is required to prevent overinterpretation; nevertheless, a half-life of elimination in eggs of about 41 days (0.693/percentage of body burden excreted daily in egg yolks) can be estimated. Comparing this to the half-life of elimination from fat of 24–27 days (Figure 1), it becomes obvious that at least 50% of the decline in HCB residues is due to elimination of parent compound in egg yolk. Other routes of HCB disappearance include metabolism and fecal excretion of parent compound. Metabolism was not evaluated, but the average weekly excrement totaled 0.85 kg/bird, so that total fecal excretion of HCB by the end of the experiment (Table VI) was only 3.8 to 5.2% of the excretion in egg yolks [100% × (concentration in feces × 0.85 kg)/(concentration in yolks × 0.013 kg × no. eggs laid)].

Finally, there is a general correlation between the total number of eggs laid up to a given time and the concentration of HCB in eggs and body fat (Table VIII). The differences are more subtle in birds with lower residue burdens and more extensive data may be required to firmly establish the relationship. In addition, it may be possible that decreased egg production resulted from a higher body burden of HCB, but there is little doubt that egg yolk is the major excretory route for HCB in laying chickens and there is a relationship between HCB residues and egg production.

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Distribution and Metabolic Fate of *trans*- and *cis*-Permethrin in Laying Hens

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Radiocarbon from ^{14}C-carbonyl- and ^{14}C-methylene-labeled preparations of (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin, administered to laying hens for 3 consecutive days at 10 mg/kg for each dose, is largely eliminated from the body within 1 day after the last dose, a portion as $^{14}CO_2$. The excreta contain all and the eggs most of the following compounds identified by thin-layer cochromatography with authentic standards and specific enzymatic hydrolysis: the unmetabolized pyrethroids; *cis*-permethrin hydroxylated at the 4′-position, at the methyl group trans to the carboxyl, and at both of these sites; the dichlorovinyl acids and their derivatives hydroxylated at the trans or cis methyl group; phenoxybenzyl alcohol, phenoxybenzoic acid and their 4′-hydroxy derivatives; sulfate, glucuronide, taurine, and other conjugates of these alcohols and acids. Residues of unmetabolized *trans*- and *cis*-permethrin in fat are 0.15 and 0.93 ppm, respectively, at 7 days after the last dose, and in eggs they reach peak levels of 0.3 and 1.2 ppm, respectively, at 3–4 days after the last dose.

The distribution and metabolic fate are defined for permethrin (Gaughan et al., 1977) and many other pyrethroids (Miyamoto, 1976) in rats and for permethrin in cows (Gaughan et al., 1978) but not for any pyrethyroid in hens. Permethrin is highly effective for housefly control and, when used as a direct spray on poultry, for northern fowl mite control (Burroughs Wellcome Co., 1978). This insecticide and its metabolites might also enter the meat and eggs of hens as a result of ingesting feed contaminated with permethrin residues.

The present investigation considers the metabolism of (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin in orally treated hens and the tissue and egg residues of the permethrin isomers and their metabolites.

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MATERIALS AND METHODS

Chemicals. Four [^{14}C]permethrin preparations with specific activities of 1.0 to 1.3 mCi/mmol and radiochemical purities of >99% were used: (1*RS*)-*trans*-permethrin (*t*-per) and (1*RS*)-*cis*-permethrin (*c*-per) each labeled in the carbonyl group of the acid moiety and the

