Distribution and Metabolism of *trans*- and *cis*-Permethrin in Lactating Jersey Cows

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Radiocarbon from ^{14}C-acid- and ^{14}C-alcohol-labeled preparations of *trans*- and *cis*-permethrin administered orally to lactating Jersey cows for three consecutive days at ~1.0 mg/kg is largely eliminated from the body within 12 or 13 days after the initial treatment. Milk and fat residues, although relatively low, are higher with *cis*- than with *trans*-permethrin and consist almost entirely of unmetabolized compound. *cis*-Permethrin hydroxylated at the methyl group trans to the ester functionality also appears in trace levels in milk. Major excreted metabolites (each 8–28% of the administered radiocarbon) from both isomers are: the esters hydroxylated at the trans methyl group; the acid moieties hydroxylated at the cis methyl group and the corresponding γ-lactones; 3-phenoxybenzyl alcohol; the glutamic acid conjugate of 3-phenoxybenzoic acid. An additional 13 excreted metabolites of *trans*-permethrin and 10 of *cis*-permethrin are tentatively identified.

The pyrethroid permethrin (Elliott et al., 1973) is highly effective in controlling insect pests of livestock and of agricultural crops used as feed and forage for lactating cows. Studies with rats establish that orally administered permethrin is rapidly metabolized by hydrolysis and by hydroxylation of the methyl groups and the phenoxy ring and that *trans*-permethrin is metabolized more rapidly and gives smaller amounts of excreted ester metabolites than *cis*-permethrin (Elliott et al., 1976; Gaughan et al., 1977a,b).

It is of interest to compare the distribution and metabolism of permethrin in a ruminating animal to that in a laboratory rodent. The distribution and excretion rates of the permethrin isomers in goats are reported without metabolite identification (Hunt and Gilbert, 1977). In the present study, *trans*-permethrin labeled with ^{14}C in the acid and alcohol moieties and comparable ^{14}C preparations of *cis*-permethrin were administered orally to lactating Jersey cows for three consecutive days, and the animals were then held for 12 or 13 days after the initial dose prior to sacrifice in order to determine the distribution and metabolic fate of the permethrin isomers.

MATERIALS AND METHODS

Chemicals. The following [^{14}C]permethrin preparations (radiochemical purity >99%) were used: *trans*-(1*RS*)-permethrin (*t*-per) labeled in the carboxyl group of the acid moiety ([^{14}C]acid-*t*-per; 1.63 mCi/mmol) and in the methylene position of the alcohol moiety ([^{14}C]alc-*t*-per; 1.0 mCi/mmol); *cis*-(1*RS*)-permethrin (*c*-per labeled at the same positions in the acid moiety ([^{14}C]acid-*c*-per; 1.33 mCi/mmol) and in the alcohol moiety ([^{14}C]alc-*c*-per; 1.0 mCi/mmol).

Standard unlabeled compounds for tentative characterization of metabolites by cochromatography are described by Unai and Casida (1977). They are designated as shown in Figure 1, i.e., Cl_2CA and PBalc refer to the acid and alcohol moieties, a 4′-HO derivative is hydroxylated at the 4′ position of the phenoxybenzyl group, *t*-HO and *c*-HO refer to the trans and cis positions, respectively, of the hydroxymethyl substituent relative to the carboxyl group, PBacid is 3-phenoxybenzoic acid, and gluc, gly, and glut are glucuronide, glycine, and glutamic acid conjugates, respectively.

Chromatography. [^{14}C]Permethrin metabolite mixtures were separated for radioassay of individual components by thin-layer chromatography (TLC) on silica gel 60 F-254 chromatoplates (0.25 mm gel thickness) using two-dimensional development as follows: for ester products, benzene–ethyl acetate (6:1) (solvent system A) in the first direction and carbon tetrachloride–ether (3:1) (solvent system B) in the second direction; for hydrolysis products and their oxidized derivatives and conjugates 1-butanol–glacial acetic acid–water (6:1:1) (solvent system C) in the first direction followed by two developments with benzene (saturated with formic acid)–ether (10:3) (solvent system D) or benzene (saturated with formic acid)–ether (1:1) (solvent system E) in the second direction. Solvent system E but not C and D adequately separate the amino acid conjugates, i.e., R_f values for *t*-Cl_2CA-glut, PBacid-gly, and PBacid-glut, respectively, are: 0.72, 0.52, and 0.52 in C; 0.06, 0.13, and 0.13 in D; 0.21 and 0.13 for the latter two compounds in E. Additional solvent systems used as specified later were: benzene–ethyl acetate–methanol (15:5:1) (F); chloroform (saturated with formic acid)–ether (10:3) (G); hexane–ether (10:1) (H); carbon tetrachloride–hexane–ether (20:2:1) (I); ethyl acetate–methanol–water (13:3:1) (J) (R_f 0.36 for 4′-HO-PBacid-sulfate).

