the strips were examined for color change. The results are given in Table III.

In similar trials the strips were used for noting the time required for aerating a ship hold and a warehouse where wheat bags were fumigated with aluminum phosphide tablets.

RESULTS AND DISCUSSION

At 0.05 and 0.1 ppm, the edges of the strip showed a thin red margin, and the body of the paper turned yellowish-red slowly (Table I). At 0.3 ppm, the change was rapid and occurred in 1 min. The color was a deeper saffron, which became red in 30 min. A concentration of 0.05 ppm in the atmospheric vault (Table I) also gave the characteristic red color in the exposed strip.

With concentration of NH_3 present along with PH_3 (Table II), the color development was suppressed, denoting the shifting of pH to the alkaline side. This was also amply demonstrated when the atmosphere in the aluminum phosphide manufacturing plant was tested (Table III). The papers turned markedly red at distances away from the tableting point as concentrations of NH_3 dwindled. At the tableting point and the immediate vicinity, high concentrations of NH_3 from the ammonium carbamate were responsible for the interference in the color development; the papers remained yellow when exposed inside the exhaust hoods where the tableting was in progress. However, it is unlikely that such high concentrations of NH_3 will be met with in actual fumigation and degassing operations.

At 0.3 ppm, which is the accepted (threshold) permissible limit for prolonged exposure (Monro, 1969), the change in color is very perceptible and can caution fumigation operators quickly. The operators can wear badges containing the indicator strips (similar to the radiation exposure badges) to signal the presence of PH_3. It is also possible to use the strips to indicate exhaustion of gas mask canisters. The strips can be used in phosphine detector tubes (Singh et al., 1967) and also to determine whether the fumigated materials (Dietrich et al., 1967) are free from residual vapors (Tornow, 1942). The strips developed are more efficient than silver nitrate-treated paper strips, as they are not affected by light.

They were subsequently effectively used to detect leaks and residual gas in ship holds offshore and in a warehouse where fumigated bagged wheat was being aired. Traces of PH_3 could be detected even after 1 hr in the interspaces of bags. The change in color is almost instantaneous at higher concentrations of PH_3 (0.3 ppm).

Detector tubes containing chromogenic reactants respond to the threshold concentrations only after several pumping operations of the aspirator bulb or bellows of the detector device. The $HgCl_2$-methyl yellow paper strips, however, are easier to prepare, less expensive, not affected by light, and more versatile in that they can be placed at various check points and left there to indicate even traces of PH_3. After this investigation was completed, another refinement was made for treating the filter paper strips: 1% mercuric chloride solution was prepared in the methyl yellow stain. The strips were dipped and dried before use.

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Dynamics of a Salt of (2,4-Dichlorophenoxy)acetic Acid in Fish, Water, and Hydrosol

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The uptake, distribution, and dissipation of ^{14}C-labeled dimethylamine salt of (2,4-dichlorophenoxy)acetic acid (DMA-2,4-D) from water by three species of fish was studied concurrently with the dissipation of DMA-2,4-D from water and hydrosol. Fish were exposed to 0.5, 1.0, or 2.0 mg/l. concentrations of herbicide for up to 84 days. Radioactive residues of 2,4-D were determined by radiometric procedures in eight or more tissues and organs. Residues of 2,4-D were determined in muscle and whole-body extracts by gas chromatography. Radioactive residues were found in all fish tissues and organs analyzed, but actual 2,4-D content was negligible in muscle, indicating that most of the ^{14}C-residue was a metabolite(s) of 2,4-D. Residues of 2,4-D declined in water to less than 0.1 mg/l. after 35 days and in hydrosol to less than 0.1 mg/kg after 14 days.

The widespread occurrence and uncontrolled growth of various aquatic plants, especially in the southeastern

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United States, have caused many problems, including blocked navigation ways, obstructed water flow, poor fishing, and impaired recreational values. In addition, the water hyacinth [Eichhornia crassipes (Mart.) Solms.] provides an ideal breeding ground for mosquitoes. Water hyacinth presently is controlled by the use of the dimethylamine salt of (2,4-dichlorophenoxy)acetic acid (DMA-2,4-D). Wojtalik et al. (1971) reported that DMA-2,4-D ap-

peared to be noncumulative when sprayed at the rate of 22.4 to 44.8 kg/ha (kg acid equivalent per hectare). Rodgers and Stalling (1972) found that the butoxyethanol ester of 2,4-D (BEE-2,4-D) was eliminated rapidly from fish exposed to the herbicide. At this writing no tolerance levels have been established for DMA-2,4-D in the edible portions of fish.

The objectives of this study were to determine the uptake, distribution, and dissipation of DMA-2,4-D in three species of fish, to determine the dissipation of DMA-2,4-D from water and hydrosol, and to determine the effect of pH on the uptake of DMA-2,4-D by fish.

EXPERIMENTAL SECTION

Chemicals. All solvents used for extractions were glass-distilled (Burdick and Jackson, Muskegon, Mich.). All other chemicals used were analytical reagent (AR) grade.