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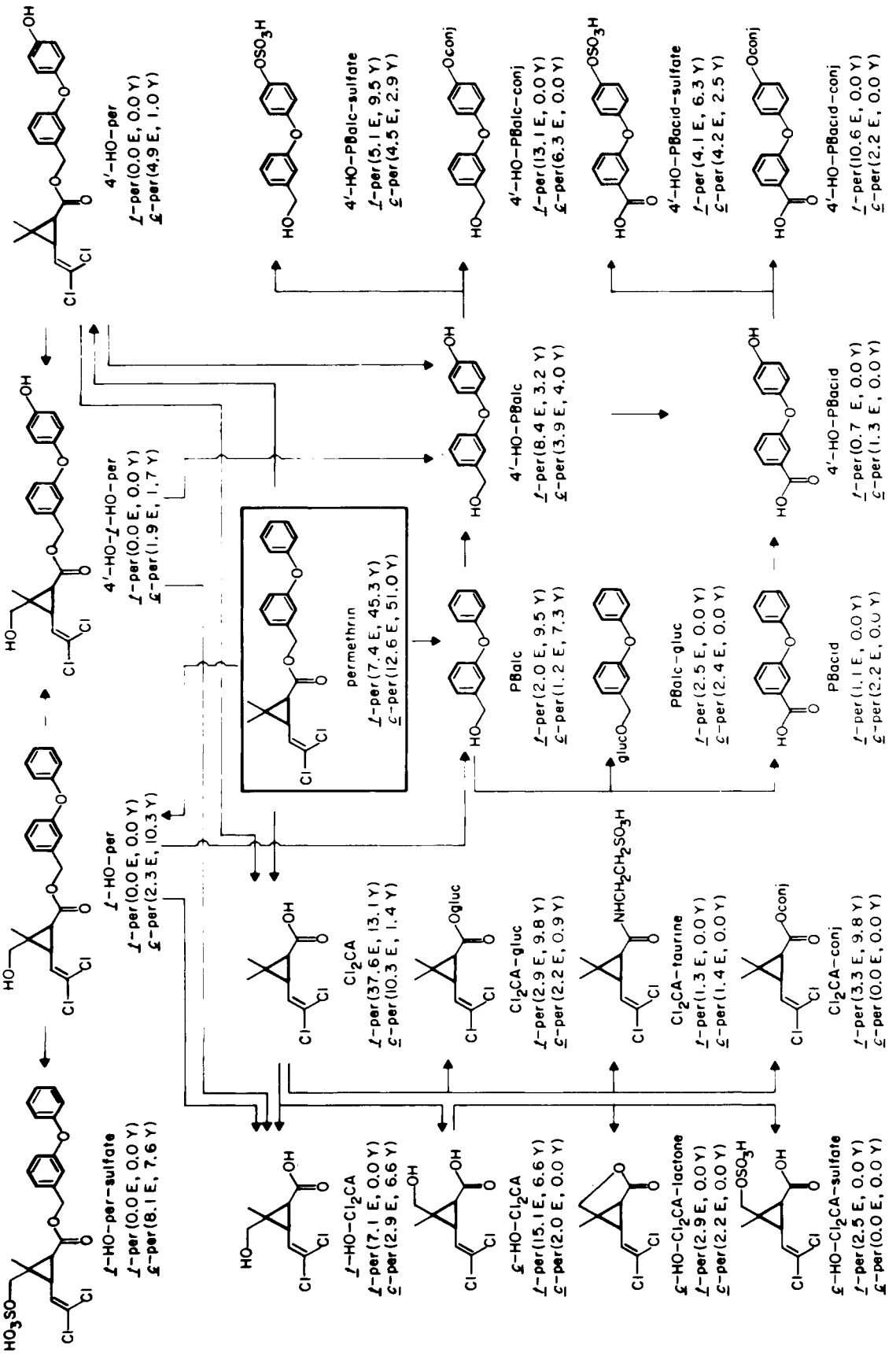

Figure 1. Metabolic pathways for (1*RS*)-*trans*- and (1*RS*)-*cis*-permethrin in hens showing abbreviations used for metabolites. Numbers in parentheses are percentage amounts of ^{14}C for each product derived from *trans*- and *cis*-permethrin as follows: E = products in excreta from the first 3 days of the treatment schedule relative to total administered ^{14}C (see Table I); Y = products in egg yolk at days 5 and 6 of the treatment schedule relative to total ^{14}C content of yolk (see Table III). Ester products are averages for ^{14}C acid and ^{14}C alcohol preparations and cleavage products are based on either ^{14}C acid or ^{14}C alcohol preparations, as appropriate.

methylene position of the alcohol moiety. The permethrin metabolites are designated as shown in Figure 1. Unlabeled standards for the metabolites are described by Unai and Casida (1977) and Shono et al. (1978b) except for the arginine and ornithine conjugates which were prepared by K. Ohsawa of this laboratory and the lysine and taurine conjugates which were provided by Shell Toxicology Laboratory (Sittingbourne Research Center, Sittingbourne, Kent, England).

Chromatography. [^{14}C]Permethrin metabolites were routinely analyzed by thin-layer chromatography (TLC) on silica gel 60 F-254 chromatoplates (0.25 mm gel thickness) with the following two-dimensional solvent systems: for ester products, benzene–ethyl acetate (6:1) (BE) in the first direction and carbon tetrachloride–ether (3:1) (CE) in the second direction; for conjugates of ester metabolites and for hydrolysis products and their oxidized derivatives and conjugates, 1-butanol–glacial acetic acid–water (6:1:1) (BAW) in the first direction, followed by two developments with benzene (saturated with formic acid)–ether (10:3) (BFE) in the second direction. Additional solvent systems were: for hydroxy acids, chloroform (saturated with formic acid)–ether (10:3) (CFE); for taurine conjugates, ethyl acetate–methanol–water (13:3:1) (EMW), giving R_f values of 0.34 and 0.37, respectively, for *t*- and *c*-Cl_2CA-taurine; for carbomethoxy derivatives of acidic metabolites, ether–hexane (10:1) (EH), benzene–ethyl acetate–methanol (15:5:1) (BEM), and hexane–ether (10:1) (HE). The chromatographic properties in these systems of all relevant compounds and metabolites are given in Table I (see Supplementary Material Available paragraph) and by Gaughan et al. (1977) and Unai and Casida (1977). Unlabeled standard compounds were detected as previously described (Gaughan et al., 1977), except for the taurine conjugates of the Cl_2CA isomers which were visualized by spraying with 0.05% aqueous $KMnO_4$.

