Comparative Metabolism and Fate of Fenvalerate in Japanese Quail (*Coturnix coturnix japonica*) and Rats (*Rattus norwegicus*)

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Adult Japanese quail were administered 100 mg/kg chlorophenyl-labeled [^{14}C]fenvalerate, α-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)isovalerate, for study of its distribution, elimination, and metabolism. Ninety percent of the administered dose was eliminated in the excreta within the first 24 h. In addition to fenvalerate, the following metabolites were present: benzeneacetic acid, 4-chloro-α-(1-methylethyl)-, cyano(3-phenoxy-4-hydroxyphenyl)methyl ester [4'-OH-fenvalerate]; benzeneacetic acid, 4-chloro-α-(1-methylethyl)-, (aminocarbonyl)(3-phenoxyphenyl)methyl ester [CONH$_2$-fenvalerate]; 4-chloro-α-(1-methylethyl)benzeneacetic acid [Cl-V acid]; 4-chloro-α-(2-hydroxy-1-methylethyl)benzeneacetic acid [4-OH-Cl-V acid]. In time course studies radiocarbon peaked at 3 h (9 μg/g) in the liver and gradually declined, while in the blood it peaked within 2 h and fell quickly to an equilibrium value of 1.5 μg/mL blood. In liver microsomal and isolated heptatocyte preparations of Japanese quail and rat, the following metabolites were identified: Cl-V acid, 4-OH-Cl-V acid, 4'-OH-fenvalerate, CONH$_2$-fenvalerate. Oxidation was found to be the predominant route of degradation either pre- or posthydrolysis of the parent compound. Rapid excretion, lesser absorption, and faster metabolism probably explain the lower toxicity of fenvalerate to birds compared to rats.

INTRODUCTION

Fenvalerate, α-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)isovalerate, is gaining wide acceptance in agricultural usage and is destined for increased use against agricultural, poultry, dairy, and household pests. Its metabolism has been studied in rats and mice (Ohkawa et al., 1979; Kaneko et al., 1981), in dogs (Kaneko et al., 1984), in ruminants (Wszolek et al., 1981), in chickens (Akhtar, 1983) and bobwhite quail (Bradbury and Coats, 1982), and in soil and sediment systems (Ohkawa et al., 1978; Caplan et al., 1984).

Fenvalerate was rapidly absorbed, distributed to tissues and organs, metabolized, and excreted from treated rats and mice (Ohkawa et al., 1979; Kaneko et al., 1981). The half-lives of excretion following treatment of rats at 7.0 or 8.4 mg/kg and mice at 30 mg/kg were 0.5–0.6 day. Most of the radioactivity was recovered in urine. Analysis of metabolites recovered revealed that fenvalerate was hydrolyzed at the ester linkage and hydroxylated in both

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alcohol and acid moieties. A small portion of the administered dose was excreted as unaltered fenvalerate. No significant differences were observed in the nature and amounts of metabolites between male and female animals, but there were differences between rats and mice. Hydroxylation at the 4'-position occurred to a larger extent in rats than in mice, and there were differences in conjugates formed.

Disposition and metabolism studies were conducted in bobwhite quail (Bradbury and Coats, 1982). Fenvalerate was incubated with chicken liver enzyme preparations; cleavage of the ester bond was found to be the predominant route of degradation. However, less than 10% fenvalerate metabolism was observed under the conditions used in this study (Akhtar, 1983).

The present study was conducted to determine the fate of fenvalerate in Japanese quail, a species used extensively in avian research (Riesenfeld et al., 1981), and to establish a "reference point" for fenvalerate metabolism studies in birds. Previously reported studies in birds have been limited in their approach. We conducted both in vitro studies with quail liver microsomes and isolated heptatocyte suspensions as well as in vivo studies. For comparative purposes metabolism studies were also done with rat

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                                              Excreta
                                                 │
                                     ┌───────────┴───────────┐
                                     │ homogenized with water (1:10)
                                     │ using a Sorval Omni Mixer
        ┌────────────────────────────┼─────────────────────────────────┐
  1/5 homogenate                3/5 homogenate                    1/5 homogenate
        │                            │                                   │
        │                            │                                   │
   lyophilized               extracted thrice                     stored frozen
        │                    with CHCl₃ under                      for future work
        │                    neutral conditions
                            ┌──────┴──────┐
 5% of the lyophile, in triplicate    CHCl₃        Aqueous
 combusted on a Harvey biological    neutral      homogenate
 oxidizer-OX400 followed by          extracts         │
 radioassay.                                           │
                                                    acidified
                                                    extracted thrice with
                                                    CHCl₃
                                              ┌────────┴────────┐
                                           CHCl₃            Spent homogenate
                                           acid extracts          │
                                                              filtered
                                                        ┌─────────┴─────────┐
                                                  Solid residues    Clear homogenous
                                                                    solution
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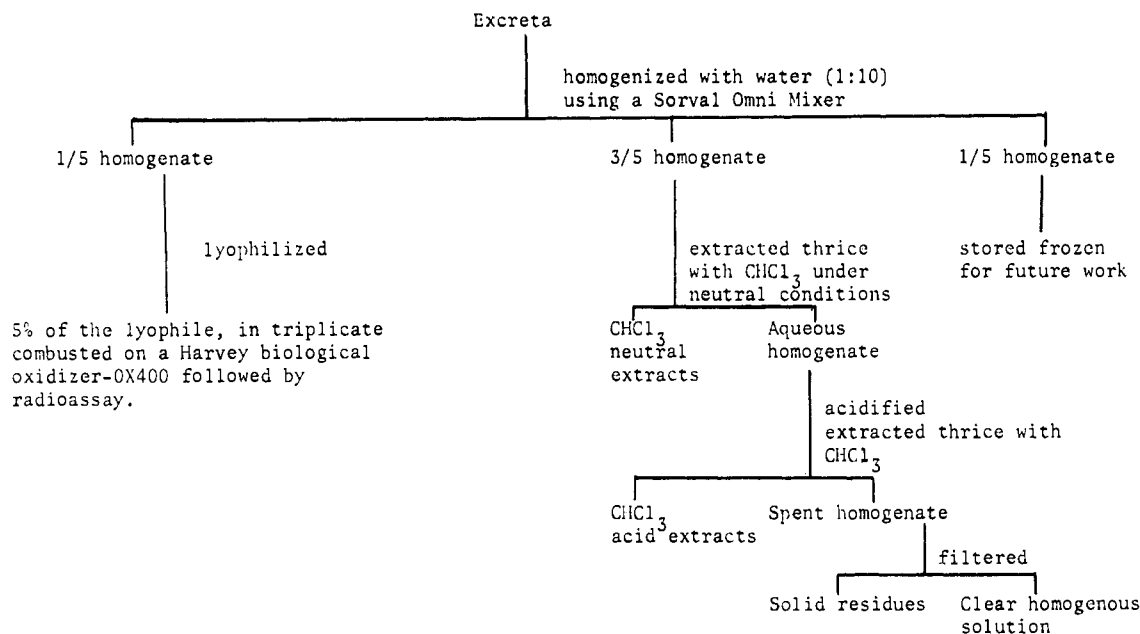
Figure 1. Extraction and analysis of excreta samples.