Uniformly ring-labeled DMA-^{14}C-2,4-D was purchased from Mallinckrodt Nuclear, St. Louis, Mo. The DMA-^{14}C-2,4-D used in the uptake and distribution study and in the metabolism study had a specific activity of 35.11 μCi/mg, while that used to measure whole-body residues had a specific activity of 0.165 μCi/mg.

Radioactive samples were counted in a Beckman model 200-L liquid scintillation counter. The scintillation cocktail contained a Beckman fluoralloy dry mix dissolved in toluene. The fluor contained 8 g/l. of butyl PBD [2-(4'-tert-butylphenyl)-5-(4''-biphenyl)-1,3,4-oxdiazole] and 5 g/l. of PBBO [2-(4'-biphenyl)-6-phenylbenzoxazole].

Uptake and Distribution Study. Channel catfish (*Ictalurus punctatus*) were provided by the Fish-Pesticide Research Laboratory, Columbia, Mo. Bluegills (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) were obtained from the National Fish Hatcheries at Corning and Mammoth Springs, Ark., respectively. The catfish had an average weight of 52 g, the bluegills 55 g, and largemouth bass 121 g. They were maintained in concrete raceways in flowing well water at 17° and fed a standard pelleted fish food for 2 weeks. Then they were weighed, treated for 10 sec in a 10 mg/l. solution of malachite green to prevent fungus infection, and stocked into plastic pools. They were held in the pools for 2 weeks before exposure to labeled DMA-2,4-D and fed *ad libitum* throughout the experiment.

Tests with bluegills and largemouth bass were conducted in plastic pools 3 m in diameter and 0.8 m deep. Channel catfish were treated in 2.4 m × 0.5 m pools. The large pools contained 3400 l. of well water (pH 7.7; total hardness 275 mg/l. as CaCO$_3$) and the small pools contained 1400 l. A 2.5-cm layer of clay-loam soil was spread over the bottom of each pool.

Fish were exposed to concentrations of 0.5, 1.0, or 2.0 mg/l. of DMA-2,4-D. Ten (10.0) milligrams of radioactive DMA-2,4-D (specific activity, 35.11 μCi/mg) were placed in each pool, and sufficient technical grade DMA-2,4-D was added to bring the concentration to either 0.5, 1.0, or 2.0 mg/l. of total DMA-2,4-D. One control pool was maintained for each species.

Two fish were removed from each pool on the day of treatment and at 1, 3, 7, 14, 21, 35, 49, 63, and 84 days thereafter. Water and hydrosol samples also were taken at these intervals.

Uptake and Dissipation Study. This study was conducted in large plastic pools. Fish were exposed to a 1.0 mg/l. concentration of DMA-^{14}C-2,4-D for 2 weeks. Then two individuals of each species were analyzed for radioactive residues in the tissues, and the remainder of the fish were transferred to herbicide-free water. Two of each species of the latter fish were analyzed at intervals of 1, 2, and 4 weeks to measure the decline of radioactive residues in their tissues with time.

Uptake and Whole-Body Residue Study. Channel cat-

fish, bluegills, and largemouth bass used in the whole-body residue study weighed 0.5-3.0 g and were provided by the Fish-Pesticide Research Laboratory. Fish were exposed to DMA-2,4-D in fiberglass tanks (0.84 m × 0.5 m × 0.64 m deep) containing 45 l. of reconstituted water (pH 7.1; alkalinity 35 mg/l.; hardness 40 mg/l. as CaCO$_3$) and maintained in a constant temperature water bath at either 17 or 25 ± 0.5°. Fish were fasted for 2 days and then exposed to DMA-^{14}C-2,4-D (specific activity, 0.165 μCi/mg) at a concentration of 2.5 mg/l. They were removed from fiberglass tanks for whole-body residue analysis at 4, 7, and 14 days after treatment.

pH Study. This study also was conducted in fiberglass tanks. Fish were exposed to a 2.5 mg/l. solution of DMA-^{14}C-2,4-D in reconstituted water maintained at 17 ± 0.5°, but the pH was adjusted to either 6 or 9. The pH was monitored daily and adjusted to the original value when necessary.

Toxicity Study. The acute toxicity of DMA-2,4-D to channel catfish and bluegills was determined at two temperatures (17 and 25°) and to fathead minnows (*Pimephales promelas*) at 17°. Tests were conducted in glass jars which contained 15 l. of reconstituted water at a pH of 7.1, alkalinity of 35 mg/l., and a hardness of 40 mg/l. Toxicity values were determined at 96 hr.

Tissue Residue Determination. Samples of fish from plastic pools were brought into the laboratory, anesthetized in a 200 mg/l. solution of tricaine methanesulfonate (MS-222), weighed, and samples of blood were collected immediately.

Blood samples (usually 200 μl) were obtained from bluegills by severing the caudal fin and collecting the blood from caudal vessels. Blood samples (0.5 to 1.0 ml) from channel catfish and largemouth bass were obtained from the caudal vessels with a syringe.

