Metabolism of *cis*- and *trans*-Resmethrin in Laying Hens[1]

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The cis-1*RS* and trans-1*RS* isomers of resmethrin, labeled with radiocarbon in either the alcohol or acid moiety, were individually administered orally to white Leghorn laying hens at a dosage of 10 mg/kg of body weight. With each isomer and label position, greater than 90% of the radiocarbon was eliminated in the excreta within 24 h posttreatment. The metabolic routes for both resmethrin isomers arise from ester hydrolysis and oxidation of the hydrolytic products. Certain of these metabolites are further conjugated with glucuronic acid, sulfate, or other unidentified compounds before excretion.

The synthetic pyrethroid insecticide resmethrin [(5-benzyl-3-furyl)methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate] exhibits excellent insecticidal activity against houseflies, mosquitos, cockroaches, and a variety of other insects. Its short-lived residual activity and low mammalian toxicity, combined with a minimal degree of acquired pest resistance, makes resmethrin a prime candidate for controlling certain arthropod pests on and around food-producing animals.

To date, in vivo metabolism studies on *cis*- and *trans*-resmethrin have been reported only in rats (Miyamoto et al., 1971; Ueda et al., 1975a) and lactating dairy cattle (Ridlen et al., 1984). The studies described herein were designed to evaluate the distribution and metabolic fate of resmethrin in the laying hen and to define the potential for transfer of residues into edible tissues and eggs after oral exposure.

MATERIALS AND METHODS

Chemicals. Four radiocarbon-labeled preparations of resmethrin were provided by the S. B. Penick Corp. (Lyndhurst, NJ): acid-labeled [^{14}C]-(1*RS*)-*cis*-resmethrin (CAC), acid-labeled [^{14}C]-(1*RS*)-*trans*-resmethrin (TAC), alcohol-labeled [^{14}C]-(1*RS*)-*cis*-resmethrin (CAL), and alcohol-labeled [^{14}C]-(1*RS*)-*trans*-resmethrin (TAL). The preparations were labeled in the carbonyl group of the acid moiety or at carbon 2 of the furyl ring of the alcohol moiety. The radiochemical purity of all four preparations was found to be greater than 98.5% as determined by thin-layer chromatography (TLC) (solvent systems D–F, vide infra).

The isomeric purity of the radiolabeled resmethrin preparations was previously established by gas–liquid chromatography/mass spectrometry (GLC/MS) (Ridlen et al., 1984). These analyses showed each of the radiochemicals to be of greater than 98% isomeric purity.

Unlabeled analogues of *cis*- and *trans*-resmethrin for use as possible metabolic standards were also supplied by Penick Corp. The chemical names of these products and acronyms used to designate them throughout this paper are as follows: 5-benzyl-3-furoic acid (BFCA), (5-benzyl-3-furyl)methanol (BFA), *cis*-chrysanthemic acid (*c*-CA), and *trans*-chrysanthemic acid (*t*-CA).

Chromatography. Radiolabeled resmethrin and its metabolites were initially analyzed by TLC on silica gel 60 F-254 chromatoplates (0.25- or 0.5-mm gel thickness, 20 × 20 cm, with fluorescent indicator; E. Merck, Darmstadt, West Germany). Plates were developed in either one or two dimensions. Visualization of the ^{14}C components was performed by exposing the plates to X-ray film (X-Omat or Direct Exposure Film; Eastman Kodak Co., Rochester, NY). Unlabeled resmethrin and the metabolite standards were visualized by viewing the plates under short-wave (254-nm) ultraviolet light. Several solvent systems were used for two-dimensional analysis. For alcohol-labeled preparations (TAL, CAL) these were (A) benzene (saturated with formic acid)–tetrahydrofuran (2:1) in the first direction and (B) benzene–acetonitrile–acetic acid (40:10:1) in the second direction. When solvent system A was used for one-dimensional analysis, plates were developed two times. For acid-labeled preparations (TAC, CAC), solvent systems were (C) benzene (saturated with formic acid)–tetrahydrofuran (5:1) in the first direction and (D) benzene (saturated with formic acid)–ether (10:3) in the second direction. Additional solvent systems used for one-dimensional analysis were (E) benzene–chloroform–ether (20:2:3), (F) benzene–ethyl acetate–methanol (18:2:1) plus 1% acetic acid, (G) benzene–ethyl acetate–methanol (15:5:1), (H) carbon tetrachloride–hexane–ether (8:1:1), (I) hexane–ether (2:1), (J) carbon tetrachloride–ether (3:1), (K) ethyl acetate–methanol–water (13:3:1), (L) 1-propanol–acetic acid–water (6:1:1), (M) methanol–water–acetic acid (8:3:1), and (N) benzene (saturated with formic acid)–tetrahydrofuran (10:1).

High-performance liquid chromatography (HPLC) was used in some cases to further resolve the [^{14}C]resmethrin metabolites. An Axxion 710 HPLC controller and a Tracor HPLC system (Model 951 pumps and Model 970A variable-wavelength detector) were used with a 5-μm Supelco C-18 column (15-cm length, 4.6-mm i.d.; Supelco, Inc.) preceded by a Whatman guard column (Pierce Chemical Co.). The UV detector was set at 240 nm for the analysis of all resmethrin metabolites. This wavelength was selected after UV absorbance curves were obtained for resmethrin and metabolite standards with a Beckman DU-7 spectrophotometer (Beckman Instruments, Inc., Irvine, CA). For optimum resolution of radiolabeled resmethrin metabolites, various mobile phases were used as follows: (HPLC-A) acetonitrile–water (70:30), (HPLC-B) acetonitrile–water (45:55), (HPLC-C) acetonitrile–water (40:60), and (HPLC-D) acetonitrile–water (30:70). Quantitation of the radiocarbon in the eluents was achieved by collection of timed HPLC eluent fractions for assay by liquid scintillation counting (LSC).

GLC/MS was used to make structural assignments of the isolated [^{14}C]resmethrin metabolites where possible. Mass spectral studies utilized a Varian Vista 6000 gas–liquid chromatograph coupled with VG Analytical System 70-250 magnetic scan spectrometer. The gas chromatograph was equipped with a 30 m × 0.25 mm (i.d.) SPB-1 fused silica capillary column (Supelco, Inc.). Chromatographic separations on the gas chromatograph were performed by temperature programming (initial column temperature at 50 °C, with temperature increasing at 15 °C/min), and a helium gas pressure of 10 psi was maintained throughout each sample run. Analysis of the samples was performed in the electron-impact (EI) mode at 70 eV. Generally, the isolated compounds were methylated with ethereal alcoholic solutions of diazomethane at room temperature for 1 h prior to further cleanup

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by TLC. The methylated metabolites were subsequently filtered through a 0.45-μm membrane filter (Bioanalytical Systems, Inc., West Lafayette, IN) prior to HPLC and/or GLC/MS analysis.

Treatment and Sample Collection. Twenty-four white Leghorn hens (average weight 1.8 kg) in good egg production (>80%) were placed in individual metabolism cages housed in an environmentally controlled room (average temperature 19 °C, relative humidity 68%) with a 15-h lighting cycle. Throughout the study, the birds were maintained on a commercial pelleted laying feed with water available ad libitum.

Each ^{14}C-labeled resmethrin preparation was diluted with the appropriate unlabeled resmethrin isomer to a specific activity of 2200 dpm/μg. The dose was dissolved in a small amount of acetone and added to a small amount of the laying diet to facilitate dispersion. The individual doses were sealed inside gelatin capsules (0.25 oz) and delivered orally to the hens. At the time of treatment, the hens were 33–37 weeks of age. The birds received a single dose of one of the four [^{14}C]resmethrin preparations equivalent to 10 mg of resmethrin/kg of body weight, with specific doses tailored to the individual weight of each bird.

