Metabolism of 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Bluegills and Water

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Bluegills (*Lepomis machrochirus*) were exposed outdoors in a plastic pool to one 2 mg/L application of ^{14}C-labeled dimethylamine salt of 2,4-dichlorophenoxyacetic acid (DMA-^{14}C-2,4-D). Fish and water samples were examined during the 12-week exposure to investigate degradation of the herbicide and potential incorporation of ^{14}C fragments into natural biochemicals. We found that ^{14}C was incorporated into both free fatty acids and the fatty acid moiety of triglycerides from fillets and head–viscera samples. In addition, ^{14}C was detected in glycogen, which was isolated from a fish liver extract. The biochemical synthesis of ^{14}C amino acids and their subsequent incorporation into proteins was inferred. The incorporation of ^{14}C in fatty acids, glycogen, and protein materials accounted for 85% of the ^{14}C activity in the samples. Also, $^{14}CO_2$ was identified as a degradation product of ring-labeled DMA-^{14}C-2,4-D in a similarly treated plastic pool containing no fish.

Schultz (1973) demonstrated that fish exposed to the dimethylamine salt of uniformly ring-labeled 2,4-dichloro[^{14}C]phenoxyacetic acid (DMA-^{14}C-2,4-D) in plastic pools contained ^{14}C residues in their tissues. Further, these ^{14}C residues increased throughout the 12-week exposure and did not appear to reach an asymptote. Yet, gas chromatographic (GC) analyses of these tissues revealed only negligible amounts of 2,4-D and he suggested that metabolism or degradation of 2,4-D had occurred. Also, 2,4-D residues in water and hydrosol from the plastic pools decreased rapidly during the exposure period. Microbial degradation of 2,4-D in water and hydrosol was presumed to be a primary factor in the disappearance of the parent molecule. This hypothesis was supported in a review by Loos (1969) on 2,4-D metabolism, which showed that many microorganisms are capable of degrading 2,4-D and sometimes completely degrade the aromatic moiety of 2,4-D.

Earlier investigations to confirm the existence of 2,4-D metabolites in fish were reported by Grant (1973) and also supported Schultz's hypothesis. The objectives of the present investigation were to confirm the existence of [^{14}C]2,4-D degradation products in fish and in water and identify any ^{14}C residues in fish tissues.

MATERIALS AND METHODS

Reagents and Apparatus. All organic solvents were glass distilled (Burdick and Jackson, Muskegon, Mich.) Silica Gel-Woelm (activity grade I, deactivated before use by adding 10% H_2O w/w) was obtained from ICN Pharmaceutical GmbH & Co., West Germany. Cation-exchange resin (Amberlite IR-120H) for cation-exchange chromatography (CEC) was purchased from Mallinckrodt Chemical Co., St. Louis, Mo. Uniformly ring-labeled DMA-^{14}C-2,4-D (sp act. 393 cpm/μg) was purchased from Mallinckrodt Chemical Co. The integrity of the [^{14}C]2,4-D standard was checked by thin-layer chromatography (TLC), silica gel chromatography (SGC), and gel permeation chromatography (GPC). The standard was 92% pure and chromatographic characteristics indicated that 2,4-dichloro[^{14}C]phenol was the major impurity. Precoated TLC plates were Kiesel Gel F-254 from Brinkman Instruments, Inc., Westbury, N.Y.

A Beckman Model 200-L liquid scintillation counter was used for most radiometric determinations. Several tissue samples were analyzed with a Packard Auto Oxidizer Model 305. Scintillation fluid contained Beckman fluoralloy dry mix, dissolved in toluene. Triton X-100 was used as a tissue and water solubilizer when necessary. All sample counts were corrected for quench.

The GPC used in this study was described by Tindle and Stalling (1972). Gels and solvents used for GPC were Bio-Beads SX-3 with ethyl acetate or Sephadex LH-20 with benzene–methanol (50:50, v/v). A 2.5 × 28 cm glass column was used with both gels.