Blood samples were put into a vial containing 5 ml of cold acidified diethyl ether and sonified for 1 min in a 50-W sonifier. The ether was decanted into a vial after the cellular debris settled, and the sediment was reextracted twice with acidified ether. Two to three drops of hydrogen peroxide (30%) was added to the combined ether extracts, and the vials were set aside until the ether evaporated. After the ether had evaporated, 10 ml of toluene-fluor, 4 ml of Triton X-100-fluor mix (2:3, v/v), and four drops of a saturated solution of ascorbic acid were added to each vial. They then were counted in the scintillation counter. The vials containing the sediment were treated with eight drops of hydrogen peroxide and were shaken while being warmed on a hot plate until the reaction subsided. If the deep red-brown color persisted, more hydrogen peroxide was added until the solution was light yellow. The vial was capped lightly and set aside for 1 to 2 days. After this time, the toluene-fluor (10 ml) and Triton X-100-fluor (4 ml) and four drops of ascorbic acid were added, and the vial was placed in the scintillation counter. Blood samples from control fish were treated in the same manner. Extraction efficiency of DMA-^{14}C-2,4-D from spiked blood samples was 87.5 ± 2.5%.

After the blood samples were taken from the fish, the spinal cord was severed and samples of tissue were dissected for analysis. Tissues were weighed, placed in vials containing 4 ml of Triton X-100-scintillation cocktail (2:3, v/v) mixture, and homogenized in a glass homogenizer with a Teflon pestle. The homogenates were transferred quantitatively to a glass scintillation vial using a total of 10 ml of toluene-fluor. The vials then were placed in a scintillation counter. Counting efficiency was 85 ± 5%. Tissues from nonradioactive fish were spiked with [^{14}C]toluene to determine quench. All samples were corrected for quench by use of internal and external standards.

Whole-Body Residue Determination. Fish were re-

Table I. Residues (mg/kg or mg/l.) of DMA-^{14}C-2,4-D in Tissues of Channel Catfish, Largemouth Bass, and Bluegills and in the Water and Hydrosol from Plastic Pools in Which the Fish Were Exposed to a 2.0 mg/l. Concentration of the Herbicide for up to 12 Weeks[a]

Sample	Weeks after treatment								
	1	7	12	1	7	12	1	7	12
Tissue[b]	Channel catfish			Largemouth bass			Bluegills		
Blood	0.10	38.74	29.95	0.13	1.06	6.02	0.67	8.57	68.05
Brain	0.17	58.63	27.25	0.12	3.63	5.12	0.27	37.39	98.63
Gill	0.16	45.32	28.06	0.14	2.20	6.27	0.45	35.47	71.51
Bile	106.49	10.97	5.04	5.68	3.47	1.35	17.34	12.28	24.75
Liver	0.77	48.31	22.17	0.30	3.40	7.29	2.62	32.12	61.78
A-kidney[c]	0.06	52.95	26.60						
P-kidney[d]	0.38	48.82	35.51	0.70	2.37	4.49	3.32	31.33	88.05
S-muscle[e]	0.06	7.33	5.63	0.08	0.22	1.32	0.13	4.16	39.59
L-muscle[f]	0.05	17.08	13.47	0.06	0.32	2.53	0.14	5.85	29.91
Storage fat				1.04	1.42	3.31			
Pyl-caeca[g]				0.32	4.29	7.14	2.80	84.94	322.74
Eggs					0.96	5.97	0.29[h]		76.47[h]
Testes				0.14[h]		4.25		22.15	65.81[h]
Water	1.68	0.02	<0.01	0.40	0.05	<0.01	0.50	<0.01	<0.01
Hydrosol	0.21	<0.01	0.02	0.10	<0.01	<0.01	0.08	0.06	<0.01

[a] Water and hydrosol residues based on glc analyses. Tissue residues based on radiometric analyses. [b] Values are means of two fish. [c] Anterior kidney. [d] Posterior kidney. [e] Striated muscle. [f] Lateral line muscle. [g] Pyloric caeca. [h] Data from one fish only.

moved from the fiberglass tanks, rinsed with water, weighed, and frozen. The frozen fish were ground with twice their weight of Dry Ice to a fine powder in a Waring blender (Benville and Tindle, 1970). The Dry Ice was allowed to sublime in a freezer over a 24-hr period. For total residue analysis, 20 g of fish powder was mixed with 80–100 g of anhydrous Na_2SO_4 and allowed to stand for 45 min with occasional stirring. This mixture was placed in a stainless steel grinding flask with 50 ml of methanol-phosphoric acid (99:1, v/v) and ground at medium high speed for 1 min. An additional 25 ml of methanol-phosphoric acid mixture was added to the flask, and the contents were ground for an additional minute. The slurry was suction-filtered through Whatman No. 1 filter paper, the flask was rinsed with methanol-phosphoric acid, and the rinse was filtered. The volume of filtrate was measured, and three 1-ml samples were taken for scintillation counting. The filtrate was placed in a 2-l. separatory funnel containing 1 l. of deionized water which was adjusted to pH 8 with sodium bicarbonate. Petroleum ether (100 ml) was added to the separatory funnel, and the mixture was shaken vigorously for 2 min. This step was repeated twice. The petroleum ether was reduced to about 10 ml in a Kuderna–Danish evaporator and saved for future analysis. The water–methanol mixture was acidified with phosphoric acid to pH 4 (CAUTION: gas evolution!). This mixture again was extracted three times with 100-ml por-

tions of petroleum ether (CAUTION: release pressure often!). The petroleum ether was treated as before. The water-methanol mixture was acidified with phosphoric acid to pH 1.5–2.0 and then extracted three times with 150-ml portions of diethyl ether. The diethyl ether was passed through a glass column containing 12 cm of anhydrous sodium sulfate. The column was rinsed and the diethyl ether was evaporated to 2 to 3 ml in a Kuderna–Danish evaporator or a rotary evaporator. Three samples of 0.5 ml were taken for radiometric determination, and the remainder of the diethyl ether extract was derivatized with diazomethane for quantification by gas–liquid chromatography (glc). Recovery from spiked samples was 95 ± 2.5%, as determined by radiometric analysis, and was 85 ± 2.5%, as determined by glc analysis.

