# Analysis and Persistence of Permethrin, Cypermethrin, Deltamethrin, and Fenvalerate in the Fat and Brain of Treated Rats

Abd El-Salam M. Marei,[1] Luis O. Ruzo, and John E. Casida*

A convenient procedure for residue analysis of permethrin, cypermethrin, deltamethrin, and fenvalerate, each at levels of 10 ppb or greater in the fat and brain of pyrethroid-treated rats, involves three steps: extraction of the fat with hexane and the brain with acetonitrile; cleanup of the extracts by partitioning with acetonitrile–hexane (2:1) and chromatography of the acetonitrile–soluble portion on a silica gel column with ether–hexane (2:1) for development and 9,9′-bifluorylidene as a marker dye; analysis by packed column GLC with an electron-capture detector (ECD). The identity of residues is readily confirmed by capillary column GLC and by conversion of the α-cyanophenoxybenzyl pyrethroids to their α-pentafluorobenzyl derivatives with enhanced ECD sensitivity. Residues in fat and brain are much higher and more persistent with *cis*-permethrin than with *trans*-permethrin or the α-cyanophenoxybenzyl pyrethroids. Fat and brain levels of *trans*- but not of *cis*-permethrin are greatly elevated on pretreatment with pyrethroid esterase and oxidase inhibitors. Phenobarbital appears to be moderately effective in lowering pyrethroid residues in fat.

The major agricultural pyrethroids at present are esters of 3-phenoxybenzyl alcohol or α-cyano-3-phenoxybenzyl alcohol used as a single isomer (deltamethrin) or as a mixture of four isomers (permethrin; fenvalerate) or eight isomers (cypermethrin) (Figure 1). No comparisons have been made on the persistence of these pyrethroids in mammals under standard biological and analytical conditions. We have therefore attempted to develop a suitable analytical method for residue analysis of pyrethroid mixtures and to use this procedure in evaluating the comparative persistence of permethrin, cypermethrin, and fenvalerate isomers and deltamethrin in the fat and brain of treated rats.

## MATERIALS AND METHODS

**Chemicals.** The pyrethroids examined (Figure 1), unless specifically stated otherwise, were the most insecticidal isomers of >98% stereochemical purity provided by Shell Development Co. (Modesto, CA) for (2*S*,α*S*)-fenvalerate and by Roussel-Uclaf (Paris, France) for (1*R*)-*cis*-permethrin, (1*R*)-*trans*-permethrin, (1*R*,α*S*)-*cis*-cypermethrin, (1*R*,α*S*)-*trans*-cypermethrin, and deltamethrin. (1*RS*,-α*RS*)-Fenvalerate was supplied by Shell and (1*RS*)-*cis*,-*trans*-permethrin and (1*RS*,α*RS*)-*cis*,*trans*-cypermethrin were supplied by FMC Corp. (Middleport, NY).

Extraction and cleanup used the following materials: chromatographic-grade hexane and acetonitrile (MCB Manufacturing Chemists, Inc., Cincinnati, OH); analytical reagent anhydrous diethyl ether (Mallinckrodt, Inc., Paris, KY); silica gel 60 (0.2–0.5 mm; 35–70-mesh ASTM) (E. Merck, Darmstadt, West Germany); 9,9′-bifluorylidene (Saleh and Casida, 1977) as a marker dye for the elution position of the relevant pyrethroids on silica gel.

Compounds used in the animal experiments were as follows: the esterase inhibitors tri-*o*-cresyl phosphate (TOCP) (Eastman Organic, Rochester, NY) (Casida et al., 1961), *S*,*S*,*S*-tributyl phosphorotrithioate (DEF) (Mobay Chemical Corp., Kansas City, MO) (Gaughan et al., 1980), 2-phenyl-1,3,2-benzodioxaphosphorinane 1-oxide or phenyl saligenin cyclic phosphonate (PSCP) (Casida et al., 1961) (prepared by S. Tawata of this laboratory), and *O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate (profenofos) (Ciba-Geigy, Greensboro, NC) (Gaughan et al., 1980); the oxidase inhibitor piperonyl butoxide (FMC Corp.); the antibiotic ampicillin trihydrate (Bristol Laboratories, Syracuse, NY); the strongly basic anion-exchange resin cholestyramine, used as Questran powder (Mead Johnson & Co., Evansville, IN).

