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Fate of Diflubenzuron in Water

G. Wayne Ivie,* Don L. Bull, and Joseph A. Veech

The fate of the insect growth regulator diflubenzuron (Dimilin, *N*-[[(4-chlorophenyl)amino]-carbonyl]-2,6-difluorobenzamide) was studied in distilled water and in acidic (pH 4.0) and alkaline (pH 10.0) buffers. Heat (121 °C) catalyzed degradation of diflubenzuron in these aqueous media at levels greatly above its solubility in water resulted in rapid degradation to as many as seven identified products: (4-chlorophenyl)urea, 2,6-difluorobenzoic acid, 2,6-difluorobenzamide, 4-chloroaniline, *N*,*N*′-bis(4-chlorophenyl)urea, a 2,4-quinazolinedione derivative that resulted from expulsion of HF from diflubenzuron with cyclization at the anilino nitrogen and the ortho carbon of the benzoyl ring, and a further reaction product of the quinazolinedione compound. Under less vigorous conditions (0.1 ppm of [^{14}C]diflubenzuron in water or buffer, 36 °C), the rate of degradation was highly dependent upon pH. At pH 10.0, the half-life of diflubenzuron was <3 days; but at pH 4.0, degradation was not detected even after 56 days. In distilled water (pH ~6.0), the half-life of diflubenzuron was about 7 days. The major degradation products were (4-chlorophenyl)urea and 2,6-difluorobenzoic acid, but small amounts of 2,6-difluorobenzamide and the quinazolinedione product were also formed. When tested as an ovicide against the boll weevil or as a mosquito larvicide against *Culex quinquefasciatus*, the quinazolinedione derivative did not exhibit appreciable diflubenzuron-like biological activity.

The insect growth regulator diflubenzuron (1, Dimilin, *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide, Figure 1) is a highly efficacious insecticide that acts by inhibiting the synthesis of cuticle chitin, thus disrupting normal growth and development processes of developing insects (Hajjar and Casida, 1978; Mulder and Gijswijt, 1973; Post et al., 1974; Verloop and Ferrell, 1977). Diflubenzuron is particularly toxic to the larval stages of certain Lepidoptera (Granett and Dunbar, 1975; Tamaki and Turner, 1974) and mosquito larvae (Mulla et al., 1974; Schaefer et al., 1975). With several other insect species, including the boll weevil (*Anthonomous grandis* Boheman), house fly (*Musca domestica* L.), and stable fly (*Stomoxys calcitrans* L.), exposure of the adult insects to 1 causes them to lay eggs that fail to hatch (Moore and Taft, 1975; Grosscurt, 1976; Wright and Harris, 1976; Wright and Spates, 1976). This effect is apparently due to an ovicidal action and not sterility of the treated adults since the larvae appear to undergo more or less normal development within the eggs but are unable to hatch (Grosscurt, 1976; Verloop and Ferrell, 1977). Secretion of unmetabolized 1 into the eggs apparently accounts for the ovicidal effects observed (Ivie and Wright, 1978).

Since 1 has considerable potential as an insect control agent, the environmental fate of the compound should be thoroughly investigated. Several workers have already reported on the interactions of 1 with various components of the environment. The fate of 1 has been studied in several species of insects (Chang, 1978; Chang and Stokes, 1979; Still and Leopold, 1978; Ivie and Wright, 1978; Metcalf et al., 1975; Verloop and Ferrell, 1977) and mammals (Ivie, 1978; Metcalf et al., 1975). The persistence and fate of 1 in and on plants and in soils has also been reported (Bull and Ivie, 1978; Metcalf et al., 1975; Schaefer and Dupras, 1977; Verloop and Ferrell, 1977).

Although 1 has low water solubility (0.2–0.3 ppm; Ferrell, 1978), water may represent a significant route through which nontarget organisms can be exposed to 1 or its degradation products, particularly if 1 is used as a mosquito larvicide. However, only very limited data are available on the fate of 1 in water. Schaefer and Dupras (1976) reported that dilute solutions of 1 were not stable in field waters and that in tap waters stability was least when both pH and water temperature were relatively high. They obtained evidence that 1 in water degrades to (4-chlorophenyl)urea, but did not determine the possible occurrence of other degradation products.

The purpose of our investigation was to obtain more definitive information on the fate of 1 in water, particularly as influenced by pH. In some studies, large amounts of

Veterinary Toxicology and Entomology Research Laboratory (G.W.I.), Cotton Insects Research Laboratory (D.L.B.), National Cotton Pathology Research Laboratory (J.A.V.), Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, College Station, Texas 77840.

