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Fluvalinate Metabolism by Rhesus Monkeys

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Four rhesus monkeys were given a single oral dose of [*trifluoromethyl*-^{14}C]fluvalinate (α-cyano-3-phenoxybenzyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate) at 1 mg/kg. After 5 days, 55 ± 16 and 37 ± 12% of the applied dose were excreted in feces and urine, respectively. The major ^{14}C-labeled residue in feces (68–69% of fecal ^{14}C) consisted of unmetabolized fluvalinate while the only significant metabolite was 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoic acid, the anilino acid resulting from hydrolysis (2–15% of fecal ^{14}C). The anilino acid was also the principal ^{14}C-labeled residue in urine, occurring as its glucuronide and hydroxymethyl derivatives (55–77 and 7–29% of urinary ^{14}C, respectively). Radioactivity peaked in blood plasma 2–3 h after dosage, with fluvalinate comprising a minor fraction of the ^{14}C-labeled residue (≤1% of plasma ^{14}C) and the anilino acid representing the majority of the radioactivity (67–90% of plasma ^{14}C).

Very few pesticides have been studied in nonhuman primates even though primates are generally regarded as better metabolic models for man than rodents. One of us (Selim and Robinson, 1982, 1983) studied the pharmacokinetic profile, excretion, and metabolism of permethrin previously in rhesus monkeys (*Macaca mulata*), finding poor absorption into blood and predominantly fecal excretion. Thus, substantial differences were suggested for metabolism of permethrin by rhesus monkeys compared to rats. We embarked upon this work in order to investigate the generality of the results with permethrin and to compare the metabolism of fluvalinate (1) in rats (Quistad et al., 1983) and a cow (Quistad et al., 1982a) with that in rhesus monkeys.

EXPERIMENTAL SECTION

Preparation of the [*trifluoromethyl*-^{14}C]fluvalinate ($\alpha RS,2RS$) has been described (Quistad et al., 1982b). All animals were selected from the colony at PRI and were certified as healthy by a battery of blood chemistry tests (Selim and Robinson, 1983). Four male rhesus monkeys (8.4–11.4 kg) were given gelatin capsules containing [^{14}C]fluvalinate (1 mg/kg) in corn oil (200 mg). Two monkeys, cannulated for facile sampling of blood and urine, were placed in restraining chairs. The other two monkeys were placed directly into metabolism cages designed for separation of urine and feces. After the first 24 h, the cannulae were removed from the restrained monkeys and they too were placed in metabolism cages. Blood, urine, and feces were removed periodically for 5 days.

Radioassay. Radiolabel was quantified by liquid scintillation counting (LSC) and combustion to $^{14}CO_2$ as described previously (Quistad et al., 1982b). The methodology for metabolite analysis by thin-layer chromatography on silica gel (TLC) and liquid chromatography in the normal- (Pirkle-Type 1-A column) and reversed-phase (LiChrosorb RP-8 column) mode has also been reported (Staiger and Quistad, 1983; Quistad et al., 1982b). The following solvent systems were used (linear gradients): SS 1 (hexane–ethyl acetate–acetic acid, 12:9:0.1); SS 2 (gradient 50–90% methanol–0.1% aqueous acetic acid over 30 min); SS 3 (gradient 55–75% methanol–0.1% acetic acid over 20 min); SS 4 (gradient 60–70% methanol–0.1%

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Table I. Radiolabel Balance after 5 Days for Rhesus Monkeys Given Single Oral Doses of [*trifluoromethyl*-^{14}C]Fluvalinate at 1 mg/kg

animal no.	% applied dose	
	urine	feces
2024	36.7	52.6
2383	26.8	68.7
2382[a]	29.9	64.6
2384[a]	54.3	34.1
mean ± SD	36.9 ± 12.3	55.0 ± 15.5

[a] Animal maintained in restraining chair from 0 to 24 h for plasma collection.

acetic acid over 15 min, 70–90% over 10 min). Authentic metabolite standards were available from previous work (Quistad et al., 1982a,b).