liver microsomes and isolated hepatocyte suspensions. Understanding the mechanistic aspects of fenvalerate metabolism in both an avian and a mammalian species will permit a greater understanding of the course of metabolism of the compound in animals that are exposed to it in their environment.

MATERIALS AND METHODS

Chemicals. Chlorophenyl-labeled [14C]fenvalerate (14.9 mCi/mmol), unlabed fenvalerate, and standard reference compounds were obtained from Dr. D. Soderlund, Cornell University, and Shell Development Co. as follows: 4'-OH-fenvalerate [benzeneacetic acid, 4-chloro-α-(1-methylethyl)-, cyano(3-phenoxy-4-hydroxyphenyl)methyl ester], Cl-V acid [benzeneacetic acid, 4-chloro-α-(1-methylethyl)-], CONH₂-fenvalerate [benzeneacetic acid, 4-chloro-α-(1-methylethyl)-, (aminocarbonyl)(3-phenoxy-phenyl)methyl ester], 4-OH-Cl-V acid [benzeneacetic acid, 4-chloro-α-(2-hydroxy-1-methylethyl)-], 2-OH-Cl-V acid [benzeneacetic acid, 4-chloro-α-hydroxy-α-(1-methyl-ethyl)-], and 2,4-OH₂-Cl-V acid [benzeneacetic acid, 4-chloro-α-hydroxy-α-(2-hydroxy-1-methylethyl)-].

Radiolabeled fenvalerate was purified to 97–99% on silica gel thin-layer plates developed in hexane–ether (1:1). The radiocarbon on the plates was detected on a Varian Aerograph series 6000 radioscanner as well as by exposing the plates to Kodak XAR-5 X-ray film. The region of the plates corresponding to fenvalerate was scraped off and extracted with ether, and the extracts were concentrated and filtered through a sintered-glass funnel. The fenvalerate thus purified was used in all experiments.

Animals. Young adult Japanese quail males (weighing approximately 110 g) and females (weighing approximately 125 g) were obtained from the Poultry Science Department, University of Maryland. The quail were acclimated in individual metabolism cages designed to prevent ingestion of excreta and permitting sample collection at designated time intervals. A 12 h–12 h dark–light photoperiod, 25 °C temperature, and 40% relative humidity were maintained in the controlled-environment room where the quail were housed. The birds had separate feed containers and watering cups and received standard starter feed and water ad libitum throughout the study period. To enhance absorption of fenvalerate the birds were de-

prived of food for about 12 h prior to dosing.

Adult male Sprague–Dawley rats (weighing 150–200 g) (Camm Research Lab Animals, Wayne, NJ) were housed in the animal facility. Twenty-four hours prior to use in in vitro experrments they were transported to the controlled-environment room and held in standard rat cages.

In Vivo Methods. *Dose Preparation.* For preliminary oral toxicity studies, solutions were prepared by dissolving fenvalerate (94% pure) in Mazola oil. For the detailed metabolism and disposition experiments, unlabeled fenvalerate and/or chlorophenyl-labeled [14C]fenvalerate, dissolved in acetone, were applied directly to 75 mg of quail feed in size 5 gelatin capsules (Eli Lilly & Co, Inc.)

Treatment of Japanese Quail. Ten male quail were weighed prior to dosing and treated through a stomach tube with fenvalerate at 4000 mg/kg. Three control quail were admininstered an equivalent volume of Mazola oil (7 μL/g of body weight). Hourly observations were made during the initial 12 h; thereafter, observations were made daily. The birds were weighed and the experiment was terminated 14 days postdosing.

For the metabolism and disposition experiments three male and three female birds were administered capsules containing fenvalerate (100 mg/kg). The capsules were placed directly in the crop of the birds. For 3 consecutive days the birds were administered capsules containing unlabeled fenvalerate while on the fourth day they were administered capsules containing unlabeled fenvalerate and 5 μCi of [14C]fenvalerate. Excreta samples were collected at 6-h intervals for the first 24 h postdosing. Two more samples were collected at 48 and 72 h, and the experiment were terminated by cervical dislocation of the birds. The following tissues and organs were removed and kept frozen at −40 °C prior to residue analysis: liver, kidneys, lungs, brain, heart, reproductive organs, the entire alimentary canal, furcular fat, breast muscle, skin.

In another experiment a group of six male birds was treated as above with 1 μCi of [14C]fenvalerate to determine the levels of radiocarbon in liver and blood over time. At 0, 2, 3, 4, 5, and 6 h postdosing, heart puncture was performed on designated birds to obtain 2–3 mL of blood.

Sample Analyses. Each of the 36 excreta samples were treated as shown in Figure 1. Aliquots representing 5% of each lyophilized excreta sample were combusted in

triplicate in a Harvey biological oxidizer followed by radioassay. Samples of liver, kidneys, lung, brain, heart, and blood were lyophilized prior to combustion analysis while fat, muscle, and skin samples were subjected to combustion analysis directly. All results of bird [^{14}C]fenvalerate residues were converted to parts per million on the basis of the original wet weight of the tissue.

In Vitro Methods. *Preparation of Liver Microsomes.* Rats were killed by a blow on the head; quail were killed by cervical dislocation. The livers were immediately removed, washed with ice-cold distilled water, blotted, and weighed. About 7 g of liver (two to three livers pooled in the case of quail) was then minced with a pair of scissors and transferred to a Potter-Elvehjem tissue grinder tube containing 4 vol of ice-cold 0.25 M sucrose solution and homogenized. As soon as possible, the homogenate was transferred to ice-cold polycarbonate ultracentrifuge tubes and the 105000g microsomal pellet was obtained. This pellet was washed by resuspension in ice-cold 0.15 M KCl solution and centrifugation at 105000g for 30 min. The supernatant was decanted, the washed microsomal pellet was finally resuspended in ice-cold phosphate buffer, pH 7.4, and the volume was adjusted to half the original volume of sucrose solution. Protein content was determined by the method of Lowry et al. (1951), and cytochrome P-45 content, by the method of Omura and Sato (1964) on a Bausch and Lomb Spectronic 2000 spectrophotometer.