Table I. Thin-Layer Chromatographic Behavior of Diflubenzuron, Its Water Degradation Products, and Their Derivatives[a]

compound	R_f in indicated solvent system[b]							
	1	2	3	4	5	6	7	8
diflubenzuron (1)	0.64	0.86	0.55	0.50	0.62	0.65	0.48	0.56
(4-chlorophenyl)urea (2)	0.10	0.45	0.09	0.13	0.24	0.43	0.08	0.24
2,6-difluorobenzoic acid (3)	0.24	0.40	0.05	0.16	0.21	0.14	0.17	0.06
3, methyl ester	0.73	0.89	0.65	0.64	0.69	0.66	0.61	0.64
2,6-difluorobenzamide (4)	0.33	0.60	0.18	0.19	0.38	0.49	0.21	0.28
4-chloroaniline (5)	0.57	0.75	0.48	0.45	0.54	0.59	0.41	0.49
N,*N*'-bis(4-chlorophenyl) urea (6)	0.44	0.75	0.39	0.30	0.47	0.66	0.39	0.43
1-(4-chlorophenyl)-5-fluoro-2,4(1*H*,3*H*)-quinazolinedione (7)	0.51	0.76	0.40	0.28	0.47	0.59	0.29	0.46
7, *N*-methyl derivative	0.66	0.91	0.61	0.56	0.70	0.64	0.50	0.61
2-[(4-chlorophenyl)amino]-6-fluorobenzoic acid (8)	0.51	0.73	0.16	0.29	0.30	0.24	0.32	0.14
8, methyl ester	0.78	0.93	0.72	0.71	0.75	0.70	0.70	0.72

[a] Brinkman Silplate F-22 (0.25-mm gel thickness, with fluorescent indicator. [b] Solvent systems as follows: (1) benzene-dioxane-acetic acid, 90:30:1; (2) chloroform-ethanol-acetic acid, 85:10:5; (3) benzene-ethanol, 9:1; (4) benzene-ethanol-acetic acid, 93:7:1; (5) benzene-ethanol-diethylamine, 10:2:1; (6) hexane-ethyl acetate-methanol, 2:2:1; (7) benzene-ethyl acetate-acetic acid, 50:25:1; and (8) benzene-methanol, 5:1.

1 in water were subjected to vigorous thermal conditions in order to generate sufficient quantities of degradation products to permit analysis by spectral methods. In other studies, the degradation patterns of 1 in water were evaluated under conditions more closely approximating those likely to occur in the environment, specifically, the exposure of very dilute solutions of 1 at moderate temperature.

MATERIALS AND METHODS

Chemicals. Both unlabeled (technical grade, >99% purity) and radiolabeled (>99% radiochemical purity) samples of 1 were supplied for these studies by the Thompson-Hayward Chemical Co., Kansas City, KS. The radioactive preparation (16.14 mCi/mM) was uniformly labeled with radiocarbon in both of the aromatic rings. The 2,6-difluorobenzoyl ring contained 50.6% of the total radiocarbon and the 4-chloroaniline ring contained 49.4%. Thompson–Hayward also supplied samples of possible degradation products of 1; these have been described in a previous report (Ivie, 1978).

Exposure Media. Three test media were used in these studies. Samples of 1 were exposed in deionized, glass distilled water (pH ~6.0), 0.1 M sodium acetate buffer (pH 4.0) or 0.1 M sodium borate buffer (pH 10.0).

Heat-Catalyzed Degradation of 1. An acetone solution of 1 (5.0 mg in 1.0 mL) was pipetted into each of several glass ampules (10-mL capacity), and sufficient ^{14}C-labeled 1 was added to each ampule via a small amount of acetone such that the specific activity of the final preparation was approximately 1000 dpm/μg of 1. The solvent was then evaporated with a gentle stream of nitrogen, and 1 was left as a crystalline deposit at the bottom of the ampule. Water or buffer (5.0 mL) was added to each sample, and the ampules were then heat sealed and held in an autoclave at 121 °C (15 psi) for periods up to 24 h. For each pH and exposure period, the samples were replicated three times. In tests to obtain additional quantities of degradation products, other samples were run identically with either labeled or unlabeled 1, except that the samples were exposed in 50-mL, screw-cap culture tubes that were sealed with Teflon-lined caps.

Degradation of Dilute Solutions of 1 at Moderate Temperature. Radiolabeled 1 (2 μg, 231 000 total dpm) in acetone solution was pipetted into each of several heat-sterilized glass ampules (50-mL capacity), and the solvent was evaporated. Heat-sterilized water or buffer (20 mL) was added to each ampule to give a 0.1-ppm concentration of 1. The ampules were then heat sealed, placed in a rack, and continuously shaken in the dark with a variable speed rotator. The samples were held as long as 56 days before analysis. Although the samples were not deliberately heated, the heat generated by the rotator increased the temperature to approximately 36 °C. All samples were run in triplicate.

Extraction and Analysis. After exposure, samples were transferred to 50-mL, screw-cap culture tubes and were acidified to pH ~2.0 with HCl. The autoclaved samples were adjusted to a 10.0-mL volume with water before pH adjustment and extraction. Each sample was then partitioned four times with 10-mL volumes of ethyl acetate, and the radiocarbon in both aqueous and organic phases was quantitated by subjecting 0.2-mL aliquots to liquid scintillation counting (LSC). If, after extraction, the aqueous phase of any sample contained 1% or more of the total radiocarbon in the sample, the aqueous phase was adjusted to pH ~12.0 with NaOH and again extracted four times with ethyl acetate. The organic fractions from both acidic and alkaline extractions were combined, dried over anhydrous Na_2SO_4, concentrated, and subjected to thin-layer chromatographic (TLC) analysis. Radiocarbon in the residual aqueous phase was quantitated but was not further analyzed.

