Table XII. Average Bioconcentration Factors for Fluridone, Its Metabolite (II), and the Total Residue in Ten Fish Species from Field Trials

	av BCF[a]		
chemical residue	edible	inedible	whole
fluridone	1.20	3.14	3.01
metabolite (II)	0.23	4.16	3.07
total	1.33	7.38	6.08

[a] Average bioconcentration factor determined from 175 samples.

species collected from the field trials is summarized in Table XI. The BCF was very low in all fish species, indicating that large residues will not accumulate in fish as a result of SONAR applications.

The average BCF for fluridone, the metabolite, and the total residue in all fish species combined is summarized in Table XII. The average BCFs for the total residue were 1.33, 7.38, and 6.08 in edible, inedible, and whole fish tissues, respectively. The edible tissue bioconcentrated fluridone 5.2 times greater than the metabolite. However, the BCF for the inedible tissue was 1.3 times higher for the metabolite, and the BCF for whole fish was nearly identical for both compounds.

The observed BCFs for fluridone correlate well with its low *n*-octanol/water partition coefficient. The log K_{ow} for fluridone was determined to be 1.87 (Lilly Research Laboratories, 1981) by using established procedures (Environmental Protection Agency, 1975). An equation relating BCFs of chemicals in whole fish to partition coefficients (Veith et al., 1979) predicts a BCF of 7.8 for fluridone. Thus, applications of SONAR to lakes and ponds would not be expected to result in a large buildup of residues in the aquatic food chain.

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LITERATURE CITED

Arnold, W. R. *J. Aquat. Plant Manage.* **1979**, *17*, 30.
Environmental Protection Agency *Fed. Regist.* **1975**, *40*, 26880.
Grant, D. L.; Warner, L. C.; Arnold, W. R.; West, S. D. *Proc., South. Weed Sci. Soc.* **1979**, *32*, 293.
Kenaga, E. E. In "Environmental Toxicology of Pesticides"; Matsummura, F.; Bousch, M. G.; Misato, T., Eds.; Academic Press: New York, 1972.
Lilly Research Laboratories "SONAR Technical Manual"; Lilly Research Laboratories: Greenfield, IN, 1981.
Marquis, L. Y.; Comes, R. D.; Yang, C. P. *Pestic. Biochem. Physiol.* **1982**, *17*, 68.
McCowen, M. C.; Young, C. L.; West, S. D.; Parka, S. J.; Arnold, W. R. *J. Aquat. Plant Manage.* **1979**, *17*, 27.
Muir, D. C. G.; Grift, N. P. *J. Agric. Food Chem.* **1982**, *30*, 238.
Muir, D. C. G.; Grift, N. P.; Blouw, A. P.; Lockhart, W. L. *J. Environ. Qual.* **1980**, *9*, 151.
Parka, S. J.; Arnold, W. R.; McCowen, M. C.; Young, C. L. *Proc. EWRS Symp. Aquat. Weeds, 5th* **1978**.
Rainey, D. P.; Berard, D. F., Lilly Research Laboratories, personal communication, 1979.
Rivera, C. M.; West, S. D.; Perez, J. *Proc. Annu. West. Soc. Weed Sci., 32nd* **1979**, *32*, 67.
Sanders, D. R.; Theriot, R. F.; Arnold, W. R.; West, S. D. U.S. Army Engineer Waterways Experiment Station Technical Report A-79-3, 1979.
Saunders, D. G.; Mosier, J. W. *J. Agric. Food Chem.* **1983**, *31*, 237.
Veith, G. D.; DeFoe, D. L.; Bergstedt, B. V. *J. Fish. Res. Board Can.* **1979**, *36*, 1040.
West, S. D. *J. Agric. Food Chem.* **1978**, *26*, 664.
West, S. D., submitted for publication in *Anal. Methods Pestic. Plant Growth Regul.*, 1983.
West, S. D.; Burger, R. O. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 1304.
West, S. D.; Day, E. W.; Burger, R. O. *J. Agric. Food Chem.* **1979**, *27*, 1066.
West, S. D.; Parka, S. J. *J. Agric. Food Chem.* **1981**, *29*, 223.