Two groups of hens were used for each radiolabeled preparation: One group of birds (group A), consisting of three birds, was killed at 12 h posttreatment; these birds were used primarily for characterization of tissue and excreta residues. A second group of three birds (group B) was maintained for 14 days for characterization of residues in excreta, egg white, and yolk samples.

Excreta samples were collected every 1–4 h and temporarily stored in the dark at 4 °C prior to pooling as 12–24-h composite samples (vide infra) held frozen at -60 °C. Beginning at 48 h posttreatment, excreta from the group B birds was collected at 12-h intervals. Excreta samples were subsequently pooled for each bird as a single 12-h sample at the time of sacrifice for the group A birds and were pooled for individual birds to result in 12- or 24-h samples for the group B birds. The composited excreta samples for each bird were thoroughly mixed, and the total weight of each sample was determined.

Eggs were collected on a daily basis. The egg whites and yolks were separated, weighed, and frozen at -60 °C for later analysis.

The birds were killed by decapitation, and samples of blood (~30 mL) were immediately transferred to heparinized tubes, shaken, and stored on ice. The following samples were then removed and stored on ice: liver, kidney, gizzard (with koilin lining removed), heart, lungs, muscle (breast and leg), fat, skin, ovarian yolk follicles, shell gland egg, and the entire gut contents posterior to the crop. Radiocarbon in aliquots (100–500 mg wet weight) of the tissues, eggs, and excreta (each homogenized and/or thoroughly mixed) was determined by oxygen combustion (Christopher et al., 1985). The remainder of each sample was stored in glass jars and held frozen at -60 °C for future use.

Sample Analysis. *Excreta.* All samples were analyzed in duplicate. Samples targeted for quantification were initially fortified with 50–100 μg of unlabeled resmethrin and available metabolite standards. Scaled-up extraction procedures (without standard fortification) were later done for subsequent isolation and mass spectral work. Radiocarbon in all solvent and residue phases generated throughout the extractions was quantified either by direct LSC or by oxygen combustion and LSC.

Analytical efforts were limited to the 12-h excreta samples. Excreta samples (3-g composite of 1 g from each of three birds within a specific treatment group) were homogenized in 25-mL volumes of methanol on a Polytron homogenizer. The samples were centrifuged sufficient to sediment particulates, and the supernatant was removed and temporarily stored in a freezer at -4 °C. The above extraction procedure was performed a total of three times. The supernatant fractions collected after each extraction were pooled for each individual sample. The combined extracts were concentrated under reduced pressure and subsequently analyzed by two-dimensional TLC for quantitative work and one-dimensional analysis for isolation of the metabolites. For additional work with other sample extracts, metabolites identified from the excreta were used as standards.

The very polar conjugates remaining at the origin following TLC analysis of excreta extracts were subjected to either enzymatic or acid hydrolysis procedures. The origin metabolites were scraped from the plates, and aliquots of this gel were incubated with bacterial (*Escherichia coli*) β-glucuronidase (Type VII, 1000 Sigma units of activity/vial) in buffered vials (phosphate buffer, pH 6.8) or with aryl sulfatase (Type VIII, from abalone entrails, 22 units of activity/mg solid) buffered in 5.0 mL of a 0.2 M sodium acetate–acetic acid buffer (pH 5.0). Enzyme samples were incubated in the dark at 37 °C for 24 h and then acidified with 3 N HCl to pH 2.0 and extracted with ethyl acetate (3 × 15 mL). Control samples were done by incubating the origin metabolites with inactivated enzyme (enzyme samples heated in water (100 °C) for 30–45 min). Following extraction, each sample was concentrated and then analyzed on TLC (A, D, G). The products released were characterized by TLC comparisons with reference compounds.

Nonspecific cleavage of the origin metabolites was accomplished by subjecting the samples to acid hydrolysis. Each sample (0.15–0.25 g of scraped TLC gel) was heated in 5.0 mL of 1 N HCl at 100 °C for 1 h. After the samples were allowed to reach room temperature, the samples were adjusted to pH 2.0 with 10 N NaOH. The samples were then extracted with ethyl acetate (4 × 15 mL). The extracts were combined for each sample and concentrated to near dryness under reduced pressure. The organic extractable radiocarbon was subsequently characterized by one-dimensional TLC analysis using solvent systems A and D with compounds of known structure.

Egg White and Yolk. Egg white samples (6-g composite of 2–3 g from each of two or three eggs for each group) were lyophilized, and the resulting powder was homogenized in methanol (2 × 30 mL) and centrifuged. The resulting extracts were concentrated and cochromatographed on TLC with standards in solvent systems A, D, G, and K.

To each egg yolk sample (3-g composite of 1–1.5 g from each of two or three eggs for each treatment group) was added anhydrous sodium sulfate (approximately 2 g). Each sample was extracted by homogenization (Polytron) with a hexane–tetrahydrofuran (1:1) mixture (3 × 20 mL), followed by centrifugation. The extracts were concentrated to an oily residue and subsequently extracted with acetonitrile (4 × 15 mL). The oily residue remaining was redissolved in 5 mL of hexane and the radiocarbon content determined by LSC. The acetonitrile fractions were concentrated and subjected to one- and two-dimensional TLC analysis. One-dimensional TLC work utilized six solvent systems (A, D, G–J), while the two-dimensional TLC work was performed on the pairs of solvent systems designed for the alcohol- and acid-labeled preparations (vide supra).

Tissue (Liver, Kidney, Fat, Skin, and Muscle). Samples of liver (1.0 g) from each bird within each treatment group were pooled to give a 3.0-g composite sample, and the samples were then homogenized in 10 mL of distilled water. The homogenate was adjusted to pH 2.0 with 6 N HCl and extracted with an ether–ethanol (3:1) mixture (4 × 15 mL). The combined extracts were concentrated, reconstituted in hexane (5–10 mL), and then partitioned with acetonitrile (4 × 15 mL). Radiocarbon residues associated with the hexane phase (containing <15% of sample radiocarbon in every case) were not studied further. The acetonitrile extracts were concentrated and then analyzed by one- and two-dimensional TLC analysis. One-dimensional TLC analysis utilized solvent systems A, D, and G, and two-dimensional studies were done as with yolk.

Samples of kidney (0.5 g from each bird) were composited, extracted, and subjected to TLC in similar manners as described for liver samples, with the exception that low lipid content in kidney extracts was such that it was not necessary to perform a hexane–acetonitrile cleanup partition.

