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

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Resmethrin labeled with radiocarbon in either the acid or alcohol moiety and administered orally to lactating Jersey cows at 10 mg/kg was rapidly absorbed, metabolized, and excreted. The cis isomer was eliminated primarily in feces, but the trans isomer was eliminated primarily in urine. Tissue residues at 48 h posttreatment were low (<1 ppm) except in liver and kidney and were generally higher with the alcohol-labeled compounds. Only very low levels of radiocarbon were secreted into milk. Unmetabolized resmethrin appeared in trace amounts in tissue and as the major residue in milk and feces. The major metabolites from both isomers arise from ester hydrolysis and subsequent oxidation of the hydrolytic products and include chrysanthemic acid (free and conjugated with glucuronic acid), chrysanthemumdicarboxylic acid, 5-benzyl-3-furoic acid (free and conjugated with glucuronic acid or glycine), and 5-(α-hydroxybenzyl)-3-furoic acid.

The synthetic pyrethroid insecticide resmethrin is a mixture of (1*RS*)-cis and (1*RS*)-trans isomers of (5-benzyl-3-furyl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate. Resmethrin combines very low mammalian toxicity (Verschoyle and Barnes, 1972; Gray and Conners, 1980) with excellent insecticidal activity (Elliott, 1971; Okuno et al., 1969). Previous studies have examined the metabolism of related pyrethroids in ruminants (Gaughan et al., 1977; Ivie and Hunt, 1980) and the metabolism of resmethrin has been studied in vitro (Ueda et al., 1975a) and in the laboratory rat (Miyamoto et al., 1971; Ueda et al., 1975b).

Among the projected uses for resmethrin is its utilization as a space spray for fly control in and around dairy facilities. The current studies were designed to obtain data on the distribution of resmethrin isomers and their metabolites in the body tissues, excreta, and milk of orally dosed lactating cattle. Remethrin metabolites have been resolved, quantitated, and identified where possible.

MATERIALS AND METHODS

Chemicals. Radiocarbon-labeled preparations of resmethrin (RES) were supplied by the S. B. Penick Corp. (Lyndhurst, NJ) as follows: acid [^{14}C]-(1*RS*)-*cis*-resmethrin (CAC) and acid [^{14}C]-(1*RS*)-*trans*-resmethrin (TAC), both labeled in the carbonyl group of the acid moiety, and alcohol [^{14}C]-(1*RS*)-*cis*-resmethrin (CAL) and alcohol [^{14}C]-(1*RS*)-*trans*-resmethrin (TAL), both labeled in the carbon 2 position of the furan ring. Analysis of each preparation by gas–liquid chromatography/mass spectroscopy (GLC/MS) showed each of the chemicals to be of >98% isomeric purity. Thin-layer chromatography (TLC) in several systems (B–G) (vide infra) showed each preparation to be of >96% radiochemical purity. The chemical identitites of the isomers were verified by mass spectroscopy. Unlabeled samples of (*RS*)-*cis*- and (*RS*)-*trans*-resmethrin and certain analogues for use as possible metabolite standards were also supplied by the Penick Corp. and included 5-benzyl-3-furoic acid (BFCA), (5-benzyl-3-furyl)methanol (BFA), *cis*-chrysanthemic acid (*c*-CA), and *trans*-chrysanthemic acid (*t*-CA).

Chromatography. Initial resolution of resmethrin metabolities from all samples was accomplished by TLC, using precoated silica gel plates (0.25 mm gel thickness, 20 × 20 cm, with fluorescent indicator, Brinkmann) and various combinations of solvent systems as follows: (A) benzene (saturated with formic acid)–tetrahydrofuran (10:1), developed 3 times; (B) benzene (saturated with formic acid)–ether (10:3), developed 2 times; (C) hexane–ether (5:1); (D) carbon tetrachloride–hexane–ether (8:1:1), developed 2 times; (E) benzene–ethyl acetate–methanol (6:1:1); (F) carbon tetrachloride–ether (3:1); (G) benzene–hexane (1:1); (H) 1-propanol–acetic acid–water (6:1:1); (I) methanol–water–acetic acid (8:3:1).

High-performance liquid chromatography (HPLC) was used in some cases to further resolve and chromatographically characterize the metabolites of the resmethrin isomers. A Waters Associates Model 440 instrument with UV detector at 254 nm and Waters chromatography pump, Model M-6000, were used with a 5-μm Supelco C-18 column (15-cm length, 4.6 mm i.d., Supelco, Inc.) preceded by a Whatman C-18 guard column (Pierce Chemical Co.). The mobile phase was varied for optimum resolution of the various metabolites and included (HPLC-A) acetonitrile–water–formic acid (30:70:0.5), (HPLC-B) acetonitrile–water–formic acid (35:65:1), and (HPLC-C) acetonitrile–water (70:30). Radiocarbon quantiation of the eluents was accomplished by collection of the appropriate