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Metabolism of *p*-Chlorobenzotrifluoride by Rats

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When rats were given a single oral dose of *p*-chloro[*trifluoromethyl*-^{14}C]benzotrifluoride at 1 mg/kg, 3–4 and 14–15% of the applied ^{14}C were excreted in feces and urine, respectively. The major urinary metabolites were glucuronides of dihydroxybenzotrifluoride and 4-chloro-3-hydroxybenzotrifluoride (each representing 3–4% of the applied ^{14}C), as well as minor amounts of a mercapturic acid conjugate of *p*-chlorobenzotrifluoride. *p*-Chlorobenzotrifluoride itself was rapidly expired by rats (62–82% of the applied dose) and was the major ^{14}C-labeled residue in feces. In general, levels of ^{14}C-labeled residues in tissues were low, but the small amount of radiolabel in the rat carcass 4 days after dosage (ca. 1% applied dose) was also identified as *p*-chlorobenzotrifluoride and was found predominantly in fat.

p-Chlorobenzotrifluoride (*p*-chloroBTF, 1) is an important intermediate in the synthesis of certain crop protection chemicals, especially herbicides (Boudakian, 1980), thereby offering a significant potential for occupational exposure. The metabolic fate of this compound is unreported although Cacco and Ferrari (1982) communicated the absorption and translocation of *p*-chloroBTF in soil and crops. We now report the metabolic fate of *p*-

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chloroBTF in rats in order to help assess its toxicological impact.

EXPERIMENTAL SECTION

Radiosynthesis. *p*-Chloro[*trifluoromethyl*-^{14}C]benzotrifluoride was synthesized by ICN (Irvine, CA). A portion of the [^{14}C]-*p*-chloroBTF (ca. 6 mg, 0.5 mCi) was purified by reversed-phase liquid chromatography (LC) with the following conditions: Waters M-6000 pump; 10-μm LiChrosorb RP-8 column, 0.46 × 25 cm; methanol–water, 60:40; ultraviolet detection at 254 nm; 1.6 mL/min. The [^{14}C]-*p*-chloroBTF was extracted from the eluate with pentane. Although care was taken to minimize losses of *p*-chloroBTF due to volatility, this procedure gave only a 39% recovery of [^{14}C]-*p*-chloroBTF (99.0% radiochemical purity by LC). The specific activity was 15.1 mCi/mmol as determined by gas–liquid chromatography–mass spectrometry (GLC–MS) by comparing the relative abundance of m/z 184 (C = 14, Cl = 37) and m/z 180 (C = 12, Cl = 35) after adjusting for the natural abundance of chlorine.

Analytical Methods. Metabolites were analyzed initially by thin-layer chromatography (TLC, silica gel GF, Analtech) with radiolabeled zones located with a Packard Model 7201 radiochromatogram scanner. In order to enhance resolution of metabolites, TLC was followed usually by reversed-phase LC analysis of TLC zones (Spectra Physics 8000 instrument; 10-μm LiChrosorb RP-8 column, 0.46 × 25 cm; ultraviolet detection at 254 nm; 35 °C; 1.6 mL/min). The following solvents containing acetonitrile or methanol in 0.1% acetic acid (v/v) were used: SS 1 (50% methanol), SS 2 (50% acetonitrile), SS 3 (60% methanol), and SS 4 (40% methanol for 5 min, gradient 40–100% methanol over 20 min). In general, metabolite quantitation was based on collection of the total column effluent in timed fractions for radioassay by liquid scintillation counting (LSC, Packard Model 2425).

Certain tissues and residual solids were quantitated for radiolabel by combustion to $^{14}CO_2$ [Harvey Biological Material Oxidizer (OX 300) with collection in Carbon-14-Cocktail (Harvey)] followed by LSC.

Mass spectra were obtained by coupled GLC–MS by using a Hewlett–Packard Model 5985 instrument in either the electron impact (EI) or chemical ionization (CI) mode. Nuclear magnetic resonance (^1H NMR) spectra were determined by using a Varian T-60 spectrometer.

Metabolite Standards. *N*-Acetyl-*S*-(4-chlorophenyl)-cysteine was synthesized in 47% yield from α-acetamidoacrylic acid and 4-chlorothiophenol according to the method of Renner et al. (1978). 4-Chloro-3-hydroxybenzotrifluoride (**2**) was obtained from Marshallton Research Laboratories, Inc., and 4-chloro-2-hydroxybenzotrifluoride was synthesized from 3,4-dichlorobenzotrifluoride by C.-H. Chuang (Zoecon). The benzoates of these phenols were prepared by reaction with benzoic acid in CH_2Cl_2 containing 1 equiv of dicyclohexylcarbodiimide and a catalytic amount of 4-(dimethylamino)pyridine.