Abdominal fat (15-g composites, 5 g from each of three hens) was homogenized in hexane (2 × 50 mL) and subsequently partitioned with acetonitrile (4 × 75 mL). The hexane layer from each of the acid-labeled samples (TAC, CAC) and the cis alcohol (CAL) sample contained in all cases <8% of the total sample radiocarbon, and these hexane fractions were not analyzed further because the absolute amounts of radiocarbon present in these fractions were exceedingly low. A large percentage of sample radiocarbon (61%), however, remained associated with the hexane layer of the trans alcohol (TAL) sample after partitioning with acetonitrile. Additional partitions of the hexane phase with acetonitrile (4 × 45 mL) recovered an additional 30% of the sample radiocarbon. Further attempts to define the nature of the radiocarbon in the TAL hexane layer were not successful

Table I. Summary of Radiocarbon Elimination and Residue Retention by Laying Hens Treated with a Single Oral Dose of [*acid*-^{14}C]- or [*alcohol*-^{14}C]-(1*RS*)-*cis*- or -(1*RS*)-*trans*-Resmethrin at 10 mg/kg of Body Weight[a]

label and isomer	cum ^{14}C rec,[b] %: excreta	egg residue, ppm		tissue residue,[c] ppm	
		white[d]	yolk[e]	liver	kidney
[*acid*-^{14}C]-*c*-RES	98.6 ± 8.7	0.34 ± 0.05	0.39 ± 0.04	3.86 ± 1.84	7.25 ± 5.19
[*acid*-^{14}C]-*t*-RES	94.0 ± 5.0	0.16 ± 0.06	0.30 ± 0.06	2.74 ± 1.98	3.12 ± 1.28
[*alcohol*-^{14}C]-*c*-RES	102.8 ± 2.0	0.08 ± <0.02	0.58 ± 0.16	2.85 ± 0.56	3.89 ± 1.68
[*alcohol*-^{14}C]-*t*-RES	108.1 ± 2.4	0.02 ± <0.02	0.62 ± 0.15	3.02 ± 1.68	10.49 ± 5.62

[a] Data summarized from Christopher et al. (1985). [b] Radiocarbon recoveries are expressed as averages ± SD from three hens treated with the individual resmethrin preparations, after 14 days. [c] Tissue residue values (ppm resmethrin equivalent) are from hens killed 12 h posttreatment. Residues in other tissues were lower. [d] Reported are peak radiocarbon residues in egg white samples collected at 1 day posttreatment from CAC- and TAC-treated birds and 2 days posttreatment from CAL- and TAL-treated birds. [e] Reported are peak radiocarbon residues in egg yolk samples collected at 4 days posttreatment from CAL-, TAL-, and CAC-treated birds and at 5 days posttreatment from TAC-treated birds.

because the very low levels of radiocarbon present could not be resolved from the lipid materials. The acetonitrile fractions were concentrated under reduced pressure and were analyzed by one-dimensional TLC using four solvent systems (A, D, G, I). The sample extracts were cochromatographed with available radiolabeled *cis*- and *trans*-resmethrin, unlabeled metabolite standards (BFCA, CA), and radioactive standards generated from excreta samples.

Skin samples (21-g composites of 7 g from each of three hens) were cut in small sections. The samples were homogenized (Polytron) in hexane (3 × 100 mL) and centrifuged at 10 000 rpm (0 °C). The residue remaining was then extracted with acetonitrile (2 × 75 mL) and centrifuged in the same manner as described above. The hexane phase was concentrated under reduced pressure to a volume of approximately 5 mL. An average of 7.4% (2–21%) of the total sample radiocarbon remained in the hexane layer after partitioning with acetonitrile. As with the TAL hexane layer of the fat extracts, the hexane portion of the TAL sample extract from the skin contained the highest radiocarbon residues after acetonitrile partitioning. The hexane phases from all samples were not studied further because of the very low levels of radiocarbon present. The acetonitrile phases were further concentrated and analyzed by one-dimensional TLC along with unlabeled standards and radiolabeled compounds of known structure from excreta using solvent systems A, D, and G, followed by autoradiography.

To 36 g of breast and leg muscle composite samples (6 g of each muscle type from each of three birds) was added 75 mL of distilled water. Each sample was acidified with 6 N HCl to pH 2.0 and then homogenized (Polytron) in an ether–ethanol (2:1) solvent mixture (3 × 150 mL). The samples were centrifuged at 0 °C to break emulsions. The combined sample extracts were dried over sodium sulfate and passed through a funnel fitted with a cotton plug to remove suspended particulates. The sample extracts were concentrated to near dryness under reduced pressure, and the resulting residue was extracted with ethyl ether (3 × 25 mL). The ether extracts were subsequently passed through activated C-18 SEP-PAK cartridges (Waters Associates, Milford, MA) prior to TLC analysis using four solvent systems (A, D, G, I). As a result of the additional sample preparation procedures, an average of 8.3% (5.7–13.3%) of the muscle sample radiocarbon was lost.

RESULTS

Excretion Patterns and Tissue Residues. The distribution and excretion patterns of the resmethrin isomers in the excreta, eggs, and tissues of orally treated hens have been reported earlier (Christopher et al., 1985). Table I briefly summarizes these data.

Within 24 h posttreatment, >90% of the acid-labeled (91–94%) and of the alcohol-labeled (92–104%) [^{14}C]resmethrin doses had been eliminated in the excreta. Radiocarbon residues in the egg white and yolk fractions of all birds were low, with peak levels observed at 1–2 and 4–5 days posttreatment in white and yolk, respectively.

Analysis of tissues from chickens killed 12 h after dosing with the individual resmethrin preparations showed highest radiocarbon residues in kidney and liver samples. No obvious differences existed, with respect to the isomer or label position, in radiocarbon retention by the tissues (Christopher et al., 1985).

Table II. Resmethrin and Metabolites in the Excreta of Laying Hens 12 h after Treatment with a Single Oral Dose of [*alcohol*-^{14}C]-*trans*- or -*cis*-Resmethrin at 10 mg/kg of Body Weight

metabolite	% of sample radiocarbon	
	trans alcohol	cis alcohol
resmethrin	3	2
BFA-*Z*-CDA	0	1
BFCA		
free	1	1
glucuronide	5	2
other conjugates[a]	34	24
α-OH-BFCA		
free	3	4
conjugates[a]	15	3
4-OH-BFCA		
free	2	<1
glucuronide	1	<1
sulfate	1	<1
other conjugates[a]	3	2
unknowns[b]		
1	5	8
2	4	4
3	3	6
4	<1	2
others[c]	11	29
unextractable	9	12

[a] As liberated by acid hydrolysis. [b] Unknowns 1–4 have corresponding R_f values on TLC-A of 0.59, 0.21, 0.05, and 0.55, respectively. [c] Refers to sample radiocarbon that did not adequately resolve after TLC analysis and includes compounds that did not move free of the origin even after enzymatic or acid hydrolysis.

Characterization of Metabolites in Excreta. Direct extraction of the excreta with methanol resulted in an average of 93% (88–98%) recovery of the radiocarbon present for all samples. Distribution of the radiolabeled compounds present in the excreta samples from the treated birds for both isomers and label positions at 12 h posttreatment are given in Tables II and III. Efforts at characterization of the resmethrin metabolites in excreta were limited to those products detected in the samples collected 12 h posttreatment. Quantitative studies with 24-h excreta sample extracts (not reported) revealed TLC patterns and proportions of metabolites similar to those obtained with the 12-h samples.