Gas Chromatography. A Perkin-Elmer Model 900 GC, interfaced with a Model 270 electron impact, electromagnetic mass spectrometer (MS) was utilized for the analysis of 2,4-D degradation products. The MS total ion monitor served as a GC detector. The GC column was coiled glass (2 mm id × 2.7 m), packed with 3% OV-7 on Chromosorb W HP (w/w). All samples were temperature programmed from 150 °C to a maximum of 250 °C at 5 °C/min. A heated GC effluent valve permitted attachment of glass capillary tubes for GC–component trapping (GC–TRAP). The capillary tubes were attached to the vent valve with a silicone septum and each tube was filled with small segments of glass wool. The end of each capillary tube was immersed in scintillation cocktail during component trapping. Another valve arrangement allowed GC interface with a radioactivity monitoring (RAM) system (Nuclear Chicago, Model 5190). The minimum detection limit for a distinct GC peak was 500 cpm.

A Micro-Tek Model 220 GC equipped with a flame ionization detector (FID) was used for confirmation of selected samples. The glass column (2 mm id × 1.8 m) was packed with 0.65% EGA on Chromosorb W HP (w/w). Injected samples were temperature programmed from 130 to 225 °C at 5 °C/min.

Fish. Bluegills (*Lepomis machrochirus*), obtained from the Carbon Hill (Alabama) National Fish Hatchery, were maintained in the laboratory in flowing well water at 17 °C for 2 weeks as described by Brauhn and Schoettger (1975).

Experimental Designs. Previously, we had conducted several experiments including in vitro incubation of [^{14}C]2,4-D with microsomal preparations of channel catfish livers and an in vivo intraperitoneal (IP) injection of 110 μg [^{14}C]2,4-D/g of body weight of bluegills. The fish were held for 24 h in flowing well water following the in vivo injection. We were unable to show that [^{14}C]2,4-D was degraded by fish in either experiment. The IP injection of 2,4-D (in vivo) was rapidly excreted, as we did not detect ^{14}C residues in fillets. An outdoor pool experiment was designed to permit longer exposure of bluegills to [^{14}C]-

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2,4-D and to characterize ^{14}C residues in samples reported by Schultz (1973). Two plastic pools similar to those used by Schultz were lined with a 2.5-cm layer of clay loam soil and each was filled with 1400 L of well water. Thirty percent of each pool was covered with dark metal screen to provide shade. The pools were located outdoors, and communities of invertebrates, algae, and other life forms were allowed to develop over a period of 2 weeks. Both pools were treated with DMA-^{14}C-2,4-D to give a concentration of 2 mg/L (393 cpm/μg). Thirty bluegills weighing 20–30 g each were placed in one pool and the other pool served as a treated control without fish. The fish were fed ad libitum throughout the experiment. Water samples were taken weekly and six fish were removed for analysis at 7, 35, 56, and 84 days postexposure. Individual fish were rinsed with water and divided into two subsamples, the fillets (edible portion) and a combination of the head–viscera. Each subsample was weighed and frozen.

Water Analysis. Radioactivity in water was determined by direct liquid scintillation counting of three 1-mL water samples from different locations in each pool. Evaporation and rainfall were approximately equal during the study period. In addition, 100 to 500 mL of water samples were analyzed by TLC and GC–RAM at 7, 35, and 84 days posttreatment. These samples were acidified to pH 1.0–2.0 with concentrated phosphoric acid and extracted twice with 100 mL of diethyl ether. The extracted pH 1 water was counted by liquid scintillation. The diethyl ether extracts were combined and percolated through a 1 × 17 cm glass column with a 100-mL reservoir filled to 17 cm depth with anhydrous Na_2SO_4. Recovery of 2,4-D from Na_2SO_4 was checked prior to this step, as some batches of Na_2SO_4 adsorbed 2,4-D and required methanol for elution. Then the dried extracts were transferred to a porcelain casserole (Coors, 275-mL capacity) and concentrated to 5 mL under a hood with a heat lamp. Afterwards, the samples were transferred to a 15 mL conical tip centrifuge tube and concentrated for spotting on TLC or derivatized with diazomethane for analysis with GC–RAM. Developer for TLC plates was a mixture of petroleum ether–diethyl ether–acetic acid (77:20:3, v/v/v).

To determine $^{14}CO_2$ content of water, we placed 100-mL samples from each pool in a three-neck distillation flask (500 mL). These samples were acidified to pH 1 by injecting 10 mL of concentrated HCl with a syringe through a silicone septum in one neck of the flask. Nitrogen was passed over the pH 1 water sample and the flask was shaken vigorously several times. The effluent containing $^{14}CO_2$ was percolated through a glass fritt immersed in ethanolamine. Aliquots of the ethanolamine were counted by liquid scintillation to determine $^{14}CO_2$ evolved and adsorbed.

