

the relative proportions of PDIC and the metabolite were approximately the same in seed extracts and external washes of leaves, it is possible that this product was formed on the leaf by photodecomposition and then absorbed and translocated to the seed. However, the metabolite was also detected in seed of plants that were treated by petiole injection and had no surface exposure of the PDIC to sunlight, suggesting that it may be a metabolic product as well.

¹⁴C-labeled PDIC was eliminated very rapidly from rats after oral administration and no significant concentrations of radiocarbon were retained by any of the tissues after 24 h. The low mammalian toxicity of PDIC and its rapid elimination by treated animals, coupled with the fact that only minor amounts of a single photochemical and/or metabolic degradation product are formed in or on the cotton plant, may tend to minimize the impact of the relatively large accumulations of PDIC in the seed. If the chemical is eventually used only as a conditioner for defoliation at the somewhat lower recommended rates of

application and at a later stage of plant maturity, residues in seed would likely occur at reduced levels.

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Uptake, Metabolism, and Elimination of the Lampricide 3-Trifluoromethyl-4-nitrophenol by Largemouth Bass (*Micropterus salmoides*)

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Largemouth bass (*Micropterus salmoides*) exposed to a 1.0- μ g/mL solution of the lampricide 3-trifluoromethyl-4-nitro[¹⁴C]phenol (TFM) for up to 24 h accumulated radioactive residues in all tissues analyzed at each of five successive sampling periods. Maximum concentrations occurred after 8 h in brain and muscle and after 12 h in blood, liver, kidney, and head plus viscera. Concentrations of radioactivity in the bile increased throughout the experiment. In a second group of fish exposed to 1.0 μ g/mL of [¹⁴C]TFM for 12 h and then transferred to lampricide-free flowing water, the concentration of radioactive materials in tissues generally decreased with time throughout a 72-h elimination period. No TFM was detected in muscle tissue 12 h after the fish were transferred to lampricide-free water. The presence of conjugated TFM in the bile was confirmed. Hexane/ether extracts contained [¹⁴C]TFM and other unidentified ¹⁴C materials from muscle and head plus viscera, whereas methanol extracts taken after the hexane/ether extraction contained only a negligible amount of [¹⁴C]TFM but large quantities of unidentified, polar ¹⁴C compounds.

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is currently applied to tributaries of the Great Lakes to control populations of the sea lamprey (*Petromyzon marinus*). Registration for the use of TFM as a lampricide in the United States requires the collection of data concerning its fate in fish and water. Sills and Allen (1975) measured residues in muscle tissues of eight species of fish exposed to TFM. Hunn and Allen (1975) investigated the effects of exposure to TFM on the renal excretion of coho salmon (*Oncorhynchus kisutch*), and Allen and Hunn (1977) assessed the effect of an intraperitoneal injection of TFM on the renal function of channel catfish (*Ictalurus punctatus*). Lech (1973) and Lech and Costrini (1972) investigated the metabolism of TFM in rainbow trout (*Salmo gairdneri*). In this study we attempted to determine the uptake, distribution, and elimination of [¹⁴C]TFM by various tissues in largemouth bass (*Micropterus salmoides*) and, using [¹⁴C]TFM, to corroborate the method of Sills and Allen (1975) for TFM residues in fish muscle.

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MATERIALS AND METHODS

Stock solutions of [¹⁴C]TFM (uniformly ring labeled, sp act. 3.66 mCi/mM, Mallinckrodt Chemical Works, St. Louis, MO) and technical grade TFM (82.4%, Hoechst Ag, Frankfurt, Germany) in methanol were used to prepare the 1.0- μ g/mL treatment solutions.

Largemouth bass were exposed to a mixture of 1:74 (w/w) of [¹⁴C]TFM and technical grade TFM (82.4%) in polyethylene tanks containing 75 L of water (pH 6.8, temperature 12.0 \pm 1.0 $^{\circ}$ C). Constant water bath temperature was maintained with a chilling unit, and oxygen content of the treatment solution was sustained by aeration.