The chromatographic properties in these systems of all standard compounds and metabolites (other than the conjugates indicated above) are give by Gaughan et al. (1977a) and Unai and Casida (1977). There are three further exceptions which are compounds not available as authentic standards but detected as metabolites, i.e., the glucuronides of *c*-HO,*t*-per, *t*-HO,*t*-per, and *t*-HO,*c*-per. On two-dimensional development with the C and D solvent systems, these conjugates of ester metabolites are not separable from each other or from 4′-HO-PBacid-sulfate (detected with [^{14}C]alc preparations only) and Cl_2CA-conj (see Gaughan et al., 1977b; this previously unknown metabolite is now identified as a mixture of *c*-HO,*t*-per-gluc and *t*-HO,*t*-per-gluc). Individual compounds within these conjugate mixtures are differentiated by the starting isomer and labeling position, by selective enzyme cleavage (glucuronidase and sulfatase) and by cochromatography

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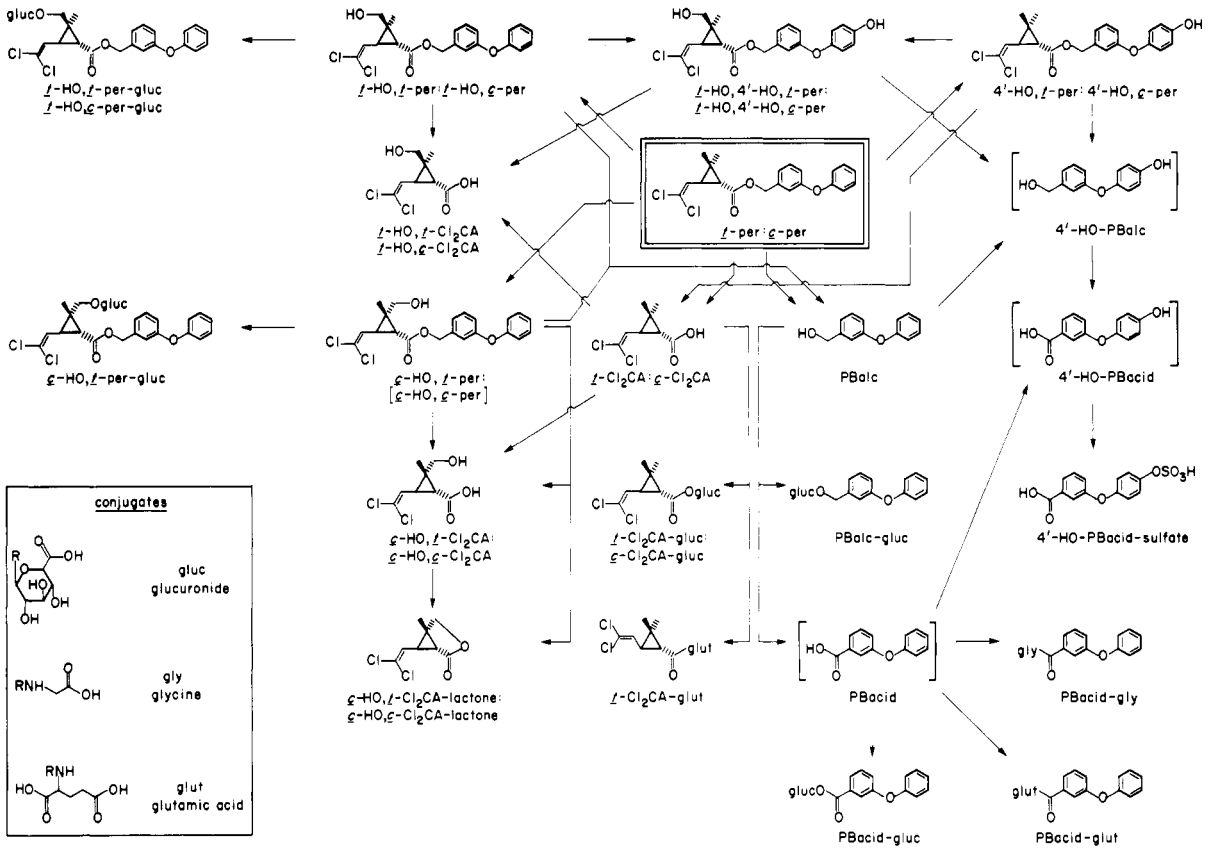

Figure 1. Metabolic pathways for *trans*- and *cis*-permethrin in cows. Although the (1*R*) isomers are shown, the metabolites are (1*RS*) isomers.

with the appropriate deconjugated products as indicated below.

