

Metabolism of [*benzyl-U-ring*-¹⁴C]Fluvalinate by Rats

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When male and female rats were given a single oral dose of [*benzyl-U-ring*-¹⁴C]fluvalinate at 0.7 and 60 mg/kg, >95% of the applied dose was excreted within 4 days. Radiolabeled residues in urine (43-56% of the applied dose) were identified as 3-phenoxybenzoic acid (free and glycine conjugate) and 3-(4-hydroxyphenoxy)benzoic acid (free and sulfate conjugate). Rats excreted 39-56% of the applied dose in the feces, with unmetabolized fluvalinate as the major fecal residue. Also identified in feces were 3-phenoxybenzoic acid, 3-phenoxybenzyl alcohol, and 3-(4-hydroxyphenoxy)benzoic acid. Radioactivity in plasma peaked at 2-4 h posttreatment, and after 4 days, residues remaining in the carcass were low, ≤2% of the applied dose. The alcohol moiety from [*benzyl*-¹⁴C]fluvalinate is metabolized in a manner generally analogous to that of other cyanopyrethroids.

The fate of fluvalinate [α -cyano-3-phenoxybenzyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate, Mavrik insecticide] labeled with ¹⁴C in the CF₃ moiety, has been studied extensively in rats (Quistad et al., 1983), as well as plants (Quistad et al., 1982b), chickens (Staiger et al., 1982), rhesus monkeys (Quistad and Selim, 1983), and a cow (Quistad et al., 1982a). In order to investigate the fate of the alcohol moiety, we prepared fluvalinate labeled with carbon-14 in the benzyl ring and now report its metabolism in rats. In addition, we compare the metabolic fate of fluvalinate in rats to that of other cyanopyrethroids.

EXPERIMENTAL SECTION

Analytical Methods. Liquid scintillation counting (LSC) was performed with a Packard 460C spectrometer. Silica gel plates (5 × 20 cm, 0.1 cm thick, Analtech) were used for thin-layer chromatography (TLC), and radioactive zones were located by using a Packard Model 7201 radiochromatogram scanner.

Reversed-phase liquid chromatography (LC) was performed with a Spectra Physics 8000A liquid chromatograph (LiChrosorb RP-8 column, 10 μm, 25 × 0.46 cm, Brownlee Laboratories; elution at 1.6 mL/min and 35 °C; SP Model 8310 ultraviolet detector, 254 nm). Solvent systems used for TLC development and LC elution are listed in Table I.

Feces and tissues were extracted with a Polytron homogenizer (Brinkmann). Quantification of radiolabeled metabolites in urine and feces extracts was achieved with gradient elution LC by coinjection of a known amount of extract together with authentic metabolite standards and collection of timed fractions for subsequent assay by LSC. Radioactivity in tissues and residual solids was quantified by combustion of aliquots to ¹⁴CO₂ (Harvey OX-300 biological material oxidizer) with collection in Carbon-14 Cocktail (Harvey) followed by LSC.

Fast atom bombardment (FAB) mass spectrometry was performed with 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.

Synthetic Standards. Wizard Laboratories (Davis, CA) provided [*benzyl-U-ring*-¹⁴C]-3-phenoxybenzaldehyde (60.0 mCi/mmol). The 3-phenoxybenzaldehyde (4.7 mCi, 0.08 mmol) was converted to its cyanohydrin by stirring 4 h at room temperature with sodium cyanide (0.08 mmol) and aqueous sodium bisulfite (0.08 mmol). The crude

cyanohydrin was reacted with (2*R*)-anilino acid (2, 0.09 mmol, 99% *R*) in the presence of *N,N'*-dicyclohexylcarbodiimide (0.09 mmol), 4-(dimethylamino)pyridine (0.01 mmol), and dichloromethane (1 mL). The resultant (α *RS*,2*R*)-[*benzyl-U-ring*-¹⁴C]fluvalinate (1) was purified by normal-phase liquid chromatography: a Lobar LiChroprep SI 60 column (size B, E. Merck) was eluted with 10% ether-pentane at 7 mL/min with ultraviolet detection at 254 nm. Analysis by LC gave a radiochemical purity of 98.9%; the specific activity of 1 was nominally 60 mCi/mmol prior to dilution with carrier.

