mylation of 4'-chloro-*o*-formotoluidide (VII). Direct cleavage of substituted phenylurea herbicides in mammals to yield substituted anilines has been postulated but apparently is not a major pathway. Therefore, it seemed likely that most of the 4-chloro-*o*-toluidine (VIII) was derived from 4'-chloro-*o*-formotoluidide (VII). This conversion has been well documented (Ahmad and Knowles, 1971a; Knowles, 1970), and an enzyme capable of catalyzing the deformylating reaction was partially purified from rat liver (Ahmad and Knowles, 1971b).

N-Formyl-5-chloroanthranilic acid (IX) was formed by oxidation of the tolyl methyl moiety of 4'-chloro-*o*-formotoluidide (VII), and the 5-chloroanthranilic acid (X) was formed by deformylation of *N*-formyl-5-chloroanthranilic acid (IX) and/or by oxidation of the tolyl methyl moiety of 4-chloro-*o*-toluidine (VIII) (Knowles, 1970; Knowles and Sen Gupta, 1970).

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Residues of Diflubenzuron [1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea] in Pasture Soil, Vegetation, and Water following Aerial Applications

Charles H. Schaefer* and Emil F. Dupras, Jr.

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] was applied to irrigated pastures using liquid (25% wettable powder in water) and granular (1% Attaclay and 1% sand) formulations. Multiple applications of the wettable powder produced high and long-lasting residues on vegetation; these residue levels were reduced but not eliminated using the 1% Attaclay granules. One percent sand granules achieved control of mosquito larvae and no high or persistent residues on vegetation resulted. None of these formulations caused high or persistent residues in soils.

Diflubenzuron, TH6040, or Dimilin [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] shows high potential as a mosquito control agent (Schaefer et al., 1975). Since diflubenzuron is projected to be used as a commercial larvicide, its persistence in mosquito breeding habitats is of high interest. It has been demonstrated that its persistence in pasture waters is limited to a few days following aerial applications of a 25% wettable powder formulation containing 0.02 to 0.04 lb of active ingredient (AI)/acre; hydrolysis, and adsorption onto organic matter, appear to limit persistence in water (Schaefer and Dupras, 1976). As it is anticipated that diflubenzuron will be used numerous times (each irrigation cycle) on the same fields in a given year, for controlling pasture mosquitoes (*Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar) in California, determinations of residues in water, soil, and vegetation following multiple applications were made.

MATERIALS AND METHODS

1975 Studies. Two 40-acre pastures were treated with four consecutive applications of diflubenzuron each during the 1975 field season. One field (Colosso pasture in western Stanislaus County) was treated at 0.02 lb of AI/acre and the other (Monteiro pasture in Tulare County) at 0.04 lb of AI/acre. These rates cover the projected range for operational use of diflubenzuron for pasture mosquito control. A 25% wettable powder formulation was mixed so that the aircraft dispersed the active ingredient in 1 gal of water/acre. No other chemical treatments were made to these fields during the study period.

Before and at 1 h, 1, 3, and 7 days following each of the first three treatments and 1 h, 1, 3, 7, 28, and 56 days following the fourth treatment, soil and vegetation samples were collected from each field. Soil cores (3 in. diameter and ca. 3 in. depth) were collected, using a sharpened steel cylinder, from areas not having vegetative cover. Cores were collected from areas over the entire field until a composite sample of 5–6 lbs was obtained. Vegetation samples were cut from areas over the entire field until 3–4 lbs, fresh weight, was obtained. Soil and vegetation samples were stored at −20 °C until analysis. Water samples (4 × 600 mL) were collected before and at 1, 24, 48, and 72 h after each treatment.

1976 Studies. A comparative study of residues in water, soil, and vegetation was made using four multiple aerial applications of 0.04 lb of AI/acre of a liquid (25% wettable powder in 1 gal of water/acre) and a dry (1% diflubenzuron on 16/30 mesh Attaclay granules) formulation. One-half of a 12-acre pasture (File pasture, Fresno County) was treated with the wettable powder and one-half with

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Table I. Residues of Diflubenzuron in Pasture Soil following Multiple Aerial Applications of a 25% Wettable Powder Formulation

