Metabolism, Distribution, and Excretion of Deltamethrin by Leghorn Hens

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Studies were conducted to determine the metabolic fate of deltamethrin in Leghorn hens. The birds were administered orally 7.5 mg of ^{14}C-labeled (*gem*-dimethyl or benzyl) deltamethrin/hen/day on each of three consecutive days, and the elimination of radiocarbon (^{14}C) in excreta and eggs was monitored for five days after the last dose. About 83% of the administered ^{14}C was eliminated during the first 24 h after dosing. Tissue residues were generally very low with the exception of those for liver and kidney. Residues derived from the *gem*-dimethyl portion of the molecule tended to be higher than those from the benzyl moiety. Egg yolks contained considerably higher levels of residues than egg albumen. Various metabolites were isolated and identified by a combination of TLC, GC, GC–MS, and high-resolution MS techniques. The compounds identified indicated that the metabolic routes of deltamethrin in laying hens include hydrolysis of ester linkage, followed by hydroxylation of one or both *gem*-dimethyl groups, hydroxylation of the 2′-, 4′-, 5-, or 6-position of the phenoxybenzyl moiety.

Deltamethrin, [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, is a synthetic pyrethroid also known as RU-22974, NRDC-161, OMS-168, decamethrin, Decis, and K-Orthin. Although it has been developed for control of insect pests of crops, livestock, and man, very little is known about its distribution and metabolism in poultry and farm animals and the potential for dietary transfer of residues to man through diet.

The metabolic fate of deltamethrin has been defined for rats (Ruzo et al., 1978), mice (Ruzo et al., 1979), mouse liver microsomal system (Shono et al., 1979), and cow and chicken liver enzyme preparations (Akhtar, 1984). The objective of the present study was to acquire information on the fate of two forms of ^{14}C-labeled deltamethrin orally administered to White Leghorn hens for three consecutive days.

MATERIALS AND METHODS

Chemicals. Radiocarbon (^{14}C)-labeled and unlabeled deltamethrin were provided by Roussel Uclaf (France) through Hoechst of Canada Ltd. Two forms of [^{14}C]deltamethrin preparations used in the study were (i) [^{14}C]-*gem*-dimethyl (>98% radiochemical purity) and (ii) [^{14}C]benzyl (>96% radiochemical purity). The radiochemical purity of each preparation was determined by thin-layer chromatography (TLC) followed by autoradiography. Metabolites 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (c-Br_2CA), 3-phenoxybenzyl alcohol (PBalc), 3-phenoxybenzoic acid (PBacid), 3-(4′-hydroxyphenoxy)benzyl alcohol (4′-HO-PBalc), and 3-(4′-hydroxyphenoxy)benzoic acid (4′-HO-PBacid) were available from a previous study (Akhtar, 1984). Dr. Hutson of Shell Research Ltd., Kent, England kindly provided 3-(2′-hydroxyphenoxy)benzoic acid (2′-HO-PBacid). The abbreviations used in the text, tables, and figures as structure designations for the products are generally the same as those used by earlier workers. Glucurase (β-glucuronidase from bovine liver) 5000 sigma unit/mL was purchased from Sigma Chemical Co., Missouri.

Preparation of Treated Feed. For treatment, individual preparations of [^{14}C]deltamethrin were diluted with purified unlabeled deltamethrin to give a specific activity as follows: (i) [^{14}C]*gem*-dimethyl, 0.4 μCi/mg; (ii) [^{14}C]-benzyl, 0.6 μCi/mg. To prepare the daily dose the insecticide was dissolved in acetone and a known volume equivalent to 7.5 mg of deltamethrin was adsorbed onto the feed particles of a standard laying hen diet (10–15 g) so that none of the solution touched the sides of the container. Feed for control chickens was treated with a similar volume of acetone. In order to remove acetone, the containers were allowed to stand at room temperature for about 28 h.

Treatment and Sample Collection. White Leghorn hens at 343 days of age were housed in individual cages and allowed to acclimatized for 14 days. The birds received a standard laying hen diet and water ad libitum. On the day of pesticide administration, the hens were weighed and divided at random into three groups after they had been starved for 6 h. One group was given [^{14}C-*gem-dimethyl*]deltamethrin-treated feed, the second group [^{14}C-*benzyl*]deltamethrin-treated feed, and the third group acetone-treated feed. The treated feeds were administered daily to birds for three consecutive days according to the precision feeding method described by Sibbald (1976). The birds were starved for 6 h prior to each dosing. All birds consumed the feed offered to them. The exaggerated level (about 50 mg kg^{-1} of daily feed intake) and repeated dosage were used in order to accumulate sufficient residues in various tissues for chemical identification of major metabolites. The protocol was similar to that used by Gaughan et al. (1978) who used 3 consecutive daily dosages to study the distribution and metabolic fate of permethrin in laying hens.

Excreta were collected at 24-h intervals until the birds were killed and stored at −20 °C until analyzed. Excreta was collected on a plastic tray covered with polyethylene film. Eggs were collected daily and stored at 4 °C until analyzed.

At 6, 18, 48, and 120 h after the final dose the hens were killed by CO_2 asphyxiation. Just prior to asphyxiation, birds were weighed and blood samples (10 mL) withdrawn from the wing vein, heparinized, and frozen. Representative samples of liver, kidney, heart, subcutaneous, and abdominal fat, breast, and leg muscle and gizzard were collected and frozen for later analysis.

Measurement of Radiocarbon. Tissue, blood, and albumen and yolks were combusted in a Tri-Carb 306 oxidizer (Packard) and counted by liquid scintillation counting (LSC) on a Beckman Model LS-235. Duplicate samples (200–300 mg wet weight) were packed between two

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layers of cellulose before combustion. Likewise fat and yolk samples (about 80–120 mg) were combusted and analyzed on the LSC.

Excreta was extracted with methanol (2.5 mL/g of wet excreta) in a Waring blender, the extract was filtered, and the residue was reextracted thrice. The ^{14}C in the methanol extract was quantitated by LSC. The residue was air-dried for 36 h at room temperature and the ^{14}C content was determined by LSC following sample combustion.

Isolation and Identification of Excreted Metabolites. The methanol extract of excreta was evaporated to near dryness in a fumehood at room temperature. The residue was dissolved in water and extracted five times with equal volumes of ether. Ether extract was dried over Na_2SO_4, filtered, concentrated, and radioassayed. The aqueous phase was concentrated at room temperature and the ^{14}C content was determined.

