Metabolism of Fenvalerate in Laying Hens[1]

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Laying hens were administered 7.5 mg of [carbonyl-^{14}C]-labeled fenvalerate after feeding for 3 days on unlabeled compound, to study its distribution, retention, elimination, and metabolism. Approximately 85% of the administered radiocarbon was eliminated in 24 h, followed by excretion of an additional 3 and 0.2% of ^{14}C in the next 2 and 6 days, respectively. Eggs laid within 24 h contained radiocarbon mainly in albumen. Radiocarbon residues in yolk peaked in 4–5 days. Radiocarbon residues were detected mainly in the liver and kidney; in other tissues and organs the residue concentration ranged between nondetectable to parts per billion. Fenvalerate was rapidly metabolized by hydrolytic cleavage of the ester bond followed by extensive hydroxylation of the acid moiety at the carbon adjacent to the carboxyl group, the methyl group, or both. Trace amounts of fenvalerate and the acid were identified in egg yolks, liver, and kidneys of treated hens. It was concluded that laying hens when accidentally exposed to fenvalerate residues in diet at concentrations ≤50 mg/kg level should not produce an appreciable concentration of residues in eggs and meat of exposed birds.

Fenvalerate, also known as Sumicidin, Belmark, and Pydrin, is a racemic mixture of α-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)-3-methylbutyrate. The pesticide is gaining wide acceptance in agricultural usage due to its high insecticidal activity against a number of insect pests, moderate mammalian toxicity, and adequate stability in the field (Ohno et al., 1977). Fenvalerate effectively controls northern fowl mites (Hall et al., 1978; Loomis et al., 1979) and reduces the populations of face fly, horn fly, and ticks in cattle (Davey et al., 1980; Knapp and Herald, 1981).

Metabolism of racemic and various other isomeric preparations of fenvalerate has been studied extensively in rats and mice (Ohkawa et al., 1979; Kaneko et al., 1981; Lee et al., 1985; Mumtaz and Menzer, 1986). However, very little information is available on the fate of fenvalerate in poultry and livestock. Wszolek et al. (1981a,b) detected the insecticide and a metabolite in milk, feces, and urine of a dairy cow fed fenvalerate-fortified grain. A chicken liver enzyme preparation degraded fenvalerate very slowly (<10%), mainly by the cleavage of the ester bond (Akhtar, 1983). Recently, Mumtaz and Menzer (1986) observed oxidation at the 4-position of the phenoxy moiety of fenvalerate (21 ± 6.8% 4′-hydroxyfenvalerate) to be the predominant metabolic route for fenvalerate in Japanese quail. Since the expected agricultural use pattern of the insecticide may contaminate feedstuffs, the present study was undertaken to determine the efficiency of excretion and distribution of fenvalerate and to identify the nature of residues in eggs and meat of laying hens that were fed fenvalerate.

MATERIALS AND METHODS

Chemicals. Pesticide- and HPLC-grade solvents were used as received. Radiolabeled (carbonyl-^{14}C) and unlabeled fenvalerate (Fen), 4′-methoxyfenvalerate (4′-MeO-Fen), and 2-(4-chlorophenyl)-3-methylbutyric acid (2-Cl-BA) were available from a previous study (Akhtar, 1983). The radiochemical purity of [^{14}C]fenvalerate was greater than 99% as determined by thin-layer chromatography, autoradiography, and liquid scintillation counting. Methylation was carried out with diazomethane, prepared from the reaction of 50% KOH with *N*-methyl-*N*-nitrosourea.

Caution: Precaution is advised in handling diazomethane and its precursor due to their carcinogenic properties.

Chicken Treatment and Collection of Samples. Ten white Leghorn hens, approximately 1 year old and at 75% egg production, were housed in individual cages within a room maintained at 20 °C with 16 h of light; water and feed were available ad libitum. The laying hens were allowed to acclimatize for 14 days. At the beginning of the experiment, the birds were weighed and randomly divided into two groups of six and four for treatment with fenvalerate or acetone (control) treated feed, respectively. The birds, whose average body weight was 1.5 kg, were starved for 6 h prior to each dosing. For three consecutive days, the six test birds were administered feed containing 7.5 mg of unlabeled fenvalerate/day by the precision feeding technique (oral gavage) described by Sibbald (1976). Twenty-four hours after the third dosing with unlabeled fenvalerate, each bird was given a single dose of feed that contained 7.5 mg (5.93 μCi total ^{14}C) of [*carbonyl*-^{14}C]-fenvalerate. The four control birds were administered feed treated only with acetone. The experimental designs were similar to those published for fenvalerate with Japanese quail (Mumtaz and Menzer, 1986).

Excreta from individual birds were collected 24, 96, and 144 h after being dosed with ^{14}C-labeled fenvalerate. The excreta were collected on a plastic tray covered with polyethylene film, and the entire collections were stored at −20 °C until analyzed. Eggs were collected daily in the morning, marked with the bird's identity and date laid, and stored at 4 °C until being analyzed. Radiocarbon content in eggs was determined within 1 month of collection.

Two hens from the [^{14}C]fenvalerate-treated group were killed 24, 96, and 144 h postdosing, whereas two hens from the control group were killed at 24 h and only one each at 96 and 144 h postdosing. The birds were weighed and blood samples withdrawn from the wing vein prior to CO_2 asphyxiation. Samples of liver, kidney, heart, subcutaneous and abdominal fats, breast and leg muscles, and gizzard were removed and frozen for later radioisotope analysis. Visceral organs were observed at necropsy for gross changes in appearance.