Water Analyses. Residues of DMA-2,4-D in water were determined by adding 100–500 ml of water to a 1-l. separatory funnel. The water was acidified to pH 1.5–2.0 with concentrated phosphoric acid and shaken vigorously for 2 min. The water was extracted three times with 100-ml portions of chloroform, and the funnel was rinsed with 20 ml of chloroform. The chloroform was evaporated just to dryness with a rotary evaporator (CAUTION: do not allow water bath temperatures to exceed 45°). The flask was cooled, and the 2,4-D was derivatized with diazomethane for analysis by glc. Recovery from spiked water samples was 95 ± 2.5%.

Hydrosol Analyses. Hydrosol (35 g) was placed into a stainless steel grinding flask. The following mixture was then added to the flask: 0.5 ml of 20% H_2SO_4; 7.5 ml of acetone; 12.5 ml of petroleum ether; 50 ml of diethyl ether; and 5 g of Celite 545. The resultant slurry was ground for 2 min, and the liquid was suction-filtered through Whatman No. 1 filter paper. This procedure was repeated again, and the soil was washed with two 25-ml aliquots of diethyl ether-petroleum ether (1:1, v/v). The filtrate was poured into a 1-l. separatory funnel and partitioned twice with 100-ml portions of 0.05 N NaOH. The aqueous phase was acidified by dropwise additions of 20% H_2SO_4 and then quantitatively transferred to a 1-l. separatory funnel. The acidified aqueous phase was partitioned three times with 50-ml portions of chloroform. The chloroform was evaporated just to dryness with a rotary evaporator and the remaining residue was derivatized with diazomethane. Recovery from spiked hydrosol samples was 87.5 ± 2.5%.

Table II. Loading Ratio, Biomass, and 2,4-D Concentration Ratio for Fish Exposed to 0.5, 1.0, or 2.0 mg/l. Concentrations of DMA-^{14}C-2,4-D

Species and number of fish	Exposure[a] level	Loading[b] level	mg of 2,4-D/g of fish
Channel catfish, 22	0.5	0.81	0.62
	1.0	0.88	1.14
	2.0	0.74	2.70
Bluegills, 20	0.5	0.28	1.79
	1.0	0.33	3.03
	2.0	0.33	6.06
Largemouth bass, 30	0.5	1.05	0.48
	1.0	1.01	0.99
	2.0	1.15	1.74

[a] Mg of 2,4-D/l. of water. [b] g of fish/l. of water.

Table III. Residues in Muscle of Fish Exposed to 2 mg/l. Concentrations of DMA-^{14}C-2,4-D in Plastic Pools for up to 84 Days (As Determined by Radiometric and glc Procedures)

Species and procedure	Residues in mg/kg after exposure for, in days								
	1	3	7	14	21	35	49	63	84
Channel catfish									
[14]C	[a]	0.018	0.022	0.014	0.036	0.360	0.262	0.403	0.953
glc	ND[b]	TR[c]	TR	0.016	ND	ND	ND	ND	ND
Bluegills									
[14]C		0.017	0.043	0.139	0.383	0.400	0.383	0.426	1.065
glc	ND	TR	TR	ND	ND	0.050	ND	TR	ND
Largemouth bass									
[14]C	0.017	0.052	0.017	0.035	0.017	0.061	0.008	0.035	0.035
glc	ND	ND	TR	TR	ND	ND	ND	ND	ND

[a] No data available. [b] ND = not detectable. [c] TR = trace, less than 5 μg/kg.

Derivatization of 2,4-D. The ether solutions of 2,4-D were derivatized by adding diazomethane (ether solution, Aldrich Chemical Co.; see precautions in Pesticide Analytical Manual, 1970) with an eyedropper until a yellow color persisted (Howard and Yip, 1971). The mixture was allowed to react at room temperature for 10 min. Most of the solvent was evaporated, 3 ml of benzene was added, and the solvent was evaporated again until the diethyl ether was removed. Silica gel (70–325 mesh ASTM, No. 7754, Brinkmann Instruments) was activated by heating at 130° for 3 hr. When cool, it was slurried with benzene and poured into a glass column (1 × 30 cm) to a height of 12 cm. The column was washed with 20 ml of benzene. The 2,4-D methyl ester was added to the column and eluted with five 10-ml portions of 2% diethyl ether in benzene. The eluate was evaporated to 3–4 ml, transferred quantitatively to a 5- or 10-ml volumetric flask, and brought up to volume with benzene. This solution was used for quantification by glc.

