abdominal fat as *p*-chloroBTF.

After removal of selected tissues, the rat carcasses were homogenized and extracted with organic solvents. The rat carcass remains contained only about 1% of the applied dose and 95% of the ^{14}C-labeled residue volatilized when the extract was evaporated to dryness. Hence, trace levels of *p*-chloroBTF in fat depots probably represent the major component of ^{14}C-labeled residues in the rat carcass.

The amount of ^{14}C in blood peaked rather quickly (1 h) and then diminished rather slowly. However, the total amount of radiolabel in blood was never very high (maximum 0.05 ppm equiv as 1) which implies that once *p*-chloroBTF is absorbed, it is rapidly transported to the lungs for exhalation since most of the *p*-chloroBTF was recovered from expired gases.

Conclusions. When *p*-chloroBTF is administered orally to rats, about 80% of the applied dose is exhaled. At a 1 mg/kg dose rate, only 16–18% of the applied ^{14}C is excreted in urine and feces. Only about 15% of the applied *p*-chloroBTF is metabolized by rats (Figure 3); the remaining 85% is unaltered *p*-chloroBTF which is either exhaled, excreted in feces, or retained in low amounts in tissues (mostly fat). Male and female rats metabolize *p*-chloroBTF similarly although females have relatively higher ^{14}C-labeled residues in tissues, but for both sexes tissue residues were quite low (Table III). *p*-chloroBTF and its metabolites are rapidly eliminated by rats.

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Fluvalinate Metabolism by Rats

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Within 4 days of receiving a single oral dose of [*trifluoromethyl*-^{14}C]fluvalinate at 1 mg/kg by gavage in corn oil, rats excreted 9–19 and 75–88% of the applied dose in urine and feces, respectively. About 45% of the applied radiolabel was excreted in feces as unmetabolized fluvalinate (**1**), while the major fecal metabolite (11% applied dose) was an anilino acid (**2**) that arose from hydrolysis of the parent ester. This anilino acid formed fecal conjugates with amino acids (glycine, serine, threonine, valine), bile acids (cholic, taurocholic, taurochenodeoxycholic), and glycerol (also oleoyl- and linoleoylglycerol). The amide of **2** was also present (3% of fecal ^{14}C). The conjugations of an acidic xenobiotic with threonine, glycerol, and monoglycerides, as well as conversion to an amide, are to our knowledge novel conversions. The major metabolites in urine were hydroxymethyl-**2** and 2-amino-3-chloro-5-(trifluoromethyl)phenol (as a sulfate conjugate), representing up to 46 and 24% of the urinary ^{14}C, respectively. Pharmacokinetic behavior (blood and bile) and numerous metabolic variables were investigated.

Fluvalinate [1, (*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate] is an insecticide with pyrethroid-like activity against numerous agriculturally important pests. As part of our program to study the environmental fate and toxicological significance of fluvalinate and its metabolites, we now report its metabolism by rats [for the previous report in this series, see Staiger and Quistad (1983)].

EXPERIMENTAL SECTION

Analytical Methods. The procedures for analysis included liquid scintillation counting (LSC), silica gel thin-layer chromatography (TLC), reversed-phase liquid chromatography (LC), combustion to $^{14}CO_2$, and spectral analysis by ^1H nuclear magnetic resonance (NMR) and electron-impact mass spectrometry as described previously (Quistad et al., 1982b). Fast atom bombardment (FAB) mass spectrometry for the sulfate conjugate of **14** used a Hewlett-Packard Model 5985A instrument fitted with a fast atom gun (Phrasor Scientific, Duarte, CA). The sample was dissolved in a glycerol matrix and then bombarded with xenon atoms at 50 μA of total ion current and 8 kV of accelerating voltage. The following solvent systems were used for TLC: hexane–ethyl acetate–acetic acid, 12:9:0.1 (SS 1) and several mixtures of ethyl acetate–hexane (SS 2, 2:1; SS 3, 1:3; SS 4, 1:1; SS 5, 1:4). Reversed-phase LC employed a 10-μm LiChrosorb RP-8 column (25 × 0.46 cm) with the following mixtures of methanol–0.1% acetic acid (all gradients linear): SS 6 (55–75% methanol over 20 min); SS 7 (60–70% methanol over 15 min, then 70–90% over 10 min, hold at 90% for 10 min); SS 8 (65% methanol for 10 min, 65–70% over 5 min, 70–80% over 5 min); SS 9 (55–75% methanol over 30 min); SS 10 (90% methanol); SS 11 (50–90% methanol over 30 min); SS 12 (10–30% methanol over 15 min). A mixture of acetonitrile–0.1% acetic acid (10–30% acetonitrile over 20 min) was used for SS 13.

Synthetic Standards. The preparation of [*trifluoromethyl*-^{14}C]fluvalinate has been reported previously (Quistad et al., 1982b). The [^{14}C]fluvalinate used in this work was a mixture of α*R*,2*R*, α*S*,2*S*, α*R*,2*S*, and α*S*,2*R* isomers (23, 25, 28, and 24%, respectively) with a combined radiochemical purity of 99% and specific activity of 48.3

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mCi/mmol prior to dilution.

Amino acid conjuguates (as methyl esters) of anilino acid **2** were prepared from the acid chloride of **2** and the methyl ester of the respective amino acid. The following general method was used to prepare the *serine conjugate* of **2** (i.e., **3** as its methyl ester): Oxalyl chloride (52 mg, 0.4 mmol) was reacted with anilino acid **2** (100 mg, 0.34 mmol) in ether (5 mL) and dimethyl formamide (1 drop) to give the acid chloride of **2** after stirring magnetically for 2 h. The ethereal solution of acid chloride was decanted into a separate flask where the solvent was evaporated to dryness and replaced with tetrahydrofuran–toluene (1:1, 10 mL). The HCl salt of the methyl ester of serine (50 mg, 0.32 mmol) was dissolved in pyridine (3 mL) and then added via syringe to the solution of acid chloride (initially at 0 °C for 0.5 h and then 1 h at room temperature). After addition of saturated aqueous NaCl solution and acidification, the crude product was extracted into ethyl acetate. The methyl ester of the serine conjugate (**3**) was purified by preparative TLC (SS 2): 11% yield; NMR ($CDCl_3$) δ 1.09 [d, 6, J = 7 Hz, $CH(CH_3)_2$], 2.37 [*m*, 2, $CH(CH_3)_2$ and OH], 3.68 and 3.74 (two s, 3, OCH_3), 3.92 (br s, 3, CH_2OH and NHCHCO), 4.65 (m, 1, $CHCO_2$), 5.0 (br s, 1, NH), 6.63 (d, 1, J = 8 Hz, ar), ca. 7.3 (m, 3, ar and NH); mass spectrum m/z (rel intensity) 396 (3, M^+), 377 (1, M – F), 252 (32), 251 (13), 250 (100), 55 (10).