**Synthesis of Internal Standards.** Two cyclopropanecarboxylates (A and B) were synthesized as internal standards for the GLC analyses. Internal standard A was prepared by treatment of 3-benzoylbenzoic acid (Aldrich Chemical Co., Milwaukee, WI) with excess thionyl chloride in benzene at reflux for 2 h, evaporation of the solvent and excess thionyl chloride, and reduction of the acyl chloride with 4 equiv of tri-*tert*-butoxyaluminum hydride in tetrahydrofuran at 25 °C for 6 h to obtain 3-benzoylbenzyl alcohol which was esterified with *cis*-chrysanthemoyl chloride and equivalent pyridine in benzene. Internal standard B was obtained on treating the appropriate cyclopropanecarboxylic acid chloride (Ruzo and Casida, 1981) with 3-phenoxybenzyl alcohol as above. The compounds were purified by TLC [silica gel; hexane–acetone (6:1) for A and carbon tetrachloride-ether (3:1) for B] and characterized by chemical ionization mass spectrometry (methane, 0.8 torr): m/e (relative intensity) 363 [$(M + 1)^+$, 56], 195 (100), and 151 (43) for A and 557 [$(M + 1)^+$, 0.5], 357 (11), and 183 (100) for B ($^{79}Br$).

**Extraction and Cleanup of Fat and Brain Samples.** One gram of fat was mixed with ~0.5 g of anhydrous sodium sulfate and extracted for 2 min with 15 mL of hexane by using the Polytron homogenizer (Kinematica GmbH, Lucerne, Switzerland). After filtration through a Büchner funnel using ~0.5 g of Celite analytical filter aid, the residue was reextracted with 15 mL of hexane for 1 min with the homogenizer prior to filtration. The combined filtrates were concentrated to 5 mL by using a rotary evaporator. This hexane extract was transferred to a 25-mL separatory funnel followed by 10 mL of acetonitrile. Vigorous shaking for 2 min and then 3–5 min of standing gave the hexane phase which was discarded and the acetonitrile phase which was concentrated in a 15-mL conical tube to 0.5 mL by using a stream of nitrogen.

Each brain (~1.9 g) was extracted for 2 min with 28 mL of acetonitrile and ~0.5 g of anhydrous sodium sulfate by

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

[1]Present address: Pesticide Chemistry Division, Plant Protection Department, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt.

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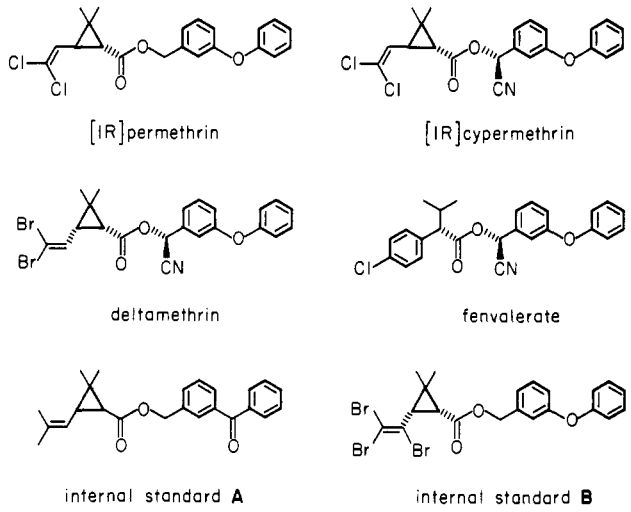


**Figure 1.** Structures of pyrethroids showing the configuration for the most insecticidal isomer. The pyrethroids were examined as various isomer mixtures. Compounds used as internal standards were 3-benzoylbenzyl *cis*-chrysanthemate (A) and 3-phenoxybenzyl *cis*-2,2-dimethyl-3-(1,2,2-tribromovinyl)cyclopropanecarboxylate (B).

using the Polytron homogenizer. The extract was filtered through ~0.5 g of anydrous sodium sulfate, and the residue was reextracted with 28 mL of acetonitrile and then filtered through sodium sulfate. The residue was finally washed by mixing with 15 mL of acetonitrile, and the extract was clarified by centrifugation and filtration (sodium sulfate). The combined acetonitrile extracts were concentrated to 20 mL and partitioned with 10 mL of hexane, and the acetonitrile phase was recovered for evaporation to 0.5 mL as above.