The aqueous phase containing polar metabolites was treated separately with 3 N HCl and refluxed for 5 h with 0.5 acetate buffer (pH 4.5) or β-glucuronidase in acetate buffer (pH 4.5) for 18 h at 37 °C. The released [^{14}C]aglycons were extracted with ether and radiocarbon content was measured.

The ether extracts (before and after hydrolyses of aqueous phases) were concentrated and, along with available standards, were applied on TLC plates. The plates were developed once in benzene saturated with formic acid–diethyl ether (10:3, v/v) and were exposed to Kodak X-Omat AR film for autoradiography. ***Note***! Diethyl ether used as TLC solvent was preserved with 2% ethanol, (Caldeon Laboratories, Georgetown, Canada). The radioactive zones were scraped off the plates directly into counting vials, 1 mL of methanol was added, the solutions were shaken gently for 2–3 min and allowed to stand overnight before being quantitated by LSC (Hutson et al., 1981). A summation of the ^{14}C content of various zones on a TLC plate gave the total extractable radioactivity which was taken as 100%.

Metabolites were identified by removing radioactive regions from the TLC plates, isolating the compounds with methanol and ether, and comparing their R_f (TLC), gas chromatographic retention time (R_t), and gas chromatography–mass spectrometry (GC–MS), as methylated derivatives where necessary, with those of authentic standards. Structural assignments of compounds for which standards were not available, were made by chemical reactions, comparison of MS fragmentation patterns with those reported for structurally related dichlorovinyl acid analogues, and by high-resolution mass spectrometry. Ruzo et al. (1979) have compared chromatographic (TLC) properties to identify deltamethrin metabolites in a study with mice.

Extraction of ^{14}C from Eggs (Albumen and Yolks), Liver, and Kidney. Composite samples of eggs (albumen and yolks from the eggs were separated, pooled from the same treatment group on a daily basis, and weighed), liver, and kidney of hens treated with [^{14}C-*gen-dimethyl*]-deltamethrin were homogenized in water and then extracted with acetonitrile followed by ether. The aqueous phases were hydrolyzed with 3 N HCl and extracted with ether. Radiocarbon in organic extracts were determined by LSC, combined, and concentrated. The oily residues were applied on TLC plates, developed in benzene saturated with formic acid–diethyl ether (10:3, v/v), and autoradiographed. The ^{14}C content in the extracted residues was determined by combustion followed by LSC.

Thin-Layer Chromatography (TLC). Silica gel TLC plates (Whatman, 0.25–2.0 mm thick with fluorescent indicator) were spotted with ether extracts and developed in benzene saturated with formic acid–diethyl ether (10:3, v/v). The R_f values of deltamethrin, *c*-Br_2CA, PBald, PBacid, PBalc, 2′-HO-PBacid, 4′-HO-PBacid, and 4′-HO-PBalc were 0.92, 0.83, 0.88, 0.71, 0.58, 0.42, 0.38, and 0.25, respectively. The metabolites were detected by (i) viewing under UV light and (ii) autoradiography. Metabolites arising from *c*-Br_2CA moiety were not easily visible under UV light. An autoradiograph of ether extracts prior to hydrolysis, ([^{14}C]-*gem-dimethyl*) is shown in Figure 1.

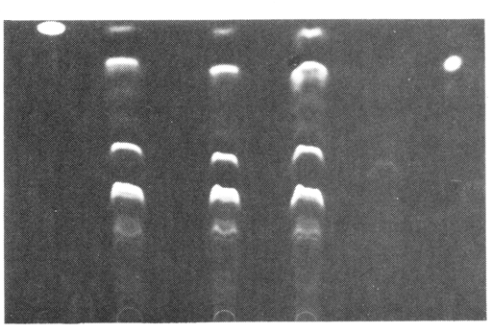

Figure 1. Autoradiographs of ether extracts, prior to hydrolysis, of excreta from laying hens fed [^{14}C-*gem-dimethyl*]deltamethrin for 3 consecutive days. TLC conditions: Whatman K6F silica gel (20 × 20 cm), 250 μ, developed once in benzene saturated with formic acid–diethyl ether (10:3, v/v). Day 1 refers to excreta collected within 24 h after the treatment commenced, etc. (see Table IV).

Gas Chromatography (GC). Extracts, after derivatization and appropriate dilution, were analyzed on a Perkin-Elmer Sigma 1 gas chromatograph equipped with a flame ionization detector (FID) and electron capture detector (ECD). Phenoxybenzyl metabolites were analyzed by a FID with a column 1.82 m × 4 mm (i.d.) packed with 5% OV-210 on 80/100 mesh Gaschrom Q; injector, column, and detector temperatures were 225, 195, and 300 °C, respectively; carrier gas (nitrogen) flow rate was 35 mL/min. The retention times were PBalc 3.03 min, methyl 3-phenoxybenzoate (Me PBacid) 3.62 min, methyl 3-(2-methoxyphenoxy)benzoate (Me 2′-MeO-PBacid) 6.77 min, 3-(4-methoxyphenoxy)benzyl alcohol (4′-MeO-PBalc) 7.17 min, and methyl 3-(4-methoxyphenoxy)benzoate (Me 4′-MeO-PBacid) 8.95 min. A 1.82 m × 4 mm (i.d.) column packed with 1.95% SP-2250 + 1.5% SP-2401 on 100/120 mesh Supelcoport connected to an ECD was used for identification of *c*-Br_2CA metabolites. Figure 2 is a gas chromatogram, obtained with an ECD, of an ether extract of combined ^{14}C zones 3–9 (Figure 1) following derivatization with diazomethane.

Gas Chromatography–Mass Spectrometry (GC–MS). The GC–MS analyses were carried out on a Finnigan Model 1020B automated quadrupole mass spectrometer system connected to a Perkin-Elmer gas chromatograph (Sigma 3) equipped with a 15 m × 0.25 mm (i.d.) SE-54 column. High-resolution mass measurements were performed on a Finnigan MAT 312 mass spectrometer and operated at a resolution of 7000. Both MS were connected to INCOS data systems. The spectra were run in electron impact (EI) mode at 70 eV.