Tentative Identification of Metabolites. Criteria for tentative identification of individual metabolites were generally those of Gaughan et al. (1977a). Compounds directly cochromatographed with authentic standards in the indicated solvent systems were: permethrin isomers and their monohydroxy and dihydroxy derivatives (A, B, and D); PBalc (A, B, D, and F); *c*-HO,Cl_2CA-lactones (D and G); Cl_2CA and HO-Cl_2CA isomers (D, F, and G); Cl_2CA derivatives after methylation (diazomethane) (D, F, H, and I); amino acid conjugates after methylation (diazomethane) (D and G); 4′-HO-PBacid-sulfate (C and J). For quantitation, PBacid-gly and PBacid-glut were separated with solvent system E.

Compounds designated as glucuronides were almost quantitatively cleaved by acid, base, and β-glucuronidase but were not cleaved by β-glucuronidase plus saccharic acid 1,4-lactone or by sulfatase (Gaughan et al., 1977a). The released aglucons were cochromatographed with the appropriate free carboxylic acids or PBalc, as above; solvent systems A, B, D, and F were used for PBacid. Metabolites designated as *c*- and *t*-HO-per-gluc were cleaved with β-glucuronidase and the deconjugated esters were cochromatographed (A and B) with authentic standards. On deconjugation of *c*-HO,*t*-per-gluc, a small proportion of *c*-HO,*t*-Cl_2CA-lactone is produced by cyclization during analysis.

Metabolites designated as "other" are not discrete spots in solvent systems C and D. They are probably in the most part the identified materials not adequately resolved or decomposition products of these compounds during sample storage or analysis, particularly with the fecal metabolites of [^{14}C]acid- and [^{14}C]alc-*c*-per.

Treatment and Handling of Animals. Each of four lactating Jersey cows in good milk production was treated with one of the four [^{14}C]permethrin preparations with three doses at 24-h intervals as follows:

Compound	Wt of cow, kg	Dose, mg/kg	Sacrifice, days
[^{14}C]acid-*t*-per	352	1.00	13
[^{14}C]alc-*t*-per	371	1.09	12
[^{14}C]acid-*c*-per	440	1.00	13
[^{14}C]alc-*c*-per	444	0.92	12

The [^{14}C]permethrin sample in absolute ethanol (33 mL) was administered via a tube through the mouth into the rumen with an additional ethanol rinse (50 mL) of the dosing tube. These treatments are equivalent to dietary levels of 33–39 ppm permethrin. The initial dose was administered after at least 4 days acclimatization of the animals in metabolism units involving head chambers for collection of $^{14}CO_2$ and a catheter inserted into the bladder for urine collection. The animals were maintained on a normal ration of high protein concentrate, grain, and hay with water ad libitum. Urine, feces, and milk were collected every 12 h for 72 h following the initial dose and every 24 h thereafter. Blood samples were taken from the jugular vein at 4, 8, and 24 h after each dose and at 24-h intervals thereafter until sacrifice. The cows suffered a weight loss of 12–16% during the experimental period except the one treated with [^{14}C]alc-*t*-per which suffered a 23% weight loss. This latter animal did not eat normally, not only during the dosing period but for several days afterwards, and the decreased food consumption was reflected in the marked reduction in milk and feces production for the fourth through the tenth days as compared with the other cows. The administered permethrin dose had no adverse effect on the cows, and on sacrifice the tissues and organs appeared normal. These

studies were carried out at the Analytical Development Corporation (Monument, Col.), and after total ^{14}C determination (by combustion) the frozen samples for analysis of individual ^{14}C components were transferred to the Pesticide Chemistry and Toxicology Laboratory (Department of Entomological Sciences, University of California, Berkeley, Calif.)

Analysis of Milk. Individual milk samples (100 mL) were extracted with hexane (50 mL) or 1:1 hexane–acetonitrile (100 mL), usually with centrifugation for phase separation, to obtain organosoluble and aqueous fractions for direct liquid scintillation counting and an occasional precipitate from the organosoluble portion analyzed by combustion.

More complete analyses were made on composite 2- and 3-day milk samples from all treated animals and also on 1- and 2-day milk samples following [^{14}C]alc-*t*-per treatment. A mixture of milk (25 mL) and potassium oxalate in water (5% w/v, 2 mL) was extracted with ether–ethanol (3:1) (40 mL × 2). The organic extracts, containing essentially all of the ^{14}C, were dried (Na_2SO_4), evaporated, and the residue was dissolved in pentane and introduced onto a column containing silica gel 60 (30–70 mesh, EM Reagents, Elmsford, N.Y.) (15 g) packed in hexane and topped with a layer of Na_2SO_4 (1–2 g). Development involved, in sequence, hexane–ether (5:1) (40 mL) (eluting permethrin and butterfat), hexane–ether (1:1) (60 mL), ether (40 mL) (eluting HO-per derivatives, if any), and methanol (40 mL), collecting 5-mL fractions for total ^{14}C determination. Following evaporation of the fractions under N_2, [^{14}C]permethrin was quantitatively recovered from the butterfat (~0.5 mL) by extracting with acetonitrile (5 mL × 2). This acetonitrile extract and ^{14}C metabolites in the ether fraction, if any, were individually analyzed by TLC using the two-dimensional system for separation of esters.