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Table I. GLC/Mass Spectral Data (Electron Impact, 70 eV) for Resmethrin and Certain of Its Metabolites and Analogues[a]

compound[b]	initial temp, °C	program, °C/min	retention, min	m/z		
				M^+	base peak	other ions
c-RES	125	15	10.4	338[c]	123	171, 143, 141, 128
t-RES	125	15	10.3	338[c]	123	171, 143, 141, 128
c-CA-Me	125	15	2.8	182	123	167, 139, 107, 81
t-CA-Me	125	15	2.8	182	123	167, 139, 107, 81
c-CDA-Me_2	130	10	6.0	226[c]	107	195, 167, 162, 151
t-CDA-Me_2	130	10	5.4	226[c]	107	195, 167, 162, 151
BFA	90	15	5.0	188	91	141, 129, 128, 115
BFCA-Me	110	10	7.6	216	128	184, 156, 155, 129
α-OH-BFCA-Me	130	10	8.9	232	105	231, 215, 200, 172
BFCA-gly-Me	125	15	9.9	273	185	242, 214, 186, 128

[a] Studies utilized a 5992-B Hewlett Packard GLC/MS system with glass column (1.8 m × 2 mm i.d.) packed with 3% OV-1 on Gas-Chrom Q, helium carrier flow rate of 20 mL/min. [b] Trivial names for compounds shown in Figure 1 and/or described in the text. [c] Molecular ion not observed.

HPLC eluent fractions for liquid scintillation counting (LSC).

GLC/MS (electron impact, 70 eV) was used where possible to verify or determine the structures of isolated metabolites. A 5992-B Hewlett-Packard system was used with a glass column (1.8 m × 2 mm i.d.) packed with 3% OV-1 on Gas-Chrom Q and a helium carrier flow rate of 20 mL/min with various temperature gradients depending on the products under study. The isolated compounds were generally methylated with alcoholic diazomethane at room temperature for 1 h prior to analysis. Retention times and mass spectral data for the resmethrin isomers, metabolite standards, and isolated metabolites are shown in Table I.

Treatment of Animals and Sample Collection. Four lactating Jersey cows in good milk production were obtained from the milking herd of the Texas A&M University Dairy Farm, College Station, TX (immediately adjacent to our laboratories), 1 day prior to experimentation. Cows were weighed, stanchioned, and catheterized for urine collection. The cows were kept on the same ration as they were accustomed to at the dairy, which consisted of 67% corn silage, 23% feed concentrate, 5% whole cottonseed, and 5% chopped coastal bermuda hay. Coastal Bermuda hay and water were available ad libitum.

For treatment, an individual ^{14}C-labeled isomer of RES was diluted with the appropriate unlabeled isomer to a specific activity of 393 dpm/μg. The dose was dissolved in a small amount of acetone, distributed on dairy ration inside a gelatin capsule, and administered orally with a balling gun. Each cow received a dose equivalent to 10 mg of RES/kg of body weight. Animals were milked at 12-h intervals after treatment, aliquots subjected to LSC, and larger portions held at −70 °C. Urine was collected into a stainless steel container kept dark and in an ice bath to minimize possible decomposition of urine metabolites. Every 12 h, urine was measured and radiocarbon quantitated by LSC of aliquots, and portions of the remaining samples were stored at −70 °C. Feces were collected every 1–4 h and stored at 4 °C in the dark. At 24-h intervals, total weight was determined and the samples were mixed thoroughly. The radiocarbon in aliquots of feces was quantitated by oxygen combustion (Oehler and Ivie, 1980) and the remainder stored at −70 °C. Tissue samples were obtained when the animals were sacrificed at 48 h after dosing, portions were analyzed for radiocarbon by oxygen combustion, and the remaining samples were stored at −70 °C.

Sample Analysis. *Urine.* Direct ether extraction of urine resulted in very poor partitioning of radiocarbon into the organic phase. Acidification of the whole urine to pH <2 increased the extraction efficiency but only to ~80%. A more efficient extraction procedure was ultimately developed that utilized a small reversed-phase silica column (Waters C-18 Sep-PAK).

Urine was acidified to pH ~1.3 with concentrated HCl and centrifuged to separate the light precipitate that was present in some samples. This precipitate contained only negligible levels of radiocarbon (<0.1% sample ^{14}C) and was discarded. A C-18 Sep-PAK was activated with 3 mL of methanol and then washed with 5 mL of distilled water. One milliliter of the acidified whole urine was passed through the column, and the eluate, which in every case contained <1% of the original sample radiocarbon, was discarded. A gentle stream of dry nitrogen was passed through the Sep-PAK for 15 min to remove residual water. The column was then placed in a freezer at −4 °C for at least 1 h and then eluted with 10 ml of acetonitrile. This resulted in essentially quantitative recovery of the radiocarbon from the Sep-PAK. The acetonitrile eluate was reduced to ~0.2 mL and applied to a TLC plate for two-dimensional TLC analysis and subsequent visualization of ^{14}C components by autoradiography (Kodak No Screen X-ray film). Some samples of urine were also subjected, prior to analysis, to β-glucuronidase (Calbiochem-Behring Corp., La Jolla, CA) hydrolysis (37 °C and pH 4 for 24 h under dark conditions) or acid hydrolysis using 1 N HCl (100 °C for 2 h).

Feces. To aliquots (5 g of wet weight) of each feces sample, 50 mL of cold methanol was added and the samples were blended with a Polytron homogenizer, then held at 4 °C for 24 h. The cold slurry was centrifuged and the methanol decanted and kept at 4 °C. The above procedure was repeated twice again and the extracts were combined and the radiocarbon content was quantitated by LSC. Radiocarbon not extracted from the feces residue was quantitated by oxygen combustion. Extracts were concentrated by vacuum distillation with absolute ethanol added to effect azeotropic distillation of the water present. The concentrate was taken to dryness and the residue was extracted with ether. The nonether soluble residue contained negligible radiocarbon levels (<1% of the sample ^{14}C) and was discarded. The ether was analyzed by two-dimensional TLC for quantitation of the metabolites.