The glycine conjugate of **2** (as the methyl ester of **4**) was obtained in 35% yield: NMR ($CDCl_3$) δ 1.09 [d, 6, J = 6 Hz, $CH(CH_3)_2$], 2.40 [m, 1, $CH(CH_3)_2$], 3.71 (s, 3, OCH_3), 4.03 (d, 2, J = 6 Hz, CH_2), 5.0 (m, 1, NH), 6.62 (d, 1, J = 8 Hz, ar), 7.00 (t, 1, $CONHCH_2$), ca. 7.4 (m, 2, ar); mass spectrum m/z (rel intensity) 366 (3, M^+ for ^{35}Cl), 347 (1, M – F), 252 (32), 250 (100), 55 (7). A portion of the methyl ester of **4** was saponified to the free acid by treatment with methanolic 0.5 M NaOH (98% yield).

The threonine conjugate of **2** (as the methyl ester of **5**) was obtained in 16% yield: NMR ($CDCl_3$) δ ca. 1.1 [m, 9, $-CCH_3$ and $CH(CH_3)_2$], ca. 2 [br s, 2, OH and $CH(CH_3)_2$], 3.61 and 3.72 (two s, 3, OCH_3), 3.68 (br s, 1, CHCO), ca. 4.4 (m, 2, CHOH and $CHCO_2$), 4.99 (br s, 1, NH), 6.61 (d, 1, J = 7 Hz, ar), 7.00 (br s, 1, NH), ca. 7.4 (m, 2, ar); mass spectrum m/z (rel intensity) 410 (2, M^+ for ^{35}Cl), 252 (31), 251 (12), 250 (100), 55 (10).

The valine conjugate of **2** (as the methyl ester of **6**) was obtained in 21% yield: NMR ($CDCl_3$) δ ca. 0.95 [m, 6, $CH(CH_3)_2$], 1.08 [d, 6, J = 7 Hz, $CH(CH_3)_2$], 2.24 [m, 2, $CH(CH_3)_2$], 3.60 and 3.71 (two s, 3, OCH_3), 3.64 (br s, 1, CHCO), 4.50 (q, 1, J = 4 Hz, $CHCO_2$), 4.97 (br s, 1, NH), ca. 6.7 (m, 2, ar, NH), ca. 7.4 (m, 2, ar); mass spectrum m/z (rel intensity) 410 (1.2, M^+), 408 (4, M^+), 389 (3, M – F), 252 (33), 251 (13), 250 (100), 55 (19).

Amide **7**. The acid chloride of **2** in ether was reacted with gaseous ammonia to give **7** in 89% yield: NMR ($CDCl_3$) δ 1.08 (d, 6, J = 6 Hz, CH_3), 2.30 [m, 1, $CH(CH_3)_2$], 3.69 (t, 1, J = 5 Hz, $HCCO_2$), 5.03 (d, 1, J = 6 Hz, NH), 6.03 (br s, 2, NH_2), 6.64 (d, 1, J = 8 Hz, ar), ca. 7.4 (m, 2, ar); mass spectrum m/z (rel intensity) 294 (9, M^+ for ^{35}Cl), 275 (5, M – F), 252 (32), 250 (100), 206 (18), 55 (19).

Cis and Trans Lactones of Hydroxy Acid **8**. These compounds were prepared by Dr. R. J. Anderson (Chemical Research Department, Zoecon): mass spectrum m/z (rel intensity) cis, 295 (26, M^+), 293 (81, M^+), 234 (87), 214 (100), 206 (64), and trans, 295 (28, M^+), 293 (93, M^+), 234 (93), 214 (100), 235 (63).

Diglycerides Containing **2** (**9** *and* **10**). Monoolein (50 mg, 0.14 mmol, 90% α-isomer, 10% β-isomer, Sigma) was reacted with the acid chloride of **2** (43 mg, 0.13 mmol) in ether (3 mL) and pyridine (34 μL, 0.42 mmol) to give **9** and its 1,3-diglyceride isomer (27 mg, 33% yield). The 1,2-diglyceride (**9**) and 1,3-diglyceride were separable by TLC (SS 5, R_f = 0.18 and 0.21, respectively), but coeluted on LC (SS 10, k' = 5.6). The 1,2-diglyceride (**9**) and its 1,3-isomer showed only minor mass spectral differences: for **9**, m/z (rel intensity) 635 (0.3, M^+), 633 (0.6, M^+), 252 (37), 250 (100), 69 (16), and 55 (21).

In a similar manner, monolinolein was reacted with the acid chloride of **2** to give **10** and monostearin gave the corresponding stearate analogue: for the 1,3-isomer of **10**, mass spectrum m/z (rel intensity) 633 (0.2, M^+), 631 (0.7, M^+), 252 (39), 250 (100), 81 (31), and 67 (31); for the 1,3-isomer of the stearate analogue of **10**, m/z (rel intensity) 637 (0.2, M^+), 635 (0.4, M^+), 252 (35), 250 (100), 129 (18), and 98 (23).

Monoglyceride of **2** (*i.e.*, **11**). Anilino acid **2** (50 mg, 0.17 mmol) was reacted with glycerol (16 mg, 0.17 mmol) in the presence of dicyclohexylcarbodiimide (39 mg, 0.19 mmol) and 4-(dimethylamino)pyridine (3 mg, 0.03 mmol) in CH_2Cl_2 (10 mL) for 3 h at room temperature. The product was purified by TLC (R_f = 0.13 in SS 4): 10% yield; mass spectrum m/z (rel intensity), of bis(trimethylsilyl) ether, 515 (0.6, M^+ for ^{37}Cl), 513 (1.1, M^+ for ^{35}Cl), 494 (0.4, M – F), 410 (0.5), 252 (33), 250 (100).