RESULTS

A daily administration of 7.5 mg of deltamethrin to laying hens for three consecutive days had little apparent effect on egg production, body weight, or feed intake when compared to controls. As expected, there was no evidence of regurgitation of the feed after its administration to the birds. In addition, none of the tissues and organs of treated birds showed any gross histological or pathological changes

Table I. Elimination of Radiocarbon in Excreta of Laying Hens during and after Three Daily Oral Administrations of 7.5 mg of [^{14}C]Deltamethrin[a]

time after treatment commenced,[b] days	*gem*-dimethyl							benzyl							
	47[a]	2	4	6	8	1	17	35	37	14	16	18	20	21	29
1	73	75	78	94	97	85	90	67	71	96	88	96	82	88	73
2	68	72	75	87	74	78	85	68	70	76	79	79	72	97	78
3 (1)	46[c]	91[d]	95[d]	102[d]	94[d]	80	89	46[c]	47[c]	69[d]	70[d]	70[d]	65[d]	98	83
4 (2)						82[e]	90							99[e]	91[e]
5 (3)							90								
6 (4)							90								
7 (5)							91[f]								

[a] Accumulative percent of administered dose. [b] Numbers in brackets refer to time (days) after the last dose. [c] Hens were killed 6 h after the final dose. [d] Hens were killed 18 h after the final dose. [e] Hens were killed 48 h after the final dose. [f] Hen was killed 120 h after the final dose. [g] Bird number.

Table II. Amount of Deltamethrin Equivalent (μg/g) in Tissues of Chickens Given Orally 7.5 mg of ^{14}C-Labeled Insecticide Daily on Each of Three Consecutive Days[a]

	gem-dimethyl									
	6 h		18 h				48 h		120 h	
tissues	15[b]	47	2	4	6	8	1	5	7	17
liver	0.27	0.49	1.89	2.43	3.95	1.89	0.52	0.44	0.58	0.12
kidney	0.43	0.79	4.59	3.75	6.85	3.36	0.86	1.05	0.25	0.26
heart	0.02	0.02	0.61	0.31	0.43	0.21	0.05	0.07	0.09	0.04
sq. fat	lost	0.14	0.47	0.12	0.26	0.39	0.17	0.12	0.04	0.19
abdominal fat	lost	0.15	0.66	0.27	0.41	0.25	0.18	0.07	0.08	0.13
breast muscle	0.03	0.02	0.14	0.09	0.21	0.08	0.18	0.09	0.01	[c]
leg muscle	0.02	0.02	0.10	0.15	0.14	0.09	0.09	0.03	0.01	[c]
ovarian yolk	0.31	0.76	3.31	1.35	1.21	2.05	0.67	0.69	[c]	0.06
gizzard	0.05	0.02	0.27	0.35	0.61	0.29	0.06	0.08	0.04	0.01
blood	0.07	0.11	0.79	1.01	1.01	0.41	0.13	0.15	0.05	0.08

	benzyl									
	6 h		18 h				48 h		120 h	
tissues	35	37	14	16	18	20	21	29	19	23
liver	0.35	0.19	0.63	0.36	0.89	0.97	0.23	0.19	0.06	0.09
kidney	0.72	0.27	1.29	1.19	2.11	2.52	0.25	0.52	0.11	[c]
heart	0.09	0.02	0.09	0.04	0.07	0.08	0.04	0.03	[c]	[c]
sq. fat	1.95	0.09	0.16	0.08	0.13	0.17	0.37	0.11	0.12	0.11
abdominal fat	[c]	0.12	0.17	0.11	0.09	0.31	0.25	0.13	0.13	0.12
breast muscle	0.04	0.02	0.03	[c]	[c]	0.15	0.03	0.03	[c]	[c]
leg muscle	0.12	0.02	0.01	0.09	0.02	0.05	0.03	0.03	[c]	[c]
ovarian yolk	0.45	0.32	0.54	0.79	0.78	0.53	0.43	0.16	0.02	0.08
gizzard	1.18	0.03	0.12	0.03	0.24	0.23	0.07	0.07	[c]	0.04
blood	0.12	0.04	0.15	0.18	0.25	0.34	0.14	0.06	0.02	0.02

[a] Value is an average of two or three determinations at the indicated time after the last dose. [b] Refers to bird number. [c] Below 5 ppb level.

when they were killed and dissected.

Elimination and Retention of ^{14}C. The elimination of ^{14}C in the excreta of the orally dosed hens is shown in Table I. In the 24-h period after the initial dose of [^{14}C]deltamethrin, about 84% (73–97%) of *gem*-dimethyl and 83% (67–96%) of benzyl ^{14}C appeared in the excreta. Approximately 92% (80–102%) of the total administered ^{14}C from *gem*-dimethyl-labeled deltamethrin was eliminated within 18–24 h after the last treatment while most of the ^{14}C (≥90%) from both labels (*gem*-dimethyl or benzyl) was eliminated within 48 h after the last dose. Only small amounts (ca<2%) of ^{14}C were eliminated within 6 h after the last dose as determined from the values given in Table I for bird no. 35, 37, and 47. The data in Table I show that deltamethrin and its metabolites are rapidly excreted by laying hens and are similar to observations with rats and mice (Ruzo et al., 1978, 1979).

The amounts of deltamethrin equivalent in blood and various tissues of chickens are shown in Table II. In general accumulation of deltamethrin equivalents in blood and most tissues except liver, kidney, and ovarian yolk is minimal. Residues were highest in tissues of the hens killed 18 h after the last dose. ^{14}C residues in tissues were consistently 2–3 times higher from the *gem*-dimethyl moiety than those from the benzyl portion.

Table III. Deltamethrin Equivalent in the Albumen and Yolks of Eggs after a Daily Dose of 7.5 mg of [^{14}C-*gem-dimethyl*] [*benzyl*-^{14}*C*]Deltamethrin on Each of Three Consecutive Days

	[^{14}C]deltamethrin equivalents, μg/g[a]			
	albumen		yolk	
time after treatment commenced,[b] days	*gem*-dimethyl	benzyl	*gem*-dimethyl	benzyl
1	0.052	[c]	0.019	0.003
2	0.115	0.10	0.121	0.069
3 (1)	0.148	0.007	0.400	0.176
4 (2)	0.193	[c]	0.577	0.251
5 (3)	0.026	[c]	0.488	0.212
6 (4)	0.005	[c]	0.436	0.222
7 (5)	[c]	[c]	0.385	0.201

[a] Average of triplicate analyses on composite samples. [b] Numbers in brackets refer to time (days) after the last dose. [c] Nondetected.