Some samples required cleanup prior to TLC analysis. This was accomplished by chromatography on silica gel columns (Gaughan et al., 1978) or on Quantum Chromaflex PLQ F chromatoplates (Kontes, Fairfield, N.J.).

Treatment and Handling of Hens. Each of 12 White Leghorn laying hens (average weight, 1.5 kg), in good egg production and acclimatized for 19 days in an individual cage, was treated with three doses of 10 mg/kg of one of the four [^{14}C]permethrin preparations at 24-h intervals. This treatment schedule involved sufficient radiocarbon for the required analyses and gave no signs of poisoning. Each dose was absorbed on cellulose powder and delivered in a gelatin capsule. The hens were maintained on standard layer ration and water ad libitum. Excreta and eggs were collected at 24-h intervals, weighed, and stored at −10 °C. Immediately prior to sacrifice at 10 days after initiating the treatment schedule, a blood sample (1.0 mL) was withdrawn from each hen. After sacrifice by cervical dislocation, the following samples were taken: the entire gizzard, gut, heart, kidneys, liver, and lungs; samples of fat (subcutaneous and visceral), muscle (breast and thigh), skin and feathers; the remaining carcass.

Two other studies were made with additional groups of hens. To determine blood levels, seven hens were acclimatized and treated as above but with a single oral dose of each labeled preparation at 10 mg/kg. Blood samples (0.5 to 1.0 mL) were taken at frequent intervals from no less than four hens in each of the four groups. To analyze expired $^{14}CO_2$, three hens (acclimatized in individual cages for 7 days) were placed in a metabolism unit equipped to trap and monitor expired gases, with a similar group for each labeled preparation. Two unlabeled oral doses (12 mg/kg each) were administered, followed by the labeled dose (12 mg/kg) at 24-h intervals, then $^{14}CO_2$ was monitored at 12-h intervals for 72 h.

These studies were carried out at the Analytical Development Corporation (Monument, Col.) by M. E. Ackerman and associates and after total ^{14}C determination (by combustion) the frozen samples of eggs and tissues and a methanol extract of the excreta (see below) 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.).

Identification of Metabolites. Criteria for tentative identification of ^{14}C metabolites by TLC cochromatography with unlabeled standards were generally those of Gaughan et al. (1977, 1978). Esters are detected with both ^{14}C acid and ^{14}C alcohol preparations and cleavage products with only one of these labeled preparations as appropriate. Compounds directly cochromatographed with authentic standards in each of the indicated solvent systems were: permethrin isomers and their monohydroxy and dihydroxy derivatives (BE, BFE, and CE); Cl_2CA, HO-Cl_2CA isomers, PBalc, PBacid, 4′-HO-PBalc, and 4′-HO-PBacid (BEM, BFE, and CFE); *c*-HO-Cl_2CA-lactones and Cl_2CA derivatives and PBacid after methylation (diazomethane) (BFE, CFE, EH, and HE); taurine conjugates, PBalc-glucuronide (abbreviated as PBalc-gluc) and 4′-HO-PBacid-sulfate (BAW and EMW).

Conjugates were cleaved nonspecifically with HCl or specifically with enzymes (Gaughan et al., 1977, 1978) and the deconjugated compounds were cochromatographed as above. The taurine conjugates were cleaved with HCl. Metabolites designated as glucuronides (gluc) were cleaved by HCl, glusulase, and β-glucuronidase but not by glucuronidase plus saccharic acid 1,4-lactone or by sulfatase. The metabolite of *cis*-permethrin designated as *t*-HO-per-sulfate (both acid- and alcohol-labeled) gave R_f 0.55 in EMW, 0.96 in ethyl acetate–ethanol–acetic acid (15:3:2) and 0.90 in ethyl acetate–ethanol–water (15:5:1). It was almost totally cleaved by sulfatase to give *t*-HO-per, whereas on treatment with HCl or glusulase (with or without saccharic acid 1,4-lactone) it gave *t*-HO-Cl_2CA (from the ^{14}C acid preparation) and PBalc and two decomposition products (from the ^{14}C alcohol preparation). The sulfate conjugates of 4′-HO-PBalc and 4′-HO-PBacid (not separated in the solvent systems used) were cleaved by sulfatase, glusulase, glusulase plus saccharic acid 1,4-lactone, and HCl but not by glucuronidase to give 4′-HO-PBalc and 4′-HO-PBacid, respectively.

Unknown metabolites or metabolite mixtures designated as conjugates were separated by the BAW system (see microfiche edition of this journal) and then individually subjected to HCl hydrolysis for identification of the cleavage products.

Analysis of Excreta. Daily excreta samples (~100 g) were homogenized (Virtis) in methanol (100 mL × 3), filtered, and the ^{14}C in the unextractable portion (after drying for 18 h at 25 °C) was determined by combustion. For analysis of composited 0–72 h extracts, 50-μL aliquots were spotted directly on TLC chromatoplates.