Chromatography. Degradation products of 1 were resolved in all tests by TLC. The plates were precoated silica gel (Brinkman F-22, 0.25 mm thick, with fluorescent indicator, 20 × 20 cm). After development in either one or two dimensions, radioactive components were visualized by exposing the plates to X-ray film (Kodak No-Screen) for 7 days. Radioactive components on the plates were quantitated by subjecting the appropriate gel regions to direct LSC analysis; a toluene-based scintillation cocktail was used. Appropriate corrections were made for quench and instrument efficiency. The TLC solvent systems, and R_f values of the water degradation products of 1 and their derivatives are given in Table I.

Milligram or submilligram quantities of the degradation products of 1 from samples subjected to heat were similarly isolated by TLC. Extracts of these samples were spotted as bands on thin-layer plates and developed in appropriate solvent systems. The compounds were visualized by radioautography or, with some compounds, simply by viewing the plates under shortwave ultraviolet light. The appropriate gel regions were scraped from the plates, and the

compounds were eluted with diethyl ether or ethyl acetate.

Characterization of Compounds. Degradation products of 1, isolated in sufficient quantity for spectral analysis, were subjected to further purification (TLC) if necessary, and attempts were made to crystallize the products from appropriate solvents. The purified materials were studied by mass spectroscopy or gas–liquid chromatography (GLC)–mass spectroscopy, before or after derivatization with diazomethane.

Since degradation products of 1 isolated from the 0.1 ppm water or buffer samples exposed at moderate temperatures were not obtained in sufficient quantities to permit spectral analysis, their characterization was based on TLC cochromatography in several solvent systems with the compounds isolated and identified from heat-treated samples or with authentic standards.

Analytical Procedures. Mass spectral studies were conducted with a Varian/MAT CH-7 magnetic scan spectrometer, either by direct insertion probe analysis or after GLC resolution of the products or their methyl derivatives with a Varian 2700 gas chromatograph coupled with the spectrometer. The glass column (2 mm i.d. × 1.8 m) was packed with 1.5% SP-2250 + 1.95% SP-2401 on 100/120 mesh Supelcoport. The column temperature varied with the compound (vide infra), and injector and detector ovens and all carrier lines were maintained at a slightly higher temperature than the column. Flow rate of the helium carrier was ~50 mL/min, and all spectra were recorded at 70 eV. High-resolution mass spectral studies were done with a CEC-110 mass spectrometer. Infrared (IR) spectra were recorded in KBr pellets on a Beckman 18-A spectrometer. Melting points were determined in open capillaries with a Tottoli-type apparatus and are uncorrected.

Biological Activity Studies. The quinazolinedione product 7, identified as a water degradation product of 1 in these studies, was tested for diflubenzuron-like activity against the boll weevil and larvae of the mosquito *Culex quinquefasciatus* Say. The boll weevils were of the ebony mutant strain developed by Bartlett (1967). Adults were collected and sexed as they emerged; males and females were held in separate groups until used. Virgin females were treated with graded doses of 1 or 7 when they were 2 days old, held for 2 more days, and then paired with untreated virgin males of the same age and confined in petri dishes (five pairs per dish). For the treatment, 1 μL of a solution of either chemical dissolved in a mixture of acetone and corn oil (95:5) was applied with a micrometer-driven syringe to the pronotum of each female. The treated insects were provided cylindrically shaped (0.75 × 1 cm) pellets of paraffin-coated diet (two per dish) that were changed daily. Beginning the third day after the sexes were combined and continuing on each of 4 consecutive days, eggs deposited in the diet pellets were removed manually under a binocular microscope and then held on moist absorbent paper for daily observations of hatch during a 7-day period. About 25 eggs were collected daily from each group of insects; these were placed individually in small holding cells (1 cm^3) made by cutting pieces of plastic light-diffusion grid to fit closely inside plastic petri dishes. This method of holding eggs restricted the movement of newly hatched larvae, prevented cannibalism, and also facilitated observations. All phases of the experiments were conducted in continuous light at 27 °C; absorbent paper was moistened daily to minimize dehydration and collapse of eggs. Tests of each dose of the two chemicals were replicated at least six times; controls processed concurrently with each replicate consisted of weevils exposed to only the solvent mixture and handled the same way.

Third-instar larvae of an insecticide-susceptible strain of *C. quinquefasciatus* were obtained from a colony maintained by J. K. Olson, Department of Entomology, Texas A&M University, College Station, TX. Groups of ten larvae were exposed to either chemical as dilute aqueous solutions (0.0003–0.03 ppm). The larvae were exposed in open 600-mL glass beakers containing 250 mL of deionized, distilled water and the appropriate amount of either chemical added via 50 μL of acetone. The larvae were fed daily (Meola and Lea, 1972), and after pupation, the beakers were covered with tissue paper to prevent escape of the emerging adults. Seven days after the exposures were begun, counts were made of emerged adults. Tests of each concentration were replicated at least four times. Controls consisted of groups of larvae that were held in an identical manner as treated larvae, but were exposed only to the acetone/water solution.