Very low ^{14}C residues were found in eggs obtained within 24 h after the first dose (Table III). However, as the treatment continued, the amounts of deltamethrin equivalent increased reaching a peak within 48 h after the last dose. The amounts of residues in albumen were much lower than in egg yolks. Again, higher residue levels were

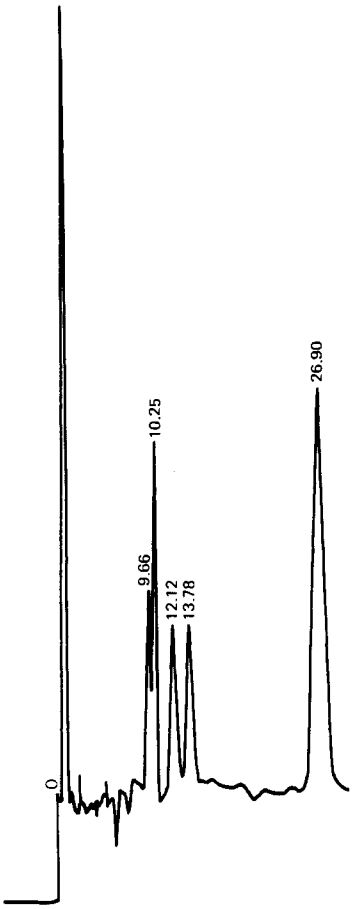

Figure 2. A gas chromatogram of a reaction mixture of ether extract of combined radioactive zone 3–9 with diazomethane. GC conditions: a 1.82 m × 4 mm (i.d.) glass column packed with 1.5% SP-2250 + 1.95% SP-2401 on 100/120 mesh Supelcoport, operated at 170 °C with carrier gas (argon + methane, 95 + 5) flow rate at 35 mL/min. Injector and detector temperatures were at 225 and 400 °C, respectively.

detected from *gem*-dimethyl than from benzyl moiety. The residue levels in the albumen dissipated much faster than in the yolks.

Nature of Excreted Metabolites. (a) [^{14}C]*gem*-Dimethyl Moiety. Laying hens treated with [^{14}C-*gem*-*dimethyl*]deltamethrin eliminated a wide range of ^{14}C-labeled compounds both in the free and conjugated forms. An autoradiograph of ether extract (prior to hydrolysis) exhibited nine radioactive zones (Figure 1).

Zones 1 (R_f 0.92) and 2 (R_f 0.83) were tentatively assigned to unchanged deltamethrin and *c*-Br_2CA, respectively, because of their TLC, GC, and GC–MS properties. Metabolite 3 (R_f 0.71) had an identical retention time in GC before and after treatment with diazomethane (R_t 13.78, Figure 2). Its mass spectra (MS) exhibited molecular ions at m/z 294, 296, 298 in 1:2:1, a distinct pattern for two bromine atoms (McLafferty, 1980) due to various isotopes of bromine atoms. Other major ions were at m/z 250, 252, 254 ($M^{+\cdot}$ – 44, lactone – COO), 237, 239, 241 ($M^{+\cdot}$ – 57), 215, and 217 (($M^{+\cdot}$ – Br). It was converted into *c*-CH_2OH-*c*-Br_2CA (metabolite 7, see below) when treated with pyridine at room temperature. These data are in agreement with a structure *c*-CH_2OH-*c*-Br_2CA-lactone. A similar MS fragmentation pattern was observed for *c*-HO-*c*-Cl_2CA-lactone by Ivie and Hunt (1980).

Compounds in zones 4 (R_f 0.62) and 5 (R_f 0.56) were methylated with diazomethane and the gas chromatograms exhibited peaks at 9.6 and 10.2 min, respectively (Figure 2). The mass spectra of methylated derivatives were

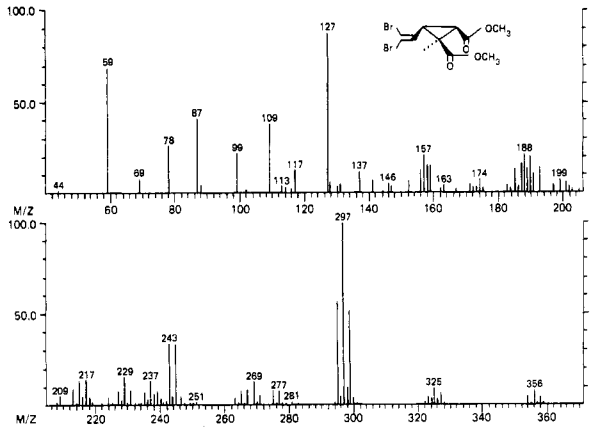

Figure 3. Mass spectrum of the metabolite in zone 5 (Figure 1) after methylation.

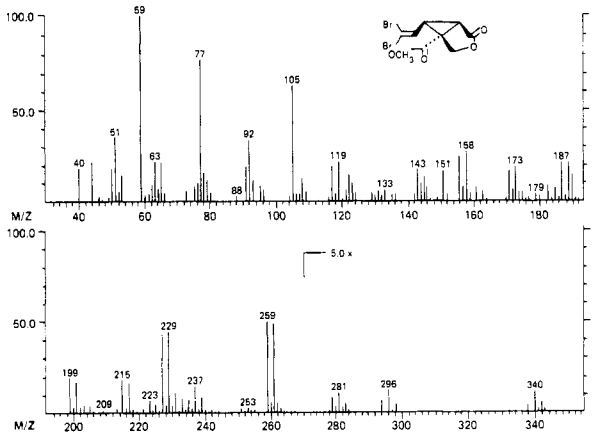

Figure 4. Mass spectrum of the metabolite in zone 6 (Figure 1) after methylation.

identical and showed ions at m/z 354, 356, 358 (1:2:1) for $C_{10}H_{12}Br_2O_4$ ($M^{+\cdot}$), 323, 325, 327 ($M^{+\cdot}$ – 31), 295, 297, 299 ($M^{+\cdot}$ – 59), 243, and 245 ($M^{+\cdot}$ – Br) (Figure 3). The exact mass of the peak at m/z 295 was 294.9019 (zone 4) and 294.8967 (zone 5) which were in good agreement for calculated value 294.8970 for $C_8H_9{}^{79}Br_2O_2$ ($C_{10}H_{12}Br_2O_4$ – $COOCH_3$). Thus, compounds 4 and 5 were isomeric in nature. The compound in zone 5 has tentatively been identified as *c*-COOH-*c*-Br_2CA since it was also formed from oxidation of *c*-CH_2OH-*c*-Br_2CA. The compound in zone 4, on the other hand, has been asigned a structure *t*-COOH-*c*-Br_2CA since it is not formed on oxidation of *c*-CH_2OH-*c*-Br_2CA (see Shono et al. 1979 for discussion on oxidation of hydroxymethyl chlorine analogues). A similar mass spectrum was reported for a methyl derivative of COOH-Cl_2CA by Roberts and Standen (1981).