Synthetic standards of 3-phenoxybenzoic acid (PBacid, 3) and 3-phenoxybenzyl alcohol (PBalc, 4) were purchased from commercial sources. The methyl ester of 3 was derived from treatment of the acid with diazomethane.

The syntheses of 3-(4-hydroxyphenoxy)benzoic acid (4'-OH-PBacid, 5), 3-(4-methoxyphenoxy)benzoic acid, and methyl 3-(4-methoxyphenoxy)benzoate, as well as the glycine conjugate of 3 (PBacid Gly, 6), have been reported (Quistad et al., 1983; Unai and Casida, 1977).

The methylated, peracetylated glucuronide conjugate of 3 (i.e., derivatized 7) was synthesized from 3 (54 mg, 0.25 mmol) and methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate (84 mg, 0.25 mmol) in the presence of dicyclohexylcarbodiimide (51 mg, 0.25 mmol) and 4-(dimethylamino)pyridine (2 mg, 0.02 mmol) in CH₂Cl₂ (2 mL). The product was purified by TLC (ether-hexane, 3:1, *R_f* = 0.38, 48% yield).

Treatment and Balance. Two male and four female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA; 178-203 g) were given single oral doses (0.7 and 60 mg/kg) of [*benzyl*-¹⁴C]fluvalinate by gavage in corn oil (0.5 mL) and maintained in all-glass metabolism chambers (Stanford Glassblowing Laboratories) with total collection of urine and feces. Animals had been fasted for 16 h prior to dosing and then were provided ground chow (S/L Custom Lab Diet G4.5, Simonsen Laboratories) and water ad libitum for 4 days prior to sacrifice with ether. Tissues were removed and frozen for subsequent analysis. The residual carcasses were finely minced and then frozen.

Two additional female rats were dosed with fluvalinate in a similar manner and housed in plastic cages. Blood was collected at intervals from the orbital sinus (Riley, 1960).

Analysis of Urine. Radioactivity in aliquots of urine (25-200 μL) was quantified by LSC, and metabolites were analyzed by checking for coelution of radiolabel with standards on LC (SS 6). PBacid and 4'-OH-PBacid were isolated by TLC (SS 1) and following methylation were shown to be coincident with authentic standards of their respective methyl esters on TLC and LC analysis (for PBacid-Me, SS 3 and SS 8; for 4'-OH-PBacid-Me and 4'-CH₃O-PBacid-Me, SS 2 and SS 8). Similar isolation of

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Table I. Thin-Layer and Liquid Chromatography (TLC and LC) of Fluvalinate and Its Metabolites

	R_f (TLC)				k' (LC)				
	SS 1	SS 2	SS 3	SS 4	SS 5	SS 6	SS 7	SS 8	SS 9
fluvalinate (1)	0.74				14.7	17.4	5.8		
PBacid (3)	0.29				5.2	12.0			5.8
PBacid-Me ^a		0.52	0.38					4.0	
4'-OH-PBacid (5)	0.24				2.4	9.9			2.9
4'-OH-PBacid-Me		0.19			8.2			2.2	
4'-MeO-PBacid-Me		0.41						4.0	
PBalc (4)					3.4	10.7			
PBacid Gly (6)				0.04	2.7	9.4			
PBacid Gly-Me				0.34	3.8				

SS 1: hexane-ethyl acetate-acetic acid, 12:9:0.1
 SS 2: hexane-ethyl acetate, 4:1
 SS 3: hexane-ethyl acetate, 10:1
 SS 4: hexane-ethyl acetate-acetic acid, 10:10:0.1

LC solvent systems all utilized methanol-0.1% aqueous trifluoroacetic acid as follows (all gradients linear):
 SS 5: 50-60% methanol over 15 min, 60-90% over 15 min
 SS 6: 20-80% methanol over 30 min, 80-90% over 10 min
 SS 7: 70-90% methanol over 20 min
 SS 8: 60-90% methanol over 30 min
 SS 9: 50-90% methanol over 45 min
 SS 10: 35-65% methanol over 60 min

^a Abbreviations are as follows: methyl 3-phenoxybenzoate (PBacid-Me); methyl 3-(4-hydroxyphenoxy)benzoate (4'-OH-PBacid-Me); methyl 3-(4-methoxyphenoxy)benzoate (4'-MeO-PBacid-Me); glycine (Gly).