Tissue Analysis. Figure 1 illustrates the general flow scheme used for the analysis of ^{14}C residues in bluegills. Tissues (2–20 g) were ground in a Waring blender with 4:1 (w/w) anhydrous Na_2SO_4–tissue and permitted to air dry (occasional stirring prevented hardening of the mixture). Three subsamples of the mixture, each weighing 500 mg, were counted by direct liquid scintillation with 4 mL of Triton X-100 and 10 mL of fluor, before and after extraction. Ground samples (Na_2SO_4 and tissue) were placed in a 2.2 cm i.d. glass column with a 250-mL reservoir and extracted with 1% phosphoric acid in methanol. Tissues weighing >10 g were extracted with 200 mL of solvent, and those weighing <10 g were extracted with 100 mL of solvent. Extraction recoveries of [^{14}C]2,4-D from spiked tissue samples after grinding with 4:1, Na_2SO_4–tissue were 98–99%.

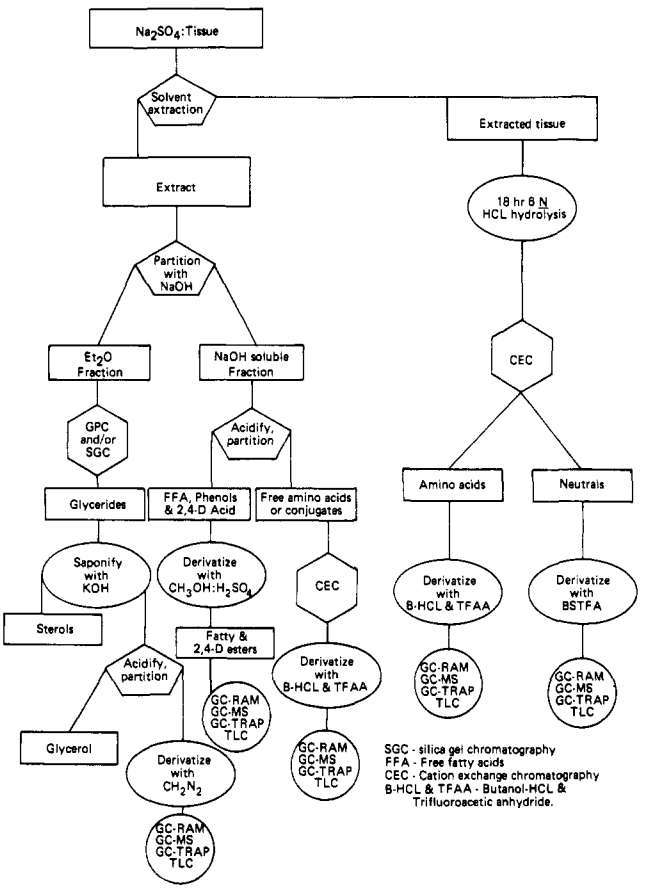

Figure 1. Analytical flow chart for possible [^{14}C]2,4-D degradation products. Additional steps were required for some samples. Abbreviations not given are in text.

Extractable ^{14}C. The sample extract (1% phosphoric acid in methanol) was made to pH 12 with 10 N NaOH after adding 50 mL of distilled water. Then the sample was transferred to a fume hood and the methanol was evaporated with the aid of a heat lamp. Afterward, the sample was placed in a separatory funnel and extracted twice with 100 mL of diethyl ether. The diethyl ether fraction contained neutral compounds, including possible ^{14}C triglycerides, while possible ^{14}C free fatty acids, -phenols, -2,4-D free acid, and -2,4-D acid conjugates would be in the aqueous base layer.

The extract containing triglycerides was concentrated to 5 mL and refluxed in 50 mL of ethanol–10 N KOH (49.5:0.5, v/v) for 2 h. After saponification, free fatty acids were purified by SGC. A 1 cm i.d. glass column (similar to Na_2SO_4 drying column) was filled to a depth of 10 cm with Woelm activity grade II silica gel. A sequential solvent elution system enabled separation of fatty acids from most impurities. Solvents and elution volumes (mL) for SGC were as follows: petroleum ether, 0–40; 10% diethyl ether in petroleum ether, 40–80; diethyl ether, 80–120; acetone, 120–140, and methanol, 140–160. Supplemental cleanup of free fatty acids (diethyl ether fraction from silica gel) was effected with GPC. The gel was Sephadex LH-20 and the solvent benzene–methanol (50:50). Radioactive eluates were derivatized with diazomethane or by H_2SO_4–methanol (0.5 mL/10 mL) reflux for 2 h. In some instances, SGC was again necessary to eliminate reagent contamination.