Microsomal Assay. In order to assess the microsomal activity the conversion of aldrin to dieldrin was measured (Opdycke et al. 1982). Fenvalerate incubations were carried out in 25-mL Erlenmyer flasks. The incubation medium contained microsomes [1 mL, 1 mg/mL of protein] and an NADPH generating system, consisting of two solutions made in 0.1 M phosphate buffer (pH 7.4) just prior to use. One solution contained 1.8 μmol of NADP, 18 μmol of glucose 6-phosphate, and 8 μmol of MgCl$_2$, in 0.9 mL of buffer, and the other contained 2 units of glucose 6-phosphate dehydrogenase in 0.1 mL of buffer. The flasks were equilibrated for 5 min before the incubations were started with the addition of [^{14}C]fenvalerate (60 000 dpm, 1.81 nmol) dissolved in 10 μL of acetone. The incubations with rat microsomes were carried out at 37 °C; those with quail microsomes, at 37 and 42 °C. The incubations were terminated at designated times by adding 5 mL of chloroform. Throughout the incubations a constant environment of Ca oxygen gas (O$_2$:CO$_2$ = 95:5) was maintained, and the incubator was set to shake at 60 cycles/min. In control incubation flasks an equal volume of phosphate buffer replaced the NADPH generating system.

Hepatocyte Isolation. Isolation of hepatocytes was accomplished by perfusion of the liver with several buffer systems. The setup consisted of circulation of warm water from a incubation through the outside water jacket of a heat-exchange coil back to the incubator. The buffer was pumped through the inside coil of the water jacket by a polystatic pump capable of flow rates of up to 40 mL/min. After the heat exchange the warm buffer passed though an air trap to a butterfly needle valve used to cannulate the liver.

The rat, anesthetized with ether, was placed on its back in a dissection tray. A U-shaped transverse incision was made carefully in the abdomen, avoiding any contact with the liver lobes. The intestines and other viscera were moved, exposing the right iliolumbar branch of the inferior vena cava. Heparin (0.25 mL of 5000 IU/mL) was injected, and the injection site was clamped off to prevent bleeding. The vena porta was then exposed, and with a surgical

Table I. TLC of Fenvalerate and Its Metabolites

metabolites	R_f on solvent system[a]				
	1	2	3	4	5
fenvalerate	0.74	0.75	0.10	0.62	0.81
4'-OH-fenvalerate	0.50	0.65	0.13	0.34	
CONH$_2$-fenvalerate	0.24	0.61	0.14	0.35	
Cl-V acid	0.36	0.65	0.69	0.42	0.70
4-OH-Cl-V acid	0.45	0.69	0.57	0.38	0.75
2,4-OH$_2$-Cl-V acid	0.03	0.12	0.83	0.00	
2-OH-Cl-V acid	0.24	0.51	0.75	0.32	0.56

[a] Solvent systems: (1) toluene–ether–acetic acid (75:25:1); (2) hexane–acetone–acetic acid (25:25:1); (3) methanol–water (8:2); (4) hexane–toluene–acetic acid (3:15:2); (5) toluene–ether–acetic acid (75:25:1). In solvent systems 1, 2, and 4, compounds were chromatographed on silica gel precoated TLC plates, and in solvent systems 3 and 5, compounds were chromatographed on C$_{18}$-bonded silica gel plates.

Table II. Buffers (Parts per Volume) Used for Liver Perfusion[a]

	Ca^{2+}-free K–H bicarbonate	collagenase	suspension
0.9% NaCl (0.154 M)	500	500	500
1.15% KCl (0.154 M)	20	20	20
1.22% CaCl$_2$ (0.11 M)		15	15
2.11% KH$_2$PO$_4$ (0.154 M)	5	5	5
3.8% Mg$_2$SO$_4$·7H$_2$O (0.154 M)	5	5	5
1.3% NaHCO$_3$[b]	105	105	105
collagenase		c	

[a] Quail livers were perfused with these buffers; rat livers were perfused with Ca^{2+}-free, collagenase, and suspension buffers prepared by the method of Seglen (1976). [b] Gassed with CO$_2$ for 1 h before mixing with other solutions. [c] Collagenase, 25 mg/50 mL of buffer dissolved in 200 mL.

probe a space was made underneath the vein. Using a haemostat, a ligature (cotton thread) was slipped underneath the vena porta and a loose knot was made. The rib cage was cut open to expose the heart. As with the vena porta a loose knot was made on the inferior vena cava. A steel canula attached to rubber tubing was heparinized and inserted through the right atrium into the inferior vena cava. The knot was tightened to anchor the cannular above the liver. The calcium-free buffer flow (Table II) was begun at 10–15 mL/min, and the needle of the butterfly valve was inserted into the vena porta about 2 mm past the ligature. The ligature was tightened to secure the needle, and the butterfly valve was pinned on the dissection tray at a fixed position to prevent any puncturing of the vessel. Perfusion of the liver in situ was thus initiated.

The quail, anesthetized with ether, was placed on its back in a dissection tray. An incision was made just underneath the keel bone. The rib cage, the keel bone, and the breast muscles were carefully dissected out to expose the heart, liver, and other viscera. The pericardium and other connective tissue around the heart were carefully detached. Thus, the heart could be freely manipulated to expose the underlying common hepatic vein. A ligature with a loose knot was put around the common hepatic vein. The calcium-free buffer flow was begun at 10–15 mL/min. The needle of the butterfly valve was inserted through the atrium and placed in the hepatic vein, a few millimeters before its bifurcation. The ligature was tightened, and all the blood vessels around the liver were severed to allow a free flow of the buffers, thus initiating the in situ perfusion of liver.