Metabolite in zone 6 (R_f 0.43) when methylated with diazomethane had a GC retention time of 26.9 min (Figure 2) and exhibited weak ions at m/z 338, 340, and 342 for a molecular composition of $C_9H_8Br_2O_4$. Other ions were at 294, 296, 298 ($M^{+\cdot}$ – 44, lactone – COO), 279, 281, 283 ($M^{+\cdot}$ – 59), 259, 261 ($M^{+\cdot}$ – Br), 235, 237, and 239 ($M^{+\cdot}$ – 44 – 59) (Figure 4). The exact mass of the peak at m/z 259 was found to be 258.9635 which was 2.8 ppm higher than that calculated for $C_9H_8{}^{79}BrO_4$ ($C_9H_8{}^{79}Br_2O_4$ – ^{79}Br), while that of the peak at m/z 261 was 260.9589 and was in excellent agreement with calculated value of 260.9590 for $C_9H_8{}^{81}BrO_4$. These data are readily interpreted in terms of assigned structure *t*-COOH-*c*-CH_2OH-*c*-Br_2CA-lactone.

The component in zone 7 (R_f 0.30) has been assigned the structure *c*-CH_2OH-*c*-Br_2CA since it was produced by the

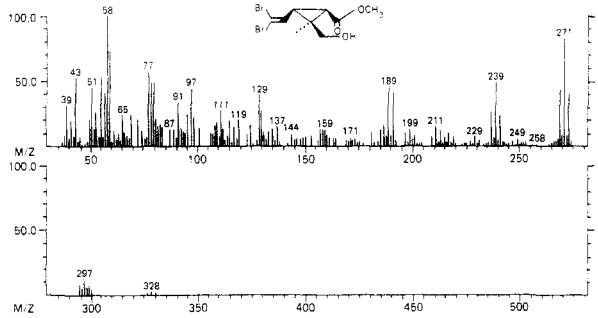

Figure 5. Mass spectrum of the metabolite in zone 7 (Figure 1) after methylation.

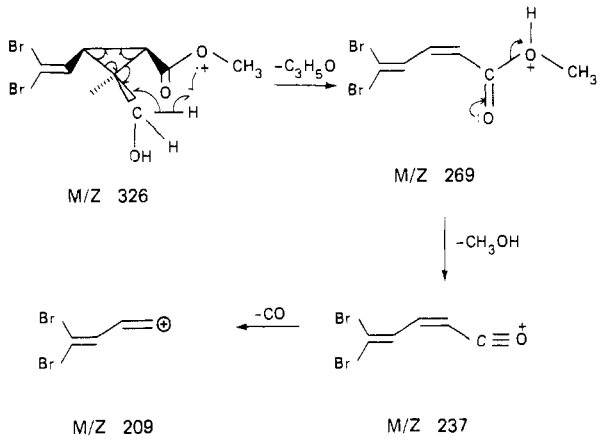

Figure 6. Proposed fragmentation pattern for the methyl ester of c-CH_2OH-c-Br_2CA.

alkaline cleavage of c-CH_2OH-c-Br_2CA-lactone (metabolite 3, Figure 1). In addition, it was totally converted into c-CH_2OH-c-Br_2CA-lactone when treated with 6 N HCl. Thus, a cis configuration of CH_2OH and COOH to each other was established (Unai and Casida, 1977). The methylated derivative of zone 7 had weak molecular ions at m/z 326, 328, and 330 for $C_9H_{12}Br_2O_3$ (Figure 5). Other major ions were at m/z 295, 297, 299 ($M^+ - 31$), 269, 271, 273 ($M^{+\cdot} - 57$), 237, 239, 241 ($M^{+\cdot} - 57 - 32$), 209, 211, and 213 ($M^{+\cdot} - 57 - 32 - 28$). The exact masses of ions at 269, 271, and 273, produced by a loss of C_3H_5O, were 268.8801, 270.8785, and 272.8781, respectively, and were in agreement with the calculated values for $C_6H_7Br_2O_2$ due to various bromine isotopes. A fragmentation pattern for c-CH_2OH-c-Br_2CA-Me resulting in major ions is shown in Figure 6. Ivie and Hunt (1980) also observed a loss of mass 57 from both t-HO-c-Cl_2CA-Me and t-HO-t-Cl_2CA-Me (molecular weight 238 for $C_9H_{12}{}^{35}Cl_2O_3$) to give a base peak at m/z 181 ($M^{+\cdot} - 57$).

Methylation of the extract of zone 8 (R_f 0.14) produced a mixture of compounds. The GC–MS of one of them exhibited ions at m/z 384, 386, and 388 for a molecular composition $C_{10}H_{10}Br_2O_6$ together with ions at 353, 355, 357 ($M^{+\cdot} - 31$), 339, 341, 343 ($M^{+\cdot} - 45$, COOH), 321, 323, 325 ($M^{+\cdot} - 57$) 305, and 307 ($M^{+\cdot} - Br$). These fragmentation patterns are consistent with a dimethyl ester of di-COOH-c-Br_2CA. The only peaks which could be tentatively identified for a second compound were at m/z 367, 369, and 371 (1:2:1). These peaks were most likely produced by a loss of a mass 31 (OCH_3) from the trimethyl ester of di-COOH-c-Br_2CA (calculated $M^{+\cdot}$ 398, 400, and 402). No other information is available presently on this compound.

Methylation of the components from a zone near the origin did not produce compound(s) which could be identified by GC–MS.

(b) [^{14}C]Benzyl Moiety. Hens administered [^{14}C]-benzyl-labeled deltamethrin excreted a complex mixture of radiocarbon compounds. Analysis of ether extracts, as detailed for the [^{14}C]*gem*-dimethyl moiety, exhibited a large number of radioactive zones. The following compounds were identified by TLC, GC, and GC–MS techniques.

Zones 1 (R_f 0.93) 2 (R_f 0.71), and 3 (R_f 0.58) were identified as unmetabolized deltamethrin, PBacid, and PBalc, respectively. A compound with R_f 0.50 (zone 4) was present in small amounts but remains unidentified.