PBacid Gly (6) by TLC (SS 4) and subsequent methylation also showed coincidence of radioactivity with an authentic standard of the methyl ester of 6 by TLC (SS 4) and LC (SS 5) analysis.

Polar radioactivity remaining at the origin on TLC (SS 1) was eluted from the silica gel with methanol and then dissolved in buffer (citrate-phosphate, pH 4.5, 5 mL); aliquots were treated with enzymes as follows: (1) buffer only; (2) buffer plus sulfatase (*Helix pomatia*, Sigma, 5 mg); (3) buffer plus β -glucuronidase (*H. pomatia*, Sigma, 5 mg); (4) buffer plus enzymes plus D-saccharic acid 1,4-lactone (10 mg). The 4'-OH-PBacid released by enzyme treatment and extracted into ether was shown to be coincident with an authentic standard on LC (SS 9). The intact sulfate conjugate of 5 (i.e., 8) was isolated from the urine of a female rat treated at 60 mg/kg. An aliquot of the urine was lyophilized, and the residual solids were adsorbed onto the top of a silica gel column (2.5 \times 7 cm) that was eluted with ethyl acetate (100 mL) followed by ethyl acetate-methanol (60:40, 100 mL). This latter fraction was treated with diazomethane, and following TLC separation (SS 1), the methyl ester of 8 (6 μ g, carboxyl group methylated) from the origin zone was purified by LC (SS 6, k' = 9.4, followed by SS 10, k' = 5.6). The structural assignment for 8 was confirmed by its FAB mass spectrum (as its methyl ester): m/z (rel intensity) 315 (81, sodium salt of methyl ester of 8 minus OCH₃), 245 (70, protonated methyl ester of 8 minus SO₃), 244 (100, methyl ester of 8 minus SO₃), 213 (46, methyl ester of 8 minus SO₃ and OCH₃), 191 (38), 173 (32), 155 (40).

Analysis of Feces. Feces were extracted with methanol and aliquots of the extracts were quantified by LSC. Residual solids were combusted to ¹⁴CO₂ for quantification of unextractable radioactivity. Fluvalinate, PBacid, PBalc, and 4'-OH-PBacid were identified by coincidence of radioactivity with that of authentic standards on LC (SS 5).

Tissues. ¹⁴C Residues in tissues and carcasses were quantified by combustion of aliquots (0.05-0.4 g) to ¹⁴CO₂ and subsequent LSC. An aliquot (1 g) of abdominal fat was extracted with acetonitrile and methanol for analysis by TLC (SS 1) and LC (SS 7).

Blood Plasma. At various intervals, blood was collected from the orbital sinus and centrifuged for 10 min. Aliquots

Table II. Balance of Radioactivity from Sprague-Dawley Rats Given a Single Oral Dose of [*benzyl-U-ring-¹⁴C]Fluvalinate and Maintained for Four Days*

	% applied dose			
	dose: sex:	0.7 mg/kg ^a female	60 mg/kg ^a female	0.7 mg/kg ^a male
urine		48.2	42.7	56.0
feces		49.5	55.9	39.4
stomach and intestines		0.2	0.3	0.1
carcass		2.0	2.0	1.4
total recovery		99.9	100.9	96.9

^a Mean for two rats.

of the resulting plasma layer (20-50 μ L) were quantified by LSC. The identity of radioactivity in the plasma was examined by LC. Aliquots (20-25 μ L) of the plasma were added to a 3-mL centrifuge tube and after addition of methanol (100 μ L) were centrifuged to remove precipitated solids. The supernatant was evaporated to an appropriate volume for injection on LC (SS 6).

RESULTS AND DISCUSSION

As shown in Table II, the recovery of radioactivity for all rats was essentially quantitative. Radioactivity was excreted efficiently in urine and feces in approximately equal amounts, primarily within 1 day. The females dosed at 60 mg/kg exhibited signs of fluvalinate toxicity (e.g., salivation and depressed activity) for ~24 h and did not feed during that time. As a result, excretion of radioactivity was delayed. This toxicity did not have a noticeable effect on the total excretion or metabolism of fluvalinate. Male rats showed slightly higher urinary excretion than female rats; however, the identity of metabolites in urine and feces of both sexes was very similar. Radiolabeled residues in tissues and carcasses were small for all rats, representing \leq 2% of the applied dose. A summary of the metabolic products from [*benzyl-U-ring-¹⁴C]fluvalinate is shown in Figure 1.*

Urinary Metabolites. Products identified from urine are listed in Table III. The most abundant urinary metabolite in all animals was the sulfate conjugate of 4'-OH-PBacid 8, representing 63-72% of urinary ¹⁴C (0-1 day).