For chromatographic cleanup, the marker dye bifluorylidene (10 μg in 0.1 mL of acetonitrile) was added to each fat and brain extract (0.5 mL). Glass chromatographic columns (1.25-cm i.d.) were prepared by adding in sequence 1 cm of anhydrous sodium sulfate, 8 g of silica gel, and 1 cm of anhydrous sodium sulfate and tapping the column to achieve appropriate settling. After a prewash of the column with 40 mL of a ether–hexane (2:1) mixture, the extract was transferred to the column along with a 5-mL ether–hexane rinse of the conical tube. The column was developed with 40 mL of a ether–hexane (2:1) mixture, retaining the eluate from ~2 mL before the first yellow dye is eluted through but not beyond the yellow region for evaporation to dryness under nitrogen.

The fat extracts were dissolved in 5 mL of hexane containing 2 μg of internal standard B and the brain extracts in 1 mL of hexane containing 1 μg of internal standard A. A 2-μL aliquot was analyzed by GLC on one of three columns: a DEGS packed column or an OV-101 capillary column in studies requiring only separation of *cis*- and *trans*-permethrin; an OV-101 packed column in all other cases.

**GLC Analysis.** GLC utilized the Hewlett-Packard Model 5840A chromatograph with a $^{63}$Ni electron-capture detector (ECD). An on-line computer provided the retention time ($t_R$) in minutes and the area of each peak. Internal standards A for brain and B for fat were used with correction for relative responses of the various pyrethroids (Table I).

For packed column GLC, the coiled glass column (180 cm × 4 mm i.d.) contained 5% OV-101 on 80–100-mesh Chromosorb G H/P except in studies requiring separation of *cis*- and *trans*-permethrin in which case 3% DEGS was used on 100–120-mesh Varaport-30. The column temperature was 250 °C for OV-101 and 200 °C for DEGS. The injector and detector temperatures were 250 and 300 °C, respectively, and the carrier gas was argon–methane (95:5) at ~30 mL/min.

Table I. GLC Retention Times on OV-101 Capillary and Packed Columns and Relative ECD Responses for Pyrethroids and Internal Standards

| compound | $t_R$, min[a] capillary | $t_R$, min[a] packed | rel response[b] |
|---|---|---|---|
| pyrethroids | | | |
| permethrin | | 7.9 | 0.24 |
| cis | 8.8 | | |
| trans | 9.2 | | |
| cypermethrin | 13.1 | 11.1 | 1.00 |
| fenvalerate | | | 1.01 |
| 2*S*,α*R* + 2*R*,α*S* | 16.6 | 14.1 | |
| 2*S*,α*S* + 2*R*,α*R* | 18.0 | 15.1 | |
| deltamethrin | 22.1 | 18.0 | 1.26 |
| internal standards | | | |
| A | 10.2 | 8.8 | 0.17 |
| B | 29.2 | 23.8 | 1.00 |

[a] Retention time ($t_R$) as illustrated in Figure 2. [b] ECD response relative to internal standard B on packed column. There is little or no isomer difference in the relative response of *cis*- and *trans*-permethrin and of (2*S*,α*S*)-fenvalerate (one peak) and (2*RS*,α*RS*)-fenvalerate (sum of two peaks).

Capillary GLC utilized a flexible fused silica capillary column (12 m × 0.2 mm i.d.; Hewlett-Packard) with OV-101 and the following operating conditions: helium as the carrier gas and argon–methane (95:5) as the makeup gas; column pressure of 0.8 kg/cm$^2$ and a 30:1 split ratio; 250 and 300 °C for injector and detector temperatures, respectively, and a 220 °C oven temperature.