In general, neat urine was analyzed by reversed-phase LC for the anilino acid **2**, the glycine conjugate of **2** (i.e., **3**), and 2-chloro-4-(trifluoromethyl)aniline (haloaniline **4**): k' = 12.1, 8.8, and 7.8, respectively, in SS 2. Since the glucuronide of **2** (i.e., **5**) and hydroxy acid **6** coeluted in this solvent system (k' = 6), the hydroxy acid was quantified as its methyl ester by TLC analysis of methylated (CH_2N_2) urine in SS 1 (R_f = 0.26). Thus, quantification of glucuronide **4** was obtained by subtracting the amount of **6** (determined by TLC) from the combined value for **5** + **6** (LC analysis of neat urine). The structure of hydroxy acid **6** was verified by conversion to cis and trans lactones (reversed-phase LC in SS 3) while glucuronide **5** was verified by (1) LC of the methylated, peracetylated metabolite with authentic standard and (2) enzymatic cleavage of the metabolite with β-glucuronidase (Quistad et al., 1982a).

An aqueous slurry of fecal samples was combusted for ^{14}C quantification. For metabolite analysis, the feces were extracted with methanol (3×) and an aliquot of the extract was analyzed by LC (SS 4). Radiolabel in the residual solids after extraction was quantified by combustion to $^{14}CO_2$. Simultaneous analysis of the four fluvalinate stereoisomers by normal-phase LC with a chiral Pirkle 1-A column has been described (Staiger and Quistad, 1983).

Aliquots of blood plasma (200 μL) were mixed with methanol (300 μL) to precipitate proteins. The precipitated solids contained negligible radioactivity. After centrifugation, the supernatant was analyzed by LC (SS 4).

RESULTS AND DISCUSSION

Within 5 days after receiving a single oral dose of [^{14}C]fluvalinate (**1**), 92 ± 4% of the applied dose was recovered in excrement with 37 ± 12 and 55 ± 16% in the urine and feces, respectively. It is evident from Table I that considerable individual variability was observed with regard to excretion in urine vs. feces although the overall excretion of ^{14}C was high for all animals.

Metabolites in Excrement. Most of the urinary metabolites were excreted in 1 day (Figure 1). The predominant metabolite was the glucuronide of **2** (i.e. **5**), representing 55-77% of the urinary ^{14}C (Table II). The only other major urinary metabolites were free anilino acid **2** and its hydroxymethyl derivative **6** which contributed 3–5 and 7–29% of the urinary ^{14}C, respectively. When hydroxy acid **6** was treated with HCl, the cis and trans lactones were formed in a 1:13 ratio. This result implies a stereoselective metabolic hydroxylation of the methyl groups in **2** [cf. Ruzo et al. (1976)]. The glycine conjugate of **2**, as well as the haloaniline **4**, were minor metabolites.

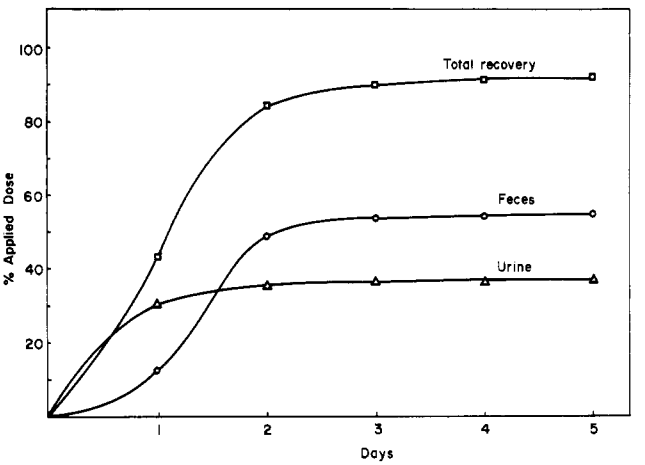

Figure 1. Excretion of ^{14}C-labeled residues from rhesus monkeys given single oral doses of [^{14}C]fluvalinate at 1 mg/kg.