Analysis of Fat. Fat samples (composite of 10 g of visceral and 10 g of kidney fat) were extracted with hexane (30 mL × 5) by grinding with a mortar and pestle to obtain the combined hexane extracts and a fibrinous residue (analyzed by combustion). These combined hexane extracts were adjusted to a total volume of 200 mL and cooled, repeating this procedure five times to remove maximum insoluble material (analyzed by combustion). The hexane extract was then evaporated to a fatty residue (~12 mL) which was extracted with acetonitrile (30 mL × 5). The acetonitrile extract was cooled, a nonradioactive precipitate removed, the extract was evaporated, and the residue was subjected to column chromatography and TLC as for milk.

Analysis of Liver. The liver was homogenized in water and the homogenate lyophilized to dryness. The residue was finely powdered and an aliquot (1 g) was added to 6 N HCl (5 mL) in an ampule. After heating (100 °C, 4 h) and cooling, water (2 mL) and $(NH_4)_2SO_4$ (1 g) were added, and the mixture was extracted with ether–ethanol (3:1) (10 mL × 2). Following TLC cleanup of the ether–ethanol soluble products on chromatoplates of 0.5-mm layer thickness (otherwise as above) using solvent system D, the ^{14}C compounds recovered by methanol extraction of the appropriate gel regions were subjected to cochromatography with suitable standards in the usual manner.

Milder extraction procedures, applied to the homogenates or the powders from lyophilization and involving a variety of solvents and enzyme treatments, recovered much lower proportions of the liver ^{14}C in a soluble form and very little of this amount was extractable with ether–ethanol as above. Thus, only the acid digestion method, which probably decomposes some metabolites, releases significant amounts of products in a suitable form for analysis.

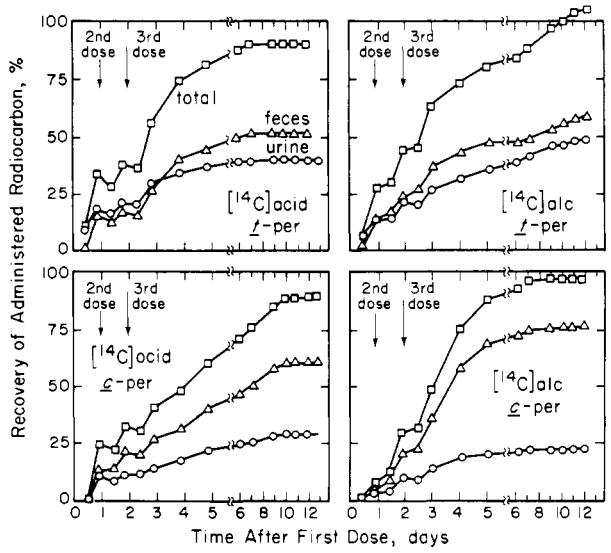

Figure 2. Radiocarbon recovery in urine and feces within 12 or 13 days after intiating a treatment schedule consisting of three daily doses of [^{14}C]acid- or [^{14}C]alcohol-*trans*- and *cis*-permethrin at ~1 mg/kg for each dose.

Table I. Radiocarbon Recovery in Excreta, Milk, Tissues, Organs, and Gut Contents 12 or 13 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [^{14}C]acid- or [^{14}C]alcohol-*trans*- and -*cis*-Permethrin at ~1 mg/kg for Each Dose

Sample	Recovery of administered radiocarbon, %			
	Trans		Cis	
	Acid	Alc	Acid	Alc
Urine	38.98	46.71	28.46	22.22
Feces	51.60	57.24	60.06	75.85
Carbon dioxide[a]	<0.01	<0.01	<0.01	<0.01
Milk	0.03	0.44	0.26	0.18
Fat	0.15	0.40	1.59	0.64
Liver	0.03	0.04	0.08	0.06
Muscle	0.01	0.04	0.03	0.11
Skin	0.01	0.07	0.04	0.06
Other tissues[b]	0.04	0.02	0.03	0.04
Gut contents[c]	<0.01	2.83	0.15	0.05
Total	90.85	107.79	90.70	99.21

[a] Collected for 6 days only. [b] See Table II and Figure 3. [c] Includes bile.

Analysis of Excreta. Aliquots (50 μL) of individual or composite urine samples were spotted directly on TLC chromatoplates (Gaughan et al., 1977a). Feces samples (30 g) were homogenized (polytron) in methanol (100 mL), the mixture filtered (Buchner), and the residue held for 24 h in an additional portion of methanol (100 mL) prior to filtration and combination of the filtrates. Aliquots (equivalent to 200–300 mg of feces) of individual or composite fecal methanol extracts were analyzed by TLC. Radiocarbon in the unextractable portion was determined by combustion.