Synthetic standards of the haloaniline (**12**) and anilino acid (**2**) were available from the Zoecon Chemical Research Department [for the synthesis of **2**, see Henrick et al. (1980)]. The synthesis of 4′-hydroxyfluvalinate (**13**), the taurine conjugate of **2**, and the methylated, peracetylated glucuronide of **2** has been reported (Quistad et al., 1982a,b; Staiger et al., 1982).

Treatment. In general, albino rats (Sprague-Dawley or Fischer 344, Simonsen Laboratories, Gilroy, CA) weighing 130–230 g (6–8 weeks old) were dosed orally by gavage with [*trifluoromethyl*-^{14}C]fluvalinate in corn oil (0.5 mL). Certain rats were bled through the orbital sinus (Riley, 1960) while three others had their bile ducts cannulated for continuous collection of bile for 6 days (Enderlin and Honohan, 1977). For some rats the volume of corn oil was varied (0.08–0.64 mL), and for certain rats the desired dose of [^{14}C]fluvalinate was adsorbed on Purina rat chow (ca. 4 g) for ad libitum consumption by the animals.

Two Sprague-Dawley male rats (7 weeks old) were used to study dermal metabolism. A shaved area on the back (ca. 4 cm^2) was treated with an acetone solution of [^{14}C]-fluvalinate (1 mg/cm^2). One of the rats was kept in a restraining harness which prevented access to the application site by either mouth or feet, thus eliminating oral exposure. The second rat was not restrained.

In a separate study, Sprague-Dawley male rats were given nonradiolabeled ($\alpha RS,2R$)-fluvalinate at 15 mg kg^{-1} day^{-1} for 15 months at International Research and Development Corp. (Mattawan, MI). These animals (plus untreated controls) were shipped to Zoecon and maintained on their previous feeding regimens prior to a single oral dose with [^{14}C]fluvalinate administered at 1 mg/kg by gavage in corn oil (ca. 1.5 mL).

Immediately after being dosed (16 h fast prior to oral dosage), the rats were housed in all-glass metabolism chambers (Stanford Glassblowing Laboratories, Palo Alto, CA) for separate collection of urine, feces, and (for some animals) bile and $^{14}CO_2$. Four days after dosage, the animals were sacrificed with removal of tissues for subsequent analysis.

Urine Analysis. The methodology for TLC and LC analysis of hydroxy acid **8**, anilino acid **2**, glycine conjugate **4**, and haloaniline **12** has been described (Quistad et al.,

1982a). Hydroxy acid 8 was converted by 1 M HCl treatment to a mixture of cis and trans lactones and the mass spectrum of the major trans lactone for the metabolite matched that of a synthetic standard. The mass spectrum of the methyl ester of metabolite 8 also verified its stuctural assignment: m/z (rel intensity) 325 (1, M^+), 267 (55), 252 (45), 208 (88), 206 (100). The glycine conjugate 4 was identified by mass spectral comparison of the metabolite (as its methyl ester) to an authentic standard. The assigned structure of haloaniline 12 was verified by conversion to its heptafluorobutyramide which was analyzed by LC (Staiger and Quistad, 1983).

Hydroxyhaloaniline 14 was present only as conjugates which remained at the origin upon silica gel TLC (SS 1). The TLC origin zone was separated into two fractions of conjugates by LC (SS 11, $k' = 0.5$ for the sulfate and 1.9 for a possible glucuronide). Both of these conjugate fractions were treated with (1) buffer only, (2) buffer plus a mixture of sulfatase with β-glucuronidase (*Helix pomatia*, Sigma), and (3) buffer plus enzymes plus 10 mM D-saccharic acid 1,4-lactone (Quistad et al., 1982a). The hydroxyhaloaniline (14) released by 1 M HCl and enzymatic treatment was analyzed by LC ($k' = 4.9$, SS 6) and TLC ($R_f = 0.56$, SS 1). The structural assignment for 14 is based on its mass spectrum: m/z (rel intensity) 213 (36, M^+), 211 (100, M^+), 194 (7, M – F), 192 (16, M – F), 182 (6, M – CHO), 175 (12, M – HCl), 160 (9, M – CF_2). This phenol was readily converted to its methyl ether by treatment with CH_2N_2: m/z (rel intensity) 227 (19, M^+), 225 (64, M^+), 212 (30, M – CH_3), 210 (100, M – CH_3), 206 (12, M – F), 184 (13), 182 (43), 147 (24). The assignment of the hydroxyl group to the ortho position of the haloaniline rather than one of the meta positions is based on the known fragmentation patterns of isomeric methoxyanilines (Budzkiewicz et al., 1967). *o*-Methoxyanilines fragment principally by loss of a methyl radical and subsequent loss of carbon monoxide, whereas *m*-methoxyanilines show negligible methyl radical loss.

The intact sulfate conjugate of 14 was isolated from the 1-day urine of a rat given 1 at 104 mg/kg. The residual solids obtained by evaporation of the urine to dryness were added to the top of a short silica gel column. An initial ethyl acetate eluate was discarded since the sulfate conjugate eluted with ethyl acetate–methanol (40:60). About 9 μg of the sulfate conjugate of 14 were obtained by successive LC purifications in SS 12 ($k' = 7$) and SS 13 ($k' = 6.3$). FAB mass spectrometry using a glycerol matrix revealed diagnostic ions at m/z 336 and 338 (relative intensity 14.6 and 5.2, respectively) for a sodium ion adduct to the sodium salt of the sulfate conjugate of 14 (M_r 313 for sodium salt, Cl = 35).