At the end of the surgical procedures the flow rate of the buffer was increased to about 25–30 mL/min. After perfusion for 10 min with calcium-free buffer, perfusion

with collagenase buffer was initiated. After about 10–12 min of perfusion with collagenase buffer the liver was flushed with 50 mL of warm suspension buffer (Table II). Lobes of liver were cut from the body, and the dissociated hepatocytes were then released in a petri dish of ice-cold suspension buffer. The connective tissue matrix of the liver was discarded. The cell suspension temperature was lowered by gently shaking the Petri dish placed in an ice bucket. The cell suspension was filtered through a 210-μm nylon mesh filter and transferred with large-bore pipets to ice-cold tubes. The tubes were centrifuged at 200 rpm for 5 min. The supernatant was aspirated off, and the loosely packed cells were resuspended and washed three times with buffer. The final cell suspension was subjected to the trypan blue test, and density and intactness of the cells were determined microscopically.

Hepatocyte Incubations. One-milliliter aliquots of cell suspension (1 × 10^6 cells/mL) were put in Erlenmeyer flasks. The flasks were equilibrated for 5 min in incubators set at 37 °C for rat and 42 °C for quail. Fenvalerate incubations were initiated by adding 10 μL of [^{14}C]fenvalerate solution in acetone (60000 dpm, 1.81 nmol). The incubator was set to shake at 60 cycles/min, and a constant environment of carbogen gas (O$_2$:CO$_2$ = 95:5) was maintained throughout the incubations. At the designated time, 5 mL of chloroform was added to the flasks to terminate the incubation. The contents of the flasks were then frozen.

Characterization of the Metabolites. *Isolation and Quantitation.* Excreta samples were homogenized with water and extracted three times with 100-mL portions of chloroform. The samples were acidified with HCl to pH 1–3 and reextracted with chloroform in the same manner. The extracts obtained for each sample were evaporated on a rotary evaporator and redissolved in 10 mL of chloroform.

After termination of the in vitro incubations, the incubation medium was extracted with 5 mL of chloroform three times each under neutral and then acidic pH conditions. To separate the two phases, the tubes were centrifuged for 5 min at 3000 rpm in a table-top centrifuge. The pooled extracts and aqueous phases were assayed for radioactivity as described above.

To assay radioactive material and determine recovery levels, a Packard Tricarb 4550 liquid scintillation spectrometer was used. Duplicate aliquots of chloroform extracts were assayed with a counting medium containing 5 g of PPO (2,5-diphenyloxazole) and 0.3 g of dimethyl-POPOP [1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene]/L of toluene. The filtered aqueous solutions were assayed using a counting medium containing 5.5 g of PPO and 0.2 g of POPOP/L of a 2:1 mixture of toluene–Triton X-100. [^{14}C]-n-Hexadecane was used for internal standardization of counting efficiency.

Major metabolites in the chloroform extracts were isolated on SEP-PAK-C$_{18}$ cartridges (Waters Associates, Cambridge, MA). The cartridge was washed with 2 mL each of 100% methanol, distilled water, and 80% methanol consecutively. The sample dissolved in 0.1 mL of 90% methanol was loaded on the cartridge. The cartridge was then washed with 1 mL of distilled water, and the sample was fractionated by eluting the cartridge with 70%, 80%, 90%, and 100% methanol. Each fraction was collected and evaporated under nitrogen.

Chromatography. Chromatographic characteristics of the parent compound and its standard hypothetical metabolites were determined in several systems given in Table I. Thin-layer chromatography (TLC) was performed on precoated 20 × 20 cm glass plates: normal-phase plates, silica gel 60 F-254, 0.25-cm thickness; reversed-phase plates, Si-C$_{18}$-F, octadecylsilane bonded to silica gel, 0.20-cm thickness (J. T. Baker).

Identification of most compounds was performed by cochromatography on thin-layer plates (silica gel 60 F-254, E. Merck). [^{14}C]Fenvalerate and its metabolites were identified by spotting authentic, unlabeled standards with radioactive extracts or fractions isolated from SEP-PAK-C$_{18}$ cartridges. After the plates were eluted in suitable solvent systems, the position of the unlabeled standards was determined under UV light. The radiocarbon spots on the plates were detected by radioautography. If the UV-observable spots and those on the radioautograph cochromatographed, the metabolites were tentatively concluded to be identical.

Mixtures of authentic unlabeled standards and radioactive extracts or fractions thereof were methylated with diazomethane. The derivatized samples were applied to TLC plates and chromatographed. Cochromatography of radiocarbon and UV-observable spots was inferred as confirmation of identity of the metabolites.

Radioactivity measurements and quantitation of metabolites on the silica gel plates were done by scraping the area of the plate corresponding to certain R_f values and assaying the scrapings for radioactivity. A nonaqueous counting medium containing Cab-O-Sil (Packard Instrument Co.) was used to radioassay the silica gel scrapings.

Mass Spectrometry. Mass spectra and selected ion monitoring (SIM) scans for the major metabolites were obtained with an HP5840A/HP5985 GC/MS system. GC conditions: 3-ft 3% OV-101 column; injection temperature 240 °C; initial oven temperature 220 or 150 °C; rate of heating 18°/min, final oven temperature 260 °C; helium flow rate 30 mL/min.

RESULTS

Chemical Identity of the Metabolites. The R_f values of fenvalerate and standard reference compounds are listed in Table I. Chloroform extracts applied to TLC plates resolved into several spots. [^{14}C]-Labeled compounds present in the extracts were tentatively identified by their R_f values in two different solvent systems. Seven compounds, which accounted for the majority of radioactivity, were identified as follows:

Compound A was presesnt in all the chloroform extracts and was one of the major [^{14}C] compounds in in vivo studies. In in vitro studies it was the majority of the radiocarbon. It cochromatographed with standard fenvalerate and could not be methylated with diazomethane. On the GC/MS system it had a retention time of 3.5 min (initial oven temperature 220 °C). Its mass spectrum exhibited a weak molecular ion at m/z 419, a base peak of m/z 125, and the following characteristic ions: m/z 225, 167, 139. Thus, compound A was confirmed as unmetabolized fenvalerate.

Compound B was one of the three major [^{14}C]-labeled compounds found in excreta extracts. It cochromatographed with standard 4'-OH-fenvalerate; it resolved from standard CONH$_2$-fenvalerate in solvent system 1 but not in solvent system 3. Under the reaction conditions employed, it did not react with diazomethane. This peak had a retention time of 5.47 min, and the mass spectrum exhibited ions of m/z 125, 167, 213, and 435. The mass spectrum of this peak confirmed metabolite B to be 4'-OH-fenvalerate.