Sample collection time	Colosso pasture (0.02 lb of AI/acre)[a] Date (1975)	% H_2O	Concn,[b] ppm	Monteiro pasture (0.04 lb of AI/acre)[a] Date (1975)	% H_2O	Concn,[b] ppm
			First treatment			
Pre	5/15	25.0	ND[c]	6/11	18.5	ND
1 h	5/16	22.5	0.013	6/12	27.5	0.066
1 day	5/17	30.0	0.020	6/13	24.0	0.044
3 day	5/19	35.0	ND	6/15	23.5	0.036
7 day	5/23	29.5	0.016	6/19	24.0	0.050
			Second treatment			
Pre	6/16	35.0	0.016	6/19	as above	
1 h	6/17	27.5	ND	6/20	23.5	0.081
1 day	6/18	25.0	ND	6/21	23.5	0.020
3 day	6/20	30.0	ND	6/23	21.0	0.065
7 day	6/24	17.5	0.016	6/27	26.0	ND
			Third treatment			
Pre	7/15	27.5	ND	6/30	20.5	0.039
1 h	7/16	25.0	ND	7/1	23.5	0.050
1 day	7/17	22.5	ND	7/2	24.5	0.051
3 day	7/19	25.0	ND	7/4	25.0	0.040
7 day	7/23	27.5	ND	7/8	18.5	0.035
			Fourth treatment			
Pre	8/14	22.5	ND	7/17	22.5	0.039
1 h	8/15	22.5	0.013	7/18	25.0	0.032
1 day	8/16	25.0	0.022	7/19	26.0	0.043
3 day	8/18	25.0	ND	7/21	10.0	0.086
7 day	8/21	22.5	ND	7/25	19.5	ND
28 day	9/12	15.0	ND	8/15	13.5	ND
56 day	10/10	20.0	ND	9/12	7.0	ND

[a] Rate per each application. [b] Corrected for percent recovery and adjusted to dry weight basis. [c] ND is not detected (<0.0038 ppm).

the granular formulation to provide side by side plots for treatment with the same amounts on the same dates. Water samples (2 × 600 mL) were taken before and at 1 h following each treatment, at 1, 4, 24, and 48 h after the first treatment (to determine decay) and at 1 and 3 days following the last treatment. Soil and vegetation samples were collected, as previously described, before each treatment, at 1 h, and 1, 3, 5, and 7 days after the first treatment, 1 day after the second and third treatment, and 1, 3, 5, 7, 14, 28, and 56 days after the last treatment from both fields.

The 1% Attaclay granules were also applied to a 12-acre pasture (Costerisan pasture, Kern County) three times during 1976. Samples of water, soil, and vegetation were collected before and after each treatment, and soil and vegetation were collected 18 days after the last treatment.

1% Sand Granules. These were prepared, using a cement mixer, as follows: to 200 lbs of 20 mesh sand slowly add 600 mL of oil (Golden Bear 1356) and mix for 30 min, then add 8 lbs of 25% diflubenzuron wettable powder and mix for 15 min, add 100 g of amorphous silica (Hi-Sil 233) and mix an additional 20 min.

Two aerial applications of the 1% sand granules were made to a 50-acre gun club, which had 6–10 in. of standing water and contained large numbers of *Culex tarsalis* larvae. Samples of water (4 × 600 mL) were collected after each application; soil was collected before treatment, at 1 and 3 days following the initial application, and at 1, 17, and 28 days following the second treatment. Vegetation was collected before treatment, at 1 h and 1 day after each treatment, and at 17 and 28 days after the second treatment.

Two additional aircraft trials were made with the 1% sand granules to obtain efficacy data at the low side of the projected rate of application (0.025 lb of AI/acre, Cunha pasture, Fresno County) and at a rate above the projected use range (0.074 lb of AI/acre, Smith's pasture, Kern County). Water, soil, and vegetation were also collected from these fields for residue analysis (sampling times shown in the tables).

Extraction and Cleanup. Water was extracted by methods previously described (Schaefer and Dupras, 1976). Soil and vegetation were extracted and cleaned up by column chromatography according to the methods of Diprima et al. (1977). For each group of seven soil or vegetation samples subjected to cleanup by column chromatography, one untreated sample, fortified with 0.05 ppm diflubenzuron, was run simultaneously to determine percent recovery. The residue concentration for each sample of each group was later corrected by percent recovery for that group.

High-Performance Liquid Chromatography (HPLC). A Varian Model 8500 HPLC having a UV photometer (254 nm) was utilized for all quantitative analyses. A Micro-Pak-CH column (octadecylsilane bonded on 10-μm particles) 8 mm × 25 cm provided reverse-phase separations using 70% acetonitrile–30% water as the mobile phase, at a flow rate of 60 mL/h, at an ambient temperature of 30 °C, diflubenzuron had a retention time of 11 min. Thirty-microliter samples of the extracts and standards were subjected to HPLC.