The methylated, peracetylated glucuronide of **2** (i.e., **3**) was synthesized by the following procedure. The potassium salt of **2** was prepared by adding 1 M KOH (1 mL, 1 mmol) to **2** (196 mg, 1 mmol) and evaporating to dryness. To this salt was added methyl tri-*O*-acetyl-α-D-glucopyranosyl bromide [1 mmol; for preparation see Bollenback et al. (1955)] in dimethylformamide (2 mL). After heating at 80 °C for 2 h, the crude products were extracted into ether. Preparative TLC on silica gel GF (ether–hexane, 4:1) gave the methylated, peracetylated β-D-glucuronide of **2** in 9% yield (R_f = 0.41). The stereochemistry at C-1 of the sugar moiety was confirmed by ^1H NMR spectroscopy.

Treatment. Four female and two male albino rats (Sprague-Dawley, Simonsen Laboratories, Gilroy, CA) weighing 162–229 g were given a single oral dose of [CF_3-^{14}C]-*p*-chloroBTF at 1 mg/kg in corn oil (0.5 mL). Two additional female rats were treated similarly at 104 mg/kg with [^{14}C]-*p*-chloroBTF. All animals were fasted for 16 h before dosing and then were housed in all-glass metabolism chambers (Stanford Glassblowing Laboratories, Palo Alto, CA) for separate collection of urine, feces, expired $^{14}CO_2$, and expired organic volatile ^{14}C. A 5% KOH solution was used to trap $^{14}CO_2$ and was monitored daily by LSC. Radiolabeled organic volatiles were trapped by passing an air stream from the metabolism chamber through Amberlite XAD-2 (~16 g, Eastman). The Amberlite resin was extracted with methanol and dichloromethane, then a portion of the extract was analyzed by LC (SS 3). After animals were maintained on rat chow (Ralston Purina) for 4 days, the rats were sacrificed with ether. Tissues were dissected, weighed, and frozen for subsequent analysis.

Two additional female rats were dosed with [^{14}C]-*p*-chloroBTF (1 mg/kg) in corn oil as described. At various intervals blood was collected in heparinized tubes from the orbital sinus according to Riley (1960) and, after being cooled in ice, was centrifuged for 10 min. Aliquots (ca. 30 μL) of the resulting plasma were quantitated by LSC.

Urine Analysis. Aliquots of urine were evaporated to dryness under vacuum (<30 °C), and the residue was dissolved in methanol. After acidification, the residue was methylated with ethereal CH_2N_2. Preparative TLC of the ^{14}C-labeled residue (hexane–ethyl acetate, 1:1) gave two fairly distinct radiolabeled zones at R_f = 0 and R_f = 0.31. Analysis of the upper TLC zone by LC (SS 1) revealed two major components at k' = 4 and k' = 8.8. The less retained component (k' = 4) was identified as *N*-acetyl-*S*-[chloro-(trifluoromethyl)phenyl]cysteine (**4**) by GLC–MS of its methyl ester: m/z (rel intensity), EI, 357 (M^+ for Cl = 37, 3), 335 (M^+ for Cl = 35, 6), 298 (40), 296 (100), 117 (27), 88 (85); m/z (rel intensity), CI, (CH_4), 398 (M + 41,4), 396 (M + 41, 9), 384 (M + 29, 28), 358 (M + H, 56), 356 (M + H, 100), 336 (M − F, 58), 179 (77), 159 (83). The mass spectral fragmentations of mercapturic acid **4** (as its methyl ester) agreed with those of methyl *N*-acetyl-*S*-(4-chlorophenyl)cysteine which was synthesized as a model.