Feces Analysis. The feces were extracted with methanol and the residual solids were combusted to $^{14}CO_2$ for quantification of radiolabel. A rather elaborate combination of TLC and LC was essential in order to separate metabolites for identification and accurate quantification. Fluvalinate and anilino acid 2 were determined readily by TLC in SS 1 (or LC in SS 7). The broad TLC zone between 1 and 2 ($R_f = 0.47$–0.76) was separated into 4′-hydroxyfluvalinate (13) and two diglyceride conjugates of 2 (i.e., 9 and 10) by LC in SS 7 ($k' = 16.5$, 23.9, and 22.7, respectively). The TLC zone (SS 1) more polar than 2 ($R_f = 0.0$–0.37) contained several less-abundant components. After methylation (CH_2N_2), the following metabolites (as methyl esters) were separated by TLC and LC: the valine conjugate of 2 (i.e., 6), $R_f = 0.89$ in SS 2, $k' = 10.5$ in SS 8; the glycine conjugate of 2 (i.e., 4), $R_f = 0.15$ in SS 3, $k' = 4.2$ in SS 8; hydroxy acid 8, $R_f = 0.26$ in SS 3, $k' = 4.8$ in SS 8; the threonine conjugate of 2 (i.e., 5), $R_f = 0.38$ in SS 4 (2×), $k' = 13.6$ in SS 9; the serine conjugate of 2 (i.e., 3), $R_f = 0.38$ in SS 4 (2×), $k' = 10.4$ in SS 9; the amide of 2 (i.e., 7), $R_f = 0.38$ in SS 4 (2×), $k' = 9.7$ in SS 9. The monoglyceride of 2 (i.e., 11) was isolated by TLC ($R_f = 0.30$ in SS 2) and LC ($k' = 6.6$ in SS 7) of the methanolic fecal extract. When a rat was dosed at 218 mg/kg, microgram quantities of 3, 4, 5, 6, and 8 (as methyl esters), as well as 7, 9, 10, 11, and 13, were purified by the TLC and LC conditions above for structure confirmation by mass spectral analysis.

Fluvalinate was isolated from several fecal extracts for analysis of individual stereoisomers by normal-phase LC with a Pirkle 1-A column (Staiger and Quistad, 1983). The analysis of bile acid conjugates in feces has been reported (Quistad et al., 1982a,c).

Tissues. Selected tissues (fat, muscle, stomach and intestine, and carcass remains) were extracted with methanol and chloroform and aliquots of the filtrate quantified by LSC. Radioactivity in the residues and remaining tissues was quantified by combustion of aliquots (0.06–0.8 g) to $^{14}CO_2$. Recoveries of radioactivity were determined by combusting samples spiked with ^{14}C. Radioactivity in bile was quantified by LSC.

Blood Plasma. At various intervals, blood was collected from the orbital sinus and, after being cooled in ice, was centrifuged for 10 min. Aliquots (50–90 μL) of the resulting plasma layer were quantified by LSC. The identity of the radiolabel in plasma samples was examined by LC. Aliquots (30–50 μL) of the plasma were added to a 1-mL centrifuge tube and after addition of methanol (1 mL) were centrifuged to removed precipitated solids. Negligible radioactivity was associated with blood solids. The supernatant was evaporated to an appropriate volume for injection on LC (SS 7).

RESULTS AND DISCUSSION

Most of the data reported herein refer to metabolism of [*trifluoromethyl*-^{14}C]fluvalinate (1) by young (ca. 7-week old) Sprague-Dawley rats. The recovery of applied radiolabel was virtually quantitative (Table I). Initial experiments demonstrated that virtually no volatile ^{14}C (<0.02% applied dose) was produced by rats, indicating that the trifluoromethyl moiety is not readily convertible by rats to $^{14}CO_2$. More than half of the applied ^{14}C was excreted within 1 day with progressively lesser amounts thereafter.

Feces. Regardless of the method of oral dosing or dose rate, radiolabel was excreted predominantly in the feces (Table I). Unmetabolized fluvalinate (1) was consistently the most abundant radiolabeled component in feces (Figure 1) and was particularly prevalent in feces during the first day. Thereafter, the free anilino acid 2 became the major fecal metabolite with diminishingly small amounts of fluvalinate. When Sprague-Dawley rats were given a single oral dose of [^{14}C]fluvalinate at 1 mg/kg in corn oil, 45 and 10% of the applied radiolabel were excreted in feces as fluvalinate and anilino acid 2.

A number of less-abundant metabolites were isolated from fecal extracts, but none of these represented more than 5% of the ^{14}C in the extract (Table II). Several miscellaneous minor metabolites were identified, including 4′-hydroxyfluvalinate (13), haloaniline 12, and hydroxy acid 8, but these each contributed only about 1% of the fecal ^{14}C. Most metabolites were conjugates of anilino acid 2 which could be conveniently divided into three classes: *conjugates with amino acids, bile acids, and glycerol.*

One metabolic pathway involved conjugation of 2 with several amino acids (Gly, Ser, Thr, and Val). We also had

Table I. Metabolism of [*trifluoromethyl*-^{14}C]Fluvalinate by Seven-Week-Old Rats

	% applied dose				
dose medium:	corn oil	corn oil	corn oil	rat chow	corn oil
rat strain:	SD[a]	SD	F344[b]	F344	SD
dose rate:	1 mg/kg[c]	1 mg/kg[c]	1 mg/kg[d]	1 mg/kg[c]	218 mg/kg[d]
sex:	male	female	female	female	female
urine	9.4	11.2	18.6	25.5	7.1
feces	88.4	77.0	74.9	66.4	88.6
carcass					
extract	1.1	2.0	2.1	2.4	0.9
residual solids	0.1	0.1	0.4	0.3	0.1
liver	1.2	1.0	1.0	1.4	0.6
selected tissues (including stomach, intestines)	1.0	1.2	1.9	1.7	1.0
total recovery	101	93	99	98	98

[a] Sprague-Dawley. [b] Fischer 344. [c] Mean for two rats. [d] Single rat only.