Measurements of Radioactivity. The radioactivity in tissues, organs, and fat were determined by combustion

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in a Packard Tri-Carb sample oxidizer, Model 306D, followed by liquid scintillation counting (LSC) in a Beckman Model LS-235 scintillation spectrometer, using an external standard and correcting the data for quenching.

Excreta were extracted with methanol, and the ^{14}C content of the methanol fraction was determined by LSC. The solid residue was air-dried and its ^{14}C content measured by combustion followed by LSC. A summation of the values from methanol extracts and residues gave the total ^{14}C content in the excreta.

Thin-Layer Chromatography (TLC). Extracts were analyzed on silica gel and reversed-phase plates (Whatman). Silica gel plates were developed in (i) toluene–ether–acetic acid (75:25:1, v/v/v) solvent system A and (ii) hexane–acetone–acetic acid (25:25:1, v/v/v) solvent system B. Reversed-phase plates were developed in methanol–water (8:2, v/v) solvent system C. TLC plates were photographed by a Berthold Beta Camera LB 292 to detect radioactive regions.

Gas Chromatography (GC). TLC extracts, with or without methylation, were analyzed on a Perkin-Elmer Sigma 1 gas chromatograph equipped with an electron capture detector. The column used was a glass column packed with 3% SE-30. For fenvalerate and 4'-methoxyfenvalerate, the column was operated at 240 °C, while for all other analyses the temperature was 165 °C, carrier gas (5% methane in argon) flow 50 mL/min. Injector and detector temperatures were maintained at 265 and 400 °C, respectively. Under the general GC conditions at 250 °C oven temperature, Fen exhibited two peaks with retention times of 6.65 and 7.11 min, whereas the retention time of the methyl ester of 2-Cl-BA at 165 °C was 1.6 min.

High-Performance Liquid Chromatography (HPLC). The individual radioactive TLC zones were analyzed by HPLC on a Waters Associates HPLC instrument equipped with a Model U6K loop injector and a Model 450 variable UV detector connected to a Berthold HPLC radioactivity monitor LB 504. A 300 × 3.9 mm μBondapak C_{18} liquid chromatography column was protected by a 70 × 2.1 mm (i.d.) Whatman "guard column" packed with C_{18}. Operating conditions: ambient temperature; acetonitrile–water (40:60, v/v) isocratic mobile phase; flow rate, 1.0 mL/min; UV detector at 215 nm with sensitivity at 0.4 AUFS; LB 504 detector sensitivity 2500 dpm full-scale deflection; chart speed, 0.33 cm/min. The radioactivity monitor was set to reject peaks at or under 35 dpm since the background level was approximately at 30 dpm. Under these conditions the retention times for Fen and 2-Cl-BA were 5.5 and 5.2 min, respectively.

Gas Chromatography–Mass Spectrometry (GC–MS). Gas chromatography–mass spectrometry was performed with MAT 312 mass spectrometer fitted with a 20 m × 0.25 mm (i.d.) DB5-H column. The spectra were run in electron impact mode at 70 eV.

Analysis of Metabolites in Excreta. The methanol extracts were evaporated to near-dryness in a fume hood at room temperature. The resulting residue was dissolved in a minimum volume of methanol, and the undissolved residues were removed by centrifugation. The clear methanol layer was applied on TLC plates along with available standards. The plates were developed in solvent system A (one development), and the radioactive regions were detected by the Beta camera. The ^{14}C content of each radioactive zone was determined both by scraping off the zones directly into vials for LSC counting and by LSC analysis of the extracts of the individual radioactive zones from preparative TLC plates. Extracts from preparative TLC were reanalyzed by TLC in various solvent systems to determine the radiopurity of each region. GC and GC–MS were performed on the extracts with or without derivatization. Structure assignments of metabolites, for which standards were not available, were made by chemical conversions to known compounds, comparison of MS data with those reported in the literature for synthesized authentic standards (Ohkawa et al., 1979; Lee et al., 1985; Mumtaz and Menzer, 1986; Soderlund et al., 1987).

Table I. Fenvalerate Equivalent in the Albumen and Yolks of Eggs of Laying Hens Given Orally a Single Dose of 7.5 mg of [*carbonyl*-^{14}C]Fenvalerate

time after single dose, h	[^{14}C]fenvalerate equiv,[a] μg/g albumen	yolk
24	0.18 ± 0.04	0.06 ± 0.01
48	0.12 ± 0.04	0.18 ± 0.04
72	0.12 ± 0.04	0.29 ± 0.10
96	0.08 ± 0.06	0.48 ± 0.03
120	0.12 ± 0.08	0.40 ± 0.08
144	nd[b]	0.22 ± 0.05

[a] The average of five analyses on composite samples. [b] nd = nondetected. Detectable limit 0.06 μg/g.

Table II. Amount of Fenvalerate Equivalent (μg/g) in Tissues of Laying Hens Given Orally a Single Dose of [*carbonyl*-^{14}C] Fenvalerate[a]

tissue	24 h	96 h	144 h
blood			
cells	0.14 ± 0.03	0.06 ± 0.02	0.04 ± 0.01
plasma	0.25 ± 0.05	0.07 ± 0.01	0.05 ± 0.01
liver	0.46 ± 0.03	0.13 ± 0.02	0.15 ± 0.03
kidney	0.50 ± 0.08	0.11 ± 0.02	0.15 ± 0.03
heart	nd[b]	0.05 ± 0.03	0.07 ± 0.03
abdominal fat	nd	0.19 ± 0.09	nd
subcutaneous fat	nd	nd	nd
leg muscle	0.05 ± 0.01	nd	0.07 ± 0.02
breast muscle	nd	nd	nd
ovarian yolk	0.11 ± 0.02	0.22 ± 0.05	nd

[a] The value is an average of five determinations for each bird at the indicated time (two birds at each time). [b] nd = nondetected. Detectable limit 0.03 μg/g for tissues and 0.06 μg/g for fat and ovarian yolk.