**Preparation and GLC Analysis of Pentafluorobenzyl Derivatives.** α-Cyanophenoxybenzyl pyrethroids were converted to their α-pentafluorobenzyl (PFB) derivatives (Saleh et al., 1980). The cleaned-up extract of 1 g of brain or fat was evaporated to dryness under nitrogen, and 1 mL of methylene chloride was added followed by 10 μL of pentafluorobenzyl bromide and 1 mL of an aqueous solution which was 0.1 M in tetrabutylammonium sulfate and 0.2 M in sodium hydroxide. After vigorous mixing for 20–25 min at 25 °C, the organic phase was recovered and evaporated to dryness, and 0.2–1 mL of toluene was added to the sample. A 2–4-μL aliquot was subjected to capillary column GLC/ECD analysis as above, but the column was isothermal at 220 °C for 20 min and then temperature programmed from 220 to 250 °C at 10 °C/min followed by 250 °C isothermal.

**Animal Experiments.** Male albino rats (240–260 g; Sprague-Dawley strain; Simonsen Laboratories, Inc., Gilroy, CA) were treated by the oral or intraperitoneal (ip) route with pyrethroids dissolved in 125 μL of glycerol formal [~75% 5-hydroxy-1,3-dioxane and ~25% 4-(hydroxymethyl)-1,3-dioxolane] (Tridom/Fluka Chemical, Inc., Hauppauge, NY). Food was withheld for 24 h before oral treatments. The pyrethroids were administered as a mixture of geometrical isomers [i.e., (1*R*)-*cis*,*trans*-permethrin] or of different compounds resolved by GLC in order to directly intercompare the distribution and persistence of the components. One study involved oral treatments with a mixture of *cis*-permethrin, *trans*-cypermethrin, deltamethrin, and (2*RS*,α*RS*)-fenvalerate (each at 3 mg/kg) in one series and a mixture of *trans*-permethrin, *cis*-cypermethrin, deltamethrin, and (2*S*,α*S*)-fenvalerate (each at 3 mg/kg) in another series, with fat analyses at 1, 3, 7, 14, and 21 days after treatment. Another investigation utilized ip administration with a

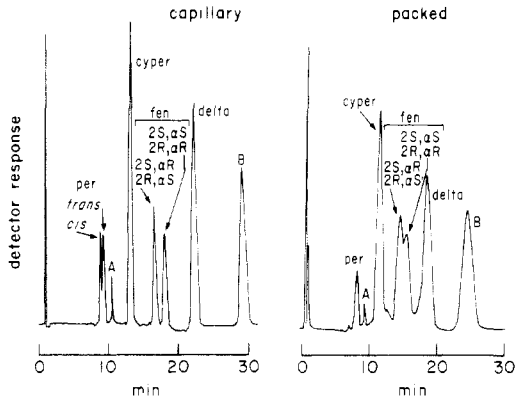


**Figure 2.** GLC analyses of pyrethroid mixture on OV-101 capillary and packed columns. A and B are internal standards. For $t_R$ values, see Table I.

mixture of (1R)-*cis*- and (1*R*)-*trans*-permethrin (each at 5 mg/kg) or with a mixture of *cis*-permethrin, *cis*-cypermethrin, deltamethrin, and (2*S*,α*S*)-fenvalerate (each at 2.5 mg/kg). Brain analyses were carried out at 3 h and 3 and 5 days after treatment with the permethrin isomers and at 1 and 3 h and 1, 3, 5, and 7 days after treatment with the other pyrethroids. A series of studies involved ip or oral pretreatment with candidate metabolic inhibitors or with ampicillin, ip, or oral administration of the (1*R*)-*cis*- and (1*R*)-*trans*-permethrin mixture (each at 5 mg/kg) and fat and brain analyses 3 h after the ip pyrethroid treatments and fat analyses 48 h after the oral pyrethroid doses. A final approach with *cis*-permethrin, *cis*-cypermethrin, deltamethrin, and (2*S*,α*S*)-fenvalerate (each administered ip at 2.5 mg/kg) utilized ip treatment with sodium phenobarbital before or after the pyrethroids with fat analyses 5 days later. Alternatively, the same pyrethroids were given orally (each at 3 mg/kg) along with orally administered cholestyramine or activated charcoal with fat analyses 2 days later.

All analytical results are the average values from two or more animals analyzed individually with duplicate GLC determinations in each case.

## RESULTS AND DISCUSSION

**Analytical Methods.** This study required modification of available procedures for pyrethroid analysis [e.g., Chapman and Simmons (1977), Fujie and Fullmer (1978), and Oehler (1979)] for application to fat and brain.