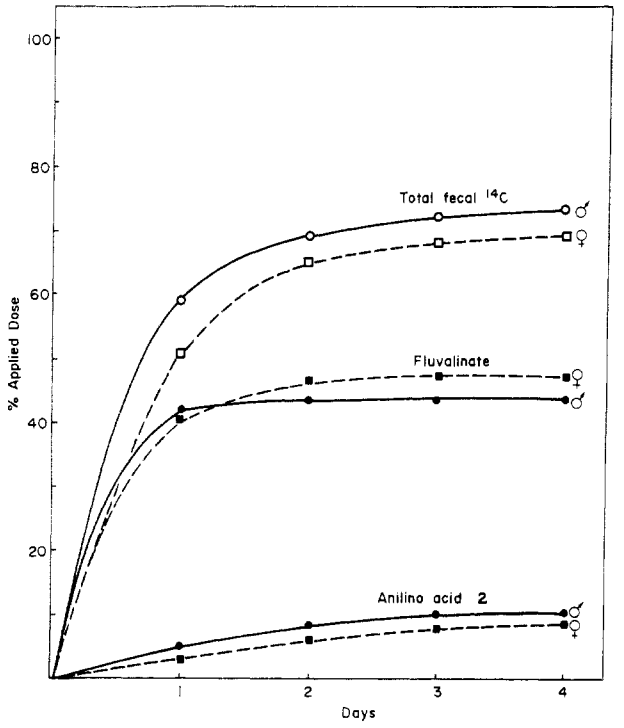

Figure 1. Identity of radiolabel in feces extract from Sprague-Dawley rats dosed orally with [*trifluoromethyl*-^{14}C]fluvalinate (1 mg/kg) in corn oil.

Table II. Quantification of Fecal Metabolites from Sprague-Dawley Rats Given a Single Oral Dose of [*trifluoromethyl*-^{14}C]Fluvalinate in Corn Oil

	% of ^{14}C in extract	
	1 mg/kg, 1-day feces, male (59.1% of applied dose)	218 mg/kg, 2-day feces, female (27.9% of applied dose)
fluvalinate (1)	64.2	68.1
4'-hydroxyfluvalinate (13)	≤0.4	0.07
haloaniline 12	0.8	0.07
anilino acid 2	12.6	7.7
amide of 2 (7)	2.9	0.43
anilino acid conjugate of		
glycine (4)	1.0	1.3
serine (3)	1.0	1.0
threonine (5)	0.6	0.2
valine (6)	0.3	0.01
oleoylglycerol (9)	0.6	0.06
linoleoylglycerol (10)	1.5	0.15
glycerol (11)	1.8	
taurocholate	5.0	
taurochenodeoxycholate	3.2	
cholate	0.5	
hydroxy acid 8		
free	1.0	0.8
unknown conjugates	2.9	1.3
total	101	81

authentic standards for Ala, Glu, and Phe conjugates of 2, but these metabolites were not found. The glycine conjugate of 2 was a major component in both urine and feces; however, in contrast to most xenobiotics, the remaining amino acid conjugates of 2 were found only in feces. The amide of 2 (i.e., 7) was also fairly abundant (3% of fecal ^{14}C). This amide is an unusual metabolite and we know of only one other precedent for amide formation from an acidic xenobiotic (Caldwell, 1982).

We have reported the presence of bile acid conjugates of anilino acid 2 previously (Quistad et al., 1982c). Since taurocholic and taurochenodeoxycholic acids are the major bile acids in rats (Haslewood, 1978), recovery of their conjugates with 2 was expected as 2 appears to conjugate with whatever bile acid predominates in a given species. The route of formation of these bile acid conjugates is unknown. We were unable to demonstrate the presence of these conjugates in bile itself, but since the bile had been stored several months (−18 °C) prior to analysis and the bile acid conjugates containing taurine are technically difficult to analyze (Quistad et al., 1982c), we are somewhat reluctant to draw conclusions. Thus, the apparent absence of the bile acid conjugates of 2 in the bile suggests that they may originate in the digestive tract (as opposed to the liver).

The isolation of glyceride conjugates, as well as other lipophilic derivatives from xenobiotics, is reported with increasing frequency (Hutson, 1982; Schooley and Quistad, 1982). When rats were dosed at 1 mg/kg with fluvalinate in corn oil, 2% of the ^{14}C in the 1-day fecal extract was identified as diglyceride conjugates of the anilino acid 2 with oleic and linoleic acids. The anilino acid was conjugated primarily as a 1,2-diglyceride, but substantial 1,3-diglyceride was also present. Diglyceride conjugates of 2 containing stearic acid were absent. Linoleic, oleic, and stearic acids contribute 34, 50, and 3% of the total fatty acids in corn oil (Altman and Dittmer, 1972). Hence, both the abundance of diglycerides containing 2 plus oleic and linoleic acids and the lack of stearate diglycerides are consistent with the conclusion that the high fat content of corn oil is a prerequisite to formation of these glyceride conjugates of 2. A retrospective analysis of the fecal extract from corn oil dosage revealed that 2% of the ^{14}C-labeled residue was a glycerol conjugate of 2. This is the first report of a monoglyceride conjugate from a xenobiotic to our knowledge.

Table III. Quantification of Urinary Metabolites from Female Rats Given a Single Oral Dose of [*trifluoromethyl*-^{14}C]Fluvalinate in Corn Oil

	% of ^{14}C in urine	
	Fischer (F344),[a] 1 mg/kg, 1-day urine (12.6% of applied dose)	Sprague-Dawley,[b] 218 mg/kg, 2-day urine (3.0% of applied dose)
hydroxy acid **8**	31.6	13.8
anilino acid **2** (free)	6.4	<1
anilino acid **2** (unknown conjugates)	7.8	7.5
glycine conjugate of **2** (**4**)	4.3	10.3
haloaniline **12**	8.1	6.3
sulfate conjugate of hydroxyhaloaniline (**14**)	19.0	21.5

[a] Mean for two rats. [b] Single rat only.

Urine. Fluvalinate was not readily absorbed as evidenced by relatively low amounts of ^{14}C in urine (Table I). 2-Chloro-4-(trifluoromethyl)aniline (**12**) contributed up to 8% of the urinary ^{14}C (Table III). The most abundant primary metabolite in urine was hydroxy acid **8** which represented up to 46% of the urinary ^{14}C and 11% of the applied dose although conjugates of **8** were apparently minor metabolites. When **8** was acidified, it cyclized to cis and trans lactones (1:3 ratio) which were used as part of the structural confirmation. Mild acidification of **8** followed by reaction with CH_2N_2 gave the methyl ester of **8** whose structure was also verified by mass spectrometry.