Gas Chromatograph. The glc determinations were done using a Perkin-Elmer Model 880 gas chromatograph equipped with a [63]Ni detector. The column was coiled glass (⅛ in. × 6 ft) packed with 1.5% OV-17 on Chromosorb G. The column temperature was 190°; injector temperature was 220°; and detector temperature was 265°. Nitrogen flow was 40 ml/min. Chart speed was 5 cm/min. Retention time of 2,4-D methyl ester was approximately 2 min. Minimal detectable amount of 2,4-D was 2 ng.

RESULTS AND DISCUSSION

A complete set of data from the Uptake and Tissue Distribution Study and from the Water and Hydrosol Analyses will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W. Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-73-186. A representative sample of the data is included in Table I.

Uptake and Tissue Distribution Study. The first tissue in which significant amounts of radioactive residue were detected was the gallbladder (bile). After exposure to a 2 mg/l. solution of DMA-^{14}C-2,4-D for 1 week, the bile of channel catfish and bluegills had radioactive residue levels of more than 17 mg/kg (Table I). After 7 weeks' exposure, levels of radioactive residue exceeded 1 mg/kg in the liver and kidney of fish exposed to a 2 mg/l. solution of herbicide (Table I). Radioactive residue was detected in all tissue analyzed, including storage fat (largemouth bass only), eggs, and testes. After 84 days' exposure, the striated muscle (S-muscle), which comprises the edible portion of fish, had lower levels of radioactive residue than any other tissue except the gallbladder. It was not possible to

quantify the actual amount of 2,4-D represented by the radioactivity, since all of the tissue was used for radiometric determinations except the liver and muscle. No fish mortalities occurred during the 84-day exposure period, nor were adverse biological effects observed at these exposure levels.

Channel Catfish. The gallbladder was the principal tissue in which large concentrations of radioactive residue were detected. This radioactivity peaked after exposure for 7 days and declined thereafter. Presumably, the radioactive residue either was eliminated from the body of the fish or was redistributed to other tissues through physiological processes. Generally, there was an increase in radioactive residues in all other tissue in fish treated in 0.5 and 1.0 mg/l. solutions. In fish treated with a 2.0 mg/l. solution, the radioactive residue in most tissues peaked at 5 weeks' exposure and declined thereafter.

Largemouth Bass. The amounts of radioactive residue in bass never reached the levels found in most channel catfish tissues at each sample time. Bass treated in 2.0 mg/l. solutions contained residues greater than 10 mg/kg in only three instances, whereas channel catfish has residue concentrations greater than 10 mg/kg in 34 instances. A partial explanation may be found by examination of the fish-loading ratios and 2,4-D concentration ratios (Table II). It was observed that bass had the highest loading ratios of the three species used and the lowest concentration ratios. The differences in the loading ratios and concentration ratios were the result of three factors: bass were in larger pools; bass were twice the weight of the channel catfish; and more bass than channel catfish were stocked in each pool.

Bluegills. Bluegills accumulated large amounts of radioactive residue during their exposure (Table I). High residue levels (from 1 to 126 mg/kg) were reached by the third week of exposure and continued to increase throughout the duration of the experiment in most tissues.

It is likely that the differences in residue levels between bluegills and the other species again were due in part to differences in the loading and 2,4-D concentration ratios. The bluegill loading ratio was low, resulting in a 2,4-D concentration ratio which was more than twice that of channel catfish and more than three times that of the largemouth bass.

The high residue level found in the pyloric caeca may have been due to concentrations of the radioactive material within that tissue prior to excretion.

Muscle samples from fish treated in 2 mg/l. solutions also were analyzed for 2,4-D content using the whole-body residue extraction procedure. Small portions of the diethyl ether extract were counted by scintillation spectrometry, and the remainder of the ether extract was derivatized for quantification by glc (Table III). When the results of this study are compared with those in Table I, it is evident

Table IV. Radioactive Residues in Tissues of Channel Catfish, Bluegills, and Largemouth Bass Following 2 Weeks' Exposure to a 1 mg/l. Concentration of DMA-^{14}C-2,4-D, and Maintenance in Fresh Water for up to 4 Weeks[a]

Species and tissue	Residues[b] in mg/kg after maintenance in fresh water for, in weeks			
	0	1	2	4
Channel catfish				
Blood	0.00	0.72	0.65	0.63
Brain	1.40	1.15	1.15	1.00
Gill	1.73	1.47	1.54	1.03
Bile	1.46	0.16	0.24	0.15
Liver	1.24	0.94	3.27	1.20
Gonads	0.34	c		
A-kidney[d]	0.40	0.75	1.14	0.61
P-kidney[e]	0.67	0.70	1.12	0.43
S-muscle[f]	0.06	0.19	0.12	0.14
L-muscle[g]	0.14	0.12	0.27	0.13
Bluegills				
Blood	5.96	10.44	4.51	8.41
Brain	10.44	10.40	14.86	14.14
Gill	8.38	7.05	18.71	9.69
Pyl-caeca[h]	21.50	14.05	13.04	10.11
Bile	9.71	2.66	10.72	3.53
Liver	9.10	16.11	22.87	7.70
Gonads	5.45			4.05
P-kidney	10.46	12.67	15.42	12.11
S-muscle	1.10	1.38	2.20	2.41
L-muscle	2.59	3.28	4.71	3.84
Largemouth bass				
Blood	1.12	0.94	0.92	0.45
Brain	1.28	1.45	1.04	1.02
Gill	1.10	1.25	1.19	0.48
Storage fat	1.08	2.43	1.11	1.16
Pyl-caeca	1.66	2.10	1.01	0.60
Bile	0.72	0.65	0.69	1.45
Liver	1.70	5.70	4.01	0.82
Eggs	1.55	1.45	2.15	
Testes	1.27			1.00
P-kidney	1.15	1.48	6.72	0.62
S-muscle	0.12	0.28	0.23	0.20
L-muscle	0.33	0.47	0.26	0.73