The broad radioactive region at R_f 0.38–0.45 (zone 5) could be due to 2′- and 4′-HO-PBacids. Methylation followed by TLC in toluene (three developments) gave two distinct radiocarbon regions with R_f 0.51 and 0.48 identical with the R_f values for Me 2′-MeO-PBacid and Me 4′-MeO-PBacid, respectively. In addition, the gas chromatogram of a methylated mixture had peaks due to both Me 2′-MeO-PBacid and Me 4′-MeO-Pbacid, as determined by cochromatography with authentic standards. Furthermore, GC–MS of methylated products were in agreement with those recorded for Me 2′-MeO-PBacid and Me 4′-MeO-PBacid (Crawford and Hutson, 1977). On the basis of GC and TLC data, it has been concluded that zone 5 contained both 2′- and 4′-HO-PBacids and their percentage was calculated by the ratio of their methyl esters.

The metabolite from zone 6 (R_f 0.32) when methylated had a GC–MS scan time lower than Me 4′-MeO-PBacids but higher than Me 2′-MeO-PBacid. Its MS had ions at m/z 258 (relative intensity 50%), 199 (100), 171 (55), 153 (33), 93 (48), and 77 (30), whereas that for Me 4′-MeO-PBacid was m/z 258 (100), 199 (5), 171 (16), 93 (0), and 77 (19). A base peak at m/z 199 ($M^{+\cdot} - 59$) suggested that the loss of $COOCH_3$ produces a very stable ion. The peaks at m/z 93 and 77 are attributed to $C_6H_5O^+$ and $C_6H_5{}^+$, respectively, strongly indicating that one of the phenyl rings is free of substitution, i.e., both OCH_3 and $COOCH_3$ groups are on the same ring. These data support a structure methyl 5- or 6-methoxyphenoxybenzoate. Thus, zone 6 has been tentatively identified as containing 5- or 6-HO-PBacid.

The compound obtained from zone 7 (R_f 0.25) behaved as 4′-HO-PBalc. Treatment with diazomethane afforded a derivative which had a GC and GC–MS identical with those obtained for synthetic methyl 3-(4-methoxyphenoxy)benzyl alcohol (Me 4′-MeO-PBalc). Thus, the presence of 4′-HO-PBalc has been authenticated.

Other zones, between the origin and 0.25 could not be identified due to small quantities. They have been collectively termed as unknowns.

Analysis of Conjugate. The reaction and incubation mixtures were extracted with ether and analyzed as detailed above.

(a) [^{14}C]*gem*-Dimethyl Moiety. The amount of ether extractable radiocarbon was slightly higher in HCl (69%) than that from β-glucuronidase treatment (61%). A considerably smaller percentage of radiolabeled material was also released by buffer (30%).

The nature of the residual water-soluble and unextractable radiocarbon was not investigated further.

(b) [^{14}C]Benzyl Moiety. Approximately 46% of the ^{14}C-labeled material in the aqueous phases was ether extractable after the mixture was hydrolyzed with HCl. Similarly, about 19 and 27% of the radioactivity in the aqueous phases was ether extractable after incubation with buffer and β-glucuronidase, respectively.

The source of the residual radioactivity in the water-soluble fraction remains unidentified.

Nature of ^{14}C in Eggs, Liver, and Kidney. In order to ascertain the nature of the extractable radiocarbon from egg (albumen, and yolk), liver, and kidney, extracts were resolved by TLC and autoradiographed.

Eggs. The recovery of radiolabel from albumen and yolks were 32–65% and 27–66%, respectively. Almost all of the radioactivity extracted from albumen was due to unchanged deltamethrin. In egg yolks, a major portion (>71%) of the extractable ^{14}C appeared to be due to deltamethrin by inference with the R_f of deltamethrin. However, its identity could not be positively ascertained by GC due to interfering peaks.

Liver. The amount of radiocarbon extracted from liver ranged from 25–57%. Greater than 90% of the radioactivity occurred in a single region with R_f similar to that for deltamethrin. Attempted analysis by GC before and after cleanup on TLC and microcolumn caused considerable loss of column and detector efficiency, presumably due to the presence of fatty material in the extracts.

Kidney. More than 80% radiocarbon was extracted. An autoradiograph of the TLC plates of the extracts gave four distinct radiocarbon regions due to unchanged deltamethrin (31–35%), *c*-Br_2CA and *c*- and *t*-COOH-*c*-Br_2CA (27–28%), *c*-CH_2OH-*c*-Br_2CA and *t*-COOH-*c*-CH_2OH-*c*-Br_2CA-lactone (19–27), and near the origin, unidentified component (0–23%). The identities of compounds in the first three regions was authenticated by GC.

DISCUSSION

The data presented in this paper with radiolabeled deltamethrin in laying hens confirm earlier reports with rats and mice (Ruzo et al., 1978, 1979) that the insecticide is rapidly metabolized and eliminated. In comparative studies with [^{14}C]*gem*-dimethyl- and [^{14}C]benzyl-labeled deltamethrin reported in the paper, most of the ^{14}C (≥90%) was excreted within 48 h after the last dose (Table I). Likewise, radiocarbon from permethrin isomers were largely eliminated from the body of laying hens within 1 day after the last dose (Gaughan et al., 1978). In fact, in laying hens the rapid excretion prevents accumulation of residues in major tissue compartments.

It was interesting to observe that the residue levels in tissues, organs (Table II), and egg yolks (Table III) for [^{14}C]*gem*-dimethyl were generally 2–3-fold higher than those for birds treated with benzyl-labeled deltamethrin. This may, in part, be due to the lipophilic nature of the *gem*-dimethyl moiety of deltamethrin.

Residues in major edible tissues, namely skeletal muscles were very low (traces to 0.21 μg/g) during the entire period of the study. However, the two vascular organs, the liver and kidneys have relatively high yet transitory levels especially shortly after the dosing period. For example, residue levels as high as 3.95 and 6.85 μg/g were found in the liver and kidneys, respectively, 18 h after the last dose. The residue levels declined gradually during the withdrawal period (Table II).

Radiocarbon levels in albumen and yolk increased with continuing dosage and reached a peak on the 4th day (48 h after the last dose) after initiating the treatment. (Table III). Moreover, the residues were considerably higher in yolk than in albumen (Table III). Similar observations were made by Gaughan et al. (1978) for laying hens treated with permethrin for three consecutive days. These workers recorded higher residue levels in yolk than in albumen with maximum levles noted 72 h after the last dose.