A second component (k' = 8.8) isolated by LC from the TLC zone (with R_f = 0.31) was identified as a glucuronide of dihydroxybenzotrifluoride (**5**). This conjugate was identified by GLC–MS of its methylated, peracetylated derivative: m/z (rel intensity), EI, no M^+ (M_r 508), 403 (M − OAc − CH_3 − OCH_3, 0.2), 389 (M − HOAc − OAc, 0.1), 375 (M − OAc − CH_3 − CO_2CH_3, 0.2), 257 (21), 197 (78), 155 (100), 127 (79); m/z (rel intensity), CI, (CH_4), 549 (M + 41, 40), 537 (M + 29, 52), 535 (48), 523 (100), 509 (M + H, 60), 495 (63), 257 (30). A portion of methylated **5** was isolated by LC (vide supra) for enzymatic cleavage to the aglycon: β-glucuronidase (5 mg, *Helix pomatia*); citrate–phosphate buffer (5 mL, pH 4.5); 20 h at 37 °C. The resultant hydroxymethoxybenzotrifluoride was too volatile for convenient manipulation so it was benzoylated (benzoyl chloride–pyridine) prior to mass spectral analysis: m/z (rel intensity), EI, no M^+ (M_r 296), 281 (M − 15, 1.0), 268 (M − OCH_3, 2), 193 (5), 177 (8), 149 (33), 105 (100), 77 (36).

Most of the urinary ^{14}C-labeled metabolites remained at the origin after TLC (hexane–ethyl acetate, 1:1). Methylation and acetylation of this polar material gave a relatively nonpolar product which was isolated by TLC (R_f

= 0.64, ether–hexane, 4:1). After further purification by LC (SS 2), this metabolite was identified as the methylated, peracetylated glucuronide of 4-chloro-3-hydroxybenzotrifluoride (3) by GLC–MS: m/z (rel intensity), EI, 393 (M – HOAc – OAc, 0.1), 333 (1.1), 317 (6), 257 (22), 215 (16), 197 (39), 155 (100), 127 (85), 43 (100); m/z (rel intensity), CI, (CH_4), 553 (M + 41, 0.3), 541 (M + 29, 1), no M + H at 513, 493 (M – F, 7), 453 (M – OAc, 5), 393 (M – HOAc – OAc, 2), 333 (M – HOAc – HOAc – OAc, 4), 317 (47), 257 (83), 163 (27), 155 (100). A synthetic standard of methylated, peracetylated 3 gave essentially the same CI mass spectrum as the derivatized metabolite. When the methylated metabolite was cleaved with β-glucuronidase, 4-chloro-3-hydroxybenzotrifluoride was released and identified by GLC–MS of its benzoate: m/z (rel intensity), EI, no M^+ (M_r 300), 281 (M – F, 2), 197 (1.3), 105 (100), 77 (31). Although we could not separate the benzoate of 4-chloro-3-hydroxybenzotrifluoride from that of 4-chloro-2-hydroxybenzotrifluoride by reversed-phase LC, the 3- and 2-benzoyl derivatives were separable by normal-phase LC: $k' = 12.8$ and 13.6, respectively: Haskel Model 28030 pump; Spectra-physics Model 8200 UV detector; Zorbax-SIL column, 0.46 × 25 cm, 5 μm; elution at 1.6 mL/min with CH_2Cl_2–pentane, 4:96 (v/v), 50% water saturated. All of the metabolite ^{14}C eluted with the benzoate of 4-chloro-3-hydroxybenzotrifluoride upon normal-phase LC.

Feces Analysis. The feces were extracted with methanol and a portion of the extract was analyzed by reversed-phase LC (SS 4). Volatile ^{14}C in the methanolic extract was trapped by slow evaporation of the solvent through Amberlite XAD-2 resin. The trapped ^{14}C was then eluted from the resin with methanol for LC analysis (SS 3). Unextractable ^{14}C-labeled residues were combusted to $^{14}CO_2$ for radiolabel quantification.

Tissue Analysis. Selected organs and tissues were combusted for quantitation of ^{14}C-labeled residues by LSC. The stomach and intestines, as well as the carcass remains, were extracted with methanol (2×) and chloroform (1×). Abdominal fat was extracted with dichloromethane and the solvent was evaporated through Amberlite XAD-2 to trap volatile organic ^{14}C which was eluted from the resin with methanol for analysis by LC (SS 3).