Alcohol-Derived Metabolites. From the cis and trans alcohol samples, at least 12 metabolites were resolved by TLC analysis (Table II). [^{14}C]Resmethrin (RES) represented 3% or less of the total sample radiocarbon in excreta samples collected 12 h posttreatment for both alcohol-labeled preparations, as indicated by cochromatographic studies with authentic standards (TLC: A × B, D, G, I, N) and mass spectral analysis (Table IV). No unmetabolized (benzylfuryl)methanol (BFA) was detected

Table III. Resmethrin and Metabolites in the Excreta of Laying Hens 12 h after Treatment with a Single Oral Dose of [*acid*-^{14}C]-*trans*- or -*cis*-Resmethrin at 10 mg/kg of Body Weight

metabolite	% of sample radiocarbon: trans acid	cis acid
resmethrin	1	2
BFA-*Z*-CDA	0	<1
CA		
free	8	1
glucuronide	<1	<1
other conjugates[a]	40	11
CHA		
E-CHA	6	3
E-CHA-glucuronide	<1	<1
E-CHA-other conjugates[a]	<1	<1
Z-CHA	3	<1
Z-CHA-glucuronide	<1	<1
Z-CHA-other conjugates[a]	<1	<1
CDA		
tE-CDA	21	2
tE-CDA-glucuronide	<1	<1
tE-CDA-other conjugates[a]	4	0
cE-CDA	0	17
cE-CDA-glucuronide	0	<1
cE-CDA-other conjugates[a]	0	18
cZ-CDA	0	4
cZ-CDA-glucuronide	0	<1
cZ-CDA-other conjugates	0	2
unknowns[b]		
1[c]	8	13
2	<1	2
3[c]	1	12
others[d]	6	8
unextractable	2	5

[a] As liberated by acid hydrolysis. [b] Unknowns 1–3 have corresponding R_f values on TLC-A of 0.64, 0.78, and 0.53, respectively. [c] Refers to a composite of as many as three metabolites and their conjugates that were resolved on TLC, but not identified. [d] Refers to sample radiocarbon that did not adequately resolve after TLC analysis and includes compounds that did not move free of the origin even after enzymatic or acid hydrolysis.

in any of the alcohol-labeled preparations. Benzylfuroic acid (BFCA), however, was identified in both CAL and TAL sample extracts by TLC cochromatography (A × B, D, G, N), by cochromatography with authentic BFCA-Me on HPLC-B (R_t = 16.0 min), and by mass spectral analysis (Table IV).

A metabolite slightly more polar than resmethrin with R_f values of 0.87 (TLC-A) and 0.71 (TLC-D) was isolated from CAL excreta and was also extracted from the cis-acid-RES (CAC) excreta (Table III). Derivatization of this metabolite yielded a single less polar compound as indicated by its R_f value on TLC-A in relation to the unmethylated metabolite. GLC/MS analysis of the methylated derivative (Table IV) supports its assignment as the methyl ester of (5-benzyl-3-furyl)methyl *cis*-2,2-dimethyl-3-[(*Z*)-2-(hydroxy carbonyl)-1-propenyl]cyclopropanecarboxylic acid (BFA-*Z*-CDA-methyl). Chromatographic behavior of the cis-*Z* isomer of this metabolite on TLC-G, as previously reported (Ueda et al., 1975b), is essentially identical with that of the metabolite isolated in these studies.

Another metabolite that is slightly more polar than BFCA was identified as 5-(4-hydroxybenzyl)-3-furoic acid (4-OH-BFCA). GLC/MS analyses of the mono- and dimethylated derivatives of this metabolite were used for confirmation (Table IV).

The metabolite 5-(α-hydroxybenzyl)-3-furoic acid (α-OH-BFCA) was identified in the TAL and CAL excreta also. This metabolite did not resolve from 4-OH-BFCA with TLC-A but is resolved when developed in TLC-D (R_f 0.21). The mass spectrum and behavior on TLC-A of the methyl ester were essentially identical with data reported earlier (Ridlen et al., 1984) for α-OH-BFCA-Me.

Table IV. GLC/Mass Spectral Data for Resmethrin and Certain of Its Metabolites and Analogues[a]

compound[b]	m/z: M^+	base peak	other peaks
c-RES	338	123	171, 143, 128
t-RES	338	123	171, 143, 128
BFA-*cZ*-CDA-Me	382	171	351, 323, 143, 128
c-CA-Me	182	123	167, 151, 139, 107, 81
t-CA-Me	182	123	167, 151, 139, 107, 81
cE-CDA-Me	212[c]	107	181, 153, 139
tE-CDA-Me	212[c]	107	181, 153, 139
cE-CDA-Me_2	226	107	195, 167, 125
cZ-CDA-Me_2	226[c]	107	195, 167, 125
tE-CDA-Me_2	226	107	195, 167, 125
tE-CHA-Me	198	69	167, 139, 121, 102
tZ-CHA-Me	198	121	167, 139, 102
cE-CHA-Me	198	69	167, 139, 121, 102
BFCA-Me	216	216	201, 184, 156, 128
α-OH-BFCA-Me	232	232	215, 172, 115, 105, 77
4-OH-BFCA-Me	232	232	217, 200, 172, 144
4-MeO-BFCA-Me	246	246	231, 215, 186, 158

[a] Data from metabolites (or their methyl derivatives) isolated from excreta and/or eggs. Analysis parameters defined in the text. [b] Trivial names for compounds shown in Figure 1 and/or described in text. [c] Molecular ion not observed.

Polar metabolites common to both the cis and trans alcohol preparations were isolated from the excreta extracts. The methylated derivatives of these metabolites were found not to be amenable to GLC/MS analysis, and thus each was analyzed by direct-insertion probe. The spectral data from these metabolites suggested that each was a conjugate of some type. Difficulties arose, however, in establishing a true molecular ion for each of these metabolites even after several attempts. Under the conditions of study, it was not possible to identify the conjugating moiety for these metabolites. Acid hydrolysis and subsequent TLC studies indicated that these metabolites were conjugates of BFCA and α-OH-BFCA.

Incubations of the excreta origin metabolites with β-glucuronidase were effective in releasing 20% and 11% of the TAL and CAL origin metabolites, respectively. BFCA was the major hydrolysis product from both isomers, representing 5% for the trans and 2% for the cis preparations, as indicated by cochromatography on TLC (A, D, G). The glucuronide of 4-OH-BFCA was also identified by TLC as indicated by the presence of the released aglycon. The inactivated enzyme control samples contained trace amounts of free 4-OH-BFCA, probably resulting from spontaneous hydrolysis of the conjugate precursor.

Aryl sulfatase treatment of the origin metabolites released only small amounts of alcohol-derived products. From both alcohol-labeled [^{14}C]resmethrin preparations, sulfatase incubations resulted in the liberation of 4-OH-BFCA, as indicated by cochromatography on TLC (A, D, G). In both activated and inactivated enzyme systems, small amounts of BFCA and 4-OH-BFCA were released. An additional product was released from TAL and CAL origin metabolite samples upon incubation with sulfatase. This metabolite was also isolated in the conjugated form from TAL and CAL excreta and is slightly more polar than the 4-OH-BFCA metabolite, as indicated by its behavior on TLC (A, D, G). The liberated product was not detected in control samples, and therefore its precursor appears to be a sulfate conjugate. Attempts to further define the nature of this metabolite from TAL and CAL excreta extracts and from enzyme hydrolysis studies were not suc-

cessful as the majority of this metabolite (>95%) decomposed during the extraction and derivatization procedures.

Acid hydrolysis of the origin metabolites resulted in more extensive cleavage of the conjugates from both TAL and CAL samples. Four products were resolved by TLC (A, D, G) from both isomers and together represented 16 and 14% of the TAL and CAL origin sample radiocarbon, respectively. BFCA was isolated from both samples, as expected, and represented 6% and 2% of the sample radiocarbon from TAL and CAL samples, respectively. In addition, conjugates of 4-OH-BFCA were released following hydrolysis and represented 2% of the sample radiocarbon from both TAL and CAL samples. Additional products released upon hydrolysis did not cochromatograph with compounds of known composition or were present in insufficient amounts to permit further analysis.