A system (GC–TRAP) was devised to trap GC components which permitted measurement of the radioactivity present in the methyl esters of fatty acids and other materials. The GC was interfaced with our MS, and a

Table I. Radioactive Residues in Water from Plastic Pools with and without Fish, Treated with 2 mg/L of DMA-^{14}C-2,4-D

Days after treatment	Pool with fish			Pool without fish		
	cpm/mL[a] (H_2O)	2,4-D equiv residue,[b] mg/L	Activity as CO_2, %	cpm/mL[a] (H_2O)	2,4-D equiv residue, mg/L	Activity as CO_2, %
0	775	(2.0)	0	790	(2.0)	0
8	505	(1.3)	0	495	(1.3)	0
17	510	(1.3)		506	(1.3)	
31	468	(1.2)		388	(1.0)	
35	474	(1.2)		318	(0.8)	22
43	540	(1.4)		273	(0.7)	
50	446	(1.1)	0	232	(0.6)	56
57	488	(1.2)		226	(0.6)	
64	485	(1.2)		188	(0.5)	
70	365	(0.9)	0	136	(0.4)	65
84	380	(1.0)	0	124	(0.3)	89

[a] Background subtracted. [b] Determined by dividing specific activity of DMA-^{14}C-2,4-D (393 cpm/μg) into total counts present in water sample, not necessarily 2,4-D residues.

Table II. Accumulation of Radioactive Residues in Tissues of Bluegills Exposed to DMA-^{14}C-2,4-D

Sample	Mean radioactive residues in cpm/g and 2,4-D equivalents[a] in μg/g after			
	7 days	35 days	56 days	84 days
Fillet	143 (0.4)	650 (1.6)	660 (1.7)	1247 (3.2)
n[b]	2	6	2	4
Head–viscera	765 (2.0)	1243 (3.2)	1612 (4.1)	2350 (6.0)
n	2	2	4	4

[a] Determined by dividing specific activity of DMA-^{14}C-2,4-D (393 cpm/μg) into cpm in tissue, not actual 2,4-D residues. [b] Number of samples.

heated valve attached to the column effluent provided a venting option for trapping. The GC retention times and temperature elution zones were determined by temperature programming various methyl esters on an OV-7 column. Glass capillary tubes (9 cm long) were filled with small segments of glass wool wetted with scintillation cocktail. One end of each capillary tube was immersed in a scintillation vial containing 10 mL of scintillation fluid. Methyl ester fractions were trapped by sequentially attaching the capillaries and vials to the GC–MS effluent valve using a silicone septum. The trapping efficiency was approximately 90%. Several samples containing ^{14}C were also chromatographed on Kiesel Gel F-245 plates. The plates were developed with 10% diethyl ether in petroleum ether. The R_f values of standards including [^{14}C]methyl stearate were compared with those of purified samples.

The base soluble ^{14}C fraction from the original tissue extract was acidified to pH 1 with 2 N HCl and partitioned twice with 100 mL of diethyl ether. Most polar materials, including free amino acids and conjugates remained in the pH 1 aqueous phase. If present, free fatty acids, phenols, and 2,4-D acid partitioned into the diethyl ether phase. The ether extract was methylated with methanol–sulfuric acid or diazomethane. SGC as described earlier was used to separate ^{14}C-methyl esters and other ^{14}C materials. GPC with Bio-Beads SX-3 and ethyl acetate were utilized for additional cleanup of ^{14}C-fatty acid methyl esters from nonlabeled contaminants. The purified ^{14}C-esters of fatty acids were analyzed by the GC–TRAP method or TLC as described earlier.

Nonextractable ^{14}C. After column extraction with 1% phosphoric acid in methanol, the Na_2SO_4–tissue was transferred to a beaker and the Na_2SO_4 was dissolved in hot distilled water. The tissue was separated from the aqueous phase by filtration through Whatman No. 1 filter paper. The aqueous phase was counted by direct liquid scintillation before it was discarded. Tissue hydrolysis, ion-exchange cleanup, and derivatization of amino acids for GC were done by the method of Gehrke et al. (1968) or Kaiser et al. (1974). Tissues (5 g) were hydrolyzed by constant boiling in 100 mL of 6 N HCl for 20 h.