Table II. Heat-Catalyzed Degradation Products of Diflubenzuron in Aqueous Media[a]

compound[b]	% each product (range) at indicated pH 10.0	6.0	4.0
diflubenzuron (1)	1-6	4-51	11-50
(4-chlorophenyl)urea (2)	0-<1	0-<1	0
2,6-difluorobenzoic acid (3)	28-35	1-9	0
2,6-difluorobenzamide (4)	18-25	26-50	30-54
4-chloroaniline (5)	23-39	15-33	14-36
N,N'-bis-(4-chlorophenyl)urea (6)	1-3	0	0
1-(4-chlorophenyl)-5-fluoro-2,4(1*H*,3*H*)-quinazolinedione (7)	5-8	2-11	0
2-[(4-chlorophenyl)amino]-6-fluorobenzoic acid (8)	1-3	0	0
origin[c]	0-2	0	0
unidentified products[d]	0-1	0	0
water soluble[e]	0-1	0-1	0-1

[a] Five milligrams of [^{14}C]diflubenzuron in 5.0 mL of sodium borate buffer (0.1 M, pH 10.0), deionized distilled water (pH ~6.0), or sodium acetate buffer (0.1 M, pH 4.0), held at 121 °C in sealed-glass ampules for 24 h. [b] As resolved by single- or two-dimensional TLC in solvent systems 1 and 8 (see Table I). [c] Radiocarbon remaining at or near the origin on TLC after plate development. [d] Products resolved by TLC but not generated with sufficient consistency or in amounts to permit structural identification. [e] Radiocarbon not extracted from the aqueous phase.

RESULTS

Products from Heat-Catalyzed Degradation of Diflubenzuron in Water. Diflubenzuron (1) was extensively degraded after relatively large quantities of the chemical were held in water or buffer at 121 °C for 24 h (Table II). As many as eight radioactive products occurred in sufficient quantity in the heat-treated samples to permit isolation and further analysis. The products were isolated from the sample extracts by TLC using solvent systems 1, 3, or 8. The products were characterized as described below and their structures are indicated in Figure 1.

Diflubenzuron (1). A product suspected to be unchanged 1 on the basis of its TLC behavior was recrystallized from ether. Analysis by mass spectrometry (Table III) confirmed that the product was indeed 1.

(4-Chlorophenyl)urea (2). Although 2 was not observed in appreciable amounts in samples held at 121 °C for 24 h (Table II), it was isolated from samples exposed for shorter periods (1–3 h). The product obtained from water was identical on TLC with an authentic sample of

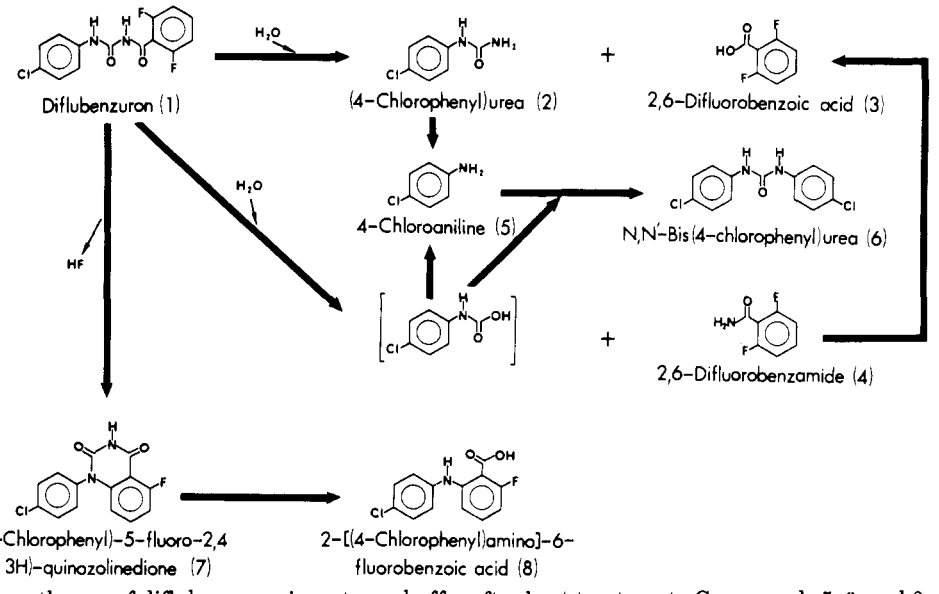


Figure 1. Degradation pathways of diflubenzuron in water or buffer after heat treatment. Compounds 5, 6, and 8 were not detected under less vigorous exposure conditions.

Table III. Mass Fragmentation Patterns of Diflubenzuron Degradation Products Formed by Heat-Catalyzed Reactions in Water or Buffer and of Their Methyl Derivatives

	m/e		
compound	molecular ion	base peak	other ions
diflubenzuron (1)	310	153 [(4-chlorophenyl) isocyanate]	141 (difluorobenzoyl)
(4-chlorophenyl)urea (2)	170	127 (4-chloroaniline	153 [(4-chlorophenyl) isocyanate], 111 (chlorophenyl), 100 (M^+ – $CNCONH_2$), 99 (M^+ – $CNHCONH_2$), 92 (aniline)
2,6-difluorobenzoic acid (3)	158	141	113 (difluorophenyl)
3, methyl ester	172	141	113
2,6-difluorobenzamide (4)	167	141	113
4-chloroaniline (5)	127	127	100, 99, 92
N,N'-bis(4-chlorophenyl)urea (6)	280	127	153
1-(4-chlorophenyl)-5-fluoro-2,4(1*H*,3*H*)-quinazolinedione (7)	290	247 (M^+ – CONH)	212 (M^+ – CONH, Cl), 199, (M^+ – CONHCO, F,H), 184 (M^+ – CONHCO, Cl), 164, 110, 106, 75
7, *N*-methyl derivative	304	247 (M^+ – $CONCH_3$)	212, 199, 184, 164, 110, 106, 75
2-[(4-chlorophenyl)amino]-6-fluorobenzoic acid (8)	265	247 (M^+ – H_2O)	212, 199, 184, 164, 110, 106, 75
8, methyl ester	279	247 (M^+ – OCH_3, H)	212, 199, 184, 164, 110, 106, 75

2, and mass spectral analysis confirmed its identity (Table III).