Table III. Products in Urine from Rats Given a Single Oral Dose of [benzyl-U-ring-¹⁴C]Fluvalinate

	% of urinary ¹⁴ C			
	female, 0.7 mg/kg	female, 60 mg/kg		male, 0.7 mg/kg,
	1-day urine (43.5% of applied dose)	1-day urine (16.1% of applied dose)	2-day urine (32.5% of applied dose)	1-day urine (57.0% of applied dose)
PBacid (3)	10.5	8.6	4.1	3.9
4'-OH-PBacid (5)	12.5	3.0	2.7	2.3
PBacid Gly (6)	2.1	9.9	15.9	9.0
4'-OH-PBacid sulfate (8)	63.3	70.4	58.4	72.0

Table IV. Products in 1-Day Fecal Extracts from Rats Given a Single Oral Dose of [benzyl-U-ring-¹⁴C]Fluvalinate

	% of ¹⁴ C in feces extract		
	female, 0.7 mg/kg (42.2% of applied dose)	female, 60 mg/kg (31.6% of applied dose)	male, 0.7 mg/kg (33.4% of applied dose)
	fluvalinate (1)	74.9	83.2
PBacid (3)	1.5	7.8	3.1
PBalc (4)	2.9	3.8	4.0
4'-OH-PBacid (5)	3.7	1.4	1.7

Table V. Balance of Radioactivity from Rats Given a Single Oral Dose of Various ¹⁴C-Labeled Pyrethroids

compd:	fluvalinate	fluvalinate	cypermethrin ^a	tralomethrin ^a	deltamethrin ^a	fenvalerate ^b
label:	[¹⁴ C]benzyl	[¹⁴ C]benzyl	[¹⁴ C]benzyl	[¹⁴ C]benzyl	[¹⁴ C]benzyl	[α- ¹⁴ C]benzyl
dose:	4 days, female 0.7 mg/kg	4 days, male 0.7 mg/kg	7 days, male 0.12 mg/kg	7 days, male 0.32 mg/kg	7 days, male 0.32 mg/kg	2 days, male 8 mg/kg
urine	48.2	56.0	62.9	42.6	65.2	63.9
feces						
extracts	44.8	36.9	29.9	47.4	29.5	28.3
residues	4.6	2.5	3.9	8.6	3.1	3.4
carcass + tissues	2.1	1.6	3.3	1.4	2.2	4.4
total recovery	100	97	100	100	100	100

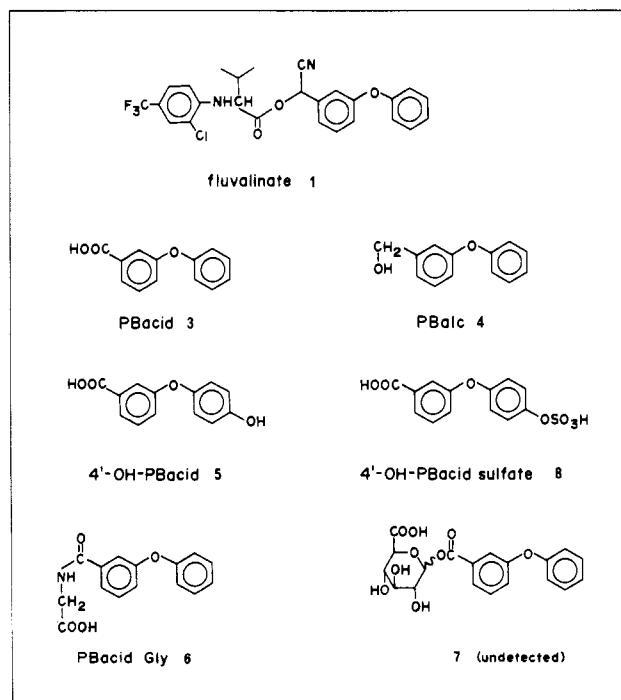
^a Cole et al. (1982). ^b Ohkawa et al. (1979).