RESULTS AND DISCUSSION

The volatility of p-chloroBTF (1) at low mass levels (i.e., <10 mg) resulted in difficulties in purification and ^{14}C-labeled residue analysis. Our normal radioassay procedure for rat doses is to dissolve the ^{14}C-labeled compound in ether (10 mL) containing corn oil (0.5 mL) and then small aliquots (10 μL) are removed for quantitation by LSC. This radioassay procedure is inadequate for [^{14}C]-p-chloroBTF since even the most careful evaporation of ether gave a 10–20% loss of [^{14}C]-p-chloroBTF because of volatility. The radioassay method which we finally developed for rat doses involved LSC analysis of triplicate, 5-μL aliquots of [^{14}C]-p-chloroBTF in 0.5 mL of corn oil. Although this method would seem to be prone to relatively large errors because of the small volume and aliquot size, overall the procedure was quite reproducible.

Radiolabel Balance. When rats were given a single oral dose of [CF_3-^{14}C]p-chloroBTF, 62–82% of the radiolabel was expired as unmetabolized p-chloroBTF (Table I; Figure 1). The [^{14}C]-p-chloroBTF exhaled by rats was trapped on Amberlite XAD-2 resin by pulling an air stream through the all-glass, rat metabolism chamber. This trapping agent is very effective since in a recovery test essentially 100% of a sample of [^{14}C]-p-chloroBTF placed on a glass Petri dish in the chamber volatilized and was trapped with this method. The identity of the volatilized ^{14}C from rats was confirmed as p-chloroBTF by LC and GLC–MS. In order to verify that the volatilized p-chloroBTF was exhaled by rats (and not evolved from excrement), the production of volatile ^{14}C was monitored over short time intervals (Figure 2) and 35% of the volatilized p-chloroBTF was trapped before any defecation occurred.

When rats were given a single oral dose of [^{14}C]-p-chloroBTF at 1 mg/kg, only 16–18% of the applied dose was excreted in urine and feces. Negligible ^{14}C was evolved as $^{14}CO_2$, evidence that the CF_3 is relatively stable to hy-

Table I. Radiolabel Balance after Four Days from Rats Given a Single Oral Dose of p-Chloro[*trifluoromethyl*-^{14}C]-benzotrifluoride

	% applied dose		
	female		
	1 mg/kg[a]	104 mg/kg[b]	male, 1 mg/kg[b]
urine	13.6	5.9	14.9
feces	2.6	2.2	3.5
methanol extract	2.3	2.0	3.0
residual solids	0.3	0.2	0.5
carcass	1.2	0.19	0.18
methanol and chloroform extracts	1.1	0.17	0.16
residual solids	0.07	0.02	0.02
$^{14}CO_2$	≤0.03		
volatile organics (p-chloroBTF)	62	82	68
total recovery	79	90	87

[a] Average for four rats. [b] Average for two rats.

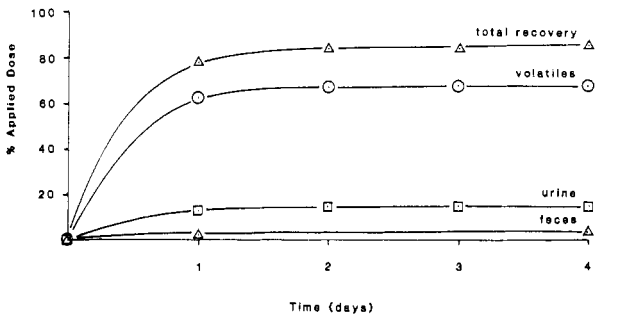

Figure 1. Elimination of radiolabel from male rats dosed orally with p-chloro[*trifluoromethyl*-^{14}C]benzotrifluoride (1 mg/kg).