RESULTS

There did not appear to be any difference in the performance factors (body weight, egg production, feed intake) between the hens given fenvalerate-treated feed (7.5 mg of fenvalerate/day) and the pesticide-free diet. Also, no unusual toxic signs were observed during the experiment. Similarly, no visible adverse effects on any of the tissues and organs were observed when examined during necropsy.

Radiocarbon was efficiently eliminated in the excreta of hens orally dosed with feed containing [^{14}C]fenvalerate. On average, about 85% (85.1 ± 9.7%) of the administered ^{14}C was eliminated in the excreta within 24 h after the hens had been dosed with [^{14}C]fenvalerate. Additional radiocarbon (0.17–9.9%, based on administered dose to individual birds) was found in the next 72 h excreta samples. Although radiocarbon continued to appear in the excreta, it accounted for only about 0.2% in the final 48-h composite samples.

Radiocarbon was detected in eggs of hens given a single oral dose of [^{14}C]fenvalerate (Table I). Initially ^{14}C was located mainly in egg albumen. Residues declined in the albumen and simultaneously increased in yolk during the first 96 h postdosing to a maximum of 0.48 μg/g. After this time, radiocarbon residues declined rapidly in the yolk to about 0.2 μg/g at 144 h after dosing.

The amounts of fenvalerate equivalents in blood (plasma and cells) and in various tissues and organs at different

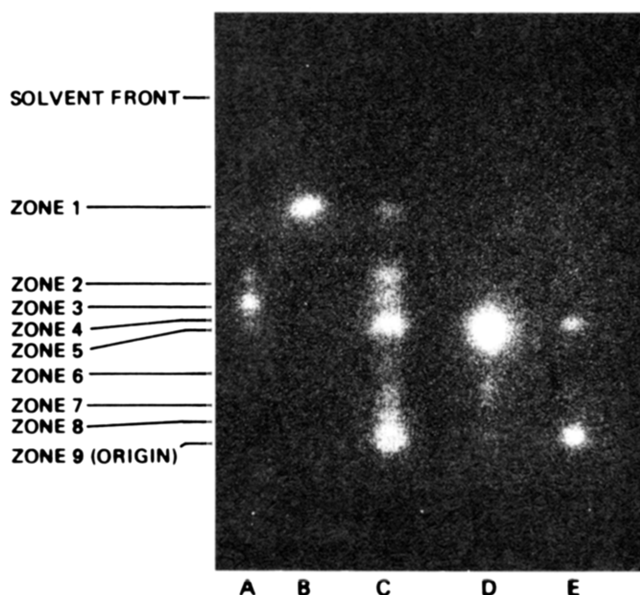

Figure 1. Autoradiograms: (A) 3-CH_2OH-2-Cl-BA-lactone; (B) fenvalerate; (C) methanol extract of excreta; (D) 2-Cl-BA; (E) 2-HO-3-CH_2OH-2-Cl-BA. (A) and (E) were isolated from the methanol extract.

time intervals after a single oral dose of [^{14}C]fenvalerate are listed in Table II. Approximately 0.37% fenvalerate equivalents (calculation assumes average blood volume of 8.3% of body weight and specific gravity of blood to be 1.054 Swenson, 1977) were found in 24-h blood but declined to 0.012 and 0.009%, respectively, in 96- and 144-h blood. The radiocarbon was found mainly in the plasma. The ratio of distribution between the plasma and the cells was 1.8 to 1 in 24-h blood and 1.2 to 1 in 96 and 144-h blood. Radiocarbon was also detected in all tissues and organs except subcutaneous fat. Residues were very low except in liver and kidneys, which contained the highest ^{14}C residues (equivalent to 0.46–0.50 μg/g) in hens killed at 24 h after the dose with [^{14}C]fenvalerate. The highest amount of fenvalerate equivalent in ovarian yolk occurred at 96 h postdosing (Table II), which agrees with the pattern observed in the yolks of eggs laid (Table I).

Autoradiographs of TLC plates (solvent system A) of methanol extracts of excreta showed nine radioactive regions (Figure 1C). Zones 1 (R_f 0.71), 2 (R_f 0.52), and 5 (R_f 0.35) were identified as Fen, 4′-HO-Fen, and 2-Cl-BA, respectively, by comparison with the TLC, HPLC, and GC–MS data of their respective standards and/or methyl derivatives, where applicable. When the metabolite in zone 2 was converted into a GC-mobile compound by a reaction with excess diazomethane and analyzed by GC–MS, the methylated product exhibited major peaks at m/z 451 ($M^{•+}$ + 2), 449 ($M^{•+}$), and 228 and 125 (base peak). The GC retention time and MS fragmentation patterns were identical with that of pure 4′-MeO-Fen prepared in our laboratories and that reported by Soderlund et al. (1987).