The anilino acid **2** was a relatively minor urinary product except in Fischer 344 rats dosed with **1** in corn oil where free **2** contributed 12% of the 1-day urinary ^{14}C. Conjugates of **2** represented about 8% of the ^{14}C in urine of this strain, but the glucuronide of **2** was not found. Treatment of urine with β-glucuronidase released only traces of **2**, and methylation of urine followed by acetylation failed to show significant radiolabel coeluting with an authentic standard of the methyl ester of the peracetylated glucuronide of **2**.

About 20% of the urinary ^{14}C consisted of conjugates of 2-amino-3-chloro-5-(trifluoromethyl)phenol (hydroxyhaloaniline **14**). Almost all of the conjugated **14** was present as the sulfate which was efficiently cleaved by a mixture of sulfatase and β-glucuronidase. The conjugates of **14** were cleaved also by these enzymes plus saccharic acid 1,4-lactone [a β-glucuronidase inhibitor (Dutton, 1980)], but they were stable to buffer alone. The sulfate conjugate was isolated by LC and the FAB mass spectrum determined in a glycerol matrix. Strong ions at m/z 336 and 338 corresponded to a sodium ion adduct of the sodium salt of the sulfate. Since the structural assignment for the sulfate of **14** was confirmed by FAB mass spectrometry, saccharic acid 1,4-lactone was indeed ineffective in inhibiting sulfatase as predicted (Dutton, 1980). The glucuronide of **14** was therefore a minor constituent (≤2% of urinary ^{14}C).

Tissues. After 4 days, about 3–4% of the applied dose was unexcreted. The majority of this retained ^{14}C seemed destined for elimination since substantial radiolabel was still being excreted at day 4. The distribution of unexcreted ^{14}C is shown in Table IV. Radiolabel was widely scattered among various tissues, but the liver and the alimentary canal contained the highest residues, again a reflection of pending excretion.

Pharmacokinetics. Radiolabel appeared and disappeared in blood relatively slowly with a maximum at ca. 7 h for 7-week-old rats (Figure 2). Analysis of ^{14}C-labeled residues in blood at 1 and 6 h revealed that anilino acid **2** comprised >86% of the total radiolabel. Fluvalinate contributed only 6 and 0.8% of the total blood ^{14}C at 1 and 6 h, respectively. Hence, it appears that fluvalinate is poorly absorbed and that hydrolysis to anilino acid **2** is the preferred route for uptake of ^{14}C-labeled residues by blood.

Three female rats were cannulated for continuous collection of bile for 4 days. Bile duct cannulation resulted in decreased urinary excretion (3 vs. 9% in unmodified

Table IV. Distribution of ^{14}C-Labeled Residues Four Days Posttreatment in Sprague-Dawley Rats Given Single Oral Doses of [^{14}C]Fluvalinate in Corn Oil

	ppm equiv as fluvalinate				
	7 week old			17-month-old males (1 mg/kg)	
	male[a] (1 mg/kg)	female[a] (1 mg/kg)	female[b] (218 mg/kg)	control[a] (no pretreatment with 1)	pretreated[c] (fed 1 at 15 mg kg^{-1} day^{-1} in diet for 15 months)
liver	0.177	0.243	24.0	0.49	0.16 ± 0.06
lung	0.015	0.040	2.8	0.20	0.06 ± 0.04
spleen	0.006	0.018	3.2	0.062	0.022 ± 0.01
heart	0.009	0.037	2.8	0.070	0.034 ± 0.016
kidney	0.020	0.050	3.5	0.15	0.06 ± 0.03
hide	0.022	0.044	4.5	0.14	0.06 ± 0.02
muscle: pectoral	0.007	0.014	1.2	0.036	0.012 ± 0.004
leg	0.017	0.011	1.0	0.034	0.011 ± 0.004
brain	0.002	0.004	0.2	0.018	0.007 ± 0.005
pancreas	0.017	0.032	4.5	0.11	0.037 ± 0.016
fat: abdominal	0.049	0.077	7.2	0.15	0.10 ± 0.07
epididymal	0.058				
pericardial		0.018	4.3	0.24	0.18 ± 0.08
subcutaneous				0.15	0.08 ± 0.04
stomach and intestines	0.021	0.125	14.4	0.69	0.27 ± 0.05
testes	0.005			0.11	0.027 ± 0.02
vas deferens, seminal vesicles, epididymus				0.076	0.027 ± 0.14
ovaries and fallopian tubes		0.047	3.5		
carcass remains	0.012	0.030	2.8	0.088	0.037 ± 0.014

[a] Mean for two rats. [b] Single rat. [c] Mean ± standard deviation for four rats.

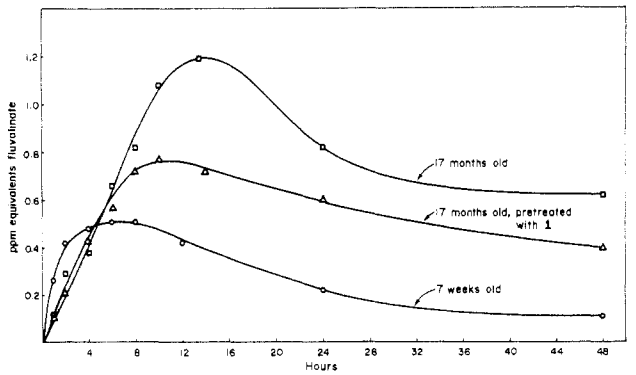

Figure 2. Profile of ^{14}C-labeled residues in blood plasma of rats after a single oral dose of [^{14}C]fluvalinate at 1 mg/kg in corn oil. Pretreated rats received nonradiolabeled ($\alpha RS,2R$)-fluvalinate at 15 mg/kg daily for 15 months.

animals). Since the bile duct cannulated rats do not have bile salts to aid in absorption of lipids in the intestine, the dosing medium can have a significant effect on absorption. Not only was more ^{14}C absorbed in cannulated rats treated with fluvalinate in propylene glycol or on rat chow compared to corn oil (14 vs. 4% of the applied dose) but also levels of radiolabel in the bile maximized sooner (4–8 vs. 14 h). Examination of the bile of the rat dosed with corn oil at its 14-h maximum revealed that anilino acid **2** was the major metabolite (57% of bile ^{14}C) but fluvalinate was absent.