Blood, Milk, and Other Tissues. Radiocarbon in whole blood samples (collected at sacrifice in tubes containing citrate anticoagulant) was determined by direct LSC of 0.2-mL aliquots. Aliquots of milk (100 mL) were acidified to pH ~1.3 and extracted 3 times with 100 mL of hexane. The hexane was reduced to 50 mL and then partitioned 2 times with 100 mL of acetonitrile. Radiocarbon in both organic phases and in the residual aqueous phase was quantitated by LSC. The acetonitrile was reduced to 0.3 mL and analyzed by HPLC.

Table II. Radiocarbon Elimination in Urine, Feces, and Milk of Cows after Administration of ^{14}C-Labeled Acid or ^{14}C-Labeled Alcohol Preparations of (1*RS*)-*cis*- or (1*RS*)-*trans*-Resmethrin as Single Oral Doses of 10 mg/kg of Body Weight

h after treatment	trans isomer,[b] % of administered radiocarbon					
	milk		urine		feces[a]	
	TAC	TAL	TAC	TAL	TAC	TAL
12	0.002	0.002	9.0	2.4		
24	0.005	0.016	6.4	10.3	3.4	4.8
36	0.010	0.019	6.2	11.1		
48	0.090	0.018	12.8	6.9	9.0	8.4
total	0.107	0.055	34.4	30.7	12.4	13.2
h after treatment	cis isomer,[b] % of administered radiocarbon					
	milk		urine		feces[a]	
	CAC	CAL	CAC	CAL	CAC	CAL
12	0.003	0.060	3.5	3.8		
24	0.035	0.030	7.7	6.4	16.6	4.3
36	0.032	0.060	5.8	6.2		
48	0.022	0.040	3.9	5.5	22.8	21.2
total	0.092	0.190	20.9	21.9	39.4	25.5

[a] Feces collected at 24-h intervals. [b] TAC = acid [^{14}C]-(1*RS*)-*trans*-resmethrin; TAL = alcohol [^{14}C]-(1*RS*)-*trans*-resmethrin; CAC = acid [^{14}C]-(1*RS*)-*cis*-resmethrin; CAL = alcohol [^{14}C]-(1*RS*)-*cis*-resmethrin.

Samples of liver and kidney (5–7 g) were homogenized in 25 mL of distilled water. Standards of unlabeled resmethrin and each of the unlabeled metabolite standards available (~100 μg each) were in some cases added to these samples to retard possible degradation of the very low levels of metabolites potentially present. The homogenate was adjusted to pH ~1.3, saturated with Na_2SO_4, and extracted with ether–ethanol (3:1). The sample was centrifuged to break up the emulsion present in some samples, and the organic portion was aspirated off. Extraction was repeated 2 more times as above. Radiocarbon in the combined organic extracts was quantitated by LSC, and then the extracts were concentrated by vacuum distillation and finally with a stream of dry nitrogen to give an oily layer that was partitioned between acetonitrile (10 mL) and hexane (5 mL). Radiocarbon in both layers was quantitated by LSC and the acetonitrile layer was analyzed by two-dimensional TLC. Residual radiocarbon in the tissue residue was determined by oxygen combustion.

Samples of fat (20 g) were homogenized in 200 mL of hexane. The hexane volume was reduced to 150 mL and extracted 3 times with 100 mL of acetonitrile. Radiocarbon in the hexane layer was quantitated. The volume of acetonitrile was further reduced to 10 mL and partioned again with 5 mL of hexane to remove residual lipid material. Radiocarbon in both layers was quantitated by LSC and the acetonitrile was reduced to ~0.3 mL and analyzed by HPLC with appropriate HPLC fractions ultimately subjected to GLC/MS analysis.

RESULTS

Distribution of Radiocarbon in Excreta, Milk, and Tissues. Cows treated with a single oral dose of TAC or TAL showed similar rates of radiocarbon elimination. Radiocarbon was excreted primarily in the urine in these cows, and >43% of the dose was eliminated within 48 h. Only very low levels (<0.2% of dose) appeared in the milk of the *t*-RES-treated cows (Table II).

Tissue levels of radiocarbon for the TAC treated cow were very low (<1 ppm) except in the kidney and liver (Table III). Radiocarbon levels in tissues of the TAL-treated cow were similarly very low (<1 ppm) except liver, kidney, and ovary. For both the TAC- and TAL-treated cows, the ^{14}C measured in the kidney was higher than that measured in the liver (Table III).

Table III. Radiocarbon Distribution in Tissues of Cows 48 h after Administration of ^{14}C-Labeled Acid or ^{14}C-Labeled Alcohol Preparations of (1*RS*)-*cis*- or (1*RS*)-*trans*-Resmethrin as Single Oral Doses of 10 mg/kg of Body Weight

tissue	ppm of resmethrin equiv			
	trans isomer[a]		cis isomer[a]	
	TAC	TAL	CAC	CAL
kidney	2.6	2.4	0.9	1.4
liver	1.4	1.9	2.6	3.4
brain	<0.1	0.3	0.1	<0.1
ovary	0.5	1.8	0.1	0.2
tongue	0.3	0.5	0.1	0.2
spleen	0.1	0.4	0.1	0.2
lung	0.3	0.9	0.2	0.3
muscle, heart	0.4	0.5	0.1	0.2
muscle, leg	0.1	0.2	0.3	0.1
muscle, longissimus dorsi	<0.1	0.2	<0.1	<0.1
udder	0.1	0.8	0.3	0.3
fat, renal	0.2	0.3	0.5	1.0
fat, omental	0.1	0.1	0.2	0.4
fat, subcutaneous	<0.1	0.2	0.3	0.3
blood	0.9	3.2	<0.1	0.5

[a] See footnote *b*, Table II.