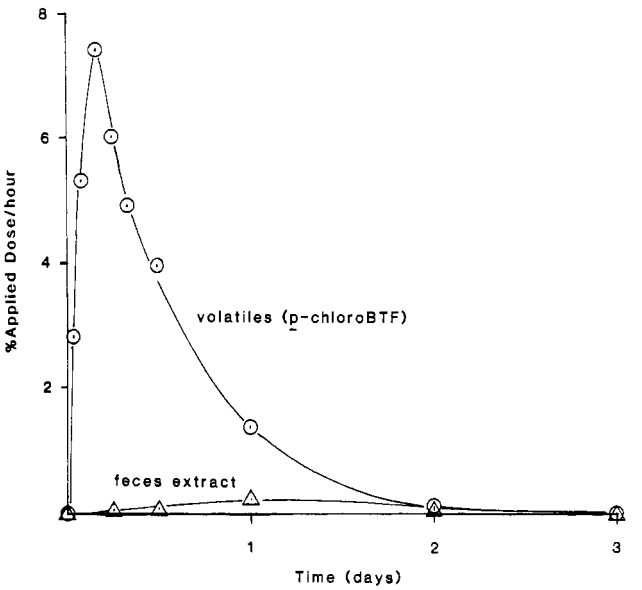

Figure 2. Elimination rate for a male rat given a single oral dose of p-chloro[^{14}C]benzotrifluoride (1 mg/kg).

Table II. Analysis of Urinary Metabolites from Rats Given a Single Oral Dose of *p*-Chloro[*trifluoromethyl*-^{14}C]-benzotrifluoride

	% applied dose				
	female				
	1 mg/kg		104 mg/kg		male, 1 mg/kg
	0-1 day	1-2 days	0-1 day	1-2 days	0-2 days
glucuronide of dihydroxybenzotrifluoride (5)	2.6	0.1	0.2	0.04	0.5
mercapturic acid conjugate of 1 (i.e., 4)	<0.1	0.1	0.1	<0.1	0.2
glucuronide of 2 (i.e., 3)	1.8	2.6	1.1	0.5	3
4-chloro-3-hydroxybenzotrifluoride (2)					0.5[a]
total ^{14}C in urine	9.4[b]	5.2[b]	3.8	1.2	17.3[b]

[a] Only sample analyzed (female urine samples not analyzed for free 2). [b] Analysis of urine from a single animal with the most urinary ^{14}C.

drolysis to a carboxyl group and subsequent decarboxylation. The relatively low total recoveries of administered ^{14}C (79–90%, Table I) are undoubtedly a reflection of the difficulties for radioassay of volatile [^{14}C]-*p*-chloroBTF since only about 1% of the applied ^{14}C remained in the rat carcass 4 days after dosage.

Urine. Glucuronides of dihydroxybenzotrifluoride (i.e., **5**) and 4-chloro-3-hydroxybenzotrifluoride (i.e., **3**) represented up to 2.7 and 4.4% of the applied dose, respectively (Table II). The presence of **5** in urine requires both oxidation and dechlorination of *p*-chloroBTF, and **5** was much less abundant at the elevated dose rate of 104 mg/kg. The glucuronide of 4-chloro-3-hydroxybenzotrifluoride (**3**) was the major identified urinary metabolite at both dose levels (Table II). By cleavage of this conjugate with β-glucuronidase the liberated aglycon (i.e., 4-chloro-3-hydroxybenzotrifluoride, **2**) was analyzed further to verify the site of metabolic hydroxylation. When **2** was benzoylated (to reduce volatility), the derivative could be separated by normal-phase LC from a benzoylated standard of 4-chloro-2-hydroxybenzotrifluoride. Hence, hydroxylation of *p*-chloroBTF occurred only at the 3-position, and the resultant phenol (**2**) was predominantly conjugated with endogenous glucuronic acid although some free **2** (0.5% applied dose) was detectable in urine. We found no evidence for the NIH-shift phenol (i.e., 3-chloro-4-hydroxybenzotrifluoride).

A mercapturic acid conjugate of *p*-chloroBTF (i.e., **4**) was also identified conclusively, but this adduct represented only 0.1–0.2% of the applied dose. As with 1,2- and 1,3-dichlorobenzene (Menzie, 1969), this mercapturic acid probably results from addition of glutathione to the aromatic ring without concomitant displacement of halogen but with subsequent conversion of the glutathione adduct to the mercapturic acid.

The unidentified ^{14}C in urine was relatively polar. Upon derivatization by methylation and acetylation, this unidentified ^{14}C was not rendered nonpolar. We could not isolate any single additional component at greater than 1–2% of the applied dose. Also, the CF_3 moiety seemed stable to hydrolysis since 4-chlorobenzoic acid (as well as its glycine and glucuronide conjugates) was not detectable in urine.