[a] Average weight of fish: channel catfish, 53.8 g; bluegills, 58.9 g; largemouth bass, 124.9 g. [b] Values are means of two fish. [c] Denotes no tissue. [d] Anterior kidney. [e] Posterior kidney. [f] Striated muscle. [g] Lateral line muscle. [h] Pyloric caeca.

Table V. Residues in Muscle of Fish Following a 2-Weeks' Exposure to a 1 mg/l. Concentration of DMA-^{14}C-2,4-D and Maintenance in Fresh Water for up to 4 Weeks (as Determined by Radiometric and glc Procedures)

Species and procedures	Residues in mg/kg after, weeks			
	0	1	2	4
Channel catfish				
^{14}C	0.018	0.022	0.022	0.018
glc	ND[a]	ND	ND	TR[b]
Bluegills				
^{14}C	c	0.139	0.148	0.191
glc	ND	ND	ND	TR
Largemouth bass				
^{14}C	0.166	0.061	0.043	0.017
glc	TR	ND	ND	ND

[a] ND = not detectable. [b] TR = trace, less than 5 μg/kg. [c] Sample lost.

study, fish were exposed to a 1.0 mg/l. concentration of herbicide for 2 weeks before being removed to herbicide-free water. The dissipation of DMA-^{14}C-2,4-D residues in the three species of fish was slow during the first 4 weeks after being placed in herbicide-free water (Table IV). There was a decline of radioactivity in most tissues at the 4-week sampling period. In some tissues, the residue level declined in quantity during the 4-week withdrawal period whereas, in others, it peaked at the 2-week harvest and declined from 2 to 4 weeks. The rise in residues in some tissues from the initial to the 1- or 2-week samples may have been due to redistribution of residues within the various tissues and organs.

When samples from this study were extracted and analyzed for residues by radiometric and glc procedures (Table V), the residue levels either were nonexistent or much lower than those found by the homogenization procedure (Table IV), again indicating that metabolism of the herbicide had occurred.

Uptake and Whole-Body Residue Study. This portion of the study was conducted to determine the uptake of DMA-2,4-D with time and to determine whether uptake differed with temperature. The highest residue found by radiometric analysis of the ether extract was 0.122 mg/kg in channel catfish exposed to a 2.5 mg/l. concentration of DMA-^{14}C-2,4-D for 14 days at 25° (Table VI). All other values, with one exception, were less than 0.1 mg/kg. The only significant differences in uptake due to temperature were between largemouth bass treated at 17 and 25° for 14 days and bluegills treated at 17 or 25° for 4 days. Bluegills treated at 17° for 14 days were the only samples containing 2,4-D residues greater than trace amounts when analyzed by glc.

pH Study. The data from the pH study are shown in Table VII. Based on radiometric analysis of the ether extracts, it was found that channel catfish and bluegills accumulated more 2,4-D residue from an acidic medium (pH 6) than from a basic medium (pH 9). The differences were significant at the 5% level in five of six cases. Presumably, DMA-2,4-D is less ionic and more lipophilic in an acidic medium and, therefore, is more readily taken up through the fish gill. However, bluegills treated at pH 9 for 7 and 14 days had significantly higher amounts of 2,4-D than those treated at pH 6. It is probable that the more basic pH suppresses the metabolism of 2,4-D.

Toxicity Study. The results of this study are shown in Table VIII. There was a striking species difference in susceptibility to DMA-2,4-D, with bluegills having a 96-hr TL_{50} value of 177 mg/l. at 17°, whereas fathead minnows had a 96-hr TL_{50} value of 335 mg/l. TL_{50} values for channel catfish and bluegills were negatively correlated with temperature.

that the much higher values in Table I for muscle tissue are not indicative of actual 2,4-D content, but reflect the fact that metabolism of the herbicide had occurred. There was an increase in 2,4-D content with time as determined by radiometric procedures (Table III). However, 2,4-D residues greater than trace amounts were detected in only 2 of 30 samples analyzed by glc. Thus, the results in Table I are indicative mainly of radiolabeled metabolites, whereas glc analyses show very negligible residues of 2,4-D in all but two instances. From examination of the results of radiometric analyses in Table III, it also is evident that some metabolite(s) was carried through the extraction process because most of the radioactive material was found not to be 2,4-D by glc analysis. Furthermore, when some of the ether extract was spotted and developed on thin-layer plates, less than 10% of the radioactivity had an R_f corresponding to that of 2,4-D (Schultz, 1971).