Compound C was a major metabolite in all chloroform extracts. It cochromatographed with standard Cl-V acid. After reaction with diazomethane it became a less polar
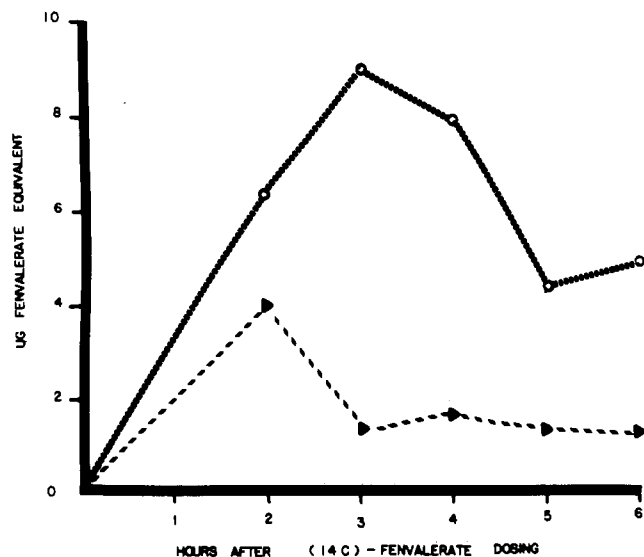
Fenvalerate Fate in Quail and Rat

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Figure 2. Time course of radiocarbon in liver (O) and blood (▲) following treatment of Japanese quail with [^{14}C]chlorophenyl-labeled fenvalerate at 100 mg/kg.

material (R_f 0.73, solvent system 1), indicative of methylation of carboxylic acid or free hydroxyl group(s). On GC/MS the methylated sample had a retention time of 1.03 min. Its mass spectrum had a molecular ion at m/z 226, a base peak of m/z 184, and the following characteristic ions, identifying it as methyl ester of Cl-V acid: m/z 167, 152, 125. Metabolite C was thus confirmed as Cl-V acid.

Compound D was a minor metabolite in the chloroform extracts and cochromatographed with $CONH_2$-fenveralate.

Compound E was the major metabolite in microsomal extracts and was also detected in excreta extracts. It cochromatographed with standard 4-OH-Cl-V acid and became less polar (R_f 0.7, solvent system 1) when methylated. It was identified as 4-OH-Cl-V acid.

Compound F was a minor metabolite barely detectable in both in vivo and in vitro extracts. It cochromatographed with standard 2-OH-Cl-V acid before methylation and with the methylated standard (R_f 0.67, solvent system 1) after methylation. It was identified as 2-OH-Cl-V acid.

Compound G was another minor metabolite present in chloroform extracts. It cochromatographed with standard 2,4-OH_2-Cl-V acid before methylation and with the methylated standard (R_f 0.53, solvent system 1) after methylation. It was identified as 2,4-OH_2-Cl-V acid.

Japanese Quail in Vivo Studies. *Oral Toxicity.* The 10 quail treated orally with fenvalerate at 4000 mg/kg started feeding normally. However, in about 90 min they appeared hyperactive and by 2 h postdosing became intensely hyperactive and ceased eating. The controls, which were administered an equivalent volume of Mazola oil, did not show any sign of intoxication up to the termination of the experiment. By about 4 h postdosing treated quail were experiencing convulsions, irregular movements, jerking, and twitching; they became progressively ataxic and uncoordinated. Two of them died at 4 and 8 h postdosing. The rest recovered slowly from the severe symptoms, and by 24 h postdosing a majority resumed feeding. These quail assumed an odd standing posture with the head held above the body, the legs extended as far straight as possible, and the wings held in an upright position, close to the body. By the end of the second day postdosing the quail were consuming water and feed regularly and appeared to be free of any toxicity symptoms. Thus, the LD_{50} of fenvalerate to Japanese quail appears

Table III. Total Combustible, Chloroform-Extractable and -Unextractable Radioactivity Present in Excreta of Japanese Quail Treated with [^{14}C]Chlorophenyl-Labeled Fenvalerate at 100 mg/kg

sample time, h postdosing	total combust radioact[a]	chloroform-extr radioact[b]	chloroform unextr radioact in solid res[a]	chloroform unextr radioact in supernants
		Male Birds		
6	74 ± 8.0	67 ± 10.0	10.0 ± 5.3	0.6 ± 0.4
12	23 ± 18.0	16 ± 10.0	3.4 ± 2.3	0.1 ± 0.03
18	3 ± 1.0	2 ± 1.0	0.4 ± 0.01	0.04 ± 0.01
24	1 ± 0.1	0.2 ± 0.1	c	c
48	1 ± 0.1	0.4 ± 0.1		
72	1 ± 0.5	0.1 ± 0.0		
total	103 ± 12.0	86 ± 2.0	13.8 ± 5.3	0.74 ± 0.47
		Female Birds		
6	77 ± 6.7	72 ± 5.0	10.2 ± 3.0	2.1 ± 1.1
12	7 ± 2.0	6 ± 3.0	1.1 ± 0.4	0.2 ± 0.1
18	2 ± 1.0	3 ± 1.0	0.3 ± 0.04	0.05 ± 0.02
24	2 ± 0.8	2 ± 0.3	c	c
48	8 ± 2.7	5 ± 2.0		
72	1 ± 0.5	c		
total	97 ± 4.5	88 ± 4.9	11.6 ± 2.8	2.35 ± 1.06

[a] Combustion efficiency correction factor 1.22. [b] Extraction efficiency correction factor 1.25. [c] Not determined.

Table IV. Qualitative and Quantitative Analyses of Radiocarbon in Chloroform Extracts of Excreta following Treatment of Japanese Quail with [^{14}C]Chlorophenyl-Labeled Fenvalerate at 100 mg/kg

compd	% administered dose male	female
fenvalerate	33.6 ± 5.8	42.3 ± 1.3
4'-OH-fenvalerate	21.0 ± 6.8	12.6 ± 1.4
Cl-V acid	20.4 ± 3.4	18.5 ± 3.2
$CONH_2$-fenvalerate	0.9 ± 0.12	1.0 ± 0.6
2-OH-Cl-V acid	1.1 ± 0.62	1.5 ± 0.5
4-OH-Cl-V acid	2.0 ± 0.20	2.0 ± 1.1
2,4-OH_2-Cl-V acid	1.2 ± 1.2	0.6 ± 0.5
unknowns	9.3 ± 4.4	12.6 ± 3.8

to be somewhat greater than 4000 mg/kg. At the termination of the experiment 14 days postdosing no weight loss was observed in the quail.