Since 45 ± 18% of the applied dose passed through the alimentary canal of rhesus monkeys as intact parent ester (enterohepatic circulation contraindicated by identity of ^{14}C-labeled residue in plasma), fluvalinate is relatively stable toward absorption and metabolic alteration. The ^{14}C-labeled residues in feces were readily extractable (93–99%) and fluvalinate was by far the most abundant ^{14}C-labeled component (68-96% of extractable ^{14}C). Anilino acid **2** was the only significant metabolite in feces (Table III, 2–23% of fecal ^{14}C). The amount of ^{14}C in feces of monkeys treated with fluvalinate was considerably less than the >80% found for rhesus monkeys dosed with permethrin (Selim and Robinson, 1982, 1983), but the permethrin-treated animals received a 50 mg/kg dose (vs. 1 mg/kg for fluvalinate) which probably contributes to the greater passage rate of permethrin. The preponderance of intact fluvalinate (vs. metabolites) in feces agrees with the permethrin data.

The individual stereoisomers of (α*RS*,2*RS*)-fluvalinate were analyzed in order to probe for possible stereoselective metabolism in monkeys. From Table IV it appears that the stereoisomers are degraded at about the same rate. Previous studies have shown that the α*S*,2*R* isomer manifests most of the insecticidal and toxic properties for fluvalinate.

Blood. The peak levels of ^{14}C in plasma occurred 2–3 h after dosage (Figure 2). Analysis of plasma at this time

Table II. Urinary Metabolites from Rhesus Monkeys Given Single Oral Doses of [*trifluoromethyl*-^{14}C]Fluvalinate at 1 mg/kg

metabolite	% of ^{14}C in urine			
animal no.:	2024	2384	2382	2383
time, h:	0-24	0-48	0-24	0-24
% applied dose:	32.3	52.6	25.9	21.2
anilino acid **2**	4.1	3.2	2.5	5.2
hydroxy acid **6**	29	11	7	11
glycine conjugate of **2** (i.e., **3**)	0.3	0.3	0.5	0.3
glucuronide of **2** (i.e., **5**)	55	75	77	71
haloaniline **4** [2-chloro-4-(trifluoromethyl)aniline]	≤2.3	≤1.1		≤1.1
total identified	89	89	87	88

Table III. Radiolabeled Residues in Feces from Rhesus Monkeys Given Single Oral Doses of [^{14}C]Fluvalinate at 1 mg/kg

animal no.	time, h	% applied dose	% of ^{14}C in extract: fluvalinate	% of ^{14}C in extract: anilino acid
2024	0-24	29.1	93.9	1.6
	24-28	20.7	77.2	14.9
2382	0-24	20.1	91.4	4.7
	24-28	43.5	84.5	8.1
2383	24-48	50.9	96.1	1.5
	48-72	13.9	88.8	6.3
2384	24-48	31.5	67.8	22.8

Table IV. Analysis of Individual Stereoisomers of ($\alpha RS,2RS$)-Fluvalinate Isolated from Rhesus Feces by LC Using a Pirkle 1-A Column

source	% isomer: $\alpha R,2R$	$\alpha S,2R$	$\alpha S,2S$	$\alpha R,2S$
control (1 before dosage)	23	24	25	28
0-24-h feces	25	25	22	28
24-48-h feces	24	21	29	26

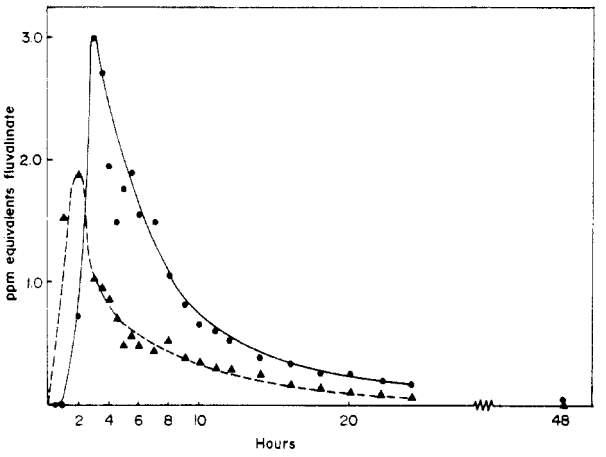

Figure 2. Radioactivity profile in blood plasma for rhesus monkeys treated with [*trifluoromethyl*-^{14}C]fluvalinate (1 mg/kg).

revealed minimal fluvalinate (0.3–1.1%) but substantial anilino acid **2** (83–90% of plasma ^{14}C). The half-life for disappearance of radiolabel from blood after reaching a peak concentration was also 2–3 h. Analysis of plasma at 8 h after dosage confirmed the abundance of anilino acid **2** (67–82%) and paucity of fluvalinate (≤1.2%) in blood. At the 50-fold higher dose rate the amount of ^{14}C-labeled residues from permethrin absorbed into rhesus blood was considerably less compared to that of fluvalinate (Selim and Robinson, 1982, 1983).

