species of lactating ruminants, indicate that this highly efficacious and selective insecticide has these desirable characteristics. It therefore seems unlikely that the proposed uses of permethrin in controlling pest arthropods will result in significant toxicological hazards to target or nontarget mammals, or in the appearance of appreciable residues in meat or milk reaching the human food supply.

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