Uptake and Persistence Study. Generally, herbicides are applied to an aquatic ecosystem once a year. Occasionally, a second application may be necessary. Thus, it is necessary to determine how long residues remain in fish after application of the herbicide has ceased. In this

Table VI. Whole-Body Residues in Channel Catfish, Bluegills, and Largemouth Bass Exposed to a 2.5 mg/l. Concentration of DMA-^{14}C-2,4-D at 17 ± 0.5° or 25 ± 0.5° for 4, 7, or 14 days (as Determined by Radiometric and glc Procedures)

Species	Exposure, days	Average[a] mg/kg (±SE) in fish exposed at			
		17°		25°	
		C-14	Glc	C-14	Glc
Channel catfish	4	0.068 ± 0.010	ND[b]	0.057 ± 0.003	ND
	7	0.065 ± 0.016	ND	0.076 ± 0.024	ND
	14	0.078 ± 0.000	ND	0.122 ± 0.040	TR[c]
Largemouth bass	4	0.052 ± 0.007	ND	0.083 ± 0.003	ND
	7	0.070 ± 0.000	TR	0.089 ± 0.005	TR
	14	0.031 ± 0.000	ND	0.094 ± 0.002	ND
Bluegills	4	0.057 ± 0.003	ND	0.116 ± 0.007	ND
	7	0.035 ± 0.000	ND	0.073 ± 0.031	TR
	14	0.067 ± 0.000	0.012	0.044 ± 0.024	ND

[a] Means of two to six experiments. [b] ND = not detectable. [c] TR = trace, less than 5 μg/kg.

Table VII. Whole-Body Residues in Channel Catfish and Bluegills Exposed to a 2.5 mg/l. Concentration of DMA-^{14}C-2,4-D at pH 6 and pH 9 (as Determined by Radiometric and glc Procedures)

Species	Exposure, days	Average mg/kg (±SE) in fish exposed at			
		pH 6		pH 9	
		C-14	Glc	C-14	Glc
Channel catfish	4	0.203 ± 0.024	TR[a]	0.051 ± 0.003	ND[b]
	7	0.175 ± 0.030	TR	0.060 ± 0.010	TR
	14	0.190 ± 0.033	TR	0.053 ± 0.005	TR
Bluegills	4	0.243 ± 0.024	TR	0.088 ± 0.010	TR
	7	0.343 ± 0.047	0.010 ± 0.003	0.081 ± 0.002	0.070 ± 0.005
	14	0.396 ± 0.017	TR	0.114 ± 0.014	0.050 ± 0.005

[a] TR = trace, less than 5 μg/kg. [b] ND = not detectable.

Water Analyses. The results of the water analyses from the plastic swimming pools are tabulated in Table I. There was some variability in 2,4-D residue throughout the sampling periods. These variations probably were due primarily to environmental factors such as evaporation and rainfall. Also, some water samples were taken immediately after the fish were harvested when the hydrosol was stirred up. At 35 days the highest level found in any of the pools was 0.05 mg/l.

Hydrosol Analyses. The amounts of radioactive residues in hydrosol samples from the plastic pools generally decreased from the seventh day of the experiment to the end (Table I). There was some fluctuation in residue amounts between various pools at different sampling dates. These fluctuations, like those in the water samples, may have been due to environmental changes or due to too much water in the hydrosol sample. It was noticed that the amount of unextractable radioactivity increased with time of harvest, indicating either metabolism of the parent compound or strong adsorption to the hydrosol particles. Extraction efficiency of spiked soil which had been refrigerated for more than 2 weeks was 97 ± 2.5%, suggesting that the unextractable radioactivity in the hydrosol probably consisted of 2,4-D metabolites. The highest amount of 2,4-D residue at any harvest time was 0.21 mg/kg at 7 days in a catfish pool treated with a 2 mg/l. concentration.

Since much of the biomass present in a pond, such as aquatic plants, insects, algae, and invertebrates, which could absorb and degrade the herbicide is not present in plastic pools, the disappearance of DMA-2,4-D from water and hydrosol must be attributed to other factors. Aly and Faust (1964) reported a photodecomposition loss of 50% in 50 min at pH 7.0 of a sodium salt of 2,4-D, while Loos

Table VIII. Toxicity of DMA-2,4-D to Channel Catfish, Bluegills, and Fathead Minnows at 17 and 25°[a]

Species[b]	96-hr TL$_{50}$ at	
	17°	25°
Channel catfish	193 mg/l.	125 mg/l.
Bluegills	177 mg/l.	160 mg/l.
Fathead minnows	335 mg/l.	c

[a] Against antimycin reference. Conducted in reconstituted water (15 l. in glass jars), pH 7.0, alkalinity 35 mg/kg, hardness 40 mg/kg. [b] Average fish weight: CCF 1.4 g, BLG 0.7 g, FHM 0.8 g. [c] No data available.

(1969), in his review of 2,4-D metabolism, reported that many microorganisms are capable of degrading 2,4-D. Hence, photodecomposition and microbial degradation undoubtedly were the primary causal factors in the dissipation of 2,4-D from water and hydrosol in the plastic pools.