Metabolic Variables. We observed no striking differences between male and female rats. Both the excretion of ^{14}C and the identity of metabolites were similar (Figure 1) as was the distribution of ^{14}C in tissues (Table IV) although females generally retained slightly higher ^{14}C-labeled residues in all tissues. Limited studies with two different rat strains demonstrated somewhat higher urinary excretion with the Fischer 344 strain than with Sprague-Dawley rats. The Fischer 344 strain seemed to both absorb more ^{14}C than Sprague-Dawley rats and also to excrete more anilino acid **2** in urine when given a single oral dose of **1** in corn oil, but the relative amount of the major urinary metabolite, hydroxy acid **8**, was similar for both strains.

The route of administration profoundly affected the metabolism of fluvalinate. When the same dose of **1** was given on rat chow vs. gavage in corn oil, the levels of radioactivity in the blood maximized at 7 h posttreatment for both dosage routes, but ^{14}C-labeled residues appeared sooner and were higher with chow treatment. Analysis of the plasma at the maximum gave a similar product profile for both treatments (85% anilino acid **2** and ca. 1% **1**). Urinary excretion of ^{14}C is higher for chow than for corn oil treatment. Not only is more radiolabel excreted in feces with corn oil treatment but also fluvalinate comprises a higher percentage of the fecal ^{14}C. The levels of ^{14}C-labeled residues in blood are inversely proportional to the amount of corn oil used for dosage. When eight rats were given **1** (4 mg/kg) in 4 different volumes of corn oil (0.4–3.2 mL/kg), the maximum ^{14}C in blood occurred at about the same time (7 h), but more ^{14}C appeared in blood with less corn oil (6.6 vs. 4.2 ppm equiv at 0.4 and 3.2 mL of corn oil/kg, respectively). Usage of corn oil as a dosing vehicle also affects the qualitative distribution of metabolites. With corn oil gavage (but not rat chow) treatment, a new class of glyceride conjugates appears, representing 4% of the applied dose for dosage of **1** at 1 mg/kg in corn oil (2.5 mL/kg). Hence, corn oil seems to protect fluvalinate from degradation during transit through rats; presumably the larger volumes of corn oil are incompletely digested.

Table V. Distribution of Radioactivity from Rats Four Days after Dermal Application of [*trifluoromethyl*-^{14}C]Fluvalinate at 1 mg/cm^2

	% of applied dose	
	restrained	unrestrained
urine	0.7	10.5
feces	0.8	59.7
skin	78.3	2.3
rinse (acetone)	57.9	1.7
extract (methanol)	20.3	0.6
carcass	2.6	10.6
total recovery	82.4	83.1

Table VI. Effect of Age and Pretreatment on Metabolism of [^{14}C]Fluvalinate Given as a Single Oral Dose in Corn Oil at 1 mg/kg to Sprague-Dawley Rats

	% of ^{14}C in sample		
		17 month old	
	7 week old	control (no pretreatment with **1**)	pretreated (fed **1** at 15 mg kg^{-1} day^{-1} in diet for 15 months)
feces (1 day)			
fluvalinate (**1**)	70.0[a]	63.8[a]	69.9 ± 6.9[b]
urine (1 day)			
anilino acid **2**	3.6[a]	0.7[a]	0.9 ± 0.3[b]
hydroxy acid **8**	27.4[a]	42.8[a]	41.5 ± 3.3[b]
plasma (at peak, i.e., 6–14 h)			
anilino acid **2**	>86[c]	70.3[c]	71.0[a]
fluvalinate (**1**)	0.8[c]	1.6[c]	1.9[a]

[a] Mean for two rats. [b] Mean ± standard deviation for four rats. [c] Single rat only.

Little absorption of fluvalinate (<5% of the applied dose) occurs when restrained rats are treated dermally (Table V). The vast majority of the dose remains at the treatment site, primarily as parent **1**. An unrestrained rat can gain access to even the most remote dermal application site and thereby remove **1** from the skin for oral consumption.

Older Sprague-Dawley rats demonstrate higher absorption of ^{14}C-labeled residues into blood than younger rats of the same strain (Figure 2) even though the maximum levels occur later (14 vs. 7 h). For both ages anilino acid **2** is the predominant ^{14}C-labeled component in blood and fluvalinate is minor (Table VI). Older rats tend to eliminate ^{14}C-labeled residues less efficiently than younger ones as evidenced by generally higher tissue residues (Table IV). While 7-week-old rats retained only 3–4% of the applied ^{14}C in the carcass 4 days posttreatment, 17-month-old rats retained 11–12%. Age had little effect on the qualitative and quantitative profile of metabolites in excreta and blood (Table VI).

Rats pretreated with **1** daily for 15 months eliminated fluvalinate more efficiently than unconditioned rats. The lower ^{14}C-labeled residues in blood (Figure 2) and urine (Table VII) of pretreated rats indicated diminished absorption. Accordingly, pretreatment with **1** resulted in lower tissue residues at sacrifice (3–7% of applied dose) than was found for untreated rats (11–12% of applied dose). Although textbook knowledge would suggest that prolonged conditioning with **1** induces enzymes to more rapidly metabolize fluvalinate, this is probably not the case. More fluvalinate is actually excreted intact by pretreated animals (58% of applied dose vs. 47%) although pretreatment has little other effect on the distribution of metabolites (Table VI).

Table VII. Distribution of Radioactivity after Four Days for a Single Oral Dose of [*trifluoromethyl*-^{14}C]Fluvalinate (1 mg/kg) in Corn Oil Given to 17-Month-Old Rats

	% of applied dose	
	control (no pretreatment with 1)	pretreated (fed 1 at 15 mg kg^{-1} day^{-1} in diet for 15 months)
urine	11.7[a]	7.2 ± 0.9[b]
feces	71.5	84.3 ± 3.5
stomach and intestines	3.3	1.5 ± 0.5
liver	1.9	0.7 ± 0.2
carcass	6.6	2.8 ± 1.1
total recovery	94.8	96.4 ± 3.6

[a] Mean for two rats. [b] Mean ± standard deviation for four rats.