Pharmacokinetics. The time course of radiocarbon in liver and blood following oral administration of fenvalerate is shown in Figure 2. The radiocarbon levels in liver reached a maximum of 9 μg/g within 3 h postdosing and by 5 h had declined to 4.8 μg/g. On the other hand, the levels of radiocarbon in blood peaked within 2 h and quickly fell to an equilibrium value of 1.5 μg/mL.

Time Course of Excretion. Total radioactivity in the excreta samples determined by combustion analyses is given in Table III. Approximately 75% of the administered [^{14}C]fenvalerate was excreted within the first 6 h. The half-life for excretion of fenvalerate in quail is less than 6 h.

Fractionation and Quantitation. Excreta samples were processed and partitioned between water and chloroform (Figure 1). On the average 86–88% of the administered radioactivity was recovered in the chloroform extracts (Table III). Chloroform-unextractable radioactivity was determined after separation of the solid residues and the supernatant by filtration prior to radioassay (Table III). A majority of the chloroform-unextractable radioactivity remained bound to the solid residues of the excreta. TLC or TLC and SEP PAK C_{18} cartridges were used to isolate, purify, and quantitate the radiocarbon present in extracts. The three major compounds found in both the male and female quail excreta were fenvalerate, 4'-OH-fenvalerate,
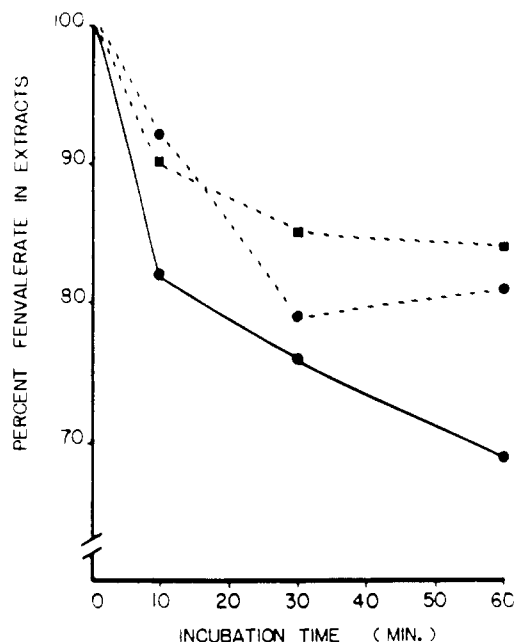
Figure 3. Metabolisum of fenvalerate by liver microsumes. Disappearance of fenvalerate (■) in rat microsomes incubated at 37 °C, (●) quail microsomes incubated at 37 °C, and (●—●) quail microsomes incubated at 42 °C.

and Cl-V acid (Table IV). Other minor compounds identified were CONH$_2$-fenvalerate and the three molecules resulting from hydroxylation of Cl-V acid at the 2- and 4-positions: 2-OH-Cl-V acid, 4-OH-Cl-V acid, and 2,4-OH$_2$-Cl-V acid. Most of the metabolites were found only in the first excreta sample; in the subsequent samples only the major metabolites could be detected.

Tissue Residues. Analyses of aliquots of selected tissues 72 h postdosing showed trace amounts of carbon-14 present in the viscera of quail (Table V). The fat, skin, and liver appeared to accumulate the radiolabel more than other tissues.

Japanese Quail in Vitro Studies. *Metabolism by Microsomes.* The extracts from each incubation of fenvalerate in quail microsomes, after measurements of radioactivity, were analyzed directly by TLC followed by
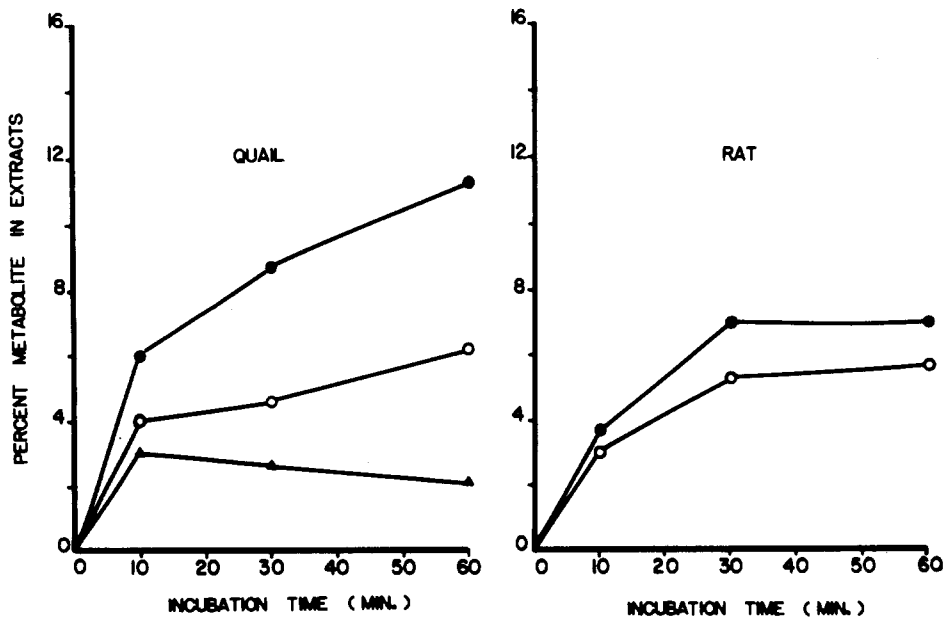
Table V. Distribution of [^{14}C]Fenvalerate Residues in Tissue Samples 72 h following Treatment of Japanese Quail with [^{14}C]Chlorophenyl-Labeled Fenvalerate at 100 mg/kg

organ	ppm[a]		organ	ppm[a]	
	male	female		male	female
liver	0.70	0.39	brain	0.02	0.02
kidney	0.19	0.19	skin	0.85	0.46
lung	0.11	0.12	fat	3.06	0.96
heart	0.23	0.14			

[a] Combustion efficiency factor 1.07–1.11.