A 2.2-cm glass column with solvent reservoir was filled with 30 g of regenerated Amberlite IR-120 H. The hydrolysate, dissolved in 10 mL of 0.1 N HCl, was placed on the resin and slowly percolated (1 mL/min) through the resin (solvent should not drain below surface of the resin). Then the column was washed with 200 mL of distilled water. The amino acids were eluted with five separate 8-mL portions of 7 N NH_4OH at 2 mL/min. Esterification and acylation using 1-butanol–3 N HCl and trifluoroacetic anhydride were as described by Kaiser et al. (1974).

The method of Montgomery (1957) was used for the isolation of [^{14}C]glycogen from liver. Autooxidation of [^{14}C]glycogen to $^{14}CO_2$ was used for determining ^{14}C content.

RESULTS AND DISCUSSION

Water Analysis. Radioactivity in the two pools declined during the 84 days after treatment, but at different rates (Table I). After 84 days, the pool without fish contained only 16% of the original radioactivity applied, and 89% of this activity was $^{14}CO_2$. Water in the pool with fish still had 49% of the initial radioactivity after 84 days, but did not contain detectable $^{14}CO_2$. Examination of non-CO_2 radioactivity in water from both pools by GC–RAM, GC–MS, and TLC during the exposure revealed only [^{14}C]2,4-D.

During the latter part of the test period, the pool with the fish contained a heavy algal bloom which was not present in the control pool. The pH of 84-day water samples from the control pool and the pool with fish averaged 8.4 and 9.8, respectively. Presence of the algal bloom in the pool with fish was probably responsible for the observed high pH. Since biological and chemical differences existed in the two pools, the populations of microorganisms established were probably different. Loos (1969) has shown that microorganisms vary in their ability to degrade 2,4-D and may produce dissimilar degradation products.

Tissue Analysis. Radioactive residues were accumulated in both head–viscera and fillets (Table II), a finding which concurred with that of Schultz (1973). The

Table III. Radioactive Residues and Distribution by Tissues of Bluegills 84 Days after Application of DMA-^{14}C-2,4-D

Sample	2,4-D equivalents[a] (μg/g) in fish no.					Percent distribution in fish no.				
	1	2	3	4	Mean	1	2	3	4	Mean
Fillet	2.5	3.9	2.9	3.7	3.2	39	32	37	41	37
Head-viscera	4.6	8.3	5.6	5.7	6.0	61	68	63	59	63

[a] Determined by dividing specific activity of DMA-^{14}C-2,4-D (393 cpm/μg) into cpm/g in tissue, not actual 2,4-D residues.

Table IV. Percentage of Extractable and Nonextractable Radioactivity[a] Found in Bluegills 84 Days after Application of DMA-^{14}C-2,4-D

Sample and fish number		Nonextractable[a] ^{14}C materials	Extractable ^{14}C materials		
		Amino acids + Glycogen	Neutral lipids or triglycerides	Water soluble activity at pH 1	Free fatty acids
Fillet	1	49			
	2	56	20	4	3[c]
	3	59	23	15	3
	4	72			
Mean		59	22	10	3
Head-viscera	1	44	21	3	32
	2	58	16	12	14
	3	39	18	11	32
	4	39	16	13	32
Mean		45	18	10	28

[a] Extraction solvent was 1% phosphoric acid in methanol and activity was expressed as percent of total sample cpm for each individual sample. [b] Suggested identity, not confirmed. [c] Free fatty acid fraction from fish no. 2 was partially spilled, resulting in activity lost.

Table V. Percent Radioactivity in Methyl Esters of Fatty Acids Liberated from Neutral Lipids Extracted from a 84-Day Head-Viscera Sample

Methyl ester	Relative peak height (RPH) %[a]	% of total trapped radioactivity (^{14}C)	% ^{14}C/RPH %	Temperature (°C) elution range of fraction
C14:0[b]	9	19	2.11	180-210
C16:+C16:1	51	12	0.24	210-225
C18:+C18:1	33	16	0.48	225-240 (1 min)
C18:3	4	31	7.75	240 (1 min)-240 (10 min)
C20:3	3	22	7.33	240 (10 min)-250 (2 min)

[a] Based on GC with FID. [b] Fatty acid chain length:number of double bonds.

^{14}C residues in head-viscera and fillets continued to increase throughout the duration of the experiment. However, we did not detect [^{14}C]2,4-D (detection limit 0.1 μg/g) in fish tissues. More ^{14}C residues were found in samples of head and viscera than in fillets (Table III).