Since the (*αRS*,2*RS*)-fluvalinate used in this work was a mixture of four isomers in roughly equal amounts, we analyzed for the preferential metabolism of individual isomers. Fluvalinate was reisolated from the 1-day feces of young rats (7 weeks old) and older rats (17 months old, with and without a 15-month pretreatment with 1). Assay of stereoisomers with a Pirkle Type 1-A column revealed no significant changes in the ratios of isomers after metabolic exposure, suggesting little stereospecificity in metabolism of isomers.

Comparison with Related Pyrethroids. In this work only the acid portion of fluvalinate was radiolabeled, but there is already ample literature precedent for the destiny of the alcohol moiety of the ester (Hutson, 1979; Casida and Ruzo, 1980). Several pyrethroids liberate the same cyanohydrin upon hydrolysis as does fluvalinate. The fate of this cyanohydrin in rats has been reported for fenvalerate (Ohkawa et al., 1979; Kaneko et al., 1981b), cypermethrin (Cole et al., 1982; Crawford et al., 1981), fenpropanate (Crawford and Hutson, 1977), deltamethrin (Ruzo et al., 1978; Cole et al., 1982), tralomethrin (Cole et al., 1982), and tralocythrin (Cole et al., 1982). The degradation of the major metabolic fragment, 3-phenoxybenzoic acid, by rats has been studied also (Huckle et al., 1981a–c; Crayford and Hutson, 1980a,b).

Rat metabolism of fluvalinate (Figure 3) was generally similar to that of other pyrethroids. As with fenvalerate (Kaneko et al., 1981b; Ohkawa et al., 1979), rats show no selective metabolism of individual stereoisomers. Male and female rats metabolize fluvalinate in the same manner. No sex-related differences were observed as in previous work with fenvalerate (Kaneko et al., 1981b) and fenpropanate (Crawford and Hutson, 1977). Dermally applied fluvalinate is poorly absorbed as noted with phenothrin (Kaneko et al., 1981a). Pretreated rats eliminate residues more efficiently as with previous reports using fenvalerate (Kaneko et al., 1981b).

When the rat metabolism of fluvalinate is compared to that of related pyrethroids, several differences are noteworthy. The ratio of fecal to urinary excretion is higher for fluvalinate than for fenvalerate (Ohkawa et al., 1979; Kaneko et al., 1981b), deltamethrin (Ruzo et al., 1978; Cole et al., 1982), fenpropanate (Crawford and Hutson, 1977), cypermethrin (Cole et al., 1982), tralomethrin (Cole et al., 1982), and tralocythrin (Cole et al., 1982). More importantly, more fluvalinate is excreted intact than for any of these other pyrethroids (45 vs. <30%). Hydroxylation at the 4′-position of the alcohol portion of other intact pyrethroids is an important metabolic route for fecal products, but with fluvalinate 4′-hydroxylation (as well as 2′-hydroxylation) appeared to be minor. With other pyrethroids, the acid arising from hydrolysis of the ester linkage was excreted as a conjugate of glucuronic acid. Such a glucuronide was relatively minor for the anilino acid 2 from fluvalinate. In this work we found several mammalian metabolic pathways for fluvalinate which have not been reported for other pyrethroids: (1) formation of an amide (i.e., 7) from a xenobiotic acid, (2) mono- and diglyceride conjugates in feces, (3) new amino acid conjugates (serine, valine, and threonine), and (4) bile acid conjugates. Moreover, to our knowledge the metabolic conjugation of a xenobiotic acid to threonine and glycerol has not been reported previously.

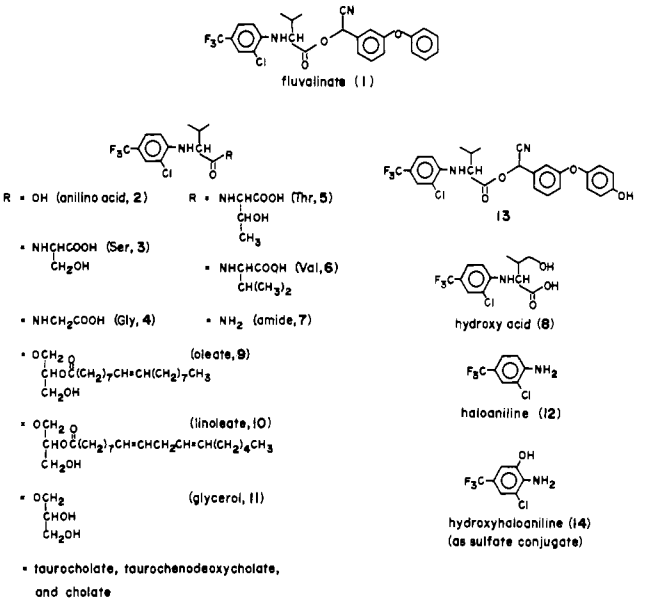

Figure 3. Rat metabolites of [*trifluoromethyl*-^{14}C]fluvalinate.

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Registry No. 1, 69409-94-5; 2, 76338-73-3; 2 (acid chloride), 76822-00-9; 2 (taurocholate), 82186-90-1; 2 (taurochenodeoxycholate), 82186-91-2; 2 (cholate), 82186-87-6; 3, 84960-05-4; 3 (methyl ester), 84960-12-3; 4, 82186-83-2; 4 (methyl ester), 84960-13-4; 5, 84960-06-5; 5 (methyl ester), 84960-14-5; 6, 84960-07-6; 6 (methyl ester), 84960-15-6; 7, 84960-17-8; 8, 85026-71-7; 9, 84960-08-7; 9 (1,3-diglyceride isomer), 84960-16-7; 10, 84986-80-1; 11, 84960-09-8; 12, 39885-50-2; 13, 82186-78-5; 14·0.5H_2SO_4, 84960-11-2; serine methyl ester, 2788-84-3; monoolein (α-isomer), 111-03-5; monoolein (β-isomer), 3443-84-3; monolinolein, 26545-74-4; monostearin, 31566-31-1; glycerol, 56-81-5.

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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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