Table VI. In Vitro Metabolism of Fenvalerate: [^{14}C]-Labeled Compounds Identified in Chloroform Extracts at 30-min Incubation Time

compd	% administered dose			
	microsomes		hepatocytes	
	rat	quail	rat	quail
fenvalerate	85.1	76.0	95.0	94.0
4'-OH-fenvalerate	ND	2.7	0.5	1.0
Cl-V acid	5.3	4.7	3.0	3.0
CONH$_2$-fenvalerate	0.5	1.0	0.8	0.8
2-OH-Cl-V acid	0.2	0.5	ND	0.5
4-OH-Cl-V acid	7.0	8.7	ND	ND
2,4-OH$_2$-Cl-V acid	0.4	0.3	ND	ND
unknown metabolites	1.5	6.4[a]	ND	ND
total	100.0	100.3	99.3	99.3

[a] R_f in solvent system 1, %: 0.24, 1.0; 0.17, 1.1; 0.14, 2.4; 0.00, 1.9. ND = not detectable.

radioautography. A majority of the metabolites resulted from the cleavage of the ester bond (Table VI). Disappearance of fenvalerate from the extracts as a function of time is shown in Figure 3. Quail microsomes when incubated at 42 °C metabolized fenvalerate faster than at 37 °C. The profiles of metabolites as a function of incubation time are shown in Figure 4. The 4-OH-Cl-V acid was the major metabolite found; other metabolites were CONH$_4$-fenvalerate, 4'-OH-fenvalerate, Cl-V acid, 2-OH-Cl-V acid, and 2,4-OH$_2$-Cl-V acid (Table VI).

Metabolism by Hepatocyte Suspensions. Most of the radioactivity recovered following incubation of fenvalerate with suspensions of hepatocytes was fenvalerate itself (Table VI). Only 6% of the fenvalerate was metabolized
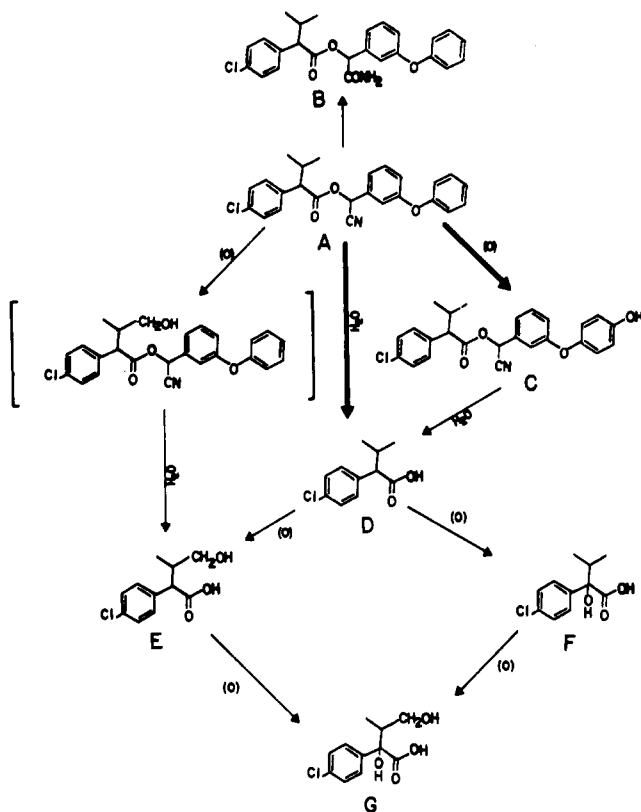

Figure 4. Metabolism of fenvalerate by liver microsomes. Appearance profiles of its metabolites, 4'-OH-fenvalerate (▲), Cl-V acid (O), and 4-OH-Cl-V acid (●).

Fenvalerate Fate in Quail and Rat

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Figure 5. Fate of fenvalerate in the Japanese quail. Key: A = fenvalerate, B = CONH₂-fenvalerate, C = 4'-OH-fenvalerate, D = Cl-V acid, E = 4-OH-Cl-V acid, F = 2-OH-Cl-V acid, G = 2,4-OH₂-Cl-V acid.

by hepatocytes, mainly to Cl-V acid. 4'-OH-Fenvalerate, CONH₂-fenvalerate, and 2-OH-Cl-V acid were also detected. Metabolism of fenvalerate by hepatocytes was only 6% compared with microsomes that metabolized 24% of the fenvalerate.

Rat in Vitro Studies. *Metabolism by Microsomes.* Of the fenvalerate 10–15% was metabolized (Table VI). The disappearance of fenvalerate and appearance of its metabolites were as shown in Figures 3 and 4. As in quail, 4-OH-Cl-V acid was found to be the major metabolite. CONH₂-Fenvalerate, Cl-V acid, 2-OH-Cl-V acid, and 2,4-OH₂-Cl-V acid were also found. 4'-OH-Fenvalerate could not be detected.

Metabolism by Hepatocyte Suspensions. A majority of fenvalerate remained unaltered (Table VI). Of the approximately 5% fenvalerate that was metabolized, Cl-V acid was the chief metabolite. This result agrees with quail data.

DISCUSSION

Fenvalerate orally administered to Japanese quail was rapidly eliminated from the body. Approximately 75% of the administered radioactivity was recovered in the first excreta sample collected 6 h postdosing. Thus, the biological half-life was determined to be less than 6 h. This value is smaller than that determined for fenvalerate in rats (Kaneko et al., 1981) and dogs (Kaneko et al., 1984). Even though the compound is distributed to various tissues and organs the levels are extremely low in all except fat.

Figure 5 shows the metabolic pathway of fenvalerate in Japanese quail. Fenvalerate underwent two major metabolic reactions: initial oxidation of the 4'-phenoxy position of the alcohol part of the molecule, yielding 4'-OH-fenvalerate, and cleavage of the ester linkage, yielding Cl-V acid. The parent molecule also underwent a minor metabolic reaction wherein the cyano group was hydrated to

yield CONH₂-fenvalerate. Quantifiable levels of 2-OH-Cl-V acid and 4-OH-Cl-V acid obtained by hydroxylation of carbon-2 or carbon-4 as well as 2,4-OH₂-Cl acid obtained by hydroxylation of both carbons were also found (Table IV). Although most of these metabolites were also found in earlier studies with rats, mice, and dogs (Ohkawa et al., 1979; Kaneko et al., 1981, 1984), quantitative differences could be observed between the avian and mammalian species when equitoxic doses are administered. In male quail excreta extracts, 4'-OH-fenvalerate was found to be 21% of the administered dose (Table IV) while it was found to range between 3.1 and 8.0% in male rats (Kaneko et al., 1981). Levels of 4-OH-Cl-V acid (free) were 5.7% in rat, but only 2% in quail. The levels of 2,4-OH₂-Cl-V acid were approximately the same (1–2%) for both species.