Analysis of Eggs. Each yolk sample (10 to 12 g; composite of 2 to 3 g from each of four to six eggs laid 5 and 6 days after initiating the treatment schedule) was homogenized (Polytron) sequentially with acetonitrile (30 mL × 3), hexane (30 mL × 2), and water (30 mL). The acetonitrile extract was used directly for TLC and the hexane was subjected to column chromatographic cleanup to remove much of the fat prior to TLC analysis. The

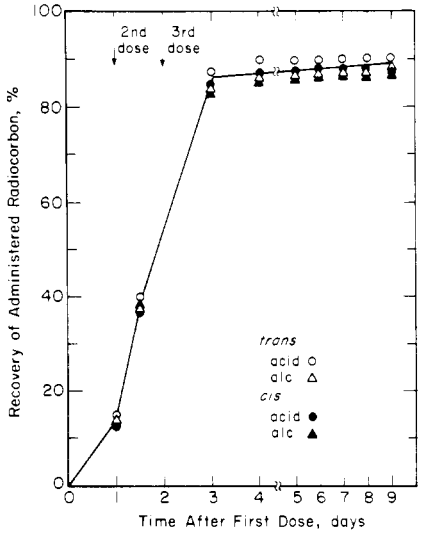

Figure 2. Radiocarbon recovery in excreta within 9 days after initiating a treatment schedule consisting of three daily doses of [*acid*-^{14}C]- or [*alcohol*-^{14}C]*trans*- or -*cis*-permethrin at 10 mg/kg for each dose.

water-soluble fraction was lyophilized to dryness, the residue was extracted with methanol (10 mL), and the methanol extract was subjected to TLC. Unextractable metabolites (the solid residue after extraction of the yolk as above) were analyzed by combustion and also by subjecting a portion (1 g) to acid hydrolysis (5 mL of 6 N HCl, 100 °C, 4 h). Water (2 mL) and $(NH_4)_2SO_4$ (1 g) were added, and the mixture was extracted with ether–ethanol (3:1) mixture (10 mL × 2). After cleanup on Quantum plates and recovery of ^{14}C from the radioactive bands by extraction with methanol, the individual products were examined by TLC.

Each white sample (12 g; composite of 4 to 6 g from each of two to three eggs laid 2 and 3 days after initiating the treatment schedule) was homogenized (Polytron) with acetonitrile (30 mL × 2) and the extract was subjected to TLC. The residue following acetonitrile extraction was hydrolyzed with HCl as above prior to TLC analysis.

Analysis of Fat. One gram of subcutaneous fat and 1 g of visceral fat from each of two or three birds were composited, ground to a uniform mixture, and extracted with cold acetonitrile (15 mL × 4) using a mortar and pestle. The acetonitrile extract was analyzed by the procedure used previously for milk, including TLC for confirmation of permethrin content (Gaughan et al., 1978).

Analysis of Skin. Skin with associated fat (3 g; composite of 1 g from each of two or three hens) was diced after holding on dry ice until sufficiently hard, then extracted in a mortar and pestle with hexane (15 mL × 3) followed by methylene chloride (15 mL × 3). The combined solvents were evaporated, the residue was extracted with cold acetonitrile (7 mL × 4), and the acetonitrile-soluble portion was analyzed by the method used previously for cow fat (Gaughan et al., 1978).

Analysis of Liver. Liver (30 g; composite of 10 or 15 g from each of two or three hens) was lyophilized to dryness and a 1-g portion was subjected to HCl hydrolysis and extracted with a ether–ethanol mixture as with cow liver (Gaughan et al., 1978). Quantum TLC plates were used for preliminary cleanup prior to regular TLC analysis.

Analysis of Kidney. Kidney (1 to 2 g; composite of equal weights from each of two or three birds) was extracted with various solvents (10 mL × 2) (acetonitrile, methanol, and methylene chloride) to recover ~16% of the ^{14}C; this extract was not suitable for TLC. Therefore, minced kidney (1 g) was hydrolyzed with 10 mL of 6 N HCl (100 °C, 4 h) followed by extraction with methylene chloride (10 mL) and ether–ethanol (3:1) mixture (10 mL × 2). Both Quantum plates and regular TLC plates were used sequentially for preliminary cleanup of the combined extracts followed by normal TLC analysis.