The compound in zone 3 (R_f 0.46) was not methylated when treated with diazomethane (see Figure 1A). TLC, HPLC, GC, and GC–MS analyses remained unaltered on treatment with diazomethane. Its retention time on a 3% SE-30 column was 4.1 min, and GC–MS consisted of peaks at m/z 212 ($M^{•+}$ + 2, 12; 35% of $M^{•+}$ at 210), 210 ($M^{•+}$, 36%), 168 (5%), 166 (17%), 153 (22%), 151 (65%), 131 (100%), 127 (6%), 125 (18%), 117 (18%), 116 (49%), 115 (56%), and 91 (27%). The GC–MS data were identical with that of a pure 3-(4-chlorophenyl)dihydro-4-methyl-2(3*H*)-furanone (3-CH_2OH-2-Cl-BA lactone) (Lee et al. 1985). Moreover, when analyzed by TLC in solvent B (two developments), the major (90–93% of the applied radioactivity) appeared as a "dumb-bell" shaped spot (R_f 0.38–0.48), indicating the presence of two compounds. On reversed phase TLC in solvent system C, the metabolite exhibited two radioactive regions at R_f 0.62 and 0.45 in a ratio of 6.5 to 3.5. The major radioactive peak on HPLC had a retention time of 7.5 min. The GC and GC–MS of compounds at R_f 0.62 and 0.45 were identical. In addition, metabolites in zones 7 and 8 also produced the same two compounds when treated with HCl (see discussion below). Thus, the identity of the radioactive compound in zone 3 was established as a mixture of α and β isomers of 3-CH_2OH-2-Cl-BA lactone. Soderlund et al. (1987) recorded R_f values of 0.46 and 0.44 for α and β isomers of 3-CH_2OH-2-Cl-BA lactone (4-HO-CMBA), respectively.

The metabolite in zone 4 (R_f 0.44) was not GC mobile. Methylation gave a GC-mobile compound with a retention time of 7.2 min on a 3% SE-30 column operated at 165 °C. Its mass spectrum contained peaks at m/z 272 (1%, approximately 40% of the peak at 270), 270 (2.5%), 240 (18%), 238 (46%), 211 (27%), 210 (70%), 180 (6%), 178 (19%), 157 (35%), 155 (100%), 117 (27%), 116 (20%), and 115 (41%) (Figure 2). The peaks at 272 and 270, with one chlorine atom pattern, are in agreement with a molecular composition of $C_{13}H_{15}ClO_4$. Furthermore, oxidation of compounds in zones 7 and 8 (see structural details below) with $K_2Cr_2O_7$ in sulfuric acid (Shono et al., 1979) followed by treatment of the reaction product(s) with excess diazomethane gave a major compound with GC–MS properties identical with those of the methyl ester of the compound in zone 4. The HPLC of the unmethylated compound exhibited a single radioactive compound with a retention time of 2.1 min, indicating a compound of a polar nature. On the basis of these data, it was concluded that the structure of the metabolite in this region was 3-carboxy-2-(chlorophenyl)butyric acid (3-COOH-2-Cl-BA).

Compound 6 (R_f 0.24) was present in very small quantities. Attempted analyses by GC and GC–MS, after methylation, did not provide any structural information. However, the R_f value (0.24) agreed closely with the reported values of 0.23 and 0.24 for 4-chloro-α-hydroxy-2-(1-methylethyl)benzeneacetic acid (α-OH-CPIA) in solvent system A (Lee et al., 1985; Mumtaz and Menzer, 1986). Thus, the metabolite in zone 6 was tentatively identified as 2-hydroxy-3-methyl-2-(chlorophenyl)butyric acid (2-HO-2-Cl-BA).

Metabolites in zones 7 (R_f 0.14) and 8 (R_f 0.07) were assigned the structure 3-(hydroxymethyl)-2-(4-chlorophenyl)butyric acid (3-CH_2OH-2-Cl-BA) since they were readily converted to 3-CH_2OH-2-Cl-BA lactone by 3 N HCl. Furthermore, their GC chromatograms prior to methylation showed a peak with a retention time identical with that of the lactone, yet their R_f values were substantially different from that of the lactone. When methylated, the GC chromatograms exhibited peaks due to the lactone (R_f 4.1 min) and one additional compound (R_f 4.72 min). However, the GC–MS spectra of the lactone and the methylated derivative were identical. A similar observation was made during the GC–MS analyses of methyl 3-(2,2-dichlorovinyl)-2-(hydroxymethyl)-2-methylcyclopropanecarboxylate (c-CH_2OH-c-Cl_2CA), which produced an MS spectrum identical with that of the corresponding lactone (Akhtar et al., 1987). Lee et al. (1985) recorded a facile ring closure during the GC–MS analysis of 2-OH-CPIA (3-CH_2OH-2-Cl-BA) but recorded a full spectrum for the methyl derivative with a molecular ion at m/z 242. Metabolites in zone 7 and 8 when analyzed by HPLC also exhibited peaks due to the lactone, in ad-