RESULTS

Distribution of Radiocarbon from [^{14}C]acid- and [^{14}C]alc-*trans*- and -*cis*-Permethrin. The ^{14}C excretion curves (Figure 2), balance sheets (Table I) and [^{14}C]-permethrin equivalents residing in the animals on sacrifice (Table II) establish consistent differences between *t*- and

Table II. [^{14}C]Permethrin Equivalents in Tissues, Organs, and Gut Contents 12 or 13 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [^{14}C]acid- or [^{14}C]alcohol-*trans*- and -*cis*-Permethrin at ~1 mg/kg for Each Dose

	[^{14}C]Permethrin equivalents, ppb[a]			
	Trans		Cis	
Sample	Acid	Alc	Acid	Alc
	Tissues			
Fat				
Kidney	<35[b]	109[b]	335[b]	119[b]
Subcutaneous		<56		101
Visceral	<35[b]	96[b]	202[b]	95[b]
Liver	72[c]	122[d]	210[e]	158[f]
	Bile and Gut Contents			
Bile	<35	588	121	64
Intestine				
Large	<35	1824	<39	<56
Small	<35	363	<39	<56
Rumen	<35	876	40	<56

[a] Both the trans and cis isomers of [^{14}C]acid- and [^{14}C]-alc-per give residues of <56 ppb permethrin equivalents in the brain, heart, kidney, lung, leg muscle, loin muscle, and skin. [b] Mostly or entirely unmetabolized permethrin. [c] 19 ppb extractable products (made up only of approximately equal amounts of *t*-per and *t*-Cl_2CA) and 53 ppb unextractable [^{14}C]permethrin equivalents. [d] 83 ppb extractable products (made up only of approximately equal amounts of *t*-per, PBalc, and an unidentified cleavage product) and 39 ppb unextractable [^{14}C]permethrin equivalents. [e] 55 ppb extractable products (made up only of approximately equal amounts of *c*-per and two unidentified cleavage products) and 155 ppb unextractable [^{14}C]permethrin equivalents. [f] 81 ppb extractable products (made up only of approximately equal amounts of *c*-per and PBalc) and 77 ppb unextractable [^{14}C]permethrin equivalents.

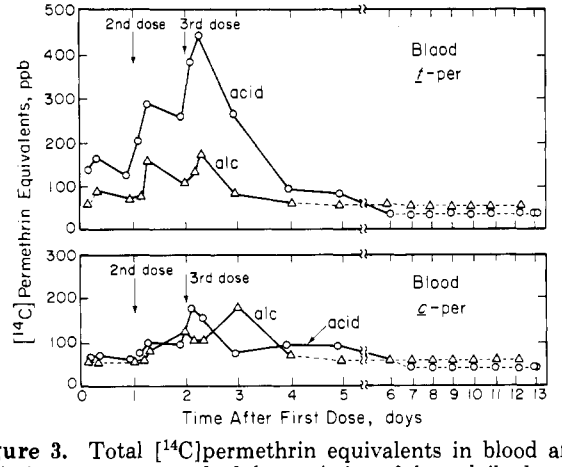

Figure 3. Total [^{14}C]permethrin equivalents in blood after initiating a treatment schedule consisting of three daily doses of [^{14}C]acid- or [^{14}C]alcohol-*trans*- and -*cis*-permethrin at ~1 mg/kg for each dose. The portion of the graphs indicated with dotted lines are the upper limits based on the sensitivity of the method.

c-per, regardless of the labeling position, as follows: more rapid elimination of *t*-per and its metabolites than of *c*-per and its metabolites; greater relative importance of the urinary excretion route for metabolites of *t*-per than of *c*-per; higher fat and liver ^{14}C levels with *c*-per than with *t*-per. None of the ^{14}C preparations give detectable $^{14}CO_2$ (Table I) or significant residues in tissues other than fat and liver (Tables I and II).