Of the four animals treated with [^{14}C]RES, the cow treated with the CAC isomer showed the highest total excretion of radiocarbon during the 48-h posttreatment period (>60%), with most elimination occurring in the feces. Radiocarbon excretion by the cow treated with CAL was >47% of the total dose, again primarily in the feces. Levels of radiocarbon in milk were very low (<0.2% of dose) for both *c*-RES-treated cows (Table II).

Similar patterns of tissue radiocarbon levels were found in the CAC- and CAL-treated cows (Table III). Levels of radiocarbon in all tissues were low (≤1 ppm) except liver and kidney. However, the levels of radiocarbon observed in the liver of these cows was more than twice those found in the kidney. Levels of radioactivity in the blood of cows treated with the *c*-RES preparations were lower than in the blood of cows treated with the *t*-RES preparations (Table III).

Metabolites in Urine. Urine metabolites were resolved by two-dimensional TLC (A,B), and the very polar metabolites were further resolved with TLC systems H and I. The TLC properties of RES, its metabolites, and derivatives are given in Table IV. The radiocarbon distribution of each resolved metabolite that constitutes 1% or more of the radiocarbon in any sample is shown in Table V. Cumulative totals for trace unidentified metabolites not shown in Table V are <2% of total sample radiocarbon in every case.

Urine from the cow treated with TAC contains no unmetabolized RES or CA as indicated by TLC analysis. Extraction and TLC resolution (B) of the urine after β-glucuronidase hydrolysis indicated that the most polar metabolite cleaves to yield a much less polar product, which was identified by cochromatography (A, B and HPLC-B) as *t*-CA. This metabolite is thus identified tentatively as the glucuronide of *t*-CA (*t*-CA-gluc).

Another metabolite from TAC urine, after methylation with diazomethane, was identified by GLC/MS as a diacid oxidation product of CA. From comparisons with previously published TLC R_f values (B, D) (Ueda et al., 1975b), the metabolite is most likely *trans*-(*E*)-chrysanthemumdicarboxylic acid [*t*-(*E*)-CDA], rather than the trans-(*Z*) isomer.

Urine from the cow treated with TAL also does not

Table IV. Thin-Layer Chromatographic (TLC) Properties of Resmethrin and Its Metabolites and Derivatives

compound[b]	R_f value in indicated solvent system[a] A	B	D	H	I
c-RES	0.85	0.80			
t-RES	0.85	0.80			
c-CA	0.74	0.70			
t-CA	0.74	0.70			
c-CA-Me		0.68	0.70		
t-CA-Me		0.68	0.70		
c-CDA	0.43	0.22			
t-CDA	0.43	0.27			
c-CDA-Me_2		0.75	0.41		
t-CDA-Me_2		0.74	0.51		
BFA	0.62	0.43			
BFCA	0.75	0.60			
BFCA-Me		0.62	0.62		
α-OH-BFCA	0.34	0.21			
α-OH-BFCA-Me		0.46	0.09		
BFCA-Gly	0.05	0.07			
BFCA-Gly-Me		0.23	0.03		
c-CA-Gluc				0.35	0.83
t-CA-Gluc				0.35	0.83
BFCA-Gluc				0.40	0.85

[a] TLC solvent systems as follows: (A) benzene (saturated with formic acid)–THF (10:1), developed 3 times; (B) benzene (saturated with formic acid)–ether (10:3), developed 2 times; (D) carbon tetrachloride–hexane–ether (8:1:1), developed two times; (H) 1-propanol–acetic acid–water (6:1:1); (I) methanol–water–acetic acid (8:3:1). [b] Trivial names for compounds shown in Figure 1 and/or described in the text.

Table V. Resmethrin Metabolites in the Urine of Lactating Cows after Administration of ^{14}C-Labeled Acid or ^{14}C-Labeled Alcohol Preparations of (1*RS*)-*cis*- or (1*RS*)-*trans*-Resmethrin as Single Oral Doses of 10 mg/kg of Body Weight

metabolite[a]	% of radiocarbon in indicated sample 12 h	24 h	36 h	48 h
Acid [^{14}C]-*trans*-Resmethrin (TAC)				
t-CA-gluc	94	91	89	88
t-(*E*)-CDA	2	4	5	6
unknown 1	3	4	5	5
unrecovered[b]	<1	<1	<1	<1
Alcohol [^{14}C]-*trans*-Resmethrin (TAL)				
BFCA-gluc	22	14	11	10
BFCA-gly	62	65	75	74
α-OH-BFCA	0	6	5	3
unknown 1	9	7	5	7
unknown 2	6	7	3	5
unrecovered[b]	<1	<1	<1	<1
Acid [^{14}C]-*cis*-Resmethrin (CAC)				
c-CA-gluc	89	80	80	78
c-(*E*)-CDA	4	6	7	6
unknown 1	5	6	8	7
unknown 2	0	2	0	2
unknown 3	0	2	0	2
unknown 4	1	3	4	4
unrecovered[b]	<1	<1	<1	<1
Alcohol [^{14}C]-*cis*-Resmethrin (CAL)				
BFCA-gluc	42	50	50	53
BFCA-gly	31	28	25	24
α-OH-BFCA	10	11	12	11
unknown 1	12	10	12	11
unknown 2	4	0	0	0
unrecovered[b]	<1	<1	<1	<1