The packed OV-101 column used for routine analysis gives each pyrethroid as a single well-resolved peak except for fenvalerate which appears as two partially resolved peaks (Figure 2; Table I). Improved resolution is obtained on the OV-101 capillary column which also partially separates *cis*- and *trans*-permethrin (Figure 2; Table I), but these advantages only partially compensate for the sensitivity loss due to the 30:1 split ratio used. An alternative procedure for analysis of *cis*- and *trans*-permethrin involves the packed DEGS column with $t_R$ values of 5.8 and 7.1 min, respectively. Internal standards A and B were selected for suitable chromatographic positions relative to the pyrethroids (Figure 2; Table I) and interfering materials.

High recovery values (86–97%; Table II) are obtained on extraction of the fat with hexane and the brain with acetonitrile, partitioning these extracts between acetonitrile and hexane, and column chromatography with bifluorylidene as a marker dye. The use of acetonitrile instead of hexane for extraction of the brain greatly minimizes interfering materials. The 2:1 acetonitrile–hexane ratio provides near complete pyrethroid recovery in the acetonitrile and almost total removal of fatty materials in the hexane. This pyrethroid recovery on acetonitrile–hexane partitioning is higher than anticipated from an earlier report on permethrin (Oehler, 1979).

Table II. Recovery Analyses of Pyrethroids from Fat and Brain of Rats

| pyrethroid | recovery, %[a] fat | brain |
|---|---|---|
| permethrin[b] | 86 | 94 |
| cypermethrin[c] | 89 | 97 |
| deltamethrin | 86 | 94 |
| fenvalerate[d] | 95 | 93 |

[a] Calculated as the peak area for pyrethroid relative to the internal standard, comparing the pyrethroid recovered from fortified tissue with the pyrethroid analyzed directly as described under GLC Analysis. [b] 1*RS*,cis,trans isomer from fat and 1*R*,cis isomer from brain. [c] 1*RS*,cis,trans-α*RS* isomer from fat and 1*R*,cis-α*S* isomer from brain. [d] 2*RS*,α*RS* isomer from fat and 2*S*,α*S* isomer from brain.

Interfering materials remaining after the partitioning step are largely removed by silica gel column chromatographic cleanup with ether–hexane (2:1), a solvent system selected because it gives similar chromatographic characteristics for bifluorylidene and the pyrethroids on TLC with silica gel chromatoplates. Permethrin appears at slightly higher $R_f$ than the marker dye and the other pyrethroids on TLC and elutes slightly before or just within the leading edge of the yellow dye on column chromatography. Accordingly, eluate collection was started just before appearance of the yellow fraction to ensure complete permethrin recovery.

The cleanup and ECD sensitivity are sufficient to analyze fat and brain pyrethroid levels as low as 10 ppb on the packed column with no interference in the analytical region except from brain where an unknown compound at $t_R$ = 23.1 min necessitates the use of A rather than B as the internal standard.

**Pentafluorobenzyl Derivatives.** α-Cyanophenoxybenzyl but not phenoxybenzyl esters react with PFB bromide to form PFB derivatives of longer $t_R$ values and enhanced ECD sensitivity (Saleh et al., 1980). Thus, the pentafluorobenzylation procedure shifts the chromatographic positions of cypermethrin, deltamethrin, and fenvalerate but not of permethrin or the internal standards. Two GLC peaks are obtained for the PFB derivatives of each of *cis*-cypermethrin, *trans*-cypermethrin, deltamethrin, and fenvalerate (Figure 3). The peak areas of the α-cyanophenoxybenzyl pyrethroids are increased 3–4-fold on pentafluorobenzylation, so this procedure is potentially useful not only for confirmation of residue identity but also for quantitation of very low residue levels. However, this potential is not fully achieved with current cleanup procedures. Interference poses no problem at 5-ppm levels, but at 0.5 ppm undesired peaks often obscure a portion of the PFB-cypermethrin region (Figure 3).