Table II. Radiocarbon Recovery in Hen Excreta, Eggs, Tissues, Organs, and Carbon Dioxide 9 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [*acid*-^{14}C]- or [*alcohol*-^{14}C]*trans*- or -*cis*-Permethrin at 10 mg/kg for Each Dose

	radiocarbon recovery, %[a]			
	trans		cis	
sample[b]	acid	alc	acid	alc
excreta	90.32	88.49	88.27	86.87
eggs				
yolk	0.13	0.12	0.38	0.47
white	0.08	<0.01	0.02	<0.01
blood	<0.01	<0.01	0.01	0.02
fat[c]	0.10	0.06	0.50	0.66
liver	0.01	<0.01	0.02	0.02
muscle[d]	<0.01	<0.01	0.02	0.02
skin	<0.01	0.01	0.10	0.09
feathers, gut and carcass	0.21		0.31	0.38
carbon dioxide[e]	0.70	4.71	1.00	3.17
total	91.55	93.39	90.63	91.70

[a] Radiocarbon recoveries are relative to total administered ^{14}C. Average of results with two or three hens for each labeled preparation. [b] Less than 0.01% of the administered ^{14}C with each label appears in gizzard, heart, kidney, and lung. [c] Average for subcutaneous and visceral fat. [d] Average for breast and thigh muscle. [e] Collected for 3 days only in a separate experiment with a single labeled dose of 12 mg/kg.

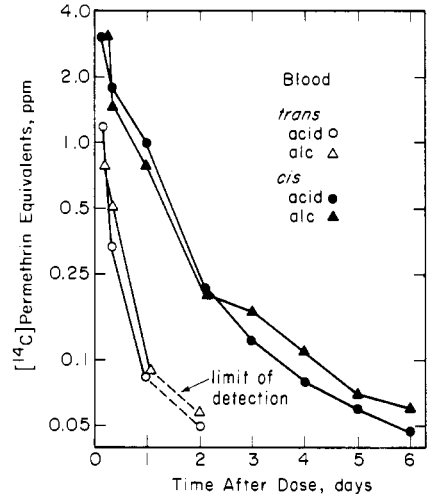

Figure 3. [^{14}C]Permethrin equivalents in blood after a single oral dose of [*acid*-^{14}C]- or [*alcohol*-^{14}C]*trans*- or -*cis*-permethrin at 10 mg/kg. Note that ppm is on a logarithmetic scale.

RESULTS

Distribution of Radiocarbon from [*acid*-^{14}C]- and [*alcohol*-^{14}C]*trans*- and -*cis*-Permethrin. Radiocarbon from each of the four [^{14}C]permethrin preparations is excreted at essentially the same rate with ~88% being eliminated within 9 days (Table II, Figure 2). The permethrin equivalent amounts in fat and skin are seven- to greater than tenfold higher with *cis*- than with *trans*-permethrin, both acid and alcohol labeled (Table II). Other tissues are generally very low in [^{14}C]permethrin equivalent amounts. The ^{14}C alcohol preparations give 3.2

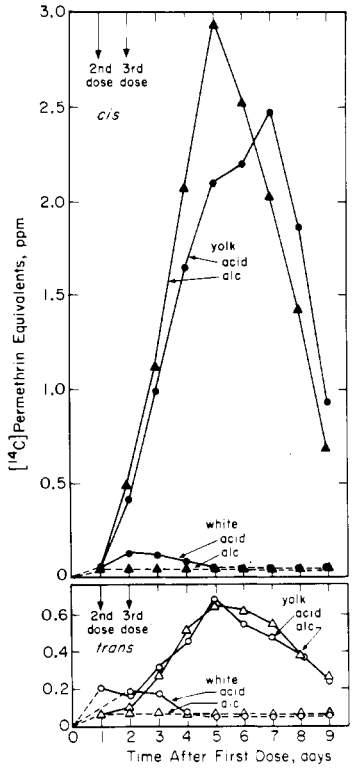

Figure 4. [^{14}C]Permethrin equivalents in yolk and white of eggs after initiating a treatment schedule consisting of three daily doses of [*acid*-^{14}C]- or [*alcohol*-^{14}C]*trans*- or -*cis*-permethrin at 10 mg/kg for each dose. The portion of the graphs indicated with dotted lines are the upper limits based on the sensitivity of the method. For composition of residues, see Tables III and IV.

to 6.7 times more $^{14}CO_2$ than the ^{14}C acid preparations (Table II).

Radiocarbon levels in blood following a single labeled dose (Figure 3) and in egg yolks with three daily doses (Table II, Figure 4) further exemplify the greater persistence of *cis*- than of *trans*-permethrin and the lack of difference between ^{14}C acid and ^{14}C alcohol preparations. Although egg whites contain much lower [^{14}C]permethrin equivalents than the yolks, it is interesting that ^{14}C from the acid moiety appears in larger amounts than that from the alcohol moiety (Table II, Figure 4).

Identity of Excreted Compounds. Detailed analyses are available on the products excreted by two hens 0–72 h after initiating the treatment schedule with each labeled preparation (Table I). The total excreted products are not directly comparable in Tables I and II since in the latter case two or three hens were examined for each labeled preparation and the values are for 9 days after the first dosing.

The 0–72 h excreta contains 1.7-fold more *cis*- than *trans*-permethrin (Table I). Hydroxylated esters are not excreted with *trans*-permethrin but four monohydroxy and dihydroxy esters including one sulfate conjugate are found with *cis*-permethrin.