Extractable Radioactivity. Radioactivity in the neutral lipid fractions (mostly triglycerides) represented 16–23% of the total in 84-day fillet and head-viscera samples from bluegills (Table IV). Several neutral lipid fractions from 84-day head-viscera samples from bluegills (Table IV) were saponified with ethanolic-KOH, and more than 90% of the radioactivity was base soluble, which suggests that most activity was associated with the presence of carboxyl groups after hydrolysis. The basic hydrolysates were acidified and free fatty acids were extracted with petroleum ether. However, after hydrolysis and fatty acid extraction, 20–32% of the radioactivity originally in the neutral lipids remained in the H_2O–ethanol (pH 1) solutions. This activity might be ascribed to glycerol but we did not confirm this hypothesis. The radioactive fractions containing free fatty acids from hydrolyzed neutral lipids were methylated by refluxing with CH_3OH–H_2SO_4 (10:0.5, v/v) for 2 h and the solutions were purified on SGC and/or GPC and analyzed by GC–MS. The methyl esters of myristic (C14:0, number of carbons:double bonds), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linolenic (C18:3) and eicosatrienoic (C20:3) acids were detected. However, we were unable to obtain satisfactory GC–RAM confirmation of ^{14}C incorporation into these fatty acids from [^{14}C]2,4-D degradation, because of the inability of the GC–RAM to detect small amounts of radioactivity in large amounts of injected methyl esters.

The incorporation of ^{14}C into fatty acids from hydrolyzed neutral lipids was confirmed by using the GC–TRAP. A 84-day head-viscera sample, which contained 2200 cpm/g of tissue (total activity), was selected for analysis by this method. Significant radioactivity (924 cpm/g of tissue) was extractable with 1% phosphoric acid in methanol. The neutral lipid fraction represented 16% of the total radioactivity in the sample. The percentage of radioactivity detected in individual methyl esters of fatty acids liberated from the neutral lipids ranged from 12 to 31% of all the activity present in neutral lipid fractions (Table V). Radioactivity was detected in all methyl esters, however, C16:0–C16:1 and C18:0–C18:1 eluted as ester pairs. The radioactivity associated with each methyl ester varied (Table V) and did not appear proportional to concentrations of the corresponding esters (Figure 2). Methyl linolenate (C18:3) had the highest specific activity and contained 31% of the activity found in the original neutral lipid fraction, but this ester constituted only 4% of all the esters, based on GC–FID relative peak height (Table V). Also, methyl eicosatrienoate (C20:3) contained

Table VI. Composition of Methyl Esters from Free Fatty Acids and Distribution of Radioactivity in an Extract of a 84-Day Head-Viscera Sample

Methyl ester	Relative peak height (RPH) %[a]	% of total trapped radioactivity (^{14}C)	% ^{14}C/RPH %	Temperature (°C) elution range of fraction
C14:0[b]	4	5	1.25	185–205
C16:0+C16:1	38	22	0.58	205–225
C18:0+C18:1	49	39	0.80	225–240
C18:3	9	29	3.22	240 (6 min)

[a] Based on GC with FID. [b] Fatty acid chain length:number of double bonds.