**Pyrethroid Persistence in Fat following Oral Administration.** Residues in fat are higher and more persistent with the cis than with the trans isomers of permethrin and cypermethrin (Figure 4), in agreement with previous reports (Crawford et al., 1981; Gaughan et al., 1977; Hutson et al., 1981). The highest levels are maintained with *cis*-permethrin in which case much of the "residue dissipation" occurs from dilution on new fat deposition as illustrated in Figure 4. The lack of difference between (2*S*,α*S*)-fenvalerate and (2*RS*,α*RS*)-fenvalerate is in agreement with earlier radiotracer studies comparing (2*S*,α*RS*)- and (2*RS*,α*RS*)-fenvalerate (Ohkawa et al., 1979). It is of particular interest to note that deltamethrin

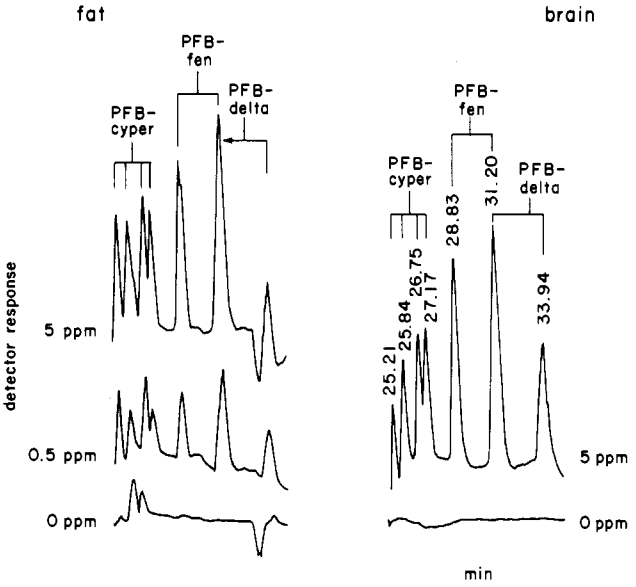


**Figure 3.** GLC analyses of pentafluorobenzyl derivatives of *cis,trans*-cypermethrin, (2*RS*,α*RS*)-fenvalerate, and deltamethrin on an OV-101 capillary column. *cis*-Cypermethrin yields the 25.21- and 26.75-min PFB derivatives and *trans*-cypermethrin the 25.84- and 27.17-min derivatives (Saleh et al., 1980). The chromatograms illustrated are from fat and brain samples fortified at 0, 0.5, and 5 ppm relative to each pyrethroid. Interfering materials preclude meaningful analyses before $t_R$ = 24–25 min. The 0- and 0.5-ppm fat analyses represent 4 μL of the 0.2-mL samples, whereas the 0-ppm brain and 5-ppm brain and fat analyses represent 3 μL from the 1.0-mL samples.

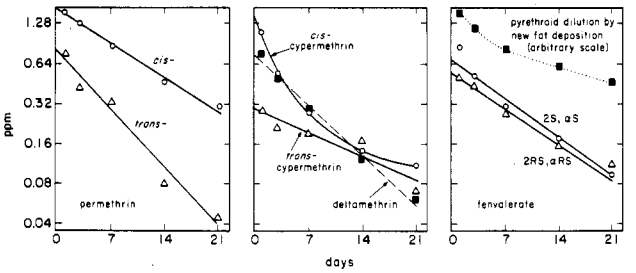


**Figure 4.** Pyrethroid levels in fat of rats treated orally at 3.0 mg/kg.

residues dissipate quite rapidly (Ruzo et al., 1978), in fact at almost an identical rate to those of *trans*-permethrin (Figure 4).

The relatively small differences in rates of residue dissipation from fat ($t_{1/2}$ = 5–6 days for *trans*-permethrin and deltamethrin and 7–10 days for the other compounds) are not surprising since the metabolism of each compound in rats involves extensive ester cleavage and methyl and 4-phenoxy hydroxylation (Casida et al., 1979). Although the major portion of an oral pyrethroid dose undergoes rapid metabolism, a small amount partitions into fatty tissues and persists in amounts detectable for at least 3 weeks. Thus, "pyrethroid esterases" or "lipases" of fat (Crawford et al., 1981; Hutson et al., 1981) act very slowly under the in vivo conditions examined. In vitro studies comparing a variety of preparation and incubation conditions in the presence and absence of esterase inhibitors confirm the low activity or absence of pyrethroid esterases in homogenates and emulsions of rat fat acting on the permethrin, cypermethrin, and fenvalerate isomers and on deltamethrin.