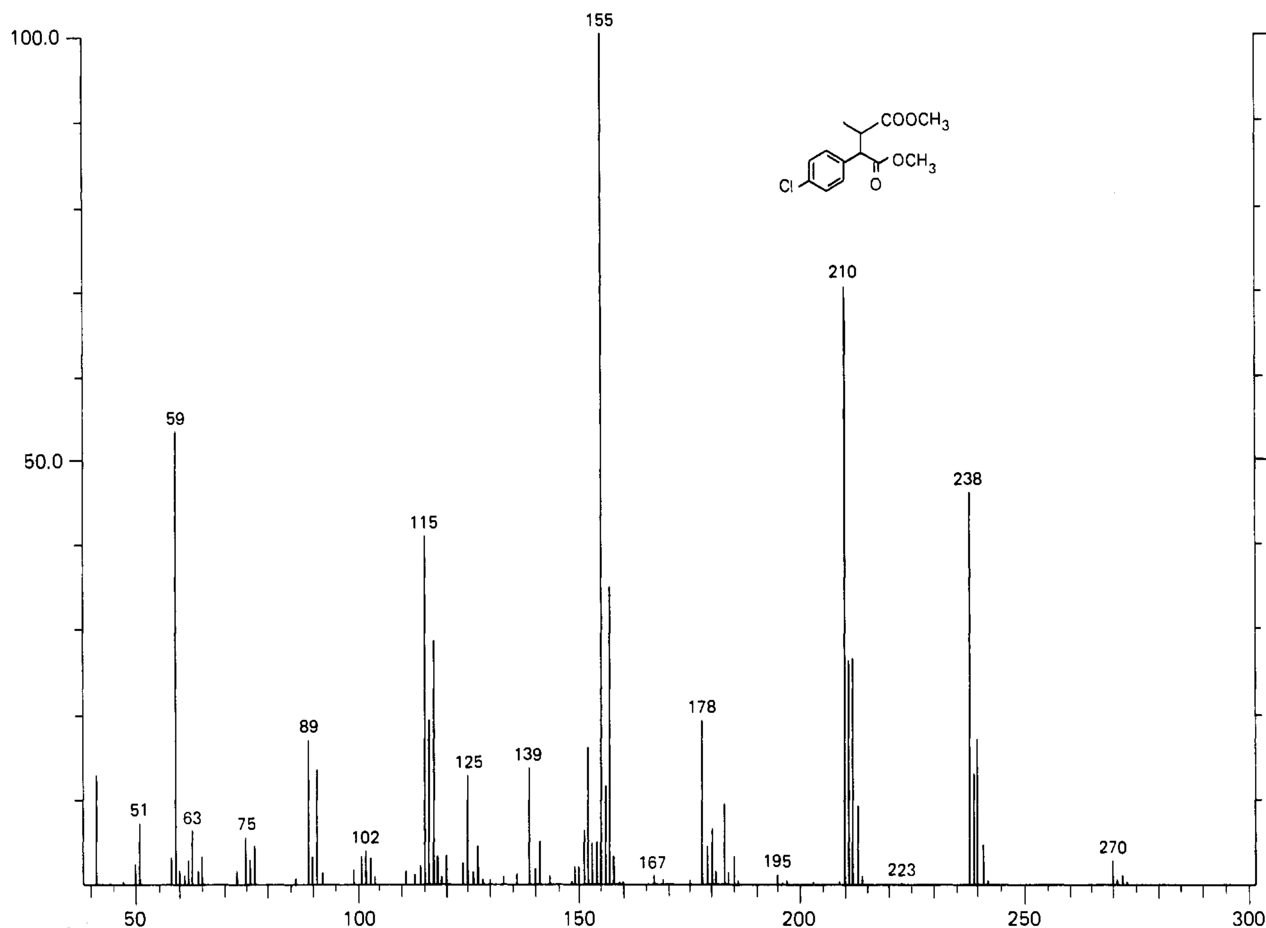

Figure 2. Mass spectrum of the methylated metabolite in zone 4.

dition to a peak with a retention time of either 1.6 or 1.7 min. Metabolites in zones 7 (R_f 0.07) and 8 (R_f 0.14) were designated as the α and β isomers, respectively, of 3-CH_2OH-2-Cl-BA by comparison of their R_f values with those reported in the literature. Both Lee et al. (1985) and Soderlund et al. (1987) recorded higher R_f values for the β isomer than the α isomer. Furthermore, the R_f values of 0.07 (for metabolite in zone 8) and 0.14 (for metabolite in zone 7) in solvent system A were identical with those observed, respectively, for standard α and β isomers of 3-CH_2OH-2-Cl-BA (Lee et al., 1985).

An extract of zone 9 (origin) was treated with excess diazomethane, and the methylated derivative(s) was taken up in hexane. Approximately 55% of the total ^{14}C in zone 9 was transferred into hexane. GC analysis of the methylated mixture indicated the presence of methyl esters of 2-Cl-BA and 3-COOH-2-Cl-BA. However, TLC of zone 1 (Figure 1E) suggested the presence of only small amounts of 2-Cl-BA and 3-COOH-2-Cl-BA. A detailed analysis of the GC–MS of the methylated mixture for the compound containing one chlorine atom confirmed the presence of the methyl esters of 2-Cl-BA and 3-COOH-2-Cl-BA and identified one additional major compound. The MS of this new compound exhibited peaks at m/z 260 (15%), 258 (42%), 223 (40%), 210 (26%), 208 (72%), 181 (49%), 153 (34%), 151 (81%), 115 (82%), and 88 (100%) (Figure 3). The ions at 260 and 258 were due to a combination of various chlorine isotopes for a molecular formula $C_{12}H_{15}ClO_4$. It appeared that ions at 210 and 208 (one chlorine atom pattern) were produced by either simultaneous or successive losses of CH_3OH and H_2O from the parent molecule, i.e., ($C_{12}H_{15}ClO_4$ − CH_3OH − H_2O). The peak at 223 was due to the loss of a chlorine atom, since an isotopic peak at 225 was not present. The MS data provided strong evidence that 2-hydroxy-3-(hydroxymethyl)-2-(4-chlorophenyl)butyric acid (2-OH-3-CH_2OH-2-Cl-BA) was the probable structure for the major radioactive component. A comparison of the R_f values of the major component with the reported values for the 2,4-dihydroxy compound provided further support for the structure. For example, the R_f of the major component was 0.04 (solvent system A), 0.09 (solvent system B), and 0.68 (on C_{18} plate, solvent system C) as compared to the standard dihydroxy compound 0.0, 0.03 (solvent system A), 0.0, 0.12, 0.24 (solvent system B), and 0.85 (solvent system C) (Lee et al., 1985; Mumtaz and Menzer, 1986; Soderlund et al., 1987). The HPLC of the unmethylated compound exhibited one major peak with a retention time of 1.6 min, indicating a highly polar compound.