Release of Diflubenzuron from Granules. Sixteen 1-gal jars containing 3000 mL of tap water each were treated with diflubenzuron granules to give an initial concentration of 0.1 ppm (eight jars with 1% Attaclay and eight with 1% sand). At the end of 4, 24, 48, and 72 h, two jars treated with each granule were filtered through a glass wool plug to remove solids and duplicate 600-mL aliquots from each jar were extracted and analyzed for diflubenzuron.

RESULTS AND DISCUSSION

1975 Results. Residues of diflubenzuron in field waters treated four times with 0.02 lb of AI/acre (Colosso pasture) and with 0.04 lb of AI/acre (Monteiro pasture) have al-

Table II. Residues of Diflubenzuron in Water from File Pasture following Multiple Aerial Applications of 0.04 lb of AI/Acre Each

Sample collection time	Date (1976)	Formulation: 25% wettable powder, Av concn, ppm[a]	Formulation: 1% Attaclay granule, Av concn, ppm[a]
Pre no. 1	7/19	ND	ND[b]
	7/19	First treatment	
1 h	7/19	0.0074	0.0045
4 h		0.0028	0.0025
1 day	7/20	0.0011	0.00053
2 day	7/21	ND	0.00059
Pre no. 2	8/1	ND	ND
	8/2	Second treatment	
1 h	8/2	0.0013	0.0020
1 day	8/3	c	0.0012
Pre no. 3	8/15	ND	ND
	8/16	Third treatment	
1 h	8/16	0.029	0.0022
Pre no. 4	8/29	ND	ND
	8/30	Fourth treatment	
1 h	8/30	0.0064	0.0011
1 day	8/31	0.00086	0.0018
3 day	9/2	c	ND

[a] Average to two duplicate 600-mL samples. [b] ND is not detected (<0.0002). [c] No standing water remained.

ready been reported (Schaefer and Dupras, 1976). Residues in soils from these fields are shown in Table I. Recoveries from fortified soil samples varied from 89–97%. The residues were generally low and it is apparent that there is no buildup with time. Diflubenzuron residues on the 1975 vegetation samples were run by the Thompson-Hayward Chemical Company, Kansas City, Kans. There was a definite increase in vegetation residues with time and these got as high as 2.3 ppm following four treatments of 0.02 lb of AI/acre (Colosso pasture) and 10.7 ppm following four treatments of 0.04 lb of AI/acre (Monteiro pasture) (Cannizzaro, 1976). These high residues on vegetation were regarded as being unreasonably high and led to 1976 attempts to utilize granules in order to lower these values.

1976 Results. *Recoveries of Diflubenzuron from Fortified Samples.* Recoveries of fortified samples were 70–103% and were similar to those reported (Diprima et al., 1977). However, using the HPLC method described herein, it was possible to detect as little as 0.0002 ppm from water and 0.0038 ppm from soil or vegetation (minimum peak was twice background).

Comparison of Residues from Wettable Powder and from 1% Attaclay Granules. Diflubenzuron residues of either formulation showed limited persistence in water (Table II) as in previous studies (Schaefer and Dupras, 1976); however, both formulations provided excellent control of *A. nigromaculis* larvae, which were present at the time of each treatment.

Residues of diflubenzuron in soil samples were generally low, and there is no evidence of buildup with time or long-term persistence (Table III). Relatively high residues on vegetation result from treatments with the liquid application of the wettable powder and significant residues persisted throughout the study period (Table IV). To verify that the peaks observed by HPLC were indeed diflubenzuron, fractions from the highest observed concentration (7-day sample following the fourth application of wettable powder) were collected, and the IR spectra of this sample and of an authentic diflubenzuron standard were run in microcells using a Beckman IR-18A; identical spectra confirm the assignment of the peak observed on the HPLC as diflubenzuron.