Samples of three fish each were removed from the treatment solution at 2, 4, 8, 12, and 24 h in the uptake experiment. For the elimination experiment, fish were exposed in the treatment solution for 12 h and then placed in lampricide-free flowing water (pH 6.8, temperature 14.0 \pm 1.0 $^{\circ}$ C). Samples of three fish each were taken immediately after treatment and at 4, 8, 12, 24, 48 and 72 h posttreatment. Fish in the elimination experiment were fed ad libitum after they had been placed in the lampricide-free water. Fish used in the uptake experiments averaged 22.6 cm in length and 171 g in weight and those in the elimination experiment, 21.5 cm and 146 g.

Table I. Average Concentrations (\pm SE) of ^{14}C Material in Exposure Water ($\mu\text{g/mL}$) and in Bile and Tissues ($\mu\text{g/g}$) from Largemouth Bass Exposed to a 1.0- $\mu\text{g/mL}$ Solution of [^{14}C]TFM for 2-24 h^a

exposure time, h	sample							
	blood	brain	bile	liver	kidney	muscle	HV ^b	water
2	0.783 (0.068)	0.940 (0.050)	14.12 (2.193)	10.79 (1.636)	4.005 (0.099)	0.469 (0.063)	0.409	0.916
4	1.022 (0.165)	1.303 (0.037)	98.42 (6.071)	12.21 (1.700)	7.412 (2.640)	0.624 (0.065)	0.896	0.898
8	1.597 (0.079)	1.827 (0.164)	476.0 (55.72)	18.13 (0.578)	10.89 (2.809)	0.895 (0.030)	1.390	0.875
12	1.708 (0.157)	1.523 (0.090)	696.4 (90.20)	22.52 (2.656)	15.35 (2.634)	0.848 (0.081)	1.566	0.848
24	1.287 (0.101)	1.463 (0.033)	1497.3 (127.0)	18.03 (1.444)	13.04 (4.867)	0.834 (0.160)	1.515	0.834

^a Each value is the average of three fish. ^b HV = head plus viscera; HV from three fish was pooled for analysis.

At each sampling interval in each experiment, blood, brain, liver, kidney, bile, muscle, head, and viscera were taken. Head and viscera from each fish were combined in a single sample (HV). Blood samples were collected by caudal puncture with a hypodermic syringe. Blood was digested and decolorized in a scintillation vial by the addition of 0.4 mL of 60% perchloric acid, followed by 0.8 mL of 30% hydrogen peroxide. The vial was capped and incubated at 70 to 75 °C until the yellow color dissipated. Scintillation cocktail (10 mL, Scintiverse, Fisher Scientific Co., Pittsburgh, PA) was then added to each sample. Individual samples (100–200 mg) of brain, liver, and kidney were dissolved in 1 to 2 mL of tissue solubilizer (Unisol, Isolab Inc., Akron, OH). Anhydrous methanol (0.5 mL) and scintillation cocktail (10–15 mL, Complement, Isolab Inc.) were then added to each sample. Bile from each fish was added directly to 10 mL of scintillation cocktail (Scintiverse).

Muscle tissue and HV were frozen and homogenized with dry ice, as described by Benville and Tindle (1970). After the dry ice sublimed, 100- to 200-mg samples were dissolved in a tissue solubilizer and counted. A sample of 25 g of fish tissue was then mixed with 100 g of anhydrous sodium sulfate and set aside for 1.5 to 2 h while moisture was absorbed from the tissues. The tissue was then placed into a glass column and extracted with 200 mL of hexane/ether (70:30, v/v) (HE), followed by 200 mL of methanol (Hesselberg and Johnson, 1972; Allen and Sills, 1974). Portions (usually 5 mL) of each extract were evaporated in a scintillation vial, 10 mL of scintillation cocktail (TLA, Beckman, Inc., Fullerton, CA) were added, and the radioactivity was determined.

The HE extracts were transferred to a separatory funnel and extracted with two 10-mL portions of 0.1 N NaOH. The alkaline extracts were combined in a 50-mL volumetric flask, and 10 mL of 1 N H₂SO₄ was added. Sufficient HE was added to yield about 0.2 μg of TFM/mL in the final solvent. The amount of HE added was based on radiometric determinations of the column extracts. The flask was shaken and a portion (usually 1 mL) of the HE extract was used to quantify TFM radiometrically, and another 1-mL portion was derivatized with diazomethane for quantification by gas chromatography (GC). Derivatization procedures and operating conditions for the gas chromatograph were those described by Allen and Sills (1974).