The pronounced differences in the residue levels of albumen and yolks were probably related to the lipid content of yolks, as well as the manner the eggs are produced (Redshaw and Follett, 1982). The data suggested that once deltamethrin and its metabolites were incorporated in the yolks, there was very little exchange from the yolk to the other body compartment. Instead the bird proceeded to encapsulate the preformed yolk containing ^{14}C residues with albumen and shell. Consequently, radiocarbon was detected in egg yolks for several days after the last dose of [^{14}C]deltamethrin. Gaughan et al. (1978) also found larger amounts of ^{14}C from permethrin in yolks than in albumen 5 days after the last dose. Residue levels in egg albumen were very low and decreased quickly when dosing was discontinued. Thus, low levels of residue in albumen were expected since albumen is produced around the yolk before the shell is formed and the final egg is laid.

Table IV. Metabolites in the Methanol Extracts of Excreta of Chickens Treated Orally with [^{14}C-*gem*-*dimethyl*]Deltamethrin for Three Consecutive Days

compound[b]	% on day[a] 1	2	3
deltamethrin	5	2	3
c-Br_2CA	12	6	6
c-CH_2OH-*c*-Br_2CA-lactone	5	3	4
c-COOH-*c*-Br_2CA	8	8	7
t-COOH-*c*-Br_2CA	4	3	3
t-COOH-*c*-CH_2OH-*c*-Br_2CA-lactone	6	4	18
c-CH_2OH-*c*-Br_2CA	11	24	7
di-COOH-*c*-Br_2CA + unknown	5	8	5
c-Br_2CA gluc	5	5	4
c-COOH-*c*-Br_2CA gluc	4	5	4
t-COOH-*c*-Br_2CA gluc	<1	1	1
t-COOH-*c*-CH_2OH-*c*-Br_2CA-lactone gluc	10	9	11
c-CH_2OH-*c*-Br_2CA gluc	4	5	4
water soluble	20	17	23

[a] Day 1 refers to within 24 h after the 1st dose. Day 2 refers to within 24 h after the 2nd dose. Day 3 refers to within 18 h after the 3rd and last dose. [b] See Figure 7 and Materials and Methods for structure and abbreviations.

The results showed that deltamethrin was metabolized by the laying hens through cleavage of the ester bond. There was no evidence for an oxidative attack on the methyl group and benzene ring prior to hydrolysis of the ester bond, a reported metabolic pathway in rats and mice (Ruzo et al., 1978, 1979). On the contrary, permethrin isomers also underwent extensive oxidation prior to hydrolysis in addition to cleavage of the ester bond (Gaughan et al., 1978). Cleavage of the ester bond produced *c*-Br_2CA and PBald via the cyanohydrin.

The relative amounts and nature of metabolites in the methanol extracts of excreta of laying hens administered [^{14}C-*gem*-*dimethyl*]deltamethrin is shown in Table IV. *c*-Br_2CA was excreted both free and as glucuronide. Furthermore, it underwent extensive hydroxylation and oxidation to give a wide variety of products which were excreted free and as glucuronides. Hydroxylation of methyl cis and trans to carboxyl produced *c*- and *t*-CH_2OH-*c*-Br_2CA, respectively, which on further oxidation produced *c*- and *t*-COOH-*c*-Br_2CA, respectively (see Figure 7). In fact, *c*-CH_2OH-*c*-Br_2CA, and *c*- and *t*-COOH-*c*-Br_2CA were present free and as their glucuronides. The absence of *t*-CH_2OH-*c*-Br_2CA may have been due to its facile oxidation to *t*-COOH-*c*-Br_2CA. In *c*-CH_2OH-*c*-Br_2CA, both hydroxymethyl and carboxyl groups are favorably positioned to undergo γ-lactonization to produce *c*-CH_2OH-*c*-Br_2CA-lactone.

Other major metabolites in excreta were *t*-COOH-*c*-CH_2OH-*c*-Br_2CA-lactone and its glucuronide, which may have been produced by a combination of steps involving the *gem*-dimethyl and carboxyl of *c*-Br_2CA. The lactone may have been produced by hydroxylation of the methyl