The ^{14}C blood levels (Figure 3) indicate that *t*- and *c*-per or their metabolites reach a transient peak shortly after each dose and drop to insignificant levels within 2 to 4 days

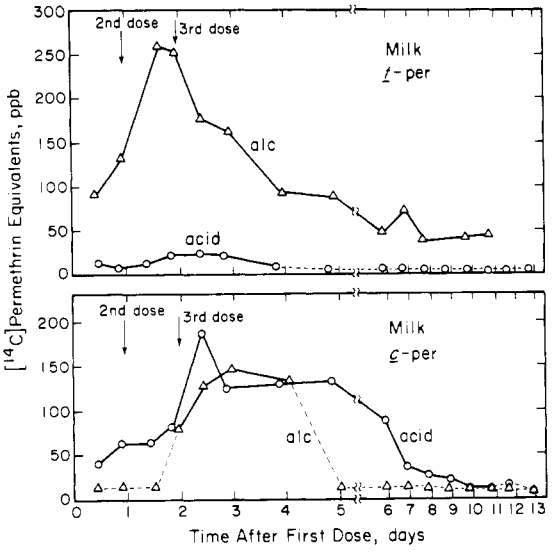

Figure 4. Total [^{14}C]permethrin equivalents in milk after initiating a treatment schedule consisting of three daily doses of [^{14}C]acid- or [^{14}C]alcohol-*trans*- and -*cis*-permethrin at ~1 mg/kg for each dose. The portion of the graphs indicated with dotted lines are the upper limits based on the sensitivity of the method.

after the last dose. Higher blood levels are attained with [^{14}C]acid-*t*-per than with [^{14}C]alc-*t*-per, a difference with labeling position not evident with *c*-per.

Milk levels of [^{14}C]permethrin equivalents are relatively low considering the total administered dose (~3 mg/kg over 3 days) (Figure 4). The lowest milk ^{14}C level is from [^{14}C]acid-*t*-per and the highest with [^{14}C]alc-*t*-per, the two labeled cis preparations giving intermediate and somewhat similar values. With all labeled preparations, the [^{14}C]-permethrin equivalent levels drop below 100 ppb within 2 to 4 days after termination of the treatment schedule.

The cow administered [^{14}C]alc-*t*-per did not eat normally during the early stages of the study and did not produce normal amounts of milk and feces. This may have contributed to the apparently high values for milk, fat, and gut contents including bile (Tables I and II).

Permethrin and Metabolites in Milk. In general, with all labeled preparations the ^{14}C compounds in milk throughout the sampling period are largely extractable (70–90%) with hexane or hexane–acetonitrile, suggesting that they are relatively apolar compounds and are possibly esters in the most part.

With milk samples at 1 to 3 days after initiating the treatment schedule, the oxalate and ether–ethanol extraction method provides essentially quantitative recoveries of the ^{14}C products. On chromatography of these extracts on silica gel columns (73–94% ^{14}C recovery), the only product recovered from [^{14}C]acid- and [^{14}C]alc-*t*-per is unmetabolized compound whereas with [^{14}C]acid- and [^{14}C]alc-*c*-per 85% of the ^{14}C is unmetabolized compound and 15% is *t*-HO,*c*-per. For confirmation, the [^{14}C]acid-per from milk was hydrolyzed with base (Gaughan et al., 1977a) and it yielded only *t*- and *c*-Cl_2CA. The abnormally high ^{14}C milk levels from [^{14}C]alc-*t*-per (see above) are probably due to permethrin per se based on analysis of both 1–2- and 2–3-day composite milk samples and since there is no time-dependent difference in the ease of extraction of the ^{14}C into organic solvent. Thus, most of the ^{14}C in milk is likely to be the unmetabolized permethrin isomer.

Permethrin and Metabolites in Fat. The ^{14}C compounds in fat from animals treated with each of the labeled preparations are recovered to the extent of 66% following

Table III. Radiocarbon Recovery as Permethrin and Its Metabolites in Excreta 12 or 13 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [^{14}C]acid- or [^{14}C]alcohol-*trans*- and -*cis*-Permethrin at ~1 mg/kg for Each Dose

Compound	Radiocarbon recovery, % for indicated isomer[a] Trans	Cis
Permethrin and Hydroxypermethrin Derivatives[b]		
Per[c]	5.6	9.3
c-HO,per[c]	2.2	0.0
t-HO,per[c]	22.4	14.4
4′-HO,per[c]	0.7	5.6
t-HO,4′-HO,per[c]	6.9	3.8
c-HO,per-gluc[d]	0.1	0.0
t-HO,per-gluc[d]	1.7	4.9
Total	39.6	38.0
Metabolites of the Acid Moiety		
Cl_2CA[d]	1.7	0.5
Cl_2CA-gluc[d]	19.2	4.1
Cl_2CA-glut[d]	0.4	0.0
c-HO,Cl_2CA[d]	7.8	1.1
c-HO,Cl_2CA-lactone[e]	5.5	7.5
t-HO,Cl_2CA[d]	1.7	4.1
Others[e]	12.3	29.5
Unextractable[c]	5.6	9.2
Total	54.2	56.0
Metabolites of the Alcohol Moiety		
PBalc[c]	9.9	7.7
PBalc-gluc[d]	1.5	0.8
PBacid-gluc[d]	1.5	0.7
PBacid-gly[d]	11.1	3.4
PBacid-glut[d]	27.8	11.9
4′-HO-PBacid-sulfate[d]	1.0	1.0
Others[e]	6.2	24.7
Unextractable[c]	2.1	4.3
Total	61.1	54.5