Samples of 500 mL each from the treatment solutions were acidified to pH 1.5 to 2.0 with concentrated H₂SO₄ and extracted in a separatory funnel with three volumes (50 + 25 + 25 mL) of HE. From each extract, 5-mL portions were evaporated to about 0.5 mL, 10 mL of TLA scintillation cocktail was added, and the radioactivity was determined. Another portion was derivatized with dia-

zomethane for quantification by GC.

Radioactivity was measured in a Beckman Model LS-200 liquid scintillation spectrometer. Each sample was counted at least three times for a minimum of 10 min, or until the preset error of <0.2% had been achieved. All radioactivity data were corrected for background, quench, and machine efficiency and were converted to $\mu\text{g/g}$ or $\mu\text{g/mL}$ on the basis of the specific activity of [^{14}C]TFM.

RESULTS AND DISCUSSION

Uptake Experiment. Radioactive material was detected in all portions of the fish analyzed at each sampling interval (Table I). The maximum concentration of ^{14}C materials in brain and muscle was found at the 8-h sampling interval and in blood, liver, kidney, and HV at 12 h. Apparently an equilibrium was reached between these tissues and the lampricide at 8 or 12 h. The bile, however, continued to accumulate radioactivity throughout the experiment, and after 24 h had concentrated the lampricide by a factor of more than 1400 times that of the exposure solution. Biliary concentration of other xenobiotics has been noted in largemouth bass (Schultz and Harman, 1978), rainbow trout (Statham et al., 1976), and goldfish, *Carassius auratus* (Kobayashi and Akitake, 1975; Kobayashi et al., 1976).

Allen and Sills (1974) reported recoveries of 75 to 100% from fish samples fortified with nonradiolabeled TFM. We found that the concentration of radioactivity was much greater in solubilized tissue samples than that in the HE extracts (Table II) and that HE had extracted less than 50% of the radioactivity from all replicates of muscle tissue and three of five replicates of HV. However, we were able to extract most of the remaining radioactivity with methanol.

An average of 73% of the HE extract was identified as TFM by GC. Most of the remaining 27% was an unidentified polar compound, which did not partition into the HE from the aqueous phase.

Elimination Experiment. Except in samples of bile and HV, the amount of radioactivity decreased almost steadily with time in fish that were transferred to lampricide-free water (Table III). After 12 h, we found no radioactivity in samples of solubilized muscle tissue (Table IV). We also found no residues of TFM greater than 0.01 $\mu\text{g/g}$ in muscle extracts after 8 h, which was in agreement with the data of Sills and Allen (1975). Though some radioactivity remained in the HE and methanol extracts of muscle even after 72 h (Table IV), there was insufficient material to determine its identity.

The amount of TFM found in the HV by GC analysis declined, but with some fluctuations over the 72-h withdrawal period (Table IV). Also, the amount of radioactivity recovered in methanol extracts of the HV was

Table II. Concentrations ($\mu\text{g/g}$) of ^{14}C Materials Found in Solubilized Tissue Samples, Hexane/Ether Extracts, and Methanol Extracts of Muscle and Head-Viscera of Largemouth Bass Exposed to a $1.0\text{-}\mu\text{g/mL}$ Solution of [^{14}C]TFM for 2-24 h

ex- pos- ure time, h	^{14}C tissue activity	^{14}C activ- ity in hexane/ ether extract	meth- anol extract	total extrac- ted ^b	GC ^a
2	0.469	0.196	0.132	c	c
4	0.624	0.246	0.176	c	c
8	0.895	0.372	0.350	0.277	0.649
12	0.848	0.282	0.191	0.303	0.585
24	0.834	0.269	0.186	0.278	0.547
Head-Viscera					
2	0.409	0.311	0.233	0.314	0.625
4	0.896	0.487	0.379	0.377	0.864
8	1.390	0.669	0.415	0.569	1.238
12	1.566	0.645	0.480	0.759	1.404
24	1.515	0.550	0.409	0.842	1.392

^a Gas chromatographic analyses were conducted on hexane:ether extracts (see Materials and Methods).

^b Total extracted = hexane-ether plus methanol extracts.

^c No data collected.

2 to 20 times higher than that in the muscle throughout the experiment. Methanol extracts contained 66 to 86% of the total radioactivity extracted from the HV and 51 to 69% of that from the muscle.