Use of the 1% Attaclay granules did reduce residues on vegetation but significant residues were still measured. This result was expected since during the applications of the 1% Attaclay granules it was observed that many fine

Table III. Residues of Diflubenzuron in Soil from File Pasture following Multiple Aerial Applications of 0.04 lb of AI/Acre Each

Sample collection time	Date (1976)	Formulation: 25% wettable powder, % H_2O	Formulation: 25% wettable powder, Concn, ppm	Formulation: 1% Attaclay granule, % H_2O	Formulation: 1% Attaclay granule, Concn, ppm
Pre no. 1	7/18	20.8	ND[b]	25.4	ND
	7/19		First treatment		
1 h	7/19	29.4	0.11	25.5	ND
1 day	7/20	23.9	0.030	23.9	0.044
3 day	7/22	18.3	ND	16.9	0.056
5 day	7/24	17.0	0.015	15.2	ND
7 day	7/26	18.6	0.015	18.1	ND
Pre no. 2	8/1	21.8	ND	18.6	ND
	8/2		Second treatment		
1 day	8/3	12.9	ND	29.6	0.32
Pre no. 3	8/15	20.0	ND	20.5	ND
	8/16		Third treatment		
Pre no. 4	8/29	22.6	0.023	20.0	0.016
	8/30		Fourth treatment		
1 day	8/31	21.9	ND	22.2	0.048
3 day	9/2	28.3	ND	20.4	0.036
5 day	9/4	17.7	ND	15.7	0.016
7 day	9/6	14.1	ND	17.0	0.025
14 day	9/13	22.7	0.016	15.2	0.039
28 day	9/27	7.4	ND	6.5	ND
56 day	10/25	5.8	ND	5.7	ND

[a] Corrected for percent recovery and adjusted to dry weight basis. [b] ND is not detected (<0.0038 ppm).

Table IV. Residues of Diflubenzuron on Vegetation from File Pasture following Multiple Aerial Applications of 0.04 lb of AI/Acre Each

Sample collection time	Date (1976)	Formulation: 25% wettable powder, % H_2O	Formulation: 25% wettable powder, Concn,[a] ppm	Formulation: 1% Attaclay granule, % H_2O	Formulation: 1% Attaclay granule, Concn,[a] ppm
Pre no. 1	7/18	74.8	ND[b]	66.4	ND
	7/19		First treatment		
1 h	7/19	70.0	0.83	76.4	0.069
1 day	7/20	63.1	0.22	77.0	0.14
3 day	7/22	69.9	0.12	71.2	0.085
5 day	7/24	72.2	1.06	72.7	0.049
7 day	7/26	67.4	1.51	72.1	ND
Pre no. 2	8/1	66.8	0.15	74.0	ND
	8/2		Second treatment		
1 h	8/2	68.5	0.23	68.6	0.084
1 day	8/3	67.6	1.49	72.2	0.11
Pre no. 3	8/15	69.6	2.12	72.2	0.027
	8/16		Third treatment		
1 h	8/16	64.1	2.13	71.9	0.080
1 day	8/17	74.2	1.11	70.1	0.053
Pre no. 4	8/29	65.9	0.34	66.4	ND
	8/30		Fourth treatment		
1 h	8/30	62.9	2.10	70.6	0.19
1 day	8/31	75.0	1.36	78.1	0.081
3 day	9/2	72.7	0.74	71.3	0.12
5 day	9/4	76.2	0.17	74.2	0.060
7 day	9/6	72.6	4.13	74.1	ND
14 day	9/13	75.1	0.28	76.6	0.043
28 day	9/27	65.4	0.24	72.5	ND
56 day	10/25	62.2	0.30	66.6	ND

[a] Corrected for percent recovery and adjusted to dry weight basis. [b] ND is not detected (<0.0038 ppm).

Table V. Residues of Diflubenzuron from Costerisan Pasture Treated with 1.0% Attaclay Granules at 0.061, 0.070, and 0.095 lb AI/Acre on July 30th, 1976, Aug 23rd, 1976, and Sept 9th, 1976, Respectively

Sample collection time	Date (1976)	% H_2O	Concn,[a] ppm
	Water samples		
1 h	7/30		0.0017
1 h	8/23		0.0015
1 h	9/9		0.0047
	Soil samples		
Pre no. 1	7/29	21.7	ND[b]
7 day	8/6	24.8	ND
Pre no. 2	8/23	24.5	ND
Pre no. 3	9/9	25.4	ND
1 day	9/10	25.7	ND
18 day	9/27	17.4	ND
	Vegetation samples		
Pre no. 1	7/29	77.8	ND
1 h	7/30	78.0	0.048
1 day	7/31	79.0	0.072
7 day	8/6	78.8	0.088
Pre no. 2	8/23	70.5	0.037
1 h	8/23	69.0	0.51
1 day	8/24	67.1	0.12
Pre no. 3	9/9	77.8	0.017
1 h	9/9	63.3	0.19
18 day	9/27	69.3	0.018

[a] Corrected for percent recovery and adjusted to dry weight basis. [b] ND is not detected (<0.0038 ppm).