Acid-Derived Metabolites. TLC work with extracts of excreta resolved at least eight metabolites from the TAC and CAC excreta. Excreta from the laying hens treated with the acid-labeled [^{14}C]resmethrin isomers contained low levels of resmethrin (1–2%), as indicated by TLC cochromatographic studies (C × D, G, I, N). The free-acid metabolite formed upon hydrolysis of the ester linkage, chrysanthemic acid (CA), was identified as a metabolite from both TAC and CAC samples by comparisons with unlabeled standards on TLC (C × D, A, G, N) and by GLC/MS analysis of their methyl esters (Table IV). Methylation of *t*-CA and *c*-CA yielded methyl esters that were volatile; significant losses (≳75%) were observed when solutions of these methylated metabolites were concentrated following cleanup stages.

Another metabolite common to both isomers, when methylated, consistently yielded two products of reduced polarity on TLC-A. GLC/MS analysis identified the least polar product as the dimethylated chrysanthemumdicarboxylic acid (CDA-Me_2) and the more polar derivative as a monomethylated chrysanthemumdicarboxylic acid (CDA-Me). We have no ready explanation as to why these diazomethane reactions did not rapidly and quantitatively methylate both carboxyls. Previously documented mass spectral data for CDA-Me_2 and CDA-Me in the EI mode (Ridlen et al., 1984) revealed fragmentation patterns essentially identical with the ones obtained in these studies. No major differences in fragmentation patterns or relative abundance of ions were detected between isomers.

On the basis of TLC comparisons with previously published TLC R_f values (D, N) (Ueda et al., 1975b), the dicarboxylic acid metabolite isolated from TAC excreta appears more likely to be the trans-*E* isomer of CDA (*tE*-CDA) rather than the trans-*Z* isomer.

A total of three diacid metabolites (in free and conjugated forms) were isolated from CAC excreta, as determined by mass spectral analysis. Both the cis-*E* and cis-*Z* isomers of CDA (*cE*-CDA, *cZ*-CDA) were tentatively identified by TLC R_f value comparisons (D, N, H) (Ueda et al., 1975a,b). The third diacid metabolite isolated from CAC excreta (*tE*-CDA) gave mass spectral data essentially identical with the cis diacid metabolites previously analyzed. This metabolite was collected along with *cZ*-CDA after development on TLC (A) and subsequent derivatization resulted in two less polar products with chromatographic properties identical with the methyl and dimethyl esters of *cZ*-CDA. Separation of the two dimethylated derivatives was unexpectedly effected on HPLC-B (R_t = 6.4, 8.0 min). The R_f value on TLC (A, D) of the *tE*-CDA metabolite, prior to methylation, and the retention time on HPLC-B of the dimethyl ester are identical with those observed with *tE*-CDA and its dimethyl ester. Therefore, it appears almost certain that this metabolite is in fact *tE*-CDA. Isomerized metabolites of resmethrin have been isolated from urine and feces of rats and have been reported to result prior to formation of the diacid (Ueda et al., 1975a). It is conceivable (though unlikely) that this metabolite may have been derived from *trans*-resmethrin impurities (<2%) in the *cis*-resmethrin preparation given to the birds.

A pair of minor hydroxylated metabolites from TAC excreta extracts were easily resolved on TLC (A). No standards or published EI mass spectral data were available for comparison, but the spectral data (Table IV) strongly support structural assignments as the *E* and *Z* isomers of 2,2-dimethyl-3-[2′-(hydroxymethyl)-1′-propenyl]cyclopropanecarboxylic acid (CHA). Similarly, both *E* and *Z* isomers of CHA were present in small amounts in CAC excreta. Previously reported TLC R_f values (Ueda et al., 1975b) support the isomeric assignments of the isolated CHA metabolites from the CAC and TAC excreta extracts.

GLC/MS analysis of polar, unidentified metabolites common to both CAC and TAC excreta extracts revealed prominent ions characteristic of the intact CDA and CA moieties. Elucidation of the conjugating moiety of these metabolites was not investigated further, but acid hydrolysis (3 N HCl, 30 min, 100 °C) of some of the isolated metabolites and subsequent TLC (D, G) did provide further evidence that conjugates of CA and CDA were present.

Incubation of TAC and CAC excreta origin metabolite samples with β-glucuronidase resulted in the release of as many as five products (Table III). TLC analysis (A, D, G) confirmed the *Z* and *E* isomers of CHA from the TAC and CAC samples as aglycons released. CDA and CA aglycons from both TAC and CAC origin samples were also released. These isolated products, however, represented <1% of the sample radiocarbon.

Acid hydrolysis of the TAC and CAC origin metabolite samples resulted in more extensive cleavage of conjugates present in these samples. Aglycons of *tE*- and *cE*-CDA conjugates were the major aglycons released and represented 4 and 18% of the total sample radiocarbon from the TAC and CAC samples, respectively. The *Z* isomer of CDA, but only from the cis preparation, was released following acid hydrolysis and represented 2% of the total sample radiocarbon.

Characterization of Metabolites in Egg Fractions. *Yolk.* The recovery of radiolabel from the yolk samples of the acid- and alcohol-labeled preparations averaged 95% (91–99%) following extraction with a hexane–tetrahydrofuran (1:1) mixture. Subsequent partitioning with acetonitrile resulted in approximately 76% (71–81%) of the total sample radiocarbon partitioning into the acetonitrile phase. Analysis of the acetonitrile extracts (TLC with appropriate authentic standards in A, D, G–I, U) showed resmethrin as the major radioactive component in the yolks, representing 62% of the total sample radiocarbon from the CAC and CAL yolk samples and 58 and 66% from the TAL and TAC samples, respectively (Table V). GLC/MS analysis of the radiocarbon associated with the resmethrin band after autoradiography confirmed the presence of resmethrin in the extracts from all four [^{14}C]resmethrin preparations.

The ester metabolite (BFA-*Z*-CDA) was tentatively identified in TAL and CAL yolk extracts on the basis of TLC (A, D). Isolation of sufficient quantities of this metabolite for confirmation on GLC/MS was not successful due to the very low amounts present. Neither BFA

Table V. Resmethrin and Metabolites in Egg Whites and Yolks of Laying Hens after Treatment with a Single Oral Dose of [*acid*-^{14}C]- or [*alcohol*-^{14}C]-*trans*- or -*cis*-Resmethrin at 10 mg/kg of Body Weight

	% of sample radiocarbon							
	egg yolk[a]				egg white[b]			
metabolite	TAL	CAL	TAC	CAC	TAL[c]	CAL	TAC	CAC
RES	58	62	66	62		61	0	0
BFA-*Z*-CDA	5	6	} 4[d]	} 3[d]		0	0	0
CA							48	8
E-CHA			0	0			18[f]	20
E-CDA			<1	9			32	26
BFCA	0	0				9		
unknown[e]	4	4	1	2	40	16	1	42
hexane soluble	29	19	28	21				
unextractable	4	9	1	3	60	14	1	4

[a] Extraction and analysis work with egg yolk samples containing peak radiocarbon residues included yolks collected at 4 days posttreatment from TAL-, CAL-, and CAC-treated birds and at 5 days posttreatment from TAC-birds. [b] Extraction and analysis work with egg white samples containing peak radiocarbon residues included whites collected at 1 day posttreatment from TAC- and CAC-treated birds and 2 days posttreatment from TAL- and CAL-treated birds. [c] Very low levels of radiocarbon associated with the TAL egg white extracts did not permit quantitation of metabolites present. [d] May represent mixtures of metabolites CA and BFA-*Z*-CDA that did not resolve on TLC analysis (A, D, G–J). [e] Refers to compounds that did not move free of the origin after TLC analysis and/or did not cochromatograph with compounds of known composition. [f] The *E* and *Z* isomers of CHA from this sample did not resolve on TLC analysis (A, D, K); thus, the reported value may represent a mixture of *E* and *Z* isomers.

nor BFCA was detected in the CAL or TAL yolk samples.