2,6-Difluorobenzoic Acid (3). This product was identical on TLC with an authentic sample of 3. It reacted rapidly with diazomethane to give a product that passed through the gas chromatograph (100 °C column, retention time 1.6 min) and whose mass spectrum (Table III) clearly indicated it to be the methyl ester of 3.

2,6-Difluorobenzamide (4). This compound was formed in relatively large amounts in the heat-treated samples. It exhibited TLC and GLC properties (150 °C column, retention time 1.5 min) identical with those of an authentic sample of 4. Recrystallization from ether–hexane, followed by mass spectral analysis (Table III), confirmed its structure as 4.

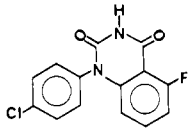
4-Chloroaniline (5). TLC studies with 5 showed that it was apparently 4-chloroaniline. GLC–mass spectral analysis of 5 (125 °C column, retention time, 1.2 min), in comparison with similar analysis of authentic 5, confirmed the structural assignment.

***N,N'*-Bis(4-chlorophenyl)urea (6).** This product was formed in small amounts in the heat-treated samples, and attempts at GLC analysis were unsuccessful. It did not react with diazomethane, as evidenced by no change in its TLC behavior. Attempts at crystallizing the TLC-purified product were not successful, but it was successfully studied by direct insertion probe mass spectrometry. The mass spectrum of 6 (Table III) showed the molecular ion at *m/e* 280 (Cl = 35) which, on the basis of the pattern of the chlorine "cluster", clearly contained two chlorine atoms. The mass spectrum also showed an ion at *m/e* 153 [one Cl, (4-chlorophenyl) isocyanate], and the base peak ion was at *m/e* 127 (one Cl, 4-chloroaniline). On the basis of the mass spectral data, the product was clearly *N,N'*-bis(4-chlorophenyl)urea.

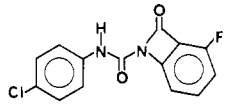
1-(4-Chlorophenyl)-5-fluoro-2,4(1*H*,3*H*)-quinazolinedione (7). This product was also formed in generally low amounts in certain of the heat-treated water samples, and after its isolation by TLC, it readily crystallized as fine white needles from ether (mp 252.0–254.0 °C). On the basis of TLC, the compound could not be identified as being any of the available diflubenzuron

analogues. Mass spectral analysis of **7** (Table III) indicated that it had an apparent molecular weight of 290 and that it contained one chlorine atom. The base peak ion, which also contained one chlorine atom, was at m/e 247, corresponding to the loss of CONH from the molecular ion. High-resolution mass spectral analysis was consistent with these assignments: calculated for $C_{14}H_8N_2O_2ClF$, 290.025815; found 290.024154; calculated for $C_{13}H_7NOClF$, 247.019975; found 247.018916. Other ions were assigned as follows: m/e 212, calculated for $C_{13}H_7NOF$ (M^+ – CONH, Cl) 212.051157, found 212.050694; m/e 199, calculated for $C_{12}H_6NCl$ (M^+ – CONHCO, H,F) 199.018873, found 199.019507; m/e 184, calculated for $C_{12}H_7NF$ (M^+ – CONHCO, Cl) 184.056247, found 184.056560. The IR spectrum of **7** exhibited two strong carbonyl stretching vibrations at 1715 and 1685 cm^{-1}. Other moderate to strong bands were at 1625, 1480, 1375, 1315, and 1195 cm^{-1}.

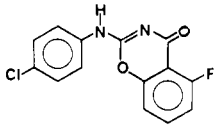
On the basis of the mass spectral data, it was apparent that **7** arose through expulsion of HF from the diflubenzuron molecule, with concomitant cyclization. Although four structures might be considered for **7**, only



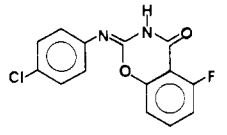
7a



7b



7c



7d

structure **7a** is consistent with the mass spectral and IR data obtained for **7**. The structure represented by **7b** would almost certainly give rise to a prominent (4-chlorophenyl) isocyanate ion (m/e 153) in its mass spectrum, but this ion was not seen in the mass spectrum of **7**. Also, structure **7b** does not permit logical assignment of the m/e 247 base peak ion (M^+ – CONH) observed. The tautomers **7c** and **7d** would exhibit only a single carbonyl stretching vibration in their IR spectra, and these structures likewise do not appear likely to fragment to a stable $C_{13}H_7NOClF$ (m/e 247) ion upon mass spectral analysis. The structure **7a** for the product was further supported by demonstration that **7** formed an *N*-methyl derivative upon reaction with diazomethane (see Table III for mass spectral data). N-Methylation is consistent with **7a** but not with **7b** or **7c** because we have shown that the amide nitrogen of diflubenzuron and related compounds is readily methylated by diazomethane, whereas the anilino nitrogen is unaffected (Bull and Ivie, 1979).