Comparative Mammalian Metabolism. The deposition of fluvalinate in rhesus monkeys is qualitatively similar to its fate in rats (Quistad et al., 1983) and a cow (Quistad et al., 1982a) although several quantitative differences are evident (Figure 3). In general, the urinary profile of metabolites in monkeys is more similar to that found for a cow than for rats. As with bovine metabolism of fluvalinate, relatively more ^{14}C appears in urine than was found for rats. Likewise, the glucuronide of anilino acid **2** was the dominant metabolite while the haloaniline **4** and glycine conjugate of **2** are minor metabolites. However, the hydroxy acid **6** is abundant in urine of both the rhesus monkey and rat.

The identity of ^{14}C-labeled residues in feces is basically similar for all three mammalian species with unmetabolized fluvalinate representing the most abundant component. Interestingly, in both rhesus monkeys and rats 45% of the applied fluvalinate survives transit through the gastrointestinal tract of the animals. Fluvalinate contributes a higher percentage of the ^{14}C-labeled residue in monkey feces than was found for rats and a cow. Fluvalinate is such a dominant ^{14}C-labeled residue that, besides anilino acid **2**, there are only traces of other metabolites. Contrary to our expectations from studies with other animals (Quistad et al., 1982c), bile acid conjugates of **2** were either minor metabolites or absent in the monkey (collectively ≤2% of fecal ^{14}C with no single conjugate >0.5%). The apparent paucity of bile acid conjugates in the monkey vs. other species (rat, cow, chicken) may be due to the lower levels of anilino acid **2** available for conjugation by the gut microbes of monkeys. If one accepts the contention that rhesus monkeys are the best model for humans and that rhesus bile acids are more suitable than those of rats for comparison (Dowling et al., 1970), then bile conjugates would be expected to be minor constituents in humans exposed to fluvalinate.

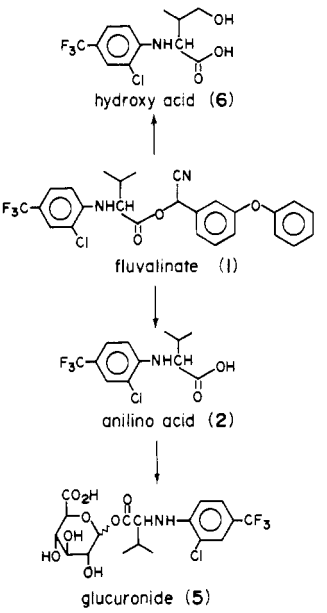

Figure 3. Rhesus monkey metabolites of [*trifluoromethyl*-^{14}C]fluvalinate.

Although the level of radiolabel in blood in rhesus monkeys peaks earlier than for rats or a cow (2–3 h vs ≥8 h), in all three species intact fluvalinate is a minor (<6%) component in blood. Thus, it appears that fluvalinate (M_r 503) is poorly absorbed from the gastrointestinal tract and anilino acid **2** is the major ^{14}C-labeled residue in blood of animals treated with [*trifluoromethyl*-^{14}C]fluvalinate. The low levels of fluvalinate in blood probably preclude enterohepatic circulation of the intact ester as an important metabolic excretion mechanism.

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Degradation and Movement of Fluvalinate in Soil

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[*trifluoromethyl*-^{14}C]Fluvalinate is rapidly degraded on sandy loam, clay loam, and clay soils under aerobic conditions with initial half-lives of 6–8 days. The primary degradation products of fluvalinate are the anilino acid (**2**) and haloaniline (**3**), the latter primarily evolved as a volatile product. Under anaerobic conditions, the degradation was slower ($t_{1/2}$ on clay soil = 15 days) but with similar products formed. The flood water above the anaerobic soils contained, in addition to the above metabolites, a diacid (**4**) and 4-amino-3-chlorobenzoic acid (**5**). A small amount of $^{14}CO_2$ was produced from all soils. Fluvalinate does not leach through soil and rapidly adsorbs to soil from water with little desorption. The anilino acid metabolite (**2**) is of low to intermediate mobility as determined by soil thin-layer chromatography plates. Lettuce, radish, and wheat plants do not accumulate appreciable ^{14}C-labeled residues when growing in soil treated with [*trifluoromethyl*-^{14}C]fluvalinate.