Metabolites derived from the acid moieties are the Cl_2CA isomers (major) and their glucuronide and taurine conjugates, the *t*-HO-Cl_2CA isomers, the *c*-HO-Cl_2CA isomers and their lactones, and the sulfate conjugate of the trans isomer of *c*-HO-Cl_2CA. In contrast to the ester metabolites of *cis*-permethrin which are hydroxylated only at the trans methyl group, the cleavage products of both isomers are hydroxylated to a greater extent at the cis than at the trans methyl substituent. A conjugate of *t*-Cl_2CA with an unidentified conjugating moiety has no counterpart conjugate derived from *c*-Cl_2CA.

Table III. Permethrin and Metabolites in the Yolk of Eggs 5 and 6 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [*acid*-^{14}C]- or [*alcohol*-^{14}C]*trans*- or -*cis*-Permethrin at 10 mg/kg for Each Dose

	[^{14}C]permethrin equivalents, ppm[a]			
	trans		cis	
compd	acid	alc	acid	alc
Permethrin and Hydroxypermethrin Derivatives				
permethrin[b]	0.31	0.25	1.18	1.28
t-HO-per[c]	0.00	0.00	0.23	0.27
4′-HO-per[d]	0.00	0.00	0.02	0.03
4′-HO,*t*-HO-per[d]	0.00	0.00	0.04	0.04
t-HO-per-sulfate[d]	0.00	0.00	0.16	0.21
Metabolites of the Acid Moiety				
Cl_2CA[d]	0.08		0.03	
Cl_2CA-gluc[d]	0.06		0.02	
Cl_2CA-conj[d]	0.06[e]		0.00	
HO-Cl_2CA[d,f]	0.04		0.14	
unknowns[g]				
water-soluble[h]	0.02		0.12	
unextractable[i]	0.04		0.19	
Metabolites of the Alcohol Moiety				
PBalc[d]		0.06		0.20
4′-HO-PBalc		0.02		0.11
4′-HO-PBalc-sulfate		0.06		0.08
4′-HO-PBacid-sulfate		0.04		0.07
unknowns[g]				
water-soluble[h]		0.04		0.10
unextractable[i]		0.16		0.36
total	0.61	0.63	2.13	2.75

[a] Average of duplicate analyses on composite samples from four to six yolks for each labeled preparation after correction for TLC recovery values of 94–109%. [b] Appears in hexane (22%) and acetonitrile (78%) extracts. [c] Appears in acetonitrile (69%) and water-soluble (31%) fractions. [d] Appear in acetonitrile extract only except for Cl_2CA and PBalc which also appear in trace levels in the water-soluble fraction. [e] The same compound as the major unidentified conjugate of Cl_2CA in excreta. [f] The cis hydroxymethyl derivative of *t*-Cl_2CA and the trans hydroxymethyl derivative of *c*-Cl_2CA. [g] Includes cleavage products and possibly some esters. [h] Mixture of unidentified conjugates. [i] Acid hydrolysis yields only PBalc from ^{14}C alcohol preparations, only *t*-Cl_2CA from [*acid*-^{14}C]*trans*-permethrin, and *c*-Cl_2CA and three lower R_f unknowns from [*acid*-^{14}C]*cis*-permethrin.

The alcohol moiety metabolites include PBalc, PBacid, their 4′-hydroxy derivatives, and the corresponding sulfates, the glucuronide of PBalc, and a variety of unidentified conjugates of 4′-HO-PBalc and -PBacid. The taurine conjugate of PBacid is not detected as a metabolite.

The amounts of identified compounds, including those for which the structure is known except for the conjugating moiety, are as follows relative to the total administered ^{14}C in the 0–72 h excreta: 80% with [*acid*-^{14}C]*trans*-permethrin; 51–58% with the other labeled preparations. The major portion of unidentified metabolites is in the unextractable fraction while some of the remainder are metabolites not adequately resolved by TLC for individual analysis.

Permethrin and Metabolites in Eggs. The yolks at 5 and 6 days after initiating the treatment schedule contain 4.4-fold more *cis*- than *trans*-permethrin and the same ester metabolites of *cis*-permethrin found in the excreta (Table III). Other metabolites in the yolk are generally the same as those in the excreta, i.e., Cl_2CA both free and conjugated and HO-Cl_2CA derivatives from the acid moiety, and PBalc, 4′-HO-PBalc, and sulfate conjugates

Table IV. Permethrin and Metabolites in the White of Eggs 2 and 3 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [*acid*-^{14}C]*trans*- or -*cis*-Permethrin at 10 mg/kg for Each Dose

compd	[^{14}C]permethrin equivalents, ppm[a] trans	cis
Permethrin and Hydroxypermethrin Derivatives		
permethrin	0.000	0.009
t-HO-per	0.000	0.018
Metabolites of the Acid Moiety		
Cl_2CA	0.054	0.036
HO-Cl_2CA	0.085	0.027
other Cl_2CA derivatives[b]	0.037	0.032
total	0.176	0.122

[a] Average of duplicate analyses on composite samples from two to three whites for each labeled preparation with TLC recovery values of >90%. [b] Acid hydrolysis of the residue following acetonitrile extraction yields only the Cl_2CA isomers.

of 4′-HO-PBalc and -PBacid from the alcohol moiety.