We recently reported these studies in preliminary form (Ivie et al., 1979) and learned at that time that others had independently isolated **7** after heat treatment of diflubenzuron in aqueous media (Verloop, 1979). Although details of the structural assignment were not given, the product was assigned structure **7a** (Verloop, 1979).

2-[(4-Chlorophenyl)amino]-6-fluorobenzoic acid (8). This product was isolated in low yield after heat treatment of **1** in pH 10.0 buffer, and it was recrystallized from ether–hexane (mp 182.0–183.0 °C). Analysis of **8** by mass spectrometry (Table III) indicated that it had a molecular ion at m/e 265 and that it contained a single chlorine atom. On the basis of the apparent molecular weight, **8** likely contained only a single nitrogen. The base peak was at m/e 247 (one Cl, $M^+ - H_2O$), and the fragmentation patterns seen below m/e 247 for **8** were essentially identical with those seen in this region for the quinazolinedione compound **7**. Therefore, **7** and **8** almost certainly were closely related compounds and both fragmented upon electron impact give the same m/e 247 [[(chlorophenyl)-amino]fluorobenzoyl] ion. The IR spectrum of **8** showed a strong carbonyl stretching vibration at 1700 cm^{-1}, with other moderate to strong bands assigned as follows: 3330 (N–H stretch), 3200–2700 (OH stretch), 1250 cm^{-1} (C–N vibration and/or C–O stretch).

Treatment of **8** with diazomethane resulted in very rapid, quantitative methylation, and the rapidity of the reaction strongly suggested that the reactive group was a carboxylic acid. The methyl derivative was successfully analyzed by GLC/mass spectroscopy (250 °C column; retention time, 4.2 min), and the mass spectrum clearly indicated the addition of one methyl group. Again, the fragmentation patterns seen below m/e 247 in the methyl derivative of **8** were essentially identical with the patterns seen in this region for both **7** and **8** (Table III).

On the basis of the spectral data for **8** and the structural assignment for the related **7**, we concluded that **8** was 2-[(4-chlorophenyl)amino]-6-fluorobenzoic acid, and that **7** was a likely precursor to **8**. Heat-catalyzed reaction of **7** in pH 10.0 buffer (~0.1 mg/mL in sealed ampules, 121 °C, 24 h) confirmed this relationship. TLC analysis of extracts of the heat-treated **7** revealed the presence of a fluorescence-quenching product with TLC behavior identical with that of **8**.

Distribution of Products from Heat-Catalyzed Reactions. Although heat treatment of **1** in aqueous media resulted in its rapid degradation, both the rate of degradation and the relative distribution of products formed varied considerably with pH (Table II). The reactions were dependent upon the presence of water because crystalline deposits of **1** that were autoclaved for 24 h in sealed ampules in the absence of water underwent no detectable degradation.

At pH 4.0, **1** degraded only to 2,6-difluorobenzamide (**4**) and 4-chloroaniline (**5**). These products were presumably generated by hydrolysis between the urea carbonyl and benzamide bonds to give **4** and an unstable carbamic acid or isocyanate derivative that subsequently degraded rapidly and quantitatively to **5**. As indicated in Table II, the reactions at pH 4.0 appeared to yield larger amounts of **4** than of **5**, a result that was not consistent with the fact that the [^{14}C]diflubenzuron used in these studies was equally labeled in each ring. This discrepancy was no doubt attributable to volatility losses of **5** during the sample concentration, TLC spotting, and plate development and exposure steps. This conclusion was supported by subsequent studies with TLC purified ^{14}C-labeled **5** that indicated that as much as 50% of this product could be lost through volatility during sample concentration and TLC analysis.

Heat-catalyzed degradation of **1** was accelerated with increasing pH, and two of the identified products, **6** and **8**, were observed only at pH 10.0 (Table II). Most likely, **6** resulted from the reaction of an unstable phenylcarbamic acid or isocyanate intermediate with **5** already present in the solution. This reaction was certainly favored by the relatively high potential concentration of reactants in these samples.

Degradation of Dilute Aqueous Solutions of 1 at Moderate Temperature. The rate of degradation of 0.1 ppm aqueous solutions of ^{14}C-labeled **1** held in sealed am-

Table IV. Degradation Products of [^{14}C]Diflubenzuron as 0.1 ppm Solutions in Aqueous Media Held at Moderate Temperature[a]