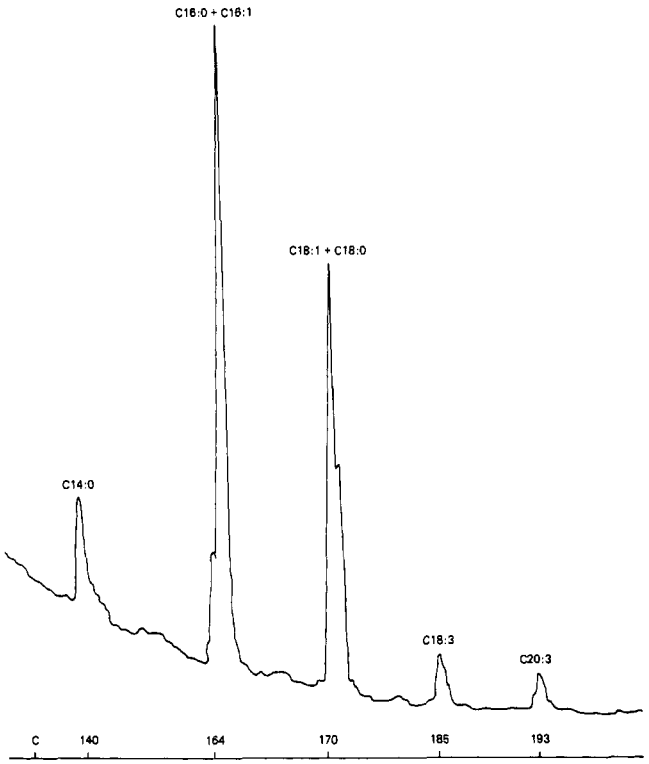

Figure 2. Fatty acids isolated from a neutral lipid fraction (triglycerides) of a head-viscera extract. Fish exposed to DMA-^{14}C-2,4-D for 84 days. GC parameters: FID, 0.65% EGA on Chromosorb W HP (w/w); temperature programmed from 100–220 °C at 5 °C/min.

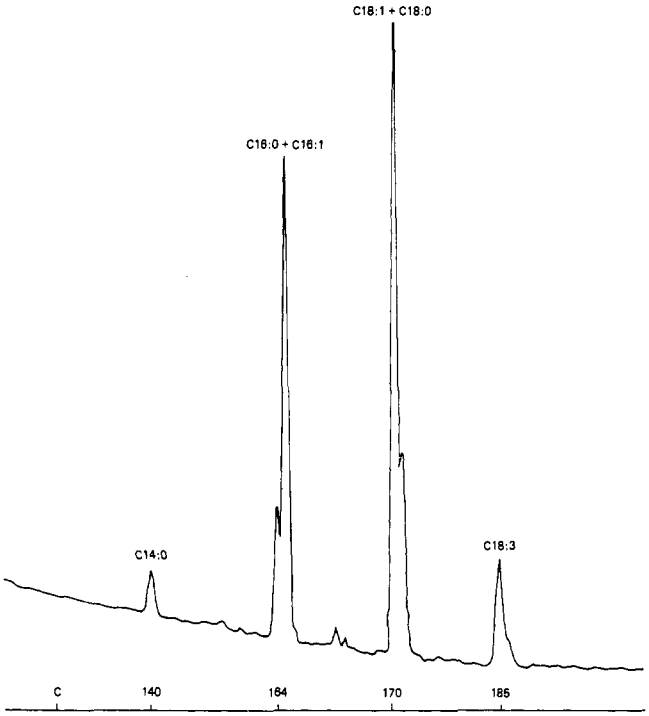

Figure 3. Fatty acids isolated from an extract of a head-viscera sample. Fish was exposed to DMA-^{14}C-2,4-D for 84 days. GC parameters same as in Figure 2.

considerable radioactivity (22% of total), but represented only 3% of all the esters (Figure 2). The radioactivity associated with all the methyl esters from neutral lipids was 13 cpm/mg.

We found that extracts of 84-day head-viscera samples also contained significant amounts of radioactive base-soluble compounds. These materials were characterized as ^{14}C-free fatty acids and represented 14–32% of the total activity in the head-viscera samples. The ^{14}C-free fatty acids were methylated by refluxing with CH_3OH–H_2SO_4 for 2 h. The methylated radioactive materials were purified with SGC and GPC. One of the purified and methylated ^{14}C-free fatty acid extracts (84-day head-viscera extract), which contained 25 cpm/mg total fatty acids, was selected for examination by GC–TRAP (Table VI). The free fatty acid methyl esters from these samples were also analyzed by GC–FID (Figure 3) and GC–MS. Linolenic (C18:3) again had the highest specific activity of the fatty acids. The C16 and C18 free fatty acids contained a greater percent of the total trapped radioactivity than did similar fatty acids liberated from triglycerides.

Nonextractable Radioactivity. Significant radioactive residues were not extractable from bluegill tissues with 1% phosphoric acid in methanol (Table IV). Therefore we analyzed four additional fish and found that nonextractable radioactivity averaged 63% for fillets and 52% for head-viscera, based on total activity in the two types of tissues. Determinations of radioactivity in extracted tissues were made by direct liquid scintillation counting of emulsified samples. The presence of radioactivity in selected samples was confirmed with an autooxidizer which measured $^{14}CO_2$ evolved. Radioactivity found in nonextractable tissues was higher (10–20%) with autooxidation than direct liquid scintillation counting, suggesting internal quench due to tissue incorporation of ^{14}C.