**Pyrethroid Levels in Brain following Intraperitoneal Administration.** The brain levels are of particular interest with α-cyanophenoxybenzyl pyrethroids since the

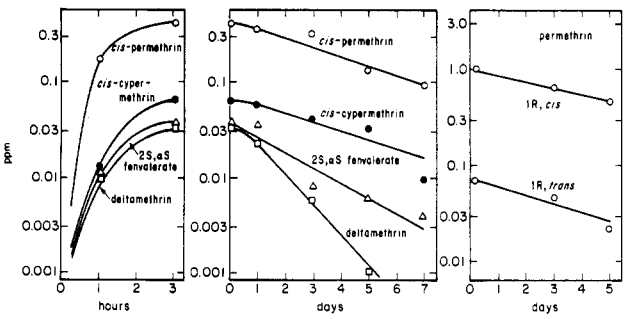


**Figure 5.** Pyrethroid levels in brain of rats treated intraperitoneally at 5.0 mg/kg with each of (1*R*)-*cis*- and (1R)-*trans*-permethrin and at 2.5 mg/kg with other pyrethroids.

Table III. Effects of Piperonyl Butoxide and Three Organophosphorus Compounds on Fat and Brain Levels of *cis*- and *trans*-Permethrin Three Hours after Intraperitoneal Treatment of Rats with (1*R*)-*cis,trans*-Permethrin at Ten Milligrams per Kilogram

| pretreatment[a] | permethrin, ppm[b] cis | trans | ratio of cis/trans |
|---|---|---|---|
| Residues in Fat | | | |
| none | 81 | 89 | 0.91 |
| piperonyl butoxide | 98 | 110 | 0.89 |
| PSCP | 107 | 121 | 0.88 |
| DEF | 94 | 98 | 0.96 |
| TOCP | 48 | 40 | 1.2 |
| Residues in Brain | | | |
| none | 0.93 | 0.061 | 15.2 |
| piperonyl butoxide | 0.50 | 0.26 | 1.9 |
| PSCP | 0.41 | 0.28 | 1.5 |
| DEF | 0.50 | 0.40 | 1.3 |
| TOCP | 0.63 | 0.55 | 1.1 |

[a] ip doses and pretreatment intervals as follows: piperonyl butoxide, 100 mg/kg, 3 h; PSCP, 10 mg/kg, 1 h; DEF, 100 mg/kg, 3 h; TOCP, 1 mL/kg, 24 h. [b] Mean for two rats with individual results averaging 6% deviation from the mean for fat and 28% for brain.

Table IV. Effects of Piperonyl Butoxide, Profenofos, and Ampicillin on Fat Levels of *cis*- and *trans*-Permethrin 48 Hours after Oral Treatment of Rats with (1*R*)-*cis,trans*-Permethrin at 10 Milligrams per Kilogram

| pretreatment[a] | permethrin, ppm[b] cis | trans | ratio of cis/trans |
|---|---|---|---|
| none | 2.6 | <0.13 | >20 |
| piperonyl butoxide | | | |
| oral | 2.7 | <0.13 | >20 |
| ip | 2.7 | 0.45 | 6.0 |
| profenofos | | | |
| oral | 4.0 | 3.1 | 1.3 |
| ip | 3.2 | 2.2 | 1.5 |
| ampicillin, oral | 4.9 | <0.24 | >20 |

[a] Profenofos (50 mg/kg) and piperonyl butoxide (100 mg/kg) administered 6 h before the pyrethroid. Ampicillin trihydrate given orally as four daily doses, each of 9 mg/kg, followed after 24 h by the pyrethroid. [b] Mean for two rats with individual results averaging 12% deviation from the mean.

poisoning signs of deltamethrin in mice are correlated with the brain levels of this compound (Ruzo et al., 1979). On ip administration, the brain levels are remarkably higher with (1*R*)-*cis*-permethrin than with (1*R*)-trans-permethrin, (1*R*,α*S*)-*cis*-cypermethrin, deltamethrin, and (2*S*,α*S*)-fenvalerate (Figure 5). The high levels and persistence of *cis*-permethrin are due to both extensive entry into the brain within 3 h and to slow dissipation between 1 and 7