[a] Trivial names for compounds shown in Figure 1 and/or described in the text. [b] Radiocarbon not recovered during the C-18 Sep-PAK column isolation procedure (see the text).

contain products that cochromatograph with standard RES, BFCA, or BFA. Treatment of the major polar metabolite with β-glucuronidase resulted in release of the aglycon, which was identified by cochromatography (B, HPLC-A) and GLC/MS (of the methyl ester) as BFCA. This metabolite is therefore identified as the glucuronide conjugate of BFCA (BFCA-gluc). A second polar metabolite (after methylation and repurification on HPLC-B) was identified by GLC/MS as the glycine conjugate of BFCA (BFCA-gly). A third metabolite from TAL urine was identified as 5-(α-hydroxybenzyl)-3-furoic acid (α-OH-BFCA). The isolated metabolite (after methylation) was confirmed as α-OH-BFCA-Me by GLC/MS analysis. The GLC behavior and MS characteristics of these metabolites are given in Table I.

Table VI. Resmethrin Metabolites in the Feces of Lactating Cows after Administration of ^{14}C-Labeled Acid or ^{14}C-Labeled Alcohol Preparations of (1*RS*)-*cis*- or (1*RS*)-*trans*-Resmethrin as Single Oral Doses of 10 mg/kg of Body Weight

metabolite[a]	% of radiocarbon in sample 24 h	48 h	24 h	48 h
	TAC[b]		TAL[b]	
t-RES	48	65	80	85
t-CA	11	10		
t-(*E*)-CDA	32	13		
BFA			8	5
unknown 1[c]	4	9	8	2
unknown 2	4	2		
unknown 3			3	7
unextracted	<1	<1	<1	<1
	CAC[b]		CAL[b]	
c-RES	56	68	73	68
c-CA	5	6		
c-(*E*)-CDA	4	3		
BFA			3	5
unknown 1[c]	0	2	0	2
unknown 2	11	5		
unknown 3	11	6		
unknown 4	12	9		
unknown 5			18	8
unknown 6			5	16
unextracted	<1	<1	<1	<1

[a] Trivial names for compounds shown in Figure 1 and/or described in the text. [b] See footnote *b*, Table II. [c] Probable ester metabolite.

No CAC or CAL urine metabolites cochromatographed with RES, CA, BFA, or BFCA. Acid hydrolysis of the CAC urine resulted in much less transformation to nonpolar products than was observed with the TAC urine. Enzyme and chromatography (HPLC-A, HPLC-B, TLC A, B) studies identified the most polar CAC metabolite as *c*-CA-gluc. The diacid of *c*-CA was identified in the CAC urine by methylation and subsequent GLC/MS. From comparison to published TLC data (B, D) (Ueda et al., 1975b), the metabolite is most likely *cis*-(*E*)-chrysanthemumdicarboxylic acid [*c*-(*E*)-CDA] rather than the cis-(*Z*) isomer.

The most polar metabolite in CAL urine is chromatographically identical with the most polar metabolite in TAL urine (A, B, H, I, HPLC-A); it yielded BFCA when treated with β-glucuronidase and so is identified as BFCA-gluc. BFCA-gly and α-OH-BFCA were also identified from the CAL urine by TLC and GLC/MS. Unknowns TAL-1 and CAL-2 in the urine appear to be chromatographically the same.

Feces. Methanol extraction of fecal samples from each of the four cows resulted in >98% recovery of the radiocarbon in every case. Results of two-dimensional TLC (A, B) are shown in Table VI. Analyses of extracts of feces from the cow treated with TAC showed a component near the solvent front that cochromatographs (A, B, HPLC-C)

Table VII. Resmethrin Metabolites in the Liver, Kidney, Fat, and Milk of Lactating Cows after Administration of ^{14}C-Labeled Acid or ^{14}C-Labeled Alcohol Preparations of (1*RS*)-*cis*- or (1*RS*)-*trans*-Resmethrin as Single Oral Doses of 10 mg/kg

	% of radiocarbon in sample							
metabolite[a]	liver[b]	kidney[b]	fat[b]	milk[c]	liver[b]	kidney[b]	fat[b]	milk[c]
	TAC[d]				TAL[d]			
t-RES	3	0	11	56	1	0	14	39
t-CA	17	32	0	0				
t-CA-gluc	19	22	0	0				
t-(*E*)-CDA	23	8	0	0				
BFA					0	0	0	0
BFCA					55	60	0	0
BFCA-gluc					6	11	0	0
unknown	4	<1	47	13	1	2	34	23
hexane soluble	28	19	41	23	19	16	51	17
unextracted	6	19	<1	8	18	11	<1	21
	CAC[d]				CAL[d]			
c-RES	0	1	34	43	4	0	32	42
c-CA	26	4	0	0				
c-CA-gluc	17	47	0	0				
c-(*E*)-CDA	3	4	0	0				
BFA					13	7	0	0
BFCA					3	12	0	0
BFCA-gluc					27	31	0	0
unknown	15	<1	32	16	8	2	27	12
hexane soluble	21	18	33	17	12	32	40	15
unextracted	18	26	<1	24	33	16	<1	31