Table VI. Residues of Diflubenzuron from Universal Gun Club following Aerial Applications of 1.0% Sand Granules at 0.044 lb of AI/Acre on Aug 20th, 1976 and 0.058 lb of AI/Acre on Sept 9th, 1976

Sample collection time	Date (1976)	% H_2O	Concn,[a] ppm
	Water samples		
1 h	8/20		0.019[b]
1 h	9/9		0.010[b]
4 h	9/9		0.0020[b]
	Soil samples		
Pre no. 1	8/20	30.8	ND[c]
1 day	8/21	31.3	0.062
3 day	8/23	31.3	0.019
Pre no. 2	9/9	32.4	ND
1 day	9/10	31.0	0.018
17 day	9/27	31.8	ND
28 day	10/28	31.3	ND
	Vegetation samples		
Pre no. 1	8/20	86.8	ND
1 h	8/20	82.5	0.38
1 day	8/21	82.8	0.11
3 day	8/23	84.0	0.31
Pre no. 2	9/9	70.2	ND
1 h	9/9	68.5	0.14
1 day	9/10	67.6	0.068
17 day	9/27	54.1	ND
28 day	10/8	70.3	ND

[a] Corrected for percent recovery and adjusted to dry weight basis. [b] ND is not detected (<0.0002 ppm for water, <0.0038 ppm for soil and vegetation). [c] Average of four 600 mL samples.

granules were present and these floated down very slowly and settled on the vegetation, rather than penetrating through to the water. Frequently the vegetation was wet, because of dew, at the time of treatment which could result in additional deposition of light granules. The fine granules apparently are gradually lost from the vegetation as long-term persistence of residues did not occur.

Higher application rates of the 1% Attaclay granules on the Costerisan pasture following three treatments showed

Table VII. Residues of Diflubenzuron from Cunha Pasture Treated with 1.0% Sand Granules on Sept 24th, 1976 to Give 0.025 lb of AI/Acre

Sample collection time	Date (1976)	% H_2O	Concn,[a] ppm
	Water samples		
1 h	9/24		0.0032
1 h			0.0050
1 h			0.0024
1 h			0.0063
		Av	0.0043
	Soil samples		
Pre	9/24	28.3	ND[b]
3 day	9/27	22.2	0.021
31 day	10/25	24.3	ND
	Vegetation samples		
Pre	9/24	82.7	ND
1 h	9/24	75.2	0.061
3 day	9/27	78.7	ND
31 day	10/25	72.1	ND

[a] Corrected for percent recovery and adjusted to dry weight basis. [b] ND is not detected (<0.0038 ppm for soil and vegetation).

Table VIII. Residues of Diflubenzuron from Smith's Pasture Treated with 1.0% Sand Granules on September 14th, 1976 to Give 0.074 lb of AI/Acre

Sample collection time	Date (1976)	% H_2O	Concn,[a] ppm
	Water samples		
Pre	9/14		ND[b]
1 h	9/14		0.0057[c]
4 h	9/14		0.0039[c]
24 h	9/15		0.0014[c]
	Soil samples		
Pre	9/14	33.6	ND
1 day	9/15	35.7	0.023
14 day	9/28	36.9	ND
	Vegetation samples		
Pre	9/14	70.5	ND
1 day	9/15	62.1	ND
14 day	9/28	72.5	ND

[a] Corrected for percent recovery and adjusted to dry weight basis. [b] ND is not detected (<0.0002 ppm water, <0.0038 ppm for soil and vegetation). [c] Average of four 600-mL samples.

a similar residue pattern (Table V), except for the values in water at 1 h which were lower and might be the result of greater water depth. Significant residues on vegetation did result from the treatments. Also, it was observed that drift of the finer granules occurred in light wind (3–5 mph); therefore, the 1% Attaclay granules must be considered as not feasible for operational use.

1% Diflubenzuron Sand Granules. Treatments of the Universal Gun Club with 0.044 and 0.058 lb of AI/acre resulted in 100% mortality of *C. tarsalis* larvae. Neither application resulted in significant soil residues (Table VI). The vegetation did show residues just after treatment, but these did not persist. The residues on vegetation are believed to be due to a portion of the wettable powder that did not adhere to the sand and settled as dust during the treatment.