Feces. Only 2–3% of the applied dose was found in the feces (Table I). The ^{14}C-labeled residue in the feces was quite volatile (98% loss when evaporated to dryness on a roto-vap). When evaporated with a resin trap for organic volatiles, at least 56% of the feces extract was identified as *p*-chloroBTF. Some of the volatilized *p*-chloroBTF trapped from the rat metabolism chamber may have come from evaporation of *p*-chloroBTF from fecal pellets; however, we would expect volatility of *p*-chloroBTF from feces to be relatively minor compared to exhalation from the rat (cf. Figure 2).

Table III. Rat Tissue Analysis Four Days after a Single Oral Dose of *p*-Chloro[*trifluoromethyl*-^{14}C]benzotrifluoride

	parts per billion (ppb) equivalents as *p*-chloroBTF		
	female		
tissue	1 mg/kg[a]	104 mg/kg[b]	male, 1 mg/kg[b]
spleen	4 ± 4	<40	<1
fallopian tubes and ovaries	14 ± 15	150	
testes			<1
pancreas	7 ± 7	60	<1
lungs	12 ± 8	371	6
kidney	6 ± 5	184	2
brain	1 ± 1	<40	<1
heart	2 ± 2	44	<1
leg muscle	1 ± 1	<40	<1
pectoral muscle	2 ± 2	<40	<1
abdominal fat	104 ± 102	1420	6
pericardial fat	2 ± 2	<40	4
liver	1 ± 1	102	2
hide	16 ± 19	146	2
stomach and intestines	5 ± 6	186	2
carcass remains	13 ± 8	186	2

[a] Average and standard deviation for four rats. [b] Average for two rats.

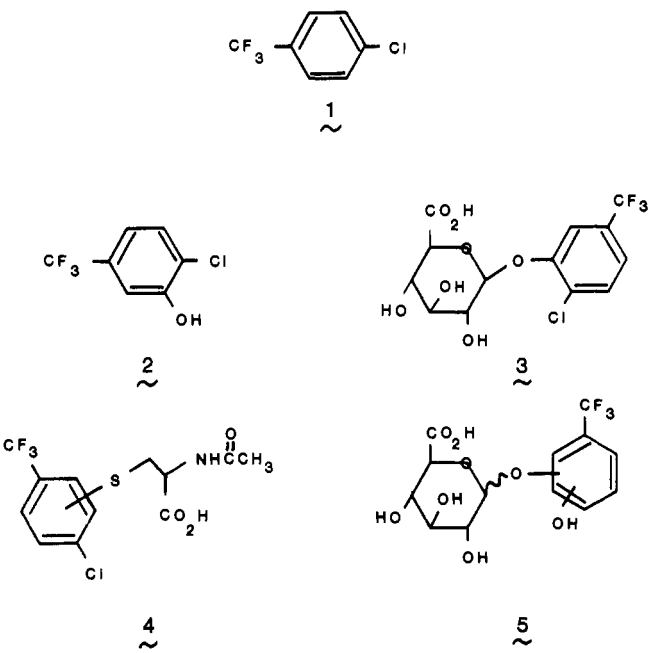

Figure 3. *p*-Chlorobenzotrifluoride and its rat metabolites.

Tissues. Four days after a single oral dose of [^{14}C]-*p*-chloroBTF, only about 1% of the applied ^{14}C remained in the rats. Analysis of selected organs and tissues (Table III) revealed low (ppb) residues. Only abdominal fat from female rats showed relatively higher ^{14}C-labeled residues. Essentially all of the ^{14}C in this fat dissolved in dichloromethane and 63% of the ^{14}C was lost when an aliquot of the extract was evaporated with a stream of nitrogen. By trapping the volatile ^{14}C from a separate aliquot on Amberlite XAD-2 resin and subsequent LC analysis, it was possible to identify >90% of the ^{14}C-labeled residue in

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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LITERATURE CITED

Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310.

Boudakian, M. M. In "Kirk-Othmer Encyclopedia of Chemical Technology", 3rd ed.; Wiley: New York, 1980; Vol. 10, p 922.

Cacco, G.; Ferrari, G. *J. Agric. Food Chem.* **1982**, *30*, 196.

Menzie, C. M. "Metabolism of Pesticides"; U.S. Department of Interior: Washington, DC, 1969; p 155.

Renner, G.; Richter, E.; Schuster, K. P. *Chemosphere* **1978**, 669.

Riley V. *Proc. Soc. Exp. Biol. Med.* **1960**, *104*, 751.

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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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