compd	% each product (±SD) at indicated time interval (days)						
	0	1	3	7	14	28	56
pH 10.0							
diflubenzuron (1)	99.2 ± 0.0	81.6 ± 0.5	31.4 ± 0.3	7.1 ± 0.8	1.7 ± 0.5	0	0
(4-chlorophenyl)-urea (2)	0	9.1 ± 0.5	32.8 ± 0.1	40.3 ± 1.4	46.2 ± 0.8	47.1 ± 1.4	45.7 ± 3.0
2,6-difluoro-benzoic acid (3)	0	8.3 ± 0.1	30.5 ± 0.3	42.9 ± 0.9	45.0 ± 0.6	44.1 ± 0.6	43.4 ± 1.8
2,6-difluoro-benzamide (4)	0	0	2.8 ± 0.1	4.7 ± 0.6	3.8 ± 0.2	4.2 ± 0.1	4.6 ± 0.4
1-(4-chlorophenyl)-5-fluoro-2,4(1*H*, 3*H*)-quinazoline-dione (7)	0.6 ± 0.0	0.9 ± 0.1	1.9 ± 0.0	2.8 ± 0.1	2.3 ± 0.2	2.5 ± 0.1	3.5 ± 0.4
origin[b]	0	0	0.4 ± 0.1	2.1 ± 1.2	0.9 ± 0.1	2.1 ± 1.1	2.8 ± 1.1
water soluble[c]	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0	0
pH 6.0							
1	99.5 ± 0.1	91.4 ± 0.1	78.4 ± 0.2	52.9 ± 1.7	28.1 ± 0.5	7.8 ± 0.7	1.2 ± 0.7
2	0	4.2 ± 0.1	9.9 ± 0.1	22.9 ± 0.7	35.2 ± 0.6	47.4 ± 0.8	49.2 ± 3.9
3	0	4.0 ± 0.1	9.7 ± 0.1	20.4 ± 0.8	32.2 ± 0.6	39.4 ± 0.4	44.3 ± 4.7
4	0	0	0.7 ± 0.1	1.6 ± 0.1	1.9 ± 0.2	2.9 ± 0.5	2.3 ± 0.1
7	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.0	1.0 ± 0.1	1.1 ± 0.2	1.5 ± 0.1	1.5 ± 0.1
origin[b]	0	0	0.2 ± 0.0	0.5 ± 0.1	0.8 ± 0.2	0.6 ± 0.0	0.8 ± 0.1
water soluble[c]	0.1 ± 0.0	0	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.7 ± 0.3
pH 4.0							
1	99.3 ± 0.1	99.4 ± 0.1	99.4 ± 0.1	99.3 ± 0.1	99.6 ± 0.1	99.4 ± 0.3	99.5 ± 0.1
7	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.3	0.5 ± 0.1
water soluble[c]	0.2 ± 0.1	0	0.2 ± 0.1	0.2 ± 0.0	0	0.1 ± 0.1	0

[a] Two micrograms of [^{14}C]diflubenzuron in 20 mL of sodium borate buffer (0.1 M, pH 10.0), deionized distilled water (pH ~6.0), or sodium acetate buffer (0.1 M, pH 4.0), sealed in 50-mL glass ampules and shaken in the dark at 36 °C. [b] Radiocarbon remaining at the origin on TLC after plate development. [c] Radiocarbon not extracted from the aqueous phase.

pules in the dark at 36 °C was highly dependent upon pH (Table IV). At pH 10.0, 1 degraded very rapidly, with a half-life <3 days. Less than 10% of the original 1 remained intact after 7 days, and none was detected by 28 days. At pH ~6.0, 1 degraded somewhat less rapidly, and its half-life under these conditions was about 7 days. However, at pH 4.0, 1 was completely stable; even after 56 days of exposure, 1 underwent no detectable degradation (Table IV).

As many as six radioactive products were seen in the sample extracts, and five of these were identified on the basis of TLC cochromatography studies in solvent systems 1–6 (Table IV). By far, the major mechanism by which 1 degraded was hydrolysis at the benzamide moiety to give (4-chlorophenyl)urea (2) and 2,6-difluorobenzoic acid (3). At both pH 6.0 and 10.0, these two compounds collectively comprised about 90% of the total radioactive degradation products present, regardless of the sampling intervals. Further, the data in Table IV indicate that, once formed, these two compounds (and the other degradation products as well) underwent essentially no further degradation. For example, the distribution of degradation products in samples held at pH 10.0 for 14 days (in which 1 was almost totally degraded) did not change appreciably even in samples held an additional 42 days (Table IV).

In addition to 2 and 3, 2,6-difluorobenzamide (4) and the quinazolinedione derivative 7 were also identified in the sample extracts. The only product(s) in these samples that was not characterized consisted of low levels of radiocarbon that remained at the origin on TLC. It is interesting that 7, in addition to being a minor water degradation product of 1 at pH 6.0 and 10.0, was also a minor contaminant of the ^{14}C-labeled 1 preparation used; thus 7 appeared in at least trace quantities in all samples (Table IV). It should be noted that, on the basis of TLC analysis, the unlabeled samples of diflubenzuron used in this study apparently did not contain 7 as a contaminant.

Because both of the aromatic rings of the ^{14}C-labeled 1 were equally radiolabeled, the occurrence of small amounts of the benzamide (4) in the water extracts necessitated the presence of one or more aniline-derived products, other than the urea (2). That, in fact, no such products were seen in the samples (Table IV) might be explained by the following: aniline-derived compounds might have indeed been formed in very low amounts but volatilized during TLC analysis to undetectable levels, or these compounds may have been present but were not resolved by TLC from the major radioactive components present. Alternatively, several aniline derived products may have been formed, of which none were in sufficient quantity to permit detection. Also, the low levels of radiocarbon remaining at the origin on TLC, or as water solubles, may represent primarily or totally compounds derived from the aniline moiety.

Data in Table IV show that dilute solutions of 1 in water or buffer did not degrade to appreciable quantities of products that were unextractable from the aqueous phase with ethyl acetate. In all samples, the extraction procedures used gave >99% partitioning of the radiocarbon present into ethyl acetate. Further, under these conditions, 1 did not degrade to appreciable amounts of highly volatile products, as evidenced by the fact that essentially all of the initial radiocarbon was accounted for in the sample extracts, even in samples exposed as long as 56 days.