TLC analysis of the TAC and CAC egg yolk extracts revealed a relatively broad radioactive band that cochromatographed with the authentic CA standard. Resolution of CA and the ester metabolite (BFA-*Z*-CDA) in this study was not apparent after TLC analysis, and it is therefore postulated that the residue associated with the radioactive band isolated may have been a mixture of CA and BFA-*Z*-CDA. The presence of BFA-*Z*-CDA in CAL and TAL egg yolks would lend support to its presence in CAC and TAC egg yolk samples as well.

The *E* isomer of CDA was also identified from TAC and CAC egg yolk extracts (TLC: A, D, G, C × D). Limited amounts of this metabolite did not enable spectral confirmation of its identity.

The balance of the radioactivity associated with the egg yolk samples did not resolve as discrete components by TLC analysis, suggesting that the composition of this radiocarbon may have included a number of very minor metabolites or decomposition products of metabolites.

Egg Whites. In samples of freeze-dried egg white, an average of 94% (86–99%) of the total sample radiocarbon was recovered by extraction with methanol for both acid-labeled resmethrin isomers (TAC, CAC) and from the CAL samples. Only 40% of the sample radiocarbon was recovered from TAL egg white samples (a phenomenon that was replicated several times).

Characterization of the radiolabeled compounds in the egg white samples was extremely difficult due to the very low levels of ^{14}C present. Extraction of large composite samples allowed quantitation of the major metabolites in both acid-labeled samples and the cis alcohol sample, but work with the TAL sample extracts was not successful. Resmethrin and BFCA were the major compounds identified from the CAL sample. The absolute percentage of metabolite distribution in the CAL egg white extracts as reported in Table V must be viewed with caution since these calculations were obtained on the basis of exceedingly low levels of radiocarbon. From the TAC and CAC samples, no detectable levels of resmethrin were found. CA and CDA were identified by cochromatography (TLC: A, D, K) with standards in both TAC and CAC egg white extracts. In addition, CHA isomers were observed by TLC comparisons (A, D, K) with standards generated from the excreta. The isomers of CHA from the TAC egg white sample did not resolve into discrete bands following TLC analysis (A, D, K) but represented (assuming both were present) 18% of the total sample radiocarbon. Only the *E* isomer of CHA was identified from the CAC egg white. A large portion of the sample radiocarbon (42%) from CAC egg white extract was not identified. Of this, however, 37% resolved from the origin on TLC analysis and appeared as a single radioactive band on TLC (A, D, K, C × D). The radiocarbon associated with this band was further resolved on HPLC-D into three radioactive components (R_t = 6.8, 8.9, 9.8 min) that were present in almost equal proportions. None of these products cochromatographed with any of the resmethrin metabolites previously identified; because only trace amounts of these products were available, they were not successfully characterized.

Table VI. Resmethrin and Metabolites in Liver and Kidney of Laying Hens 12 h after Treatment with a Single Oral Dose of [*alcohol*-^{14}C]-*trans*- and -*cis*-Resmethrin at 10 mg/kg of Body Weight

	% of sample radiocarbon			
	liver		kidney	
metabolite	TAL	CAL	TAL	CAL
resmethrin	14	17	<1	<1
BFA-*Z*-CDA	0	<1	0	<1
BFCA				
free	44	4	26	7
conjugates[a]	2	5	6	12
α-OH-BFCA				
free	5	3	11	9
conjugates[a]	<1	<1	4	5
4-OH-BFCA				
free	4	2	<1	<1
unknowns[b]				
1	<1	<1	4	4
2	0	0	6	0
3	0	0	5	0
4	0	2	0	1
others[c]	4	10	22	33
hexane soluble	15	9		
unextractable	12	48	16	29

[a] As liberated by acid hydrolysis. [b] Unknowns 1–4 have corresponding R_f values on TLC-A of 0.26, 0.21, 0.03, and 0.51. [c] Refers to sample radiocarbon that did not resolve into discrete components after TLC analysis (A × B, A, D, G) and includes compounds that did not move free of the origin.

Characterization of Metabolites in Tissues. *Liver and Kidney.* Ether–ethanol extraction of acidified samples of liver and kidney from each of the [^{14}C]resmethrin-treated laying hens recovered an average of 80% (52–91%) of the sample radiocarbon from liver and 84% (71–91%)

Table VII. Resmethrin and Metabolites in Liver and Kidney of Laying Hens 12 h after Treatment with a Single Oral Dose of [*acid*-^{14}C]-*trans*- or -*cis*-Resmethrin at 10 mg/kg of Body Weight

	% of sample radiocarbon			
	liver		kidney	
metabolite	TAC	CAC	TAC	CAC
resmethrin	6	7	<1	<1
BFA-*Z*-CDA	0	0	0	0
CA				
free	40	12	14	<1
conjugates[a]	4	<1	18	8
CHA				
E-CHA	2	12	7	6
Z-CHA	3	<1	4	<1
CDA				
tE-CDA	5	<1	20	2
cE-CDA	0	24	0	34
cZ-CDA	0	4	0	2
unknowns[b]				
1[c]	2	12	7	15
2	2	3	0	2
3	0	0	0	4
4	0	0	0	2
others[d]	19	13	21	15
hexane soluble	7	4		
unextractable	10	9	9	10

[a] As liberated by acid hydrolysis. [b] Unknown 1 has an R_f value on TLC-A of 0.64, while unknowns 2–4 have R_f values of 0.36, 0.09, and 0.25 on TLC-D, respectively. [c] Refers to a composite of as many as three metabolites resolved as a single component on TLC-A, but not identified. [d] Refers to sample radiocarbon that did not resolve into discrete components after TLC analysis (C × D, A, D, G) and includes compounds that did not move free of the origin.

from kidney samples (Tables VI and VII). Radiocarbon not extracted from the liver and kidney residue from the CAL-treated hens comprised 48 and 29% of the total sample radiocarbon, respectively. Acid hydrolysis of the CAL liver residue, followed by ether–ethanol extraction, recovered only an additional 3–5% of the radiocarbon. TLC studies with the acid-hydrolyzed CAL liver extract were unsuccessful due to low radiocarbon levels present and large quantities of interfering materials; thus, the nature of the radiocarbon in this phase is unknown.

TLC analysis of liver and kidney extracts (A × B, C × D) revealed the presence of 8–10 metabolites from each sample (Tables VI and VII). Resmethrin was present in the liver samples of all treated birds (TLC: A, D, G), but only trace amounts were detected in the kidney samples. BFCA and α-OH-BFCA, but not BFA, were identified in liver and kidney samples of both CAL- and TAL-treated hens. Resmethrin represented the major component in CAL liver. Unconjugated α-OH-BFCA and BFCA represented the major metabolites in CAL kidney. The liver extracts also contained 4-OH-BFCA, but only trace levels were detected in TAL and CAL kidney extracts. Conjugates of BFCA and α-OH-BFCA were also detected in the liver and kidney of both alcohol-treated groups, as indicated by cochromatography studies (TLC: A, D, G) with available radiolabeled standards.

The major products identified in extracts of liver from TAC- and/or CAC-treated birds included RES, CA, CHA, and CDA (Table VII). Both the *E* and *Z* isomers of CHA were identified in TAC and CAC liver.