This metabolite gave quantitative cleavage to 4'-OH-PBacid (5) upon treatment with sulfatase, as well as with sulfatase plus a glucuronidase inhibitor (D-saccharic acid 1,4-lactone). Treatment of the polar TLC origin with β-glucuronidase did release extractable ¹⁴C; however, this was probably due to known sulfatase activity in the commercially available β-glucuronidase. Methylation of the polar radioactivity followed by acetylation failed to show significant amounts of product coeluting with an authentic standard of the derivatized glucuronide of 3 (i.e., methylated, peracetylated 7). PBacid (3), 4'-OH-PBacid (5), and PBacid Gly (6) were also significant urinary metabolites, representing up to 11, 13, and 16% of the urinary ¹⁴C, respectively (Table III).

Fecal Metabolites. All fecal metabolites were quantified by LSC of radioactivity coeluting with authentic standards on LC. The major product in the feces of all animals was fluvalinate (1, 75–83% of the ¹⁴C in 1-day feces). As shown in Table IV, other metabolites present in small amounts included PBacid (3), PBalc (4), and 4'-OH-PBacid (5). Unextractable fecal radioactivity represented ≤5% of the applied dose.

Although bile acid conjugates of the acid moiety of fluvalinate were found in feces of rats dosed with [trifluoromethyl-¹⁴C]fluvalinate (Quistad et al., 1983), there was no indication of the corresponding conjugates of PBacid from the alcohol moiety. We synthesized conjugates of PBacid with cholic, taurocholic, and taurochenodeoxycholic acids and specifically looked for these adducts as metabolites in feces but failed to detect these bile acid conjugates (<0.5% of fecal ¹⁴C).

Tissues. Only 1–2% of the applied dose remained in the carcass 4 days posttreatment. Radiolabeled residues in all tissues were low, with abdominal fat and hide showing the highest concentrations (0.1 and 0.03 ppm, respectively, for 0.7 mg/kg dose). Analysis of radioactivity in fat revealed fluvalinate (1) to be the major component

Figure 1. Rat metabolites of [benzyl-U-ring-¹⁴C]fluvalinate.

(≥69% of fat ¹⁴C). As expected, an increase in the dose rate resulted in a concomitant and proportional increase in tissue residues.

Pharmacokinetics. The peak of radioactivity in blood plasma from females dosed at 0.7 mg/kg occurred 2–4 h posttreatment. This is sooner than that for rats dosed with [trifluoromethyl-¹⁴C]fluvalinate where the peak occurred at ~7 h posttreatment. Analysis of the peak ¹⁴C residues by LC revealed the major component to be PBacid (3) (68–71% of plasma ¹⁴C). Also present were fluvalinate