Fluvalinate [α-cyano-3-phenoxybenzyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate, active ingredient of Mavrik 2E insecticide] is an experimental insecticide with pyrethroid-like activity currently being developed by Zoecon Corp. for use against insect pests on various crops. Due to the likely exposure of soil to the chemical, it is necessary to determine the metabolic fate of fluvalinate in soils under various conditions. The present study deals with the degradation of fluvalinate on three soil types under aerobic and anaerobic conditions as well as the mobility of fluvalinate and a major metabolite in three soils [for the preceding report in this series, see Staiger et al. (1982)].

EXPERIMENTAL SECTION

Radioassay and Chromatography. Radioactivity was quantified by liquid scintillation counting (LSC) alone or in conjunction with sample combustion to $^{14}CO_2$ (Quistad et al., 1982). A Polytron homogenizer (Brinkman) was used to facilitate extraction of fluvalinate and degradation products from soil. The quantification of radiolabeled metabolites in extracts was achieved by using gradient-elution, reversed-phase liquid chromatography (LC) by coinjection of a known amount of radiolabeled extract together with authentic metabolite standards and collection of timed fractions for subsequent assay by LSC. The conditions for reversed-phase LC analysis of fluvalinate and metabolites have been described (Quistad et al., 1982). The following mixtures of methanol–0.1% acetic acid were used for elution: SS 1 (gradient 60–70% methanol over 15 min, 70–90% over 10 min, isocratic at 90% for 10 min); SS 2 (gradient 70–90% methanol over 20 min, isocratic at 90% for 10 min); SS 3 (isocratic at 50% methanol for 6 min, gradient 50–80% over 4 min, isocratic at 80% for 10 min); SS 4 (isocratic at 70% methanol). Purification of metabolites utilized thin-layer chromatography (TLC) on silica gel GF (Analtech, Newark, DE) with radioactive zones located with a radiochromatogram scanner (Packard Instruments, Downers Grove, IL).

Synthetic Standards. The preparation of [*trifluoromethyl*-^{14}C]fluvalinate (**1**) has been reported previously (Quistad et al., 1982) and was a mixture of *αR*,2*R*, *αS*,2*S*, *αR*,2*S*, and *αS*,2*R* stereoisomers. The radiochemical purity of combined isomers was 99.7% as judged by reversed-phase LC (SS 1) with a specific activity of 48.3 mCi/mmol determined by mass spectrometry.

Authentic samples of fluvalinate (**1**), the anilino acid (**2**), and the haloaniline (**3**) were synthesized by the Zoecon Chemical Research Department. Authentic standards of 4′-hydroxyfluvalinate, diacid **4**, desphenylfluvalinate (removal of the distal phenoxy ring), and an amide analogue of fluvalinate (resulting from addition of water to the cyano moiety) were available for chromatographic comparison.

The synthesis of 4-amino-3-chlorobenzoic acid (**5**) was performed as follows: haloaniline **3** (100 mg) was heated to 90 °C in a capped, conical vial with 1 M methanolic KOH (1 mL) for 24 h. Isolation of the product was by TLC (three plates 20 × 20 cm silica gel GF, 1 mm thick, R_f = 0.27, hexane–ethyl acetate–acetic acid, 110:110:1, 2% yield), and following methylation (CH_2N_2) the structural assignment of **5** was verified by mass spectrometry: *m*/*z* (rel intensity) 187 (M^+, 13), 185 (M^+, 45), 156 (33), 154 (100), 128 (5), 126 (14), 90 (26).

Soil. Keeton sandy loam soil from Alameda Co., California, was supplied by Sam Keeton Loam and Gravel, San Jose, CA. Clay soil from Cameron Co., Texas, and clay loam soil from Yuma Co., Arizona, were supplied by field

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