Eggs. About 65–70% of the total ^{14}C in the composite egg yolk samples was extracted with organic solvents as described previously (Akhtar et al., 1985). Only Fen and 2-Cl-BA were positively identified in the organic extracts.

Liver and Kidney. Only 50% of the ^{14}C was removed from liver and kidney with ether and ethanol as reported earlier (Akhtar et al., 1987). Again the major identifiable components were Fen and 2-Cl-BA.

DISCUSSION

Orally administered fenvalerate to laying hens was rapidly absorbed, extensively metabolized, and eliminated in excreta. Similar results were reported with rats and mice (Ohkawa et al., 1979; Kaneko et al., 1981; Lee et al., 1985), bobwhite quail (Bradbury and Coats, 1982), and Japanese quail (Mumtaz and Menzer, 1986). The efficiency of the elimination of fenvalerate by laying hens is similar to permethrin (Gaughan et al., 1978), fluvalinate (Staiger et al., 1982), deltamethrin (Akhtar et al., 1985),

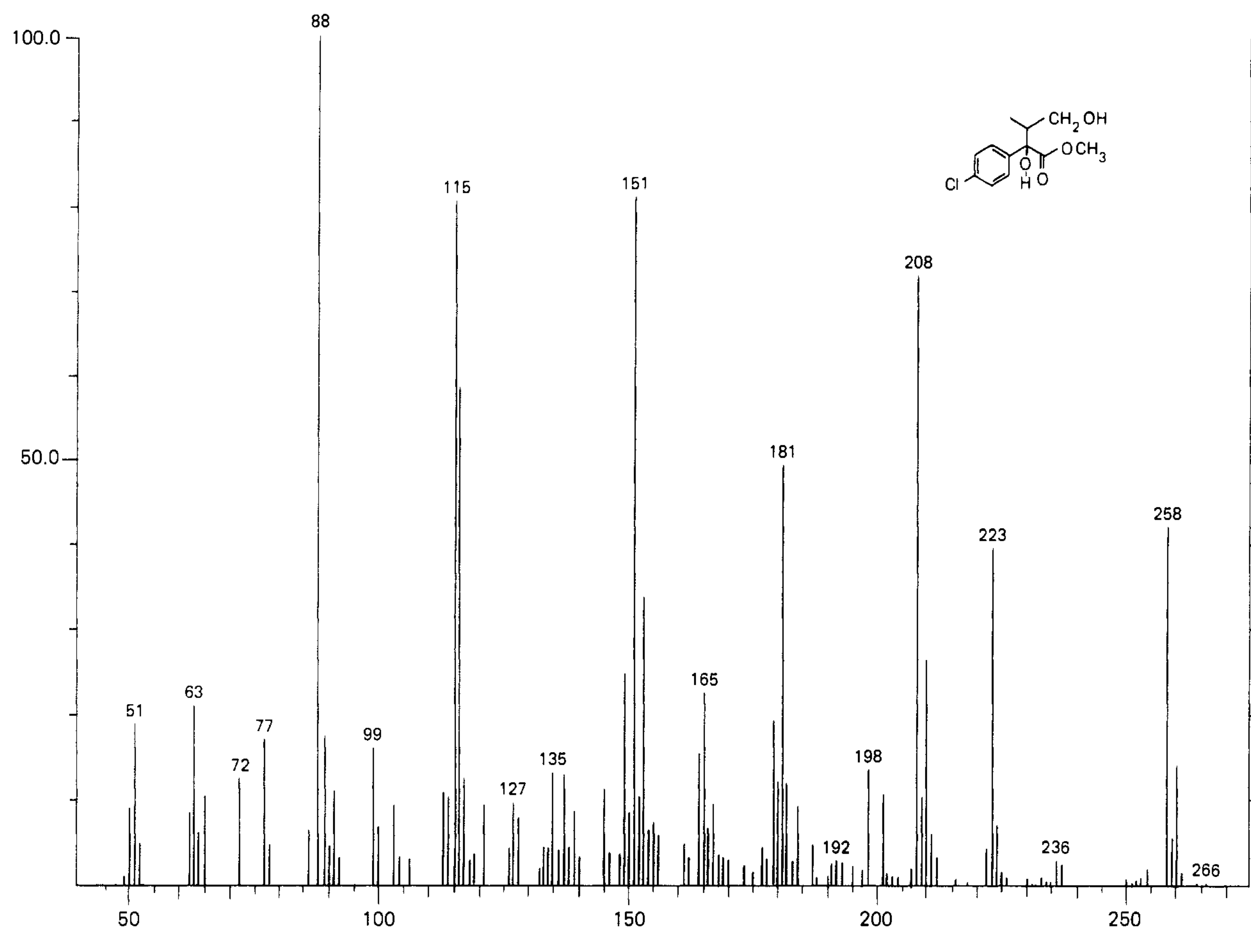

Figure 3. Mass spectrum of the major methylated metabolite in zone 9.

resmethrin (Christoper et al., 1985), and cypermethrin (Akhtar et al., 1987; Hutson and Stoydin, 1987). The rapid elimination of the fenvalerate equivalents is expected to prevent an accumulation of residues in major tissues.

Radiocarbon was detected within 24 h in the albumen and yolk of fenvalerate-treated hens. In the albumen the maximum residue concentration was found within 24 h, while in the yolk it required 96 h to reach the maximum residues level. The ovarian yolk also showed the highest residue concentration at 96 h after the treatment. These observations are in full agreement with data reported for other pyrethroids. For example, the peak residue in egg yolk of hens treated with permethrin or fluvalinate was reached in 4–5 days (Gaughan et al., 1978; Staiger et al., 1982).