The whites at 2 and 3 days after the first dose contain small amounts of *cis*-permethrin and its trans hydroxy derivative but not any esters derived from *trans*-permethrin (Table IV). With both isomers, they also contain Cl_2CA and its conjugates and HO-Cl_2CA.

Permethrin and Metabolites in Tissues and Organs. The fat and skin (including fat) contain largely unmetabolized permethrin, more from the cis than from the trans isomer, while the liver and kidney contain mostly material only released on HCl hydrolysis to give unknown degradation products (liver) or a variety of identified hydroxy derivatives of the acid and alcohol moieties (HO-Cl_2CA isomers, 4′-HO-PBalc and -PBacid) (kidney) (Table V).

DISCUSSION

Following oral administration to laying hens, *cis*-permethrin appears at higher levels than *trans*-permethrin in egg yolk, fatty tissues, and excreta. Radiocarbon from [^{14}C]*cis*-permethrin preparations also persists longer in blood than that from [^{14}C]*trans*-permethrin preparations. This isomer difference is consistent with previous findings on the milk of cows and the fat and feces of rats and cows (Gaughan et al., 1977, 1978). It probably results from more rapid esterase cleavage of the trans than the cis isomer based on the relative amounts of hydrolysis products from the two isomers in hen excreta and on analogy with the relative reactivity of the permethrin isomers in mouse and rat liver microsomal esterase systems (Shono et al., 1978a; Soderlund and Casida, 1977).

The permethrin isomers are not only hydrolyzed in hens but they also undergo extensive oxidation (Figure 1) as previously noted with rats and cows (Gaughan et al., 1977, 1978). 4′-Hydroxy- and *trans*-hydroxymethyl ester derivatives are detected in egg yolk and excreta with *cis*-permethrin but not with *trans*-permethrin. This indicates that if hydroxy derivatives of *trans*-permethrin are formed they undergo rapid hydrolysis and when *cis*-permethrin is hydroxylated at the cis methyl group it also is cleaved prior to excretion. A similar situation exists relative to the excreta with rats whereas with cows both isomers are excreted in part as monohydroxy and dihydroxy esters. The preference in hydroxylation site based on identified metabolites is the same with hens and rats, i.e., phenoxy > cis methyl > trans methyl with *trans*-permethrin and phenoxy > trans methyl > cis methyl with *cis*-permethrin. In contrast, with cows, both *trans*- and *cis*-permethrin have

Table V. [^{14}C]Permethrin Equivalents in Tissues and Organs 9 Days after Initiating a Treatment Schedule Consisting of Three Daily Doses of [*acid*-^{14}C]- or [*alcohol*-^{14}C]*trans*- or -*cis*-Permethrin at 10 mg/kg for Each Dose

sample	[^{14}C]permethrin equivalents, ppm[a] trans acid	trans alc	cis acid	cis alc
fat	0.21[b]	0.18[b]	1.03[b]	1.36[b]
skin	<0.05[c]	<0.06[c]	0.47[d]	0.41[d]
liver	0.14[e]	0.08[e]	0.27[e]	0.20[e]
kidney	0.31[f]	0.24[f]	0.34[f]	0.25[f]

[a] Average of two or three analyses on composite samples from two or three hens for each labeled preparation. Analyses which are not tabulated showed that both the trans and cis isomers of [*acid*-^{14}C]- and [*alcohol*-^{14}C]permethrin give residues of <0.06 ppm permethrin equivalents in the gizzard, heart and muscle (breast and thigh). The lung levels are 0.09 and 0.11 ppm permethrin equivalents from [*acid*-^{14}C]- and [^{14}C]alcohol-*cis*-permethrin, respectively, and <0.06 ppm from the corresponding [^{14}C]*trans*-permethrin preparations. [b] Equal weights of subcutaneous and visceral fat. The ^{14}C recovery with each labeled preparation is as follows (%): extractable and cochromatographing with unmetabolized permethrin, 78; extractable but not appearing in major permethrin peak on column chromatography, including losses, 13; unextractable, 9. [c] Not analyzed by TLC. [d] Radiocarbon recovery with each labeled preparation is as follows (%): extractable and cochromatographing with unmetabolized permethrin, 82; extractable but not appearing in major permethrin peak on column chromatography, including losses, 7; unextractable, 11. [e] Radiocarbon recovery of 96% after acid hydrolysis and ether–ethanol extraction. The extractable material contains no permethrin nor its hydrolysis products or PBacid but consists only of low R_f materials (BFE). [f] Radiocarbon recovery of 81% with [*acid*-^{14}C]*cis*-permethrin and 48–52% with the other labeled preparations after acid hydrolysis and methylene chloride and ether–ethanol extractions. The extractable products are as follows (% of total recovered after TLC cleanup): >90% *c*-HO-Cl_2CA from [*acid*-^{14}C]*trans*-permethrin; ~65% *t*-HO-Cl_2CA and ~35% of an unknown of higher R_f (BFE) from *cis*-permethrin; ~70% 4′-HO-PBalc, ~25% 4′-HO-PBacid, and ~5% of an unknown of lower R_f (BFE) from [*alcohol*-^{14}C]*trans*-permethrin; ~35% of each of 4′-HO-PBalc and 4′-HO-PBacid and ~30% of an unknown of lower R_f (BFE) from [*alcohol*-^{14}C]*cis*-permethrin.