In extracts of kidney from birds treated with the acid-labeled resmethrin preparations, major metabolites included CA, CDA, and CHA (Table VII). In CAC kidney, however, only trace residues of CA were found. An acid-labile conjugate of CA, as indicated by TLC cochromatography of the aglycon (A, D, G), was also identified in

Table VIII. Resmethrin and Metabolites in Skin, Fat, and Muscle of Laying Hens 12 h after Treatment with a Single Oral Dose of [*alcohol*-^{14}C]-*trans*- or -*cis*-Resmethrin at 10 mg/kg of Body Weight

	% of sample radiocarbon					
	trans alcohol			cis alcohol		
metabolite	skin	fat	muscle	skin	fat	muscle
resmethrin	7	51	4	43	58	<1
BFA-*Z*-CDA	<1	<1	0	19	33	<1
BFA	<1	0	<1	5	<1	<1
BFCA	47	0	34	10	<1	19
4-OH-BFCA	<1	0	0	<1	<1	<1
α-OH-BFCA						
free	1	0	13	6	<1	26
conjugates[a]	0	0	18	0	0	14
unknown	9	6	26[b]	5	<1	30[b]
hexane soluble	21	31		3	7	
unextractable	15	12	5	9	2	11

[a] As liberated by acid hydrolysis. [b] Values for unknown from muscle extracts includes percent of total sample radiocarbon lost during cleanup stages.

Table IX. Resmethrin and Metabolites in Skin, Fat, and Muscle of Laying Hens 12 h after Treatment with a Single Oral Dose of [*acid*-^{14}C]-*trans*- or -*cis*-Resmethrin at 10 mg/kg of Body Weight

	% of sample radiocarbon					
	trans acid			cis acid		
metabolite	skin	fat	muscle	skin	fat	muscle
resmethrin	19	72	<1	12	45	6
BFA-*Z*-CDA	<1	<1	0	<1	<1	0
CA	26	<1	41	13	32	6
CHA						
E-CHA	10	0	26[a]	17	<1	41[a]
Z-CHA	10	0		<1	<1	
CDA						
tE-CDA	6	0	12	5	<1	<1
cE-CDA	0	0	0	16	<1	20
cZ-CDA	0	0	0	13	<1	17
unknown	6	7	15[b]	5	<1	7[b]
hexane soluble	3	6		2	6	
unextractable	20	15	6	17	17	3

[a] Isomers were not adequately resolved by TLC. [b] Value for unknown from muscle extracts includes percent of total sample radiocarbon lost during cleanup stages.

both TAC and CAC kidney extracts.

Fat. Extraction of abdominal fat with hexane resulted in an average of 89% (83–98%) recovery of the radiocarbon from all samples. TLC analysis (A, D, G, I) of the acetonitrile fractions revealed the presence of only one or two major metabolites from each sample. At least 45% (45–72%) of the sample radiocarbon from the fat of all treatment groups was in the form of resmethrin (Tables VIII and IX). CA and the ester metabolite of resmethrin (BFA-*Z*-CDA) were other major metabolites tentatively identified in CAC and CAL fat by cochromatography on TLC (A, D, G, I). The presence of BFA-*Z*-CDA from CAL fat was also verified by HPLC-A (R_t = 7.5 min for the methyl ester) in comparison with the available radiolabeled compound from excreta of known identity. No other simple hydrolysis products or further oxidized hydrolysis products occurred in other than trace amounts in the abdominal fat from any of the treated birds.

Skin. An average of 85% (80–91%) of the sample radiocarbon from all treated groups was recovered from skin by the extraction method described. As compared to fat, a smaller percentage of the total sample radiocarbon was in the form of resmethrin, as indicated by TLC (A, D, G) with authentic resmethrin. However, resmethrin was a major component of the skin samples from all treated

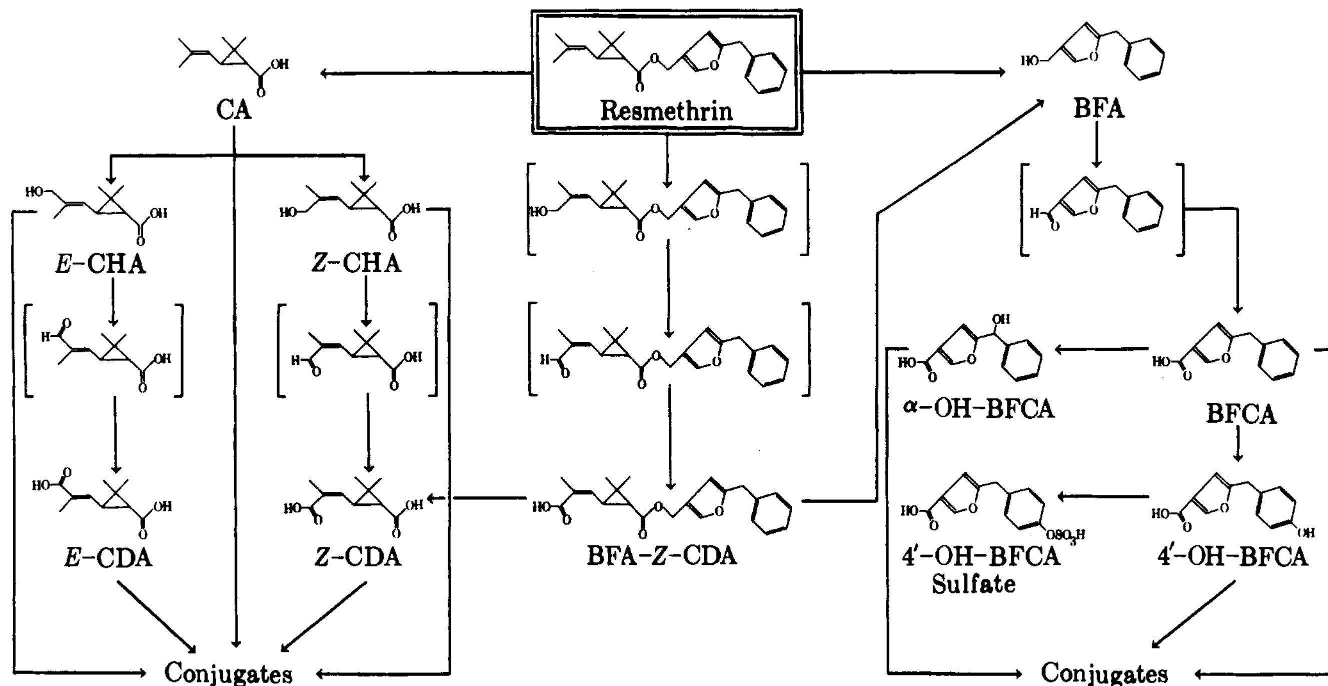

Figure 1. Proposed metabolic pathway for (1*RS*)-*trans*- and (1*RS*)-*cis*-resmethrin in laying hens. Acid moiety metabolites are shown as the trans isomer, but similar pathways were identified for *cis*-resmethrin as well. Structures enclosed by brackets were not isolated but represent logical intermediates leading to identified metabolites. Conjugates include glucuronides and other acid-labile products. The acid moiety metabolite, *cZ*-CDA, and its conjugates were generated in the hen from *cis*-resmethrin, but *tZ*-CDA or its conjugates were not detectable metabolites of *trans*-resmethrin. Although not indicated in the pathway, limited isomerization of cis acid moiety metabolites to trans derivatives may have occurred. Trivial names of metabolites are defined in the text.

birds, and it represented the major ^{14}C residue in CAL skin (Table VIII). BFA-*cZ*-CDA was detected as a major metabolite in CAL skin, but only traces were observed in skin from other treated birds. The α-OH-BFCA and BFCA metabolites were also identified in TAL and CAL samples, with BFCA representing the major portion of sample radiocarbon in TAL skin. In addition, BFA was found in the CAL sample but was present in only trace amounts in TAL skin.