[a] Trivial names for compounds shown in Figure 1 and/or discussed in the text. [b] Samples collected 48 h after dosing. [c] Samples collected 36 h after dosing. [d] See footnote *b*, Table II.

with RES. Further, this product (after precipitation in submilligram quantities from acetonitrile by addition of water) was identified as RES by direct insertion probe MS analysis. Another TAC fecal metabolite was identified as *t*-CA by TLC with the authentic standard (A, B, and HPLC-B). (Although TLC does not adequately resolve *c*-CA and *t*-CA, no studies have yet shown any biochemical isomerization of CA.) The metabolite *t*-(*E*)-CDA was also identified in TAC feces by cochromatography (A, B, E) with the same metabolite isolated and identified from urine of the TAC-treated cow. A minor unidentified feces metabolite from the TAC-treated cow that migrates just below RES on TLC (A, B) is identical in chromatographic behavior with a metabolite resolved by TLC from TAL feces and thus appears to be an intact ester derivative. Extracts of TAL feces contain unchanged RES as the major ^{14}C component and also BFA as evidenced by TLC cochromatography (A, B). No BFCA was found in feces of the TAL-treated animal.

TLC resolution of feces extracts from the CAC treated cow showed the presence of at least seven metabolites. Unchanged RES and *c*-CA were identified on the basis of TLC cochromatography studies (A, B, E). A minor CAC metabolite has identical R_f values on TLC (A, B) to a CAL metabolite and thus appears to be an unknown ester metabolite of RES. Feces from the CAL-treated cow contains RES and BFA, identified by cochromatography (A, B, HPLC-A). No BFCA was found in feces of the CAL-treated cow.

Tissues. Extraction of acidified liver and kidney samples with the ether–ethanol system gave an average of 80% radiocarbon recovery (68–95%). However, as much as 25% of the extracted radiocarbon partitioned from acetonitrile into hexane, and the nature of the radiocarbon in the hexane could not be resolved due to the presence of relatively large quantities of interfering materials. In the acetonitrile phase, metabolites were resolved well by TLC (A, B) (Table VII), but radiocarbon levels were low and percentages calculated were erratic. Metabolites in tissues were identified by cochromatography on TLC (A, B) as RES, CA, BFA, and CDA. The glucuronides of BFCA and CA were characterized by cochromatography with the identified urine metabolites on TLC (H, I). The TAC-treated cow showed a trace of unmetabolized RES in the liver but not in the kidney sample. CA, CDA, and CA-gluc were identified in both liver and kidney from the TAC-treated cow. The liver extract from the cow treated with TAL showed a trace of RES, but no RES was found in the kidney extract. The major metabolite in both tissues is BFCA. No BFA was found in either liver or kidney from the TAL-treated cow.

Extracts of the liver and kidney from the CAC-treated cow both contain *c*-CA, *c*-CA-gluc, and *c*-(*E*)-CDA. A trace of unchanged RES was found in the CAC kidney extract. Both liver and kidney from the CAL-treated cow contain BFA, BFCA, and BFCA-gluc. A trace of RES was found in the liver but not in the kidney of the CAL-treated cow.

Although the nature of the radiocarbon in liver and kidney samples from these cows that partitions into hexane is unknown, it likely consists in part of unmetabolized RES. RES does not partition totally into acetonitrile from hexane; thus, the occurrence of RES in some of the acetonitrile fractions of the liver and kidney extracts virtually assures that the hexane fraction of these samples likewise contains some of the unmetabolized parent compound.

The major ^{14}C component in renal fat in the CAC- and CAL-treated cows is intact RES as determined by cochromatography (HPLC-B, HPLC-C) and verified by GLC/MS. Renal fat from the TAC- and TAL-treated cows showed a considerably lower percentage of total radiocarbon as intact RES (Table VII). Neither BFA nor CA was present in detectable amounts in any renal fat sample.

Analysis of the milk extracts from each of the four cows using HPLC-B and HPLC-C for RES, CA, and BFA identified only unchanged *c*-RES or *t*-RES in any sample (Table VII).

DISCUSSION

The resmethrin isomers are rapidly metabolized in cows by cleavage at the ester bond and further oxidation to yield primarily acid metabolites and their conjugates. The metabolism of *c*-RES and *t*-RES as determined in the