[a] Corrected for average TLC recoveries of 90%, a value found to be independent of the isomer, labeling position, and type of sample analyzed. Thirteen days with [^{14}C]-acid-*t*- and -*c*-per with 0.0 and 0.3%, respectively, of the administered ^{14}C excreted during the last day. [b] Average of results with [^{14}C]acid and [^{14}C]alc preparations. [c] Appear only in feces. [d] Appear only in urine. [e] Appear in both feces and urine in the following amounts, respectively: *c*-HO,Cl_2CA-lactone, 4.7 and 0.8% from [^{14}C]acid-*t*-per and 5.1 and 2.4% from [^{14}C]acid-*c*-per; others, 5.9 and 6.4% from [^{14}C]acid-*t*-per, 20.2 and 9.3% from [^{14}C]acid-*c*-per, 5.0 and 1.2% from [^{14}C]alc-*t*-per, and 23.2 and 1.5% from [^{14}C]alc-*c*-per.

extensive cleanup involving extractions, precipitations, and column chromatography. All except 3% of the losses occur equally at the steps of removing hexane-insoluble materials on cooling and of extraction of the fatty residue with acetonitrile. More than 90% of the recovered ^{14}C is unmetabolized permethrin. Since the losses are independent of isomer and labeling position, it appears likely that they also represent unmetabolized permethrin (Table II).

Permethrin and Metabolites in Liver. The acid digestion and extraction procedure releases 26–27% of the total liver ^{14}C from [^{14}C]acid-per and 51–68% of the total liver ^{14}C from [^{14}C]alc-per. Much of the liberated ^{14}C is unmetabolized permethrin and its cleavage products (Cl_2CA and PBalc; a portion of the cleavage may result during acid digestion) (Table II). There are also unidentified cleavage products from [^{14}C]alc-*t*-per and [^{14}C]acid-*c*-per (Table II), and these compounds, which appear at low R_f but move free of the origin with solvent system D, do not cochromatograph with any of the authenic standards.

Identity of Excreted Metabolites. As shown in Table III, the identified excreted products account for a high proportion of the administered dose (75.9% with [^{14}C]-acid-*t*-per; 92.4% with [^{14}C]alc-*t*-per; 63.5% with [^{14}C]-alc-*c*-per) except with [^{14}C]acid-*c*-per (55.3%) where problems may have been encountered on metabolite stability during sample storage. The permethrin isomers, their mono- and dihydroxy derivatives, and PBalc appear only in the feces while the *c*-HO-Cl_2CA-lactones appear in both feces and urine. The remaining metabolites (Cl_2CA,*c*- and *t*-HO-Cl_2CA and various conjugates) appear only in the urine. Although a slightly larger proportion of *c*-per than of *t*-per is excreted without metabolism, there are similar amounts of ester metabolites with both isomers. These large amounts of ester metabolites are hydroxylated at the trans or cis methyl position of the geminal dimethyl group or at the 4′ position of the phenoxybenzyl group or at both the geminal dimethyl and phenoxy groups. The preferred hydroxylation site with both isomers is the trans methyl group. The major ester metabolite is the *t*-HO derivative of *t*- and *c*-per present both free and as the glucuronide. No *c*-HO,*c*-per or its glucuronide is detected, this hydroxy metabolite possibly undergoing lactonization in the cow or its feces. The major metabolites of the acid moiety from both isomers are the corresponding *c*-HO,-Cl_2CA derivatives and their γ-lactones and the Cl_2CA-gluc derivatives while *t*-HO,*c*-Cl_2CA is also a major metabolite of *c*-per. The major metabolites from the alcohol moiety from both *t*- and *c*-per are PBacid-glut, PBalc, and PBacid-gly.

DISCUSSION

The metabolic pathways for the permethrin isomers in cows (Figure 1) illustrate the variety of mechanisms available to convert these compounds into more polar derivatives for excretion. Eighteen metabolites are identified from *t*-per and 15 from *c*-per. In comparison with rats (Elliott et al., 1976; Gaughan et al., 1977a), cows excrete a larger proportion of ester metabolites including their glucuronides, are unique in utilizing glutamic acid for conjugation of the carboxylic acid metabolites, and carry out more extensive hydroxylation on the trans methyl group and less on the phenoxy group. One effect of these differences is that 4′-HO-PBacid-sulfate is a major metabolite in rats but not cows.

The permethrin isomers, although fat-soluble materials, are rapidly metabolized and excreted by cows so that relatively little of these compounds or their metabolites appear in milk or are retained in tissues for more than a few days. Similar results are obtained with goats (Hunt and Gilbert, 1977). In cows, the unmetabolized permethrin isomers appear in milk and fat, but only in very small amounts. The dosage levels in these studies are very high relative to likely exposure conditions with normal use for permethrin or its individual isomers. Suitable precautions in the use of permethrin should minimize or avoid milk and meat contamination with this selective and biodegradable insecticide.