the same preference order of trans methyl > cis methyl = phenoxy. Metabolites detected with hens but not with rats or cows are the cis isomer of *t*-HO-per-sulfate, the trans isomer of *c*-HO-Cl_2CA-sulfate, 4′-HO-PBalc-sulfate, and the Cl_2CA-taurine isomers. Both hens and rats extensively utilize glucuronic acid and sulfate conjugates in excretion of permethrin metabolites while with cows glutamic acid conjugates are most significant. Previous studies with hens also show the importance of sulfate conjugation in excretion of phenolic metabolites of other compounds (Andrawes et al., 1972; Paulson and Zehr, 1971; Paulson et al., 1970, 1972). Taurine conjugates are previously known to occur in birds (James et al., 1972) and the taurine conjugate of PBacid, although not detected in hens, is the major metabolite of this acid in mice (Hutson and Casida, 1978). Hens also form several unidentified conjugates of permethrin metabolites, possibly with conjugating moieties not used by rats or cows.

Larger amounts of $^{14}CO_2$ are liberated on metabolism of the alcohol- than of the acid-labeled permethrin preparations but the mechanism for these oxidations and

their localization in the gut or the body are not known.

Almost half of the residues in eggs are unmetabolized *trans*- and *cis*-permethrin in the yolk. The remainder is a great variety of metabolites in the yolk and white including most of those also detected in the excreta. Residues of permethrin-derived products in yolk reach peak levels 3 or 4 days after those in white in a similar manner to the metabolites of other xenobiotics (Andrawes et al., 1972; Davison, 1976; Paulson and Zehr, 1971). Radiocarbon from dermally applied ^{14}C-methylene-labeled (1*R*)-*cis*,*trans*-permethrin reaches maximum yolk levels at the fifth day after treatment (Hunt et al., 1978) in agreement with the present findings on oral administration of the individual trans and cis isomers.

The ease of permethrin hydrolysis and oxidation in hens suggests that rapid detoxification may contribute to the relative insensitivity of hens and other birds to pyrethroid poisoning (Casida, 1973; FMC Corporation, 1977).

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Supplementary Material Available: Complete tabulation of individual unidentified metabolites including amounts, TLC R_f values, and HCl cleavage products (2 pages). Table I of this paper utilizes this information in abbreviated form. Ordering information is given on any current masthead page.

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Toxicity of *Alternaria* Metabolites Found in Weathered Sorghum Grain at Harvest

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No evidence of toxicity was observed in rats or chicks when *Alternaria* metabolites alternariol monomethyl ether (AME), alternariol (AOH), and altenuene (ALT) were fed for 21 days at levels up to 24, 39, and 10 μg/g, respectively, which were about six times as great as those found in heavily weathered sorghum grain at harvest. Two of four isolates of *Alternaria alternata* were toxic when corn–rice–*Alternaria* culture constituted half the diet for chicks and rats. Although all the isolates produced AME, AOH, and ALT, the two that also produced tenuazonic acid and altertoxin I were lethal. Tenuazonic acid was not found in any of the 12 samples of weathered sorghum grain analyzed by gas chromatography.

When grain sorghum, *Sorghum bicolor* (L.) Moench, is exposed to wet weather before harvest, the seeds are often discolored by fungal growth. Two *Alternaria* metabolites, alternariol (AOH) and alternariol monomethyl ether (AME), were found in Kansas sorghums in amounts that correlated with the degree of grain discoloration and with the number of rainy days during September and October (Seitz et al., 1975). The abundance of weathered, discolored sorghum in various areas in recent years has caused concern about possible toxicity to livestock.

Christensen et al. (1968) reported that 53 of 60 isolates of *Alternaria* from foods and feeds were lethal when corn–rice cultures were fed to weanling rats. Doupnik and Sobers (1968) grew 96 isolates of *A. longipes* on cracked corn and fed them to 1-day-old chicks. During the 2-week trial, 53 isolates had no apparent effect, 12 depressed weight gains, and 31 were lethal. Toxic effects were noted when *Alternaria*-infested grain was fed to chicks (Forgacs

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