Treatment of the Cunha pasture at 0.025 lb of AI/acre gave 100% control of *A. nigromaculis* larvae, with only negligible residues occurring in soil or on vegetation (Table VII). This result establishes efficacy at this relatively low application rate as well as demonstrates the potential for reducing vegetative residues through use of the sand granules.

Table IX. Release of Diflubenzuron from 1% Attaclay and 1% Sand Granules in Water Treated with 0.1 ppm AI

Formulation	Hour after treatment			
	4	24	48	72
1% Attaclay (lot 1)	0.011[a]	0.015	0.021	0.025
1% Attaclay (lot 2)	0.012	0.0098	0.011	0.016
Av	0.012[b]	0.012	0.016	0.020
1% Sand (lot 1)	0.014	0.026	0.030	0.030
1% Sand (lot 2)	0.016	0.024	0.028	0.047
Av	0.015	0.025	0.029	0.038

[a] Average of two replicates, in ppm. [b] Grand average of both lots.

Application of 1% sand granules at 0.075 lb of AI/acre on Smith's pasture provided 100% control of *A. nigromaculis* larvae, as would be expected at this high rate, but did not generate significant residues in soil or on vegetation (Table VIII). The lack of measurable residues on vegetation was surprising and may be due to use of the upper part of a container of 1% diflubenzuron on sand, with the probable settling of dust into the lower section and therefore not having been placed into the aircraft hopper.

These experiments demonstrate that diflubenzuron can be formulated to provide mosquito control without the production of undesirable residues on vegetation.

Release of Diflubenzuron from Granules. There was greater variation in the release of diflubenzuron from lots of the 1% Attaclay than from lots of the sand granules. It is apparent that release from the sand is faster and also that a greater amount is released during the same period (Table IX). These release properties of the sand granules are considered to be favorable for mosquito control.

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Uptake and Metabolism of Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid by Fish

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Bluegills and channel catfish removed less than 0.5% of dimethylamine salt of ^{14}C-ring-labeled 2,4-dichlorophenoxyacetic acid (DMA-[^{14}C]-2,4-D) when exposed in aquaria to water containing 2 ppm of the herbicide. The maximum concentration of 2,4-D in the fish was reached within 24 h of treatment; thereafter it did not change significantly up to 7 days. Catfish removed a smaller amount of the herbicide from the water than bluegills. No evidence for bioaccumulation of 2,4-D in the fish was noted during the duration of the experiment. A major portion of the radioactivity absorbed by the fish was associated with the head and viscera portions with relatively low concentrations in the edible flesh. The fish did not metabolize 2,4-D during the 7 days following treatment. Bluegills administered DMA-[^{14}C]-2,4-D by intraperitoneal injection excreted 90% of the herbicide within 6 h of treatment.

The dimethylamine (DMA) salt of 2,4-dichlorophenoxyacetic acid (2,4-D) is used extensively for controlling aquatic plants such as water hyacinth and Eurasian watermilfoil. This form of the herbicide is relatively nontoxic to fish; the 96-h TL_{50} values for bluegills (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) are 160 and 125 ppm, respectively (Schultz, 1973). A knowledge of the degree of accumulation of this herbicide by fish is important if they are to be used for human consumption. The herbicide, if accumulated by fish, may undergo metabolic transformation. The nature of these metabolites must be known in order to assess their possible toxicity to fish and man.

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Presently, very little information is available on the uptake and metabolism of 2,4-D by fish. Rodgers and Stalling (1972) studied the uptake and elimination of the ^{14}C-labeled butoxyethanol ester (BEE) of the herbicide in three species of fish. They reported that the maximum residue concentrations were in the fish within 1 to 2 h of exposure. Schultz (1973) examined the uptake and distribution of DMA-[^{14}C]-2,4-D by three species of fish. In these studies, the fish were exposed to the herbicide in plastic pools containing water and a layer of soil at the bottom. The concentration of ^{14}C residues in the edible portion of the fish continued to increase up to 84 days after treatment, but the actual 2,4-D content was negligible, indicating that most of the ^{14}C residue was a metabolite(s) of 2,4-D. When the fish were exposed to DMA-2,4-D in water containing the herbicide but not soil, low levels of non-2,4-D residues were found in them. Since 2,4-D is readily degraded by microorganisms (Loos, 1969) and by photochemical processes (Aly and Faust, 1964; Crosby and