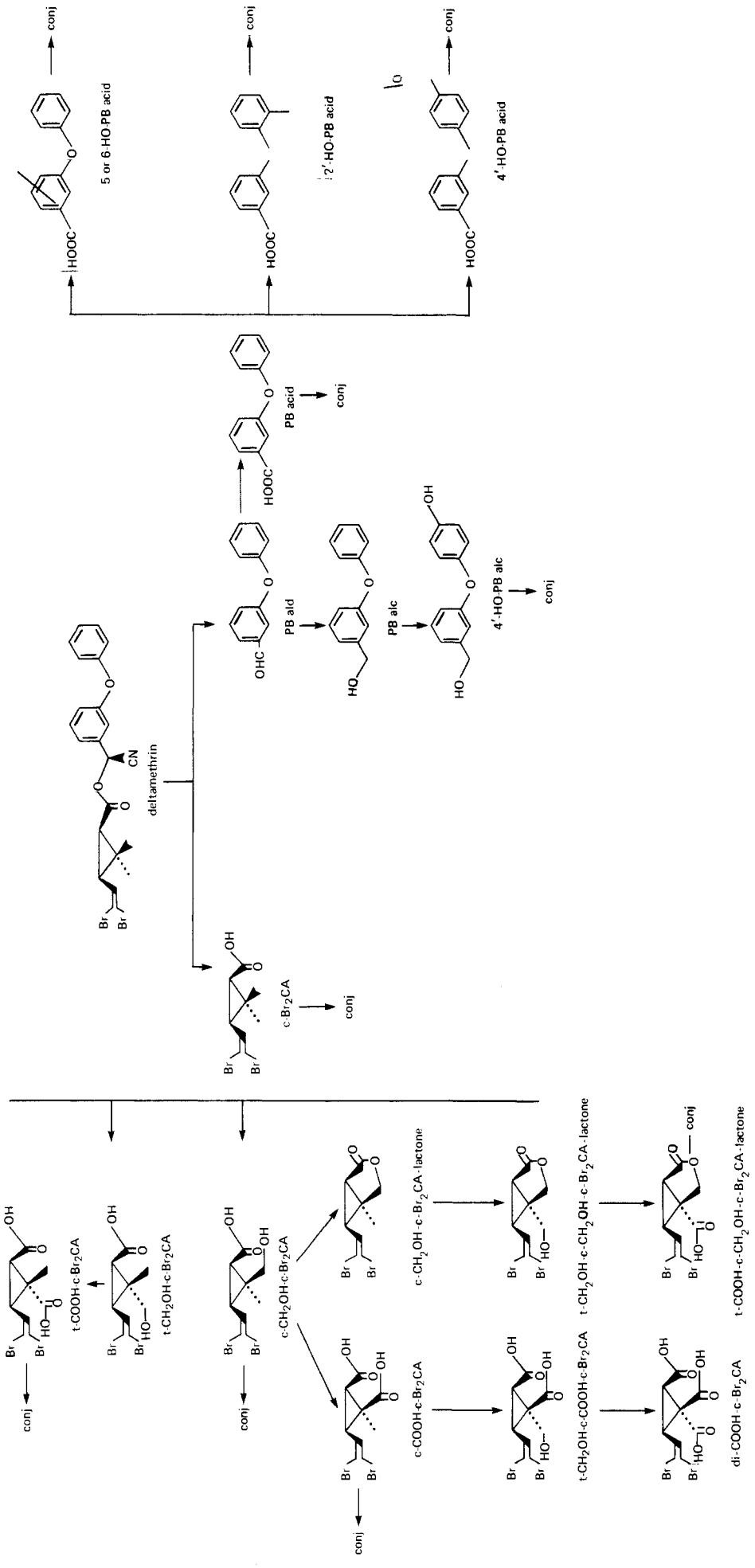

Figure 7. Proposed metabolic pathway of deltamethrin in laying hens.

Table V. Metabolites in the Methanol Extracts of Excreta of Chickens Dosed with [^{14}C-*benzyl*]Deltamethrin for Three Consecutive Days

compound	% on day[a] 1	2	3
deltamethrin	2	1	2
PBacid	4	3	4
PBalc	1	1	1
2′-HO-PBacid	5	7	7
4′-HO-PBacid	7	10	7
5 OR 6-HO-PBacid	1	1	1
4′-HO-PBalc	2	2	2
PBacid conj	1	1	1
2′-HO-PBacid conj	10	6	4
4′-BO-PBacid conj	10	9	12
5 OR 6-OH-PBacid conj	3	4	4
4′-HO-PBalc	3	4	4
unknown	16	17	12
water soluble	35	34	39

[a] See footnote Table IV.

group in c-CH_2OH-c-Br_2CA-lactone followed by oxidation, or hydroxylation of methyl in t-COOH-c-Br_2CA-lactone followed by γ-lactonization. However, the latter alternative would appear less likely since it would produce a stable compound t-COOH-c-CH_2OH-c-Br_2CA which was not detected. On the other hand, hydroxylation of methyl in c-CH_2OH-c-Br_2CA and its lactone would have produced a *trans*-hydroxymethyl moiety which would have been very susceptible to facile oxidation, again by inference with the absence of t-CH_2OH-c-Br_2CA. Although no intermediary metabolite was detected, none of the steps may be totally ruled out.

Small amounts of di-COOH-c-Br_2CA and its glucuronide are also found in the excreta. This is the product of complete oxidation of both methyl groups by a combination of routes.

The excretion pattern of the acid moiety (c-Br_2CA) by laying hens was quite different from those of rats and mice. From rats and mice (Ruzo et al., 1978, 1979) c-Br_2CA is excreted mainly as the free form and its glucuronide along with small amounts of t-CH_2OH-c-Br_2CA and its conjugates. Hens, on the other hand, excreted c-Br_2CA together with its oxidized products both as free compounds and their glucuronides. Hydroxylation of *cis*-methyl was preferred by birds, but not by rats and mice. In contrast, Gaughan et al. (1978) observed preferred hydroxylation of *trans*-methyl of *cis*-permethrin and *cis*-Cl_2CA by laying hens. Moreover, hens also efficiently modify both methyl groups and excrete them as glucuronides. This appears to be the first case in which metabolites due to hydroxylation and oxidation of both methyl groups have been isolated and identified.

The phenoxybenzyl moiety of deltamethrin was excreted in the present study after undergoing extensive structural modification (Table V). Phenoxybenzaldehyde produced after ester cleavage via the cyanohydrin was readily oxidized to PBacid, but reduction to PBalc also occurred to a small extent. In addition, hydroxylation of PBacid at 2′, 4′, 5, and 6 took place to produce polar metabolites and are excreted free and as their conjugates (glucuronides, sulfates, and others), 2′- and 4′-hydroxylation predominating (Table V). Although the nature of conjugating acid(s) other than glucuronic acid was not established, evidences for involvement of other amino acids was obtained from the amount of radioactivity released during HCl hydrolysis compared to β-glucuronidase hydrolysis. On the contrary, Ruzo et al. (1978, 1979) observed that the phenoxybenzyl moiety from rats and mice was eliminated as glucuronides of PBacid, 4′-HO-PBacid, and 5-HO-PBacid (mice only), sulfates of 2′- and 4′-HO-PBacid, and traces of PBacid-glycine (rat only); 4′-HO-PBacid and its conjugates dominated. Thus, hens utilize other amino acid(s) to eliminate the phenoxybenzyl moiety. Evidence in support of involvement of other amino acids(s) was obtained from a recent study by Huckle et al. (1982) on PBacid with chickens (Warren hens). They report that PBacid was efficiently metabolized and excreted as PBacid, 4′-HO-PBacid, PBacid-glycine, PBacid glycylvaline, PBacid acetylornithine, PBacid glucuronide, 4′-HO-PBacid glucoronide, 4′-HO-PBacid O-sulfate, and other unidentified compounds.

The nature of metabolites in various tissues, organs, and eggs could not be fully authenticated since the amounts of these residues were too small. Residues from various tissues depleted during the posttreatment period. By the end of 5 days postdosing there remained only traces of deltamethrin equivalent in tissues. The data presented here indicate that a holding period of 7–10 days would result in very low residue levels.

Based on the metabolites identified, a metabolic pathway for deltamethrin in laying hens is proposed in Figure 7.

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Registry No. c-Br_2CA, 53179-78-5; c-Br_2CA glucuronide, 66855-97-8; c-CH_2OH-c-Br_2CA lactone, 69321-12-6; c-COOH-c-Br_2CA, 96021-60-2; t-COOH-c-Br_2CA, 96093-40-2; t-COOH-c-CH_2OH-c-Br_2CA lactone, 96021-61-3; t-COOH-c-CH_2OH-c-Br_2CA lactone glucuronide, 96021-63-5; c-CH_2OH-c-Br_2CA, 96094-22-3; di-COOH-c-Br_2CA, 96021-62-4; PB acid, 3739-38-6; PB alc, 13826-35-2; 2′-HO-PB acid, 35101-26-9; 4′-HO-PB acid, 35065-12-4; 5 (or 6)-HO-PB acid, 96021-64-6; 4′-HO-PB alc, 63987-19-9; deltamethrin, 52918-63-5.

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