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Supplementary Material Available: Complete tabulation of each excreted product separately for each labeled preparation and for urine and feces at 0–2, 2–5, 5–12 (or 13), and 0–12 (or 13) days after initiating the treatment schedule (3 pages). Table III of this paper is derived from this supplementary material. Ordering information is given on any current masthead page.

LITERATURE CITED

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

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

Gaughan, L. C., Unai, T., Casida, J. E., *J. Agric. Food Chem.* **25**, 9 (1977a).

Gaughan, L. C., Unai, T., Casida, J. E., *ACS Symp. Ser.* **42**, 186 (1977b).

Hunt, L. M., Gilbert, B. N., *J. Agric. Food Chem.* **25**, 673 (1977).

Unai, T., Casida, J. E., *J. Agric. Food Chem.* **25**, 979 (1977).

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Possible Influence of Sex and Embryonic Content on Accumulation of Some Organochlorine Pesticides in Broilers

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Low levels of hexachlorobenzene (HCB), α-, β-, and γ-hexachlorocyclohexane (HCH), heptachlor, p,p'-DDT, and dieldrin have been administered via the feed to broilers for 6 weeks. The treatments did not influence growth rate or feed conversion. The mean accumulation ratios (concentration of pesticide in the fat to its concentration in the diet) found were: HCB = 11, α-HCH = 3, β-HCH = 14, γ-HCH = 2, heptachlor(epoxide) = 5, DDT (total) = 10, dieldrin = 11. Male broilers had significantly higher accumulation ratios than females. Organochlorine pesticides carried over from parents through the egg did not influence the accumulation ratios. At the end of rearing (6 weeks), loss in feces as a proportion of daily intake was: HCB = 12%, α-HCH = 5%, β-HCH = 12%, γ-HCH = 4%, heptachlor (epoxide) = 1.5%, DDT (total) = 7%, and dieldrin = 14%.

The accumulation of organochlorine pesticides in broilers was studied rather extensively by de Vos et al. (1972) and by Onley et al. (1975). In these studies no attention was paid to possible sex differences in accumulation. However, recently Siegel et al. (1976) reported a difference in accumulation pattern of dieldrin between male and female broilers.

As a continuation of a trial on accumulation of broiler breeders (Kan and Tuinstra, 1976a,b), we investigated whether or not organochlorine pesticides transferred to the embryo would alter accumulation during rearing. A large amount in the embryo could alter the capacity of the drug-metabolizing enzymes in the liver and thus the accumulation rate.

In the published work, few broilers were used, so that effects of low levels of organochlorine pesticides on growth, feed conversion, and mortality were not demonstrated. An effect on feed conversion was detected during rearing of our broiler breeders (Kan and Tuinstra, 1976a).

We therefore set up a trial with over 1000 broilers hatched from broiler breeders of the control group and that group which received the highest dosage of organochlorine pesticides (Kan and Tuinstra, 1976b). The pesticides and concentrations used there were identical with those in the present experiment (Table I). We studied accumulation ratios (concentration of pesticide in tissue to its concentration in the diet) and possible influences of sex and amount of pesticides present in the embryo on these ratios.

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In the same trial growth feed conversion and mortality were recorded. Preliminary results have already been published (Kan and Tuinstra, 1975).

The occurrence of interactions during residue formation was not investigated, as de Vos et al. (1972) did not find these interactions in their broiler study.

MATERIALS AND METHODS

Animal Experiment. The trial was factorial with three factors: four treatments (Table I), two parental treatments, and two sexes. There were four replicate cages in each of the 16 (4 × 2 × 2) experimental groups.

Hatching eggs were collected from the broiler breeders (Kan and Tuinstra, 1976a) for 14 days in August 1974. Of 1600 chickens hatched, 256 male and 256 female day-old chicks from the two parent groups I and IV [control and highest dosed group, respectively, identical with this experiment (Table I)], were taken at random. The 1024 chicks were distributed over 64 cages in groups of 16 chicks/cage. Over the 6 weeks of the trial, average room temperature was lowered from 30 °C during the first week to 20 °C in the last 2 weeks. The broilers were kept in continuous artificial light, and relative humidity was maintained at about 60%. They were all given the basic diet specified in Table I, at 600 kg per treatment. The ingredients were checked for residues and were mixed with organochlorine pesticides as described previously (Kan and Tuinstra, 1976b) to obtain the contents as specified in Table I.

Measurements. Unincubated eggs and hatched chicks were sampled for residue analyses.

For each feeding group, residues in the feed were analyzed in four samples.

At the age of 3 and 6 weeks, each broiler was weighed.

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