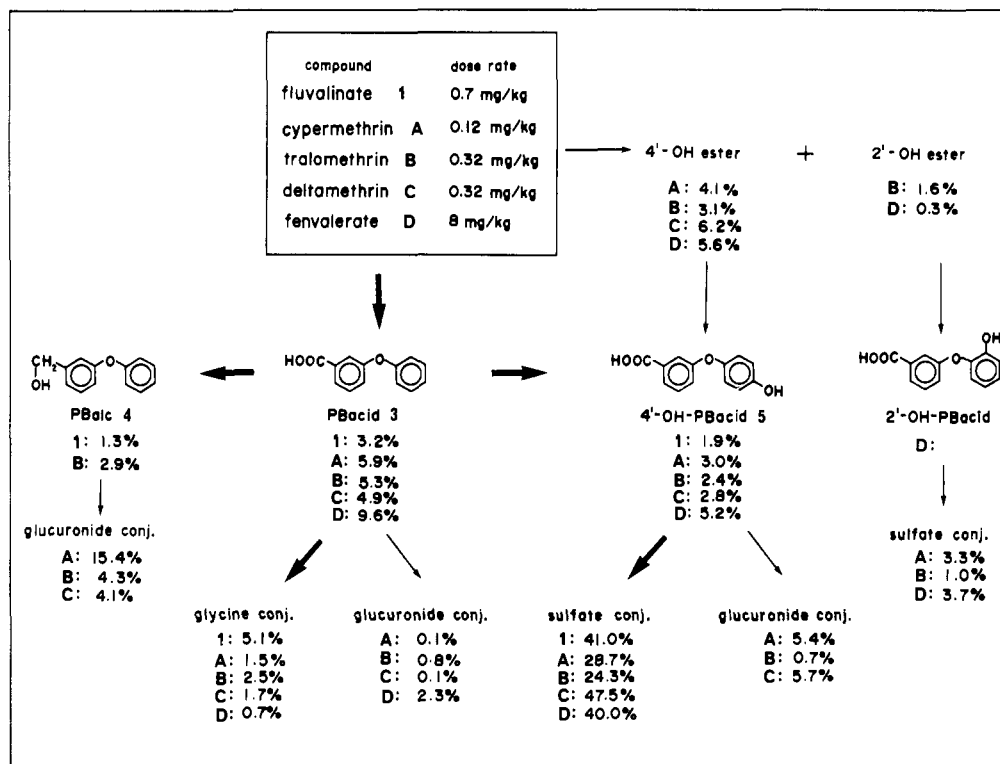


Figure 2. The metabolism of [benzyl-¹⁴C]fluvalinate and other cyanopyrethroids by male rats. Numerical values represent percent of applied dose in urine and feces combined.

(4–12%), 4'-OH-PBacid (5) (0.1–2%), and 4'-OH-PBacid sulfate (8) (13–15% of plasma ¹⁴C).

Comparison with Other Pyrethroids. A number of rat metabolism studies of other pyrethroids containing a cyano moiety and labeled with carbon-14 in the alcohol portion of the molecule (benzyl or α -carbon) have been reported. As shown in Table V, fluvalinate appears to have an excretion profile similar to that of these compounds. In addition, the metabolite profile also appears similar (Figure 2). As with fluvalinate, the sulfate conjugate of 4'-OH-PBacid is the major urinary metabolite for fenvalerate (Ohkawa et al., 1979), deltamethrin (Cole et al., 1982), and cypermethrin (Cole et al., 1982). The major ¹⁴C residue in the feces of rats treated with these pyrethroids is the parent compound. Also present in small amounts in the feces are PBacid and 4'-OH-PBacid; however, one difference is the presence of a 4'-hydroxylated parent ester in the case of other pyrethroids, which was not found with fluvalinate. Following treatment with fenvalerate, deltamethrin, and cypermethrin, 4–6% of the applied dose was excreted as the 4'-hydroxylated parent ester (Ohkawa et al., 1979; Cole et al., 1982). No 4'-hydroxyfluvalinate was found in this study nor was it a major product when rats were treated with [trifluoromethyl-¹⁴C]fluvalinate (Quistad et al., 1983). Since 4'-OH-PBacid (as the free acid and sulfate conjugate) is the major product of [benzyl-¹⁴C]fluvalinate this would imply the likelihood of ester cleavage prior to hydroxylation of the phenoxy ring.

The similarity of the metabolism of [benzyl-U-ring-¹⁴C]fluvalinate to that of other cyanopyrethroids is also evident in the amount and distribution of radioactivity in tissues. As shown in Table V, only 1–4% of the applied dose remained in the carcass at sacrifice for all of the compounds listed. Although residues in individual tissues are low for all compounds, hide (or skin and hair) and fat exhibit the highest residues. The major ¹⁴C-labeled residue in fat from rats dosed with fenvalerate was the parent ester (Ohkawa et al., 1979), a result consistent with fluvalinate in this study.

The occurrence of residues in hide is noteworthy due to the identification of ¹⁴C-labeled triglycerides in the skin of rats dosed with 3-phenoxy[¹⁴C]benzoic acid (Crayford and Hutson, 1980). When rats were given a single oral dose of 3-phenoxybenzoic acid at 0.76 mg/kg, 1–3% of the applied dose was found in the skin after 4 days, and when female rats were dosed with 3-phenoxybenzoic acid at 100 mg/kg for 7 days, (3-phenoxybenzoyl)dipalmitin was isolated from the skin and identified by mass spectrometry (Crayford and Hutson, 1980). No attempt was made to identify the ¹⁴C-labeled residues in hide in the present study; however, it is possible that a portion of these residues could be 3-phenoxybenzoic acid incorporated into triglycerides.

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