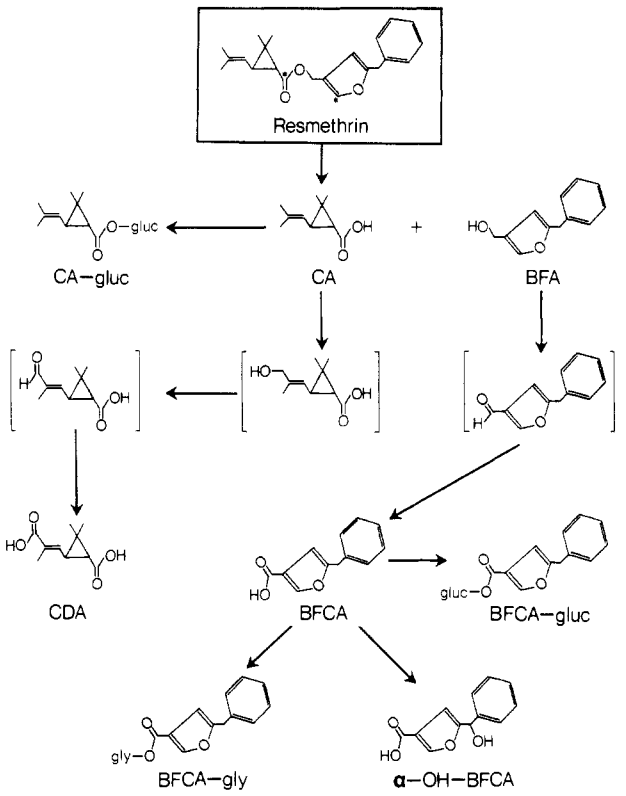

Figure 1. Proposed metabolic pathway for (1*RS*)-*trans*- and (1*RS*)-*cis*-resmethrin in lactating cows. Structures shown indicate pathways for the (1*RS*)-trans isomer—pathways for the (1*RS*)-cis isomer are identical. Trivial name designations for the metabolites are defined in the text. Compounds shown in brackets were not isolated in these studies but are logical intermediates in the pathways defined. In the resmethrin structure, sites of radiocarbon incorporation for acid- or alcohol-labeled preparations are designated by the asterisk (*).

current study is summarized in Figure 1. Eight metabolites from each isomer have been characterized, and several others (in brackets) are indicated as logical intermediates. Five principal sites of metabolic attack are identified and include ester cleavage, oxidation of the carbinol to the corresponding carboxylic acid, oxidation of the α-carbon of the alcohol moiety, oxidation of the trans methyl group of the isobutenyl side chain, and conjugation of the carboxylic acids formed, including those formed by ester cleavage.

No unmetabolized RES or simple hydrolysis products of RES are excreted in urine of cows treated with either *c*-RES or *t*-RES. The acid moiety from both isomers is extensively conjugated with glucuronic acid or to a lesser extent oxidized to the diacid. The alcohol moiety is almost quantitatively oxidized to BFCA that is then conjugated with glucuronic acid or glycine or less extensively oxidized to α-OH-BFCA.

In feces, the primary ^{14}C residue is intact RES. Less extensive metabolism in feces than in urine is indicated by the presene of the primary hydrolysis products CA and BFA and a trace of an unidentified ester metabolite. No conjugates were identified as metabolites in feces from any of the four cows.

Radiocarbon from the *t*-RES treatments is excreted primarily in the urine, but that from *c*-RES treatments is eliminated mostly in the feces. The total amount of radicarbon eliminated from the body averaged about 50% after 48 h for the four cows, varying from 44% for the alcohol-labeled trans isomer to 60% for the acid-labeled cis isomer. Considering that levels of radiocarbon in the 48-h urine and feces samples were quite high (Table II), it is clear that a considerable portion of the administered radiocarbon remained in the gastrointestinal tract at the time of sacrifice.

The cow appears to excrete resmethrin more rapidly than the rat. Although cows require only 2 days to excrete about half of an oral dose, rats require 6 days to excrete from 53 to 73% (Ueda et al., 1975b). Both animals eliminate the alcohol-labeled trans isomer most slowly and show the same general distribution of radiocarbon between urine and feces.

The major metabolite identified in the urine of rats treated with acid-labeled *c*-RES or *t*-RES is *c*-(*E*)-CDA or *t*-(*E*)-CDA while in cows the major metabolite is CA conjugated with glucuronic acid. The major metabolites from alcohol-labeled *c*-RES and *t*-RES in both species are BFCA conjugates. Both α-OH-BFCA and 4-OH-BFCA have been isolated from the urine of rats, but only α-OH-BFCA was identified from the urine of cows. No properties of metabolites from cows were observed that are similar to the published TLC properties of 4-OH-BFCA in the solvent systems used by Ueda et al. (1975b). Only metabolites derived from ester hydrolysis are found in urine of rats and cows, and the alcohol moiety is always oxidized further to the carboxylic acid. The extent or nature of conjugation has not been investigated thoroughly in the rat, but when urine was treated with β-glucuronidase and sulfatase, the efficiency of ether extraction was nearly doubled (Ueda et al., 1975b). Our study has shown that the cow excretes the large majority of the RES metabolites in urine as glucuronide or glycine conjugates.

The major ^{14}C component found in the feces of cows is intact RES. This is in sharp contrast to the rat, where, even at a dose 50 times greater than that used in the present study, no RES was found in rat feces at 24 h or later (Miyamoto et al., 1971). The diacid is a major metabolite in feces for both the rat and cow.

Resmethrin appears to be well suited for its projected use as a space spray for dairy facilities. The results of our studies show that resmethrin and its metabolites are rapidly eliminated from the cow with only minimal retention of residues in body tissues or secretion into milk. Resmethrin is a highly efficacious and selective insecticide that is rapidly biodegradable and that is minimally toxic to mammals. Data from our work with lactating cattle are consistent with the supposition that resmethrin poses little if any toxicological hazards to dairy cattle or to the general public through consumption of meat and dairy products from animals subjected to resmethrin sprays.