To simulate normal feeding activity, we fed the fish while they were in the lampricide-free water (except for those removed immediately after the 12-h exposure). In some fish, bile was emptied into the duodenum and so little bile remained in their gallbladders that it was difficult to measure. The variation in the amount of radioactivity found in the HV corresponded with variations in the bile. At withdrawal intervals when gallbladders had little bile, we found that the concentration of radioactive material in the HV had increased from the previous sampling interval (8 and 24 h). Conversely, when the radioactive content of the bile increased from one sampling interval to the next, that of the HV decreased (4 and 12 h).

The decrease in radioactivity in most tissues may have been the result of passive diffusion from the fish to the water or renal excretion (Hunn and Allen, 1975). However, the increased radioactivity in the bile probably resulted from conjugation of TFM in the liver and subsequent secretion of the conjugate into the gallbladder. Lech (1973)

isolated and identified a TFM-glucuronic acid conjugate in the bile of rainbow trout exposed to TFM. To determine whether bass also form a TFM conjugate, we pooled the bile from three fish exposed to [^{14}C]TFM. A small amount of bile was counted by liquid scintillation spectrometry, another portion was spotted on TLC plates, and the remainder was acidified to pH 1.7 and extracted with $3 \times 25\text{ mL}$ portions of HE (70:30). After extraction, the acidic solution was adjusted to pH 7 with 0.1 N NaOH, and 25 mL of 0.05 M phosphate buffer was added. The buffered solution was then incubated with 1000 Sigma units of bacterial β -glucuronidase (Sigma Chemical Co., St. Louis, MO) for 2 h at 30 °C. After incubation, the buffered solution was acidified and extracted with $3 \times 25\text{ mL}$ HE, and the sample was concentrated by partial evaporation. A portion of the sample was then derivatized with diazomethane for GC, and the rest was spotted on a TLC plate. The TLC plate was developed in benzene/methanol/acetic acid (75:25:0.5, v/v/v), dried, and scanned for radioactivity on a radiochromatogram scanner. The scan of nonincubated bile showed a major peak at an R_f of 0.1 and a minor peak at an R_f of 0.51 (the latter similar to authentic [^{14}C]TFM). The scan of the HE extract after incubation showed only a major peak at R_f 0.51. Inasmuch as GC analysis of the HE extract also confirmed the presence of TFM, it is clear that largemouth bass, like rainbow trout, are capable of conjugating TFM.

To determine if all of the TFM was extracted from muscle tissue by HE, we examined the methanol extracts from three samples previously extracted with HE. The methanol extracts were first cleaned up by making them basic (pH 11) and then partitioned with $3 \times 25\text{ mL}$ of HE (50:50) which was discarded. The extract was then acidified (pH 1.7) and partitioned again with $3 \times 25\text{ mL}$ HE (50:50), followed by $3 \times 25\text{ mL}$ ether. The ether was discarded since it contained no radioactivity. The HE was derivatized with diazomethane and quantified by GC. In the three samples analyzed, we found only $0.008\text{ }\mu\text{g/g}$ of TFM, indicating that the HE column extracts were highly efficient for removing TFM. We also extracted additional muscle tissue with methanol only. The methanol was evaporated, and the remaining aqueous solution was acidified and extracted with $3 \times 50\text{ mL}$ HE (70:30). The HE extracts were combined and extracted twice with 10 mL of 0.1 N NaOH. The combined NaOH extracts were acidified and extracted with HE (70:30). The final HE extract was derivatized with diazomethane and the TFM quantified by GC. The amount of TFM was 35, 12, and 9% of the total radioactivity in the methanol extract taken

Table III. Average Concentrations ($\mu\text{g/g}$; $\pm\text{SE}$) of ^{14}C Materials in Bile and Tissues of Largemouth Bass Exposed to a $1.0\text{-}\mu\text{g/mL}$ Concentration of [^{14}C]TFM for 12 h and then Transferred to Lampricide-Free, Flowing Water for up to 72 h^a