To understand the mechanisms involved in metabolism of fenvalerate, detailed in vitro experiments were conducted employing liver microsomes of rat and quail. Microsomes with the NADPH generating system degrade fenvalerate more than microsomes without the NADPH generating system, indicating that NADPH-dependent oxidation reactions were occurring in the incubation mixtures. The major metabolites found in both rat and quail microsomal extracts were 4-OH-Cl-V acid and Cl-V acid. The other metabolites identified were CONH₂-fenvalerate, 2-OH-Cl-V acid, and 2,4-OH₂-Cl-V acid.

4'-OH-Fenvalerate could be detected only in quail microsomal extracts. The profile of appearance of metabolites (Figure 4) revealed that 4'-OH-fenvalerate reached a maximum level within the initial 10 min and decreased thereafter, while the levels of Cl-V acid and 4-OH-Cl-V acid continuously increased. The fact that 4'-OH-fenvalerate did not appear in rat liver microsomal incubations may be due to more facile metabolism through the alternative pathways leading to Cl-V acid and 4-OH-Cl-V acid.

It is possible that in quail initial oxidation of fenvalerate yielded 4-OH-fenvalerate and 4'-OH-fenvalerate, two metabolites with their ester bonds intact (Figure 5). These metabolites could be very susceptible to esteratic attack on the basis of production of 4-OH-Cl-V acid and Cl-V acid, respectively, which were major metabolites in vitro. The presence or absence of 4-OH-fenvalerate could not be demonstrated; no authentic reference compound was available. No other metabolite was detected that had chromatographic behavior characteristic of such a compound. Thus, a sequence of reactions could be occurring wherein a combination of oxidation and hydrolysis control the fate of fenvalerate (Figure 5). Ohkawa et al. (1979) postulated that the initial oxidation to yield 4'-OH-fenvalerate is followed by further reactions to yield 3-(4-hydroxyphenoxy)benzoic acid.

There are significant differences between the quantities of metabolites determined in vivo experiments vs. in vitro experiments. 4-OH-Cl-V acid is a minor metabolite in the excreta extracts while 4'-OH-fenvalerate is a major metabolite. Microsomal metabolism leads to higher levels of 4-OH-Cl-V acid production and lesser quantities of 4'-OH-fenvalerate production. It is possible that hydroxylases are more active when purified as microsomal enzymes, but in the whole animal other factors control the rate of metabolism such as transport of fenvalerate from the alimentary canal to the liver or, following absorption, penetration through the cell wall to the endoplasmic reticulum. Another explanation could be that organs other than liver, like intestine, kidney, etc., could be involved in hydroxylation of fenvalerate at the 4'-position, resulting in an increased level of 4'-OH-fenvalerate in vivo compared to in vitro.

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It was established in this study that incubation temperature is a critical factor in the in vitro metabolism of fenvalerate. Quail microsomes incubated at 37 °C metabolized fenvalerate at a slower rate than when incubated at 42 °C, the body temperature of birds (Figure 3). Since most of the in vitro methods for birds are adapted from mammalian methods, the temperature factor is overlooked (Opdycke et al., 1982; Akhtar, 1983). However, incubation temperature could have a bearing on the outcome of results, particularly in comparative metabolism studies between different species, and should be included in the design of such experiments.

Akhtar (1983) reported less than 10% metabolism of fenvalerate by chicken liver microsomes, mostly by the initial cleavage of the ester bond. In the present study, however, 34% fenvalerate was metabolized by Japanese quail liver microsomes and both the hydrolytic and oxidative pathways were involved. Species differences between Japanese quail and chickens may account for this apparent difference, but lack of optimization of incubation conditions in the chicken study could also be a factor.

The finding that only 5% fenvalerate is metabolized by hepatocytes compared to 34% by microsomes indicates that the reaction rate in hepatocytes is limited by factors that do not operate in incubations with microsomes. It is possible that fenvalerate does not penetrate the cell membrane of the hepatocyte and is not available to the enzymes present inside. Other investigators (Billings et al., 1977) have also found that hepatocytes metabolize certain drugs more slowly than microsomes.

Several studies in vivo and in vitro have shown that by decreasing the rate of metabolism, pyrethroid toxicity can be increased. In the present study and in an earlier one with rats (Ohkawa et al., 1979) it was observed that the half-life of fenvalerate in quail is shorter than in rats (6 h vs. 12–14 h). Once fenvalerate is absorbed and reaches the liver, it is metabolized faster in quail than in rats (Figure 3). Thus, because of faster excretion, lesser absorption, and faster metabolism fenvalerate is less toxic to quail ($LD_{50} > 4000$ mg/kg) than to rats ($LD_{50} = 450$ mg/kg).

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LITERATURE CITED

Akhtar, M. H. *J. Agric. Food Chem.* **1983**, *31*, 1080.
Billings, R. E.; McMahon, R. E.; Ashmore, J.; Wagle, S. R. *Drug. Metab. Dispos.* **1977**, *5*, 518.
Bradbury, S. P.; Coats, J. R. *J. Toxicol. Environ. Health* **1982**, *10*, 307.
Caplan, J. A.; Isensee, A. R.; Nelson, J. O. *J. Agric. Food Chem.* **1984**, *32*, 166.
Kaneko, H.; Izumi, T.; Matsuo, M.; Miyamoto, J. *J. Pestic. Sci.* **1984**, *9*, 269.
Kaneko, H.; Ohkawa, H.; Miyamoto, J. *J. Pestic. Sci.* **1981**, *6*, 317.
Lowry, O. H.; Rosenbrough, N. J.; Farr, H. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Ohkawa, H.; Kaneko, H.; Tsuji, M.; Miyamoto, J. *J. Pestic. Sci.* **1979**, *4*, 143.
Ohkawa, H.; Nambu, K.; Inui, H.; Miyamoto, J. *J. Pestic. Sci.* **1978**, *3*, 129.
Omura, T.; Sato, R. *J. Biol. Chem.* **1964**, *239*, 2370.
Opdycke, J. C.; Miller, R. W.; Menzer, R. E. *J. Agric. Food Chem.* **1982**, *30*, 1227.
Riesenfeld, G.; Wals, P. A.; Golden, S.; Katz, J. *J. Biol. Chem.* **1981**, *256*, 9973.
Seglen, P. O. *Methods Cell Biol.* **1976**, *13*, 29.
Wszolek, P. C.; Hogue, D. E.; Lisk, D. J. *Bull. Environ. Contam. Toxicol.* **1981**, *27*, 869.