ACKNOWLEDGMENT

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Registry No. *trans*-CA-gluc, 92078-98-3; *cis*-CA-gluc, 92142-94-4; *trans*-(*E*)-CDA, 22413-50-9; *cis*-(*E*)-CDA, 54984-64-4; BFCA-gluc, 37744-75-5; BFCA-gly, 92078-99-4; α-OH-BFCA, 37744-70-0; *trans*-CA, 827-90-7; *cis*-CA, 15259-78-6; BFA, 20416-09-5; BFCA, 24313-22-2; *cis*-RES, 10453-56-2; *trans*-RES, 10453-55-1.

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Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable. From a dissertation by R.L.R. "Distribution and metabolism of *cis*- and *trans*-resmethrin [(5-benzyl-3-furyl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate] in lactating cattle", College of Veterinary Medicine, Texas A&M University, College Station, TX, May 1983, pp 1–78.

Superoxide-Mediated Monodehalogenation of Cyclodiene Insecticides

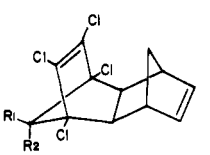
Prem Dureja, S. Walia, and S. K. Mukerjee*

The reaction of superoxide anion in Me_2SO with cyclodiene insecticides has been studied to examine its dehalogenating effect. Aldrin (1), dieldrin (2), and endrin (3) gave with this reagent only the monohalogenated products 4, 5 and 6, respectively, having *syn*-chloro orientation at the methano bridge C atom. Endosulfan ether (7), although stable photochemically, gave a similar product (8) in high yield. Excess reagent did not cause further dehalogenation even under vigorous conditions.

Superoxide ion ($O_2^{-}\cdot$) is now well-known because of its involvement in biological oxidations, but interest in its organic chemistry has increased very much only in recent years. It is known to have redox properites and is an excellent nucleophilic (basic) reagent alone or in combination with crown ethers (Yoshihiko and Christopher, 1976). These chemical reactions have been reviewed comprehensively (Lee-Ruff, 1977). More recently, this reagent has been shown to cause dehalogenation of organohalogen compounds like CCl_4, $CHCl_3$, and *p,p'*-DDT with ease in aprotic media (Roberts and Sawyer, 1981). Dureja et al. (1982), on the other hand, observed that this reagent causes facile dehydrohalogenation of the dichlorovinyl side chain of the synthetic pyrethroid permethrin in preference to ester cleavage. In this paper we report a novel stereospecific dechlorination reaction of superoxide ion on some cyclodiene insecticides and related metabolites.

MATERIALS AND METHODS

Chemicals. Aldrin, dieldrin, and endrin were procured from M/s Shell Chemicals (U.K.) and purified before use. Endosulfan was obtained from M/s Excel Industries, Ltd. (India). Endosulfan ether was prepared by the method of Lindquist and Dahm (1957). Photoendosulfan was prepared according to the method of Dureja and Mukerjee (1982). Authentic monodechlorination products 4, 5, and 6 of aldrin, dieldrin, and endrin, respectively, were prepared by treatment of the cyclodiene insecticides with sodium methoxide in methanol and Me_2SO according to the method of Adams and Mackenzie (1969). Potassium superoxide was purchased from Sigma chemicals (Switzerland). Chemical ionization mass spectrometry (CI-MS) was recorded on a Finnegan-3200 spectrometer using methane (0.7–0.9 torr) as the reagent gas at an ionization voltage of 70 eV. ^1H NMR spectra were recorded on a EM-360L spectrometer using Me_4Si as the internal reference. ^{13}C NMR spectra were recorded on a Brucker 330-MHz (FT) spectrometer.



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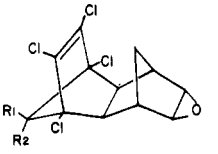
4. $R_1 = Cl, R_2 = H$



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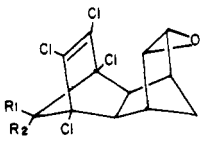
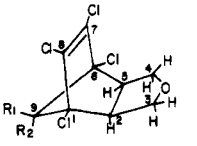


3. $R_1 = R_2 = Cl$
6. $R_1 = Cl, R_2 = H$



7. $R_1 = R_2 = Cl$
8. $R_1 = Cl, R_2 = H$
9. $R_1 = R_2 = H$
10. $R_1 = H, R_2 = Cl$

Action of Superoxide Ion on Cyclodiene Insecticides. (i) Aldrin (1) (100 mg) dissolved in dry Me_2SO (10 mL) was stirred with KO_2 (50 mg) for 2 h at 25 °C. The reaction was monitored (TLC) and on completion was terminated by adding cold water (10 mL). The mixture was extracted with hexane. The extract was dried over anhydrous Na_2SO_4 and the solvent distilled off. The product contained a bit of the unreacted material and was purified by column chromatography. It crystallized from hexane–methylene chloride as colorless needles: mp alone or when mixed with an authentic sample of 4 92–94 °C.

(ii) Reaction of KO_2 with dieldrin (2) under the above conditions and chromatographic purification gave 5 as colorless cubes from hexane–chloroform; mp alone or when mixed with an authentic sample 138–140 °C.

(iii) Similar reaction of endrin (3) with KO_2 gave 6 as colorless needles from hexane chloroform: mp alone or when mixed with an authentic sample 182–184 °C.

Action of Superoxide Ion on Endosulfan Ether (7). Endosulfan ether (200 mg) in Me_2SO (10 mL) and KO_2 (200 mg) was stirred at 25 °C for 2 h when the reaction

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