Table V. Effects of Phenobarbital, Cholestyramine, and Charcoal on Fat Levels of Pyrethroids

| | reduction in level, % | | | | |
|---|---|---|---|---|---|
| | permethrin | | | | |
| treatment | cis | trans | *cis*-cypermethrin | deltamethrin | fenvalerate |
| | Five Days after ip Pyrethroid Dose | | | | |
| phenobarbital ip | | | | | |
| before pyrethroid[a] | 47 ± 6 | 46 ± 7 | 45 ± 8 | 46 ± 10 | 40 ± 12 |
| after pyrethroid[b] | 43 ± 6 | 33 ± 4 | 36 ± 3 | 40 ± 1 | 34 ± 2 |
| | Two Days after Oral Pyrethroid Dose | | | | |
| cholestyramine oral[c] | 14 | | 11 | 30 | 20 |
| charcoal oral[c] | 27 | | 39 | 45 | 40 |

[a] Average and standard deviation for results from eight rats administered 60 mg/kg phenobarbital. Two of these rats received phenobarbital 3 days before the pyrethroids, two at 2 days, and two at 1 day. The other two rats received 15 mg/kg at each of 4, 3, 2, and 1 days before the pyrethroids. [b] Average and standard deviation for results from six rats administered 60 mg/kg phenobarbital. Two of these rats received phenobarbital 1 day after the pyrethroids, two at 2 days, and two at 3 days. [c] Mean for two rats with individual results averaging 46% deviation from the mean. Three doses of 75 mg/kg at each of 1, 7, and 24 h after the pyrethroids.

days. $t_{1/2}$ values in the brain range from 1 to 4 days during which time the brain weight is essentially constant. Pyrethroid esterases are reported from mouse brain homogenates (Casida et al., 1979; Ruzo et al., 1979), but their substrate specificity has not been examined.

**Effects of Oxidase and Esterase Inhibitors on Pyrethroid Levels in Fat and Brain.** Pyrethroid levels initially attained in fat and brain (Figures 4 and 5) are probably inversely related to their relative rates of metabolism in the liver. Compound-dependent differences in fat and brain levels are more prominent with oral treatment than with ip administration because in the former case more time is available for expression of differences in detoxification rates. Metabolic inhibitors are also useful in examining the influence of oxidases and esterases on pyrethroid persistence and residue levels (Casida et al., 1979; Gaughan et al., 1980).

Ip administration of *cis*- and *trans*-permethrin yields similar fat residues of the two isomers 3 h later, and this ratio is not greatly altered by an oxidase inhibitor (piperonyl butoxide) or esterase inhibitors (PSCP, DEF, and TOCP) (Table III). Brain levels of *cis*-permethrin are changed relatively little while those of *trans*-permethrin are increased by 4–9-fold on treatment with either the oxidase or esterase inhibitors, resulting in a large increase in the *cis*-permethrin/*trans*-permethrin ratio in the brain (Table III).

Oral treatment with *cis*- and *trans*-permethrin magnifies differences in fat levels due to metabolic inhibitors (Table IV). Piperonyl butoxide is much more effective with ip than with oral administration, whereas profenofos, an exceptionally potent pyrethroid esterase inhibitor (Gaughan et al., 1980), is highly effective with both ip dosing and oral dosing. Ampicillin treatment does not have a marked effect on the fat levels of the permethrin isomers.

**Effects of Phenobarbital, Cholestyramine, and Charcoal on Fat Levels of Pyrethroids.** Phenobarbital moderately lowers the fat residues when given before or after the ip-administered pyrethroids (Table V). It is a known inducer of liver monooxygenases, and a greater effect on pretreatment than on posttreatment with phenobarbital suggests that enzyme induction may be responsible. Cholestyramine and charcoal have less influence on fat levels of the orally administered pyrethroids (Table V). Phenobarbital, cholestyramine, and charcoal are known to reduce fat levels of chlorinated hydrocarbon insecticides (Boylan et al., 1978; Cook, 1973; Cook and Wilson, 1971; Ferguson et al., 1981).

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