The residue levels of fenvalerate equivalents in blood, skeletal muscle, internal organs, and ovarian yolks provided information on the distribution of metabolites in laying hens. Radiocarbon residues were found mostly in blood plasma and were higher in 24-h than those in 96- and 144-h samples. There was approximately 1.8 times more fenvalerate equivalents in the 24-h blood plasma than the cells, while there was 1.2 times more in both 96- and 144-h blood plasma. This was not unexpected since lipophilic insecticides tend to partition preferentially in the plasma. For example, Moss and Hathway (1964) found 2 times more dieldrin in the blood plasma than erthrocytes in rats after intraperitoneal injection of dieldrin. The radiocarbon residues in leg and breast muscles were very low. It is interesting to note that no residues were detected in the subcutaneous fat, and only one bird killed at 96 h had detectable residual ^{14}C in the abdominal fat. This is contrary to previous observations with other pyrethroids in laying hens (Gaughan et al., 1978; Staiger et al., 1982; Akhtar et al., 1985, 1987; Hutson and Stoydin, 1987) and in particular with fenvalerate administered to Japanese quail. Mumtaz and Menzer (1986) reported 3.06 ppm residues in the fat of Japanese quail at 72 h after treatment with a single dose of [*chlorophenyl*-^{14}C]fenvalerate at 100 mg/kg. Similarly, the residues in liver and kidneys were low and transitory in nature because after reaching maximum concentrations 0.46 and 0.50 $\mu g/g$, respectively) at 24 h they decreased rapidly thereafter but showed slight increase between 96 and 144 h.

Table III. Metabolites in the Methanol Extracts of Excreta of Laying Hens Treated Orally with a Single Dose of 7.5 mg of [*carbonyl*-^{14}C] Fenvalerate

compound[c]	% ^{14}C in methanol[a,b]
Fen	8
4′-HO-Fen	2
3-CH_2OH-2-Cl-BA Lactone	8
2-Cl-BA	37
2-HO-2-Cl-BA	<1
3-CH_2OH-2-Cl-BA (α)	7
3-CH_2OH-2-Cl-BA (β)	8
2-HO-3-CH_2OH-2-Cl-BA	15
unidentified	10

[a] Average of determination of three separate hens. [b] Based on a direct count of ^{14}C regions by LSC. [c] See Figure 4 and text for structure and abbreviations.

Table III summarizes the proposed identity and the average quantity of the various compounds found in the methanol extracts of excreta. Approximately 8% of the radiocarbon was associated with the unchanged fenvalerate, which suggests rapid absorption and metabolism of fenvalerate. Other workers (Gaughan et al., 1978; Staiger et al., 1982; Akhtar et al., 1985, 1987; Hutson and Stoydin, 1987) have also reported rapid absorption and extensive

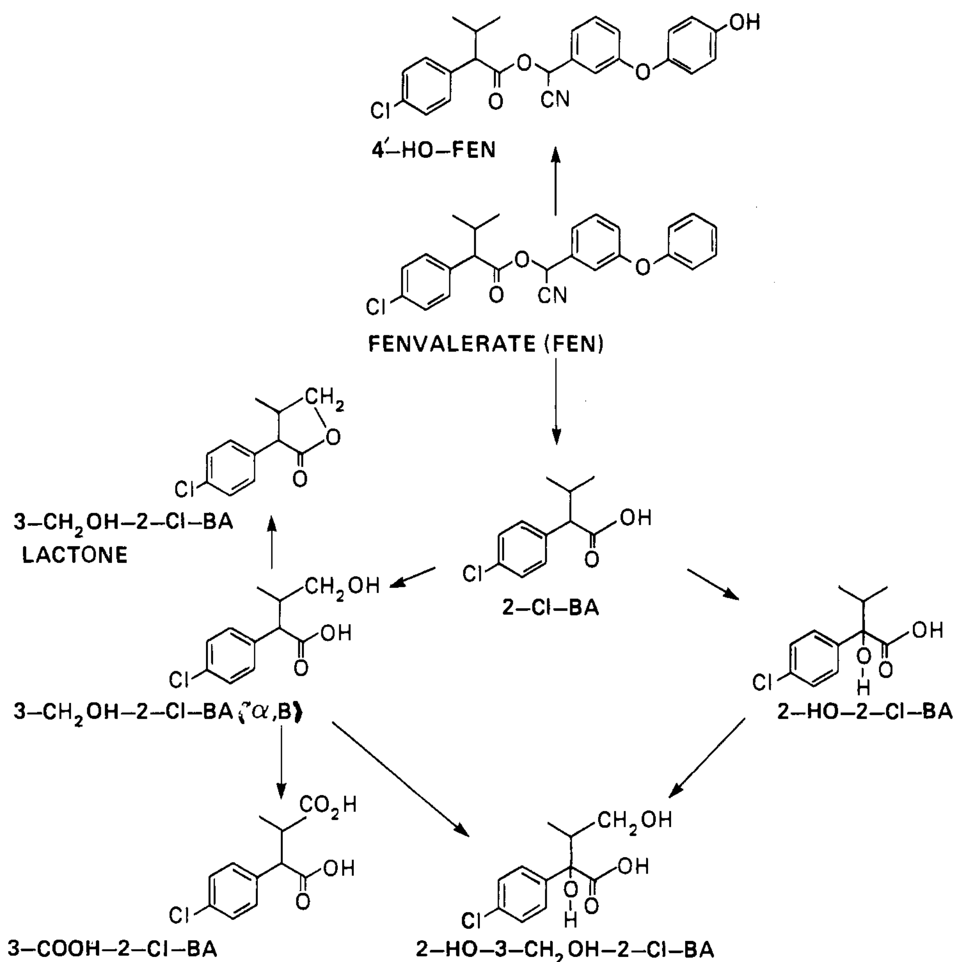

Figure 4. Proposed metabolic pathway of fenvalerate in laying hens.