As mentioned earlier, less than 10% of this residue is due to the parent molecule. In a preliminary investigation, using whole fish, the major metabolite was characterized as a 2,4-D-glucuronic acid conjugate on the basis of acid and enzymatic hydrolysis. There was also strong evidence for a phenol-type compound and possibly a 2,4-D-sulfate conjugate (Schultz, 1971). Current investigators have found at least six metabolites of 2,4-D in fish (Stalling, 1972). Thus, in contrast to many of the organochlorine pesticides which undergo biomagnification through the food chain, DMA-2,4-D is metabolized in fish without significant accumulation of the parent compound. This

may be due to the polar, hydrophilic characteristics of
DMA-2,4-D.

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Immunochemical-Cytological Study of Proteins from Partially Defatted Peanuts

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To help open new markets for peanuts and pea-
nut products, Southern Regional Research Labora-
tory is developing various processing conditions
to obtain low-fat peanuts (Pominski et al., 1970;
Vix et al., 1967). In this study, we report inte-
grated analyses correlating cytological ultrastruc-
ture with immunochemistry and electrophore-
sis of proteins of seeds that were partially defat-
ted by hydraulic pressing and reconstituted with
hot water. Pressing alone does not appreciably
affect the qualitative disk and immunoelectro-
phoretic patterns of the proteins; however, the
hot water treatment does. Protein solubility is
negligibly reduced by pressing out the oil but re-
constitution with hot water reduced it twofold.
Electron micrographs of the pressed and recon-
stituted seeds showed progressive disruption of
subcellular organelles and membranes. Trypsin
inhibitors were inactivated after the hot water
treatment.

The conventional approach to preparing peanuts for
snack use is either dry or oil roasting. For peanuts and
some of the edible tree nuts, the process achieves desir-
able texture and flavor, and in some cases mild heat im-
proves nutrition. A simple step to remove excess oil from
peanuts, while increasing protein content, is one way of
providing nutritious products for the general public.
Though it is recognized that vitamins and minerals are
also essential to good nutrition, high-quality protein foods
are a special case where people are demanding more op-
tions at moderate prices.

Laboratory-prepared peanut flours subjected to heat,
pressure, and steaming under several conditions of time
and temperature have received much attention in recent
years (Anantharaman and Carpenter, 1969; McOsker,
1962; Neucere et al., 1972; Woodham and Dawson, 1968).
Changes in physicochemical properties of peanut proteins
under various processing conditions and general charac-
terization of the proteins have also been reported (Daus-
sant et al., 1969; Dawson, 1971; Dieckert et al., 1962;
Neucere, 1972; Neucere et al., 1969; Tombs, 1965). All of
these studies revealed the complexity of peanut proteins
as evidenced by results from several different types of
analyses.

In this article we report an integrated study of cellular
ultrastructure and electrophoretic-immunochemical prop-
erties of proteins from seeds that were deoiled by hydrau-
lic pressing followed by reconstitution with hot water.
Solubility differences and trypsin inhibition affected by
processing are also discussed.

MATERIALS AND METHODS

Virginia peanut cotyledons were hydraulically pressed
and reconstituted with hot water as described by Vix *et*

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al. (1967). Seeds were pressed out at 2000 psi for 60 min
and then placed in hot water from 3 to 8 min. The deoil-
ing process removed about 75% of the oil, leaving seeds
with 40-45% protein. The expansion step (hot water) re-
sulted in seed containing about 40% moisture; the fin-
ished product is then achieved by roasting to remove most
of the moisture.

For chemical analyses: (1) full-fat seeds were used as
the control; (2) the seeds were pressed; and (3) the recon-
stituted deoiled seeds were defatted with equal volumes of
cold acetone (5 g of seed per 20 ml of acetone). The pea-
nut flours were then extracted in phosphate buffer, pH 7.9,
ionic strength 0.2, with a hand homogenizer at room tem-
perature (50 mg of flour per 1 ml of buffer). The homoge-
nate was centrifuged at 35,000 × *g* for 20 min and the
final supernatants were used in the chemical analyses.

Protein contents were determined by the method of
Lowry et al. (1951). Immunoelectrophoresis on microscope
slides was carried out according to Grabar and Williams
(1953) in 1.5% Ionagar (Oxoid Ltd., London) in 0.025 *M*
veronal buffer, pH 8.2, at room temperature with a volt-
age gradient of 4 V/cm for 2 hr. Disk electrophoresis was
performed according to Steward et al. (1965) using 7.5%
acrylamide in the running gel, and a modification of the
standard Canalco procedure (Canalco Industrial Corpora-
tion, Bethesda, Md.) using 7.5% Cyanogum 41 (Fisher
Scientific Company) gelling agent in the running gel and
3.0% of same in the stacking gel; tris-glycine buffer, pH
8.4, was employed. Fixation for electron microscopy was
performed according to Luft (1956) and tissue embedding
in epoxy resin was performed according to Spurr (1969).

RESULTS

The morphological changes induced on the fine struc-
ture of the peanut by processing are shown in Figures 1, 2,
and 3. A typical cell in the native dormant seed (Figure 1)
shows the major subcellular organelles—aleurone grains,
spherosomes, and starch grains. Spherosomes, which con-