withdrawal interval, h	sample						
	blood	brain	bile	liver	kidney	muscle ^b	HV ^c
0	1.707 (0.204)	2.470 (0.302)	823.6 (117.6)	14.219 (2.796)	17.379 (0.960)	0.862 (0.114)	1.878 --
4	0.960 (0.360)	0.871 (0.083)	1263.1 (104.4)	4.209 (0.467)	6.613 (1.611)	0.228 (0.085)	0.816 --
8	0.329 (0.056)	0.522 (0.040)	1069.4 (135.6)	2.205 (0.589)	3.307 (0.923)	0.014 --	1.567 --
12	0.191 (0.001)	0.483 (0.064)	1316.5 (59.38)	3.383 (1.944)	1.917 (0.371)	ND --	0.540 --
24	0.142 (0.023)	0.226 (0.035)	796.3 (383.2)	2.347 (1.158)	0.538 (0.089)	ND --	2.530 --
48	0.122 (0.023)	0.204 (0.010)	621.9 (551.3)	1.041 (0.523)	0.560 (0.198)	ND --	0.788 --
72	0.135 (0.017)	0.172 (0.026)	827.5 (321.8)	0.829 (0.140)	0.586 (0.117)	ND --	0.487 --

^a Each value is the average for three fish. ^b ND = not detectable; dashes indicate too few data to calculate SE. ^c HV = head plus viscera; HV samples from three fish were pooled for analysis.

Table IV. Concentrations ($\mu\text{g/g}$) of ^{14}C Materials Found in Solubilized Tissue Samples, Hexane/Ether Extracts, and Methanol Extracts of Muscle and Head-Viscera of Largemouth Bass Exposed to a $1.0\text{-}\mu\text{g/mL}$ Solution of [^{14}C]TFM for 12 h and then Transferred to Lampricide-Free, Flowing Water for Up to 72 h

with-drawal interval, h	^{14}C tissue activity	^{14}C hexane/ether extract	methanol extract	total extracted ^b
Muscle				
0	0.862	0.331	0.352	0.492
4	0.228	0.102	0.079	0.225
8	0.014	0.048	0.007	0.090
12	ND	0.040	0.001	0.058
24	ND	0.025	0.001	0.026
48	ND	0.018	ND	0.024
72	ND	0.019	ND	0.021
Head-Viscera				
0	1.878	0.555	0.481	1.061
4	0.816	0.286	0.118	0.742
8	1.567	0.247	0.080	1.460
12	0.540	0.139	0.040	0.451
24	2.530	0.411	0.176	1.519
48	0.788	0.172	0.067	0.348
72	0.487	0.160	0.056	0.608

^a Gas chromatographic analyses were conducted on hexane/ether extracts (see Materials and Methods).

^b Total extracted = hexane-ether plus methanol extracts.

at 0, 12, and 24 h after withdrawal. The total amount of TFM in replicate samples extracted with HE followed by methanol was 30, 7, and 9% of the total radioactivity in the combined extracts. On the basis of these two ex-

periments, we concluded that HE extracted most of the TFM from tissue.

Lech and Costrini (1972) found that fish liver is able to reduce TFM and acetylate the reduced form in vitro. Further studies would be required to determine if the radioactive materials we found, other than TFM, were reduced or N-acetylated, reduced TFM.

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Mothproofing Wool and Wool Blends with Permethrin

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Permethrin, a synthetic pyrethroid insecticide, can be applied to wool and wool-blend fabrics and yarn in the dye bath in a concentration of 0.003-0.005% and a wool-liquor ratio of 1:20 for 15 and 45 min at the boil with several dye types including acid premetalized, chrome, fiber reactive, acid leveling, and neutral premetalized. It was also found to be compatible with several flameproofing and shrinkproofing treatments for wool. Permethrin is absorbed nearly quantitatively by wool from many dye bath solutions. The described finishing treatments, which require only readily available equipment, appear easily adaptable to commercial use.

The excellent textile properties of wool are well-known. Nevertheless, wool has certain limitations. If it is not properly cared for, it is subject to laundering shrinkage and insect damage. Furthermore, although compared to other fabrics it is relatively resistant to ignition, it will burn. Thus it is more useful when it has been made resistant to

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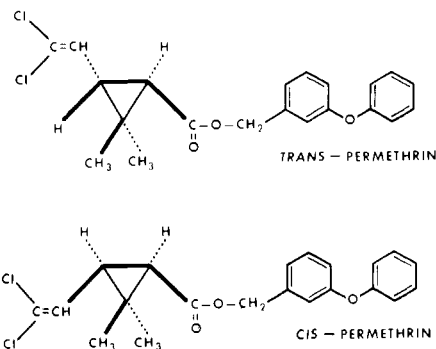


Figure 1. Structure of permethrin isomers.