metabolism of other synthetic pyrethroids in laying hens. The major metabolite identified in the extract was 2-Cl-BA (37%) compared to about 2% for 4′-HO-Fen (Table III). These data strongly indicated cleavage of the ester bond to be the predominant metabolic route and a very small contribution due to hydroxylation at the 4′-position of the phenoxybenzyl moiety of the intact insecticide. Observations support the previous reports identifying ester cleavage as the main metabolic pathway in the degradation of other pyrethroids in laying hens (Gaughan et al., 1978; Staiger et al., 1982; Akhtar et al., 1985, 1987). However, this was contrary to a recently reported metabolic pathway for fenvalerate in Japanese quail (Mumtaz and Menzer, 1986). They identified hydroxylation at the 4′-position (21% of the intact insecticide to be a major metabolic pathway) closely followed by the ester cleavage (20%). Mumtaz and Menzer (1986) also recorded a 34% conversion of fenvalerate by Japanese quail liver microsomes at 42 °C compared to less than 10% by chicken liver microsomes at 37 °C (Akhtar, 1983). Both the in vivo and in vitro studies indicated that Japanese quail have much more effective oxidative enzyme systems than chicken. Thus, the variances observed between the metabolic pathways of Japanese quail and laying hens could be interpreted as mainly due to species differences.

Other metabolites identified in the methanol extract of excreta included compounds derived from the hydroxylation of the methyl group and/or carbon adjacent to carboxyl group. Hydroxylation at the methyl group gave 3-CH_2OH-2-Cl-BA (α and β isomers), while 2-HO-2-Cl-BA was produced from hydroxylation at carbon bearing the carboxyl group. Further hydroxylation of the individual hydroxy metabolites was responsible for 2-HO-3-CH_2OH-2-Cl-BA. The α and β isomers of 3-CH_2OH-2-Cl-BA lactone also occurred in the excreta. These isomers were probably produced by nonenzymatic lactonization of their respective 3-CH_2OH-2-Cl-BA.

A compound identified as 3-COOH-2-Cl-BA was also found in excreta. This compound was formed by the oxidation of 3-CH_2OH-2-Cl-BA. The identity of this compound has not been reported in any of the previous studies with avian or mammalian systems. However, its formation was not unexpected. Recently, Akhtar et al. (1987) demonstrated that 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (Cl_2CA) was converted into 3-COOH-Cl_2CA (3-carboxy-2-(2,2-dichlorovinyl)-3-methylcyclopropanecarboxylic acid and derivatives).

On the basis of tentative identification of metabolites, it appears that fenvalerate was cleaved to produce 2-Cl-BA and 3-phenoxybenzoic acid (3-PBA) and that 2-Cl-BA was further extensively metabolized by a combination of steps (Figure 4). The nature of metabolites in various tissues, organs, and eggs was not fully determined due to a limited quantity of material in tissues. As indicated above, the identifiable compounds included the unchanged fenvalerate and 2-Cl-BA. Irrespective, the residue levels declined quickly during the withdrawal period.

The data presented here show that fenvalerate is readily detoxified by hydrolysis in laying hens, and as such, the pesticide does not produce any toxic signs even at a very high level of about 50 mg/kg of feed. In addition, under the experimental conditions, the residue concentrations in tissue and eggs were very low. It is thus concluded that, under expected exposure levels of fenvalerate in diet, namely those much lower than the dose level used in this study, the pesticide should not produce residues of toxic

concern in eggs and meat of laying hens.

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Registry No. FEN, 51630-58-1; 4′-OH-FEN, 67882-25-1; (±)-*cis*-3-CH_2OH-2-Cl-BA lactone, 117606-18-5; (±)-*trans*-3-CH_2OH-2-Cl-BA lactone, 117606-19-6; 3-COOH-2-Cl-BA (isomer 1), 117606-20-9; 3-COOH-2-Cl-BA (isomer 2), 117606-21-0; 2-OH-2-Cl-BA, 97635-01-3; 3-CH_2OH-2-Cl-BA (isomer 1), 97634-99-6; 3-CH_2OH-2-Cl-BA (isomer 2), 97635-00-2; 2-OH-3-CH_2OH-2-Cl-BA, 72041-48-6.

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Structure–Activity Studies of Tetrazole Urea Herbicides

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The Hill inhibition activity of a novel class of (heteroaryloxy)urea derivatives has been found to be related to the partitioning characteristics of these compounds, as measured by their 1-octanol/water partition coefficient (P). A parabolic relationship between pI_{50} and log P revealed that optimum in vitro activity is exhibited by compounds that have a log P approaching 4. This result contrasts with the herbicidal activity shown by these phenylureas. No clear relationship exists between log P and herbicidal activity. However, the most phytotoxic (heteroaryloxy)urea has log $P \approx 2$, a value significantly lower than those reported for some of the most successful photosynthetic herbicides.

Of the amide-type herbicides (acylanilides, phenylureas, biurets, *N*-phenylcarbamates, uracils) the phenylurea group has been of particular interest in connection with its relationship to photosystem II (PS II) inhibition. A number of structure–activity relationships (SAR) in the literature (Hansch, 1969; Seewald et al., 1978; van den Berg and Tipker, 1982; Kakkis et al., 1984; Takemoto et al., 1984; Mitsutake et al., 1986; Camilleri et al., 1987) have shown that hydrophobic, electronic, and steric factors are the physicochemical parameters that define the ability of this class of molecules to inhibit the Hill reaction. Al-

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