CA, CHA, and CDA were major metabolites isolated from both TAC and CAC skin (Table IX). CA represented the major metabolite from TAC skin. In CAC skin, CA, *cE*-CHA, and the *E* and *Z* isomers of CDA were present in almost equal proportions.

Breast and Leg Muscle. Extraction of acidified breast and leg muscle composite samples with the ether–ethanol system gave an average of 94% radiocarbon recovery (89–97%). However, an additional 6–13% of the sample radiocarbon was lost after C_{18} Sep-Pak cleanup. The presence of large amounts of interfering materials, even after steps were taken to remove these substances, contributed to poor resolution of some of the components in the extracts. As a result, the percentages calculated for metabolites in muscle extracts after TLC resolution were sometimes erratic. The relative amounts and chemical nature of some of the metabolites isolated from the muscle extracts of laying hens treated with the individual resmethrin isomers are shown in Tables VIII and IX.

TLC studies (A, D, G, I) of the TAL and CAL muscle samples showed the presence of at least five metabolites from each isomer. Intact RES, BFCA, and α-OH-BFCA were identified in both TAL and CAL muscle. TLC analysis of TAL and CAL muscle revealed the presence of an acid-labile conjugate of α-OH-BFCA (R_f 0.37, TLC-A). An unidentified polar metabolite (R_f 0.59, TLC-A) was also visualized and represents a portion of the unidentified sample radiocarbon reported. Attempts to identify this metabolite from other sample extracts were unsuccessful due to instability problems associated with it.

In extracts of muscle from birds treated with both acid-labeled resmethrin preparations, RES was identified (Table IX). CA, CDA, and CHA were also identified in TAC and CAC muscle by cochromatography (TLC: A, D, G, I).

DISCUSSION

The metabolic pathways of (1*RS*)-*cis*- and (1*RS*)-*trans*-resmethrin, as tentatively or definitively elucidated in the current study, are indicated in Figure 1. In laying hens, the resmethrin isomers are rapidly absorbed and then metabolized via hydrolytic, oxidative, and conjugative mechanisms. The trans isomer of resmethrin appears to first undergo hydrolysis of the ester linkage, followed by oxidation of the liberated acid and alcohol fragments, as has been demonstrated in rats (Ueda et al., 1975a) and cows (Ridlen et al., 1984). Metabolism of the cis isomer, however, may involve initial attack by either oxidative or hydrolytic mechanisms, as indicated by a higher percentage of the ester metabolite (BFA-*cZ*-CDA) in CAL and CAC sample extracts. Previous in vivo metabolism studies with resmethrin did not isolate or fully characterize ester metabolites; however, in vitro work with *cis*-resmethrin (Abernathy et al., 1973; Ueda et al., 1975b) demonstrated ester metabolites and supports this hypothesis.

Only a small portion (<3%) of the sample radiocarbon in the excreta of laying hens at 12 h after treatment was resmethrin. The alcohol moiety of resmethrin, once released, is rapidly oxidized to BFCA, which undergoes further oxidation or is excreted in the free or conjugated form. In dairy cows treated with *cis*- or *trans*-resmethrin, the glucuronide conjugate of BFCA represented a major metabolite present in urine and tissue (i.e., liver and kidney) samples. Although glucuronide conjugates of BFCA and 4-OH-BFCA were identified from laying hens, glucuronic acid conjugation of resmethrin metabolites did not appear to be a major metabolic reaction in laying hens

treated with resmethrin isomers. The further oxidation products of BFCA (α-OH-BFCA, 4-OH-BFCA) were excreted both in the free and conjugated form.

The acid moieties of *cis*- and *trans*-resmethrin underwent extensive oxidation in laying hens, as has previously been noted in rats (Ueda et al., 1975a). With *cis*- and *trans*-resmethrin, oxidation occurred at methyl groups of the isobutenyl side chain both cis-*Z* and trans-*E* to the cyclopropane ring. Although the preferred site of oxidation varies with the optical configuration of the chrysanthemate moiety (Elliott, 1977), a mixture of the *Z* and *E* isomers of oxidized products (i.e., CHA, CDA) was identified in this study.

The isolation of isomerized CDA metabolites from CAC excreta samples lends support to previous findings of isomerized metabolites in rats (Ueda et al., 1975a). However, it cannot be definitely concluded from this study with laying hens that isomerization did occur; it is possible (though unlikely) that the isomerized products were derived from isomeric contaminants in the resmethrin preparations used.

Unconjugated CA was a major metabolite in the excreta of hens treated with acid-labeled *t*-RES but represented only 1% of the sample radiocarbon from hens treated with *c*-RES. Conjugates of CA, of which the glucuronic acid conjugate is a minor product, were found in the excreta from both resmethrin isomers. The hydroxylated products of CA (i.e., CHA) in laying hens were excreted, underwent further oxidation, and, to a lesser extent, were conjugated with glucuronic acid or other unidentified conjugates. The major metabolite identified in CAC excreta of treated hens was *cE*-CDA. *tE*-CDA was the major metabolite in TAC excreta and represented 21% of the sample radiocarbon. In rats treated with acid-labeled *cis*- or *trans*-resmethrin, *cE*- and *tE*-CDA were the major metabolites isolated from the feces (Ueda et al., 1975a). Based on the excretion patterns and distribution of metabolites in excreta of hens 12 h after treatment, there did not appear to be any major differences in the rates of metabolism of the isomers.

More than 58% of the radiolabeled components in egg yolks was in the form of *cis*- or *trans*-resmethrin. Metabolites included traces of an ester metabolite (BFA-*Z*-CDA), CA, and minor unidentified polar radiolabeled products.

In CAL egg whites, resmethrin represented the major radiolabeled component, but BFCA was also identified in these samples. CA, CHA, and CDA were the major metabolites identified in egg whites of hens treated with acid-labeled *cis*- or *trans*-resmethrin. No detectable levels of resmethrin were found in CAC or TAC egg whites. Even though steps were taken to minimize decomposition of resmethrin present in the sample extracts, spontaneous hydrolysis of trace amounts of resmethrin may explain the lack of unchanged resmethrin in TAC and CAC extracts.

Although appreciable radiocarbon associated with hexane cleanup of some samples (Tables V–IX) is listed as unidentified hexane soluble, some of this likely existed as resmethrin. As previously reported (Ridlen et al., 1984), resmethrin does not partition completely from hexane into acetonitrile.

The resmethrin isomers are rapidly metabolized and excreted by laying hens with very little retention of resmethrin or its metabolites in tissues. Resmethrin was found in the liver of birds treated with both *c*-RES and *t*-RES, but only traces of resmethrin were found in the kidney samples. The residues in these tissues included simple hydrolysis products or further oxidized hydrolysis products, including CA, CHA, and CDA from TAC- and CAC-treated birds and BFCA, 4-OH-BFCA, and α-OH-BFCA from TAL- and CAL-treated birds. Unidentified conjugates of these metabolites were also detected in liver and kidney.

Resmethrin appears to be well suited for its projected use as a spray in poulty houses. Resmethrin, once absorbed, was rapidly metabolized and excreted, and the transfer of significant residues into edible tissues and eggs was minimal.

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