A fillet from the 56-day sample period was hydrolyzed and chromatographed on a cation-exchange column to determine the nature of the nonextractable ^{14}C residues. The radioactivity eluted in the amino acid fraction and a subsample of the radioactive eluate was prepared for GC. GC–FID of the derivatized sample confirmed 15 or more amino acids in this radioactive fraction.

Glycogen was isolated from an 84-day liver sample and analyzed by autooxidation. Of the total nonextractable radioactivity (1950 cpm, 5 μg) in the liver, approximately 12% could be attributed to [^{14}C]glycogen. After glycogen was removed the remaining liver tissue was hydrolyzed, and radioactivity in the hydrolysate again eluted from a cation-exchange column in the amino acid fraction, strongly suggesting ^{14}C-amino acids. Additional charac-

terization of these radioactive eluates was not possible because of the low amount of ^{14}C incorporated per milligram of amino acid. Detection would require injection of 25–50 mg of derivatized material into the GC–TRAP if a single amino acid contained all the radioactivity.

CONCLUSIONS

The results of our investigations strongly suggest that a significant amount of the aromatic moiety of DMA-^{14}C-2,4-D was completely degraded, probably by microorganisms and other factors within the pools. These ^{14}C fragments were then metabolically incorporated by bluegills during the 84-day exposure into specific natural biochemicals. This hypothesis is based on our preliminary in vitro and in vivo experiments which showed that 2,4-D was taken up by bluegills, but that radioactive metabolites could not be detected and are probably not produced by fish liver microsomes. However, in outdoor pools with other elements of the aquatic biotic community present, the fish took up significant amounts of radioactive materials, presumably ^{14}C fragments from [^{14}C]2,4-D. Portions of the ^{14}C intermediates were converted into fatty acids, probably through chain elongation, and some ^{14}C-fatty acids were incorporated into triglycerides. Also, the fish apparently synthesized ^{14}C-amino acids from ^{14}C intermediates and incorporated them into proteins. We were unable to detect ^{14}C intermediates in the degradation of [^{14}C]2,4-D in water or fish, which suggests rapid decomposition of the parent molecule in natural aquatic communities.

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Residue Analysis of β-Naphthoxyacetic Acid and β-Naphthol by High-Pressure Liquid Chromatography

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A method is described for the rapid, accurate, and sensitive quantitative measurement of β-naphthoxyacetic acid and β-naphthol by high-resolution liquid chromatography. The method sensitivity is 0.05 ppm for both compounds. Recovery studies of β-naphthoxyacetic acid fortified in strawberries in concentrations ranging from 0.05 to 0.25 ppm averaged 90.4 ± 4.0%. Recovery studies of β-naphthol fortified in strawberries in concentrations ranging from 0.05 to 0.25 ppm averaged 93.8 ± 4.2%. The percent deviation of the chromatographic method was established for β-naphthoxyacetic acid by measurement of peak areas for concentrations of 1.5, 3.6, and 12 ng/20-μL sample loop as 6.3% deviation and for β-naphthol by measurement of peak areas for concentrations of 1.5, 3, and 6 ng/20-μL sample loop as 7.8% deviation.

The failure of certain types of fruit to set on the early flower clusters is a common complaint of California growers who produce for the spring and summer market. Poor fruit-set is usually ascribed to low night temperatures. In each spring-market area, after the danger of winter frost, there follows a period of 6 weeks to 3 months when day temperatures are conducive to good vegetative growth, but night temperatures may drop too low for proper fruit setting. Various plant growth-regulating hormone-type chemicals such as β-naphthoxyacetic acid, 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid have been investigated on strawberries and tomatoes (Mann and Minges, 1948) for promoting early fruit-set. In California, β-naphthoxyacetic acid is currently registered for use on tomatoes as a tomato blossom fruit-set and in Oregon it is used on a limited basis on strawberries to increase fruit size. The Environmental Protection Agency Compendium of Registered Pesticides lists tolerances, dosages, and limitations for use of β-naphthoxyacetic acid on pineapple, strawberries, and tomatoes (EPA Compendium of Registered Pesticides, 1968).

No residue method has been reported in the literature for β-naphthoxyacetic acid or β-naphthol on strawberries. Only a preliminary report has been presented by Davidson (1970) on a spectrophotofluorometric determination of β-naphthoxyacetic acid in tomato products.

The purpose of the present manuscript is to describe a sensitive residue method for the analysis of β-naph-

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