Biological Activity Studies. Studies with the quinazolinedione compound 7 showed that it does not have significant diflubenzuron-like insect growth regulator activity against either the boll weevil or *C. quinquefasciatus* larvae (Table V). Although topical treatment of female boll weevils with as little as 0.1 μg of 1 reduced the hatch of eggs subsequently laid, 7 had no appreciable activity at levels as high as 2 μg/weevil. Likewise, third-instar *Culex* larvae were quite susceptible to very dilute concentrations of 1, but 7 was virtually nontoxic at concentrations in water

Table V. Activity od Diflubenzuron (1) and Its Water Degradation Product 1-(4-Chlorophenyl)-5-Fluoro-2,4-(1*H*,3*H*)-Quinazolinedione (7) as Ovicides against the Boll Weevil (*Anthonomus grandis*) and as Mosquito Larvicides against Third-Instar *Culex quinquefasciatus* Larvae

compound	dose level	% egg hatch (boll weevil) or % adult development (*Culex*)
boll weevil[a]	μg/weevil	
control[b]	0	78
1	0.1	54
	1.0	32
	2.0	25
7	0.1	74
	1.0	70
	2.0	68
Culex larvae[c]	ppm	
control[d]	0	93
1	0.0003	95
	0.001	85
	0.003	2
	0.01	0
7	0.001	98
	0.003	90
	0.01	90
	0.03	88

[a] Compounds applied in 1 μL of 5% corn oil in acetone to the pronotum of adult weevils. [b] Weevils treated with solvent-corn oil mixture only. [c] Compounds in acetone added to water such that the final acetone concentration was 0.02%. [d] Larvae exposed to solvent-water mixture only.

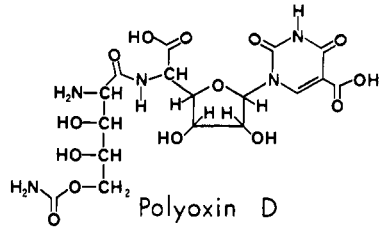
as high as 0.03 ppm (Table V).

DISCUSSION

The studies reported here have shown that diflubenzuron (1) degrades in aqueous media to a number of products, arising both through predictable hydrolytic mechanisms and, to a lesser extent, unanticipated reactions, including a rather novel cyclization. The reactions catalyzed by heat would appear to have no direct environmental implications, but they were extremely useful in providing sufficient quantities of degradation products to allow definitive characterization by spectral analysis and assay for biological activity. Fortunately, and perhaps predictably, the degradation of very dilute solutions of 1 under more normal exposure conditions proceeded along generally the same pathways seen at elevated temperatures. The exceptions were that 5, 6, and 8 were seen only in the heat-treated samples. *N,N'*-Bis(4-chlorophenyl)urea (6) occurred as the result of a bimolecular reaction clearly facilitated by the high levels of 1 present in the heated samples; therefore, predictably, 6 would probably not be seen after exposure of very dilute solutions of 1 in aqueous media. Although 8 was not found after exposure of 1 as dilute aqueous solutions at moderate temperature, its precursor 7 was found in all samples, and very low amounts of 8 possibly were, in fact, present but in quantities insufficient to be detected.

Although 4-chloroaniline (5) was a major degradation product of 1 in heat-treated samples (Table II), it was not seen in samples exposed to less vigorous reaction conditions (Table IV). (4-Chlorophenyl)urea (2) generated in the autoclaved samples was apparently converted rapidly by heat to 5, but 2 remained as a stable end product of the degradation of 1 at moderate temperature. Studies with authentic 2 in water confirmed that 2 is rapidly and almost quantitatively degraded by heat to 5, and similar studies with the benzamide (4) resulted in its conversion to the benzoic acid (3) (Figure 1), although the reaction proceeded more slowly.

The occurrence of 7 as a degradation product of 1 in water was unanticipated because it resulted from the cleavage of a C–F bond. Elucidation of the structure of 7 stimulated thoughts regarding its potential biological activity because it retains structural characteristics somewhat similar to those of 1, but also because it has seemingly significant structural similarities to the polyoxins, a group of highly efficacious fungicides whose mode of action is known to involve the inhibition of chitin synthetase, the terminal enzyme in the biosynthesis of chitin (Endo et al., 1970). However, assay of 7 for diflu-



Polyoxin D

benzuron-like insect growth regulator activity against the boll weevil and *Culex* larvae indicated that 7 apparently has little or no biological activity of significance, at least against these insects. Therefore, 7 apparently offers no insights regarding structure–activity relationships among the benzoylurea chitin synthesis inhibitor insecticides.

On the basis of the current studies, and others (Schaefer and Dupras, 1976, 1977), it is clear that diflubenzuron degrades rapidly in neutral or alkaline waters and, without taking into account its potential biodegradation in aqueous environments, the compound has potential for persistence or accumulation in water only under quite acidic conditions. Because the pH of most natural waters ranges between 6 and 9 (Stumm and Morgan, 1970), the use of diflubenzuron as an insect control agent should not result in persistent residues in environmental waters except in most unusual circumstances.

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Phosphine Sorption and Desorption by Stored Wheat and Corn

Theodore Dumas

The sorption and desorption of PH_3 from wheat treated with concentrations of 0.5–5 mg/kg of wheat was determined at 25, 45, and 85 °C. The desorbed PH_3 was extracted from the air by passing through a cold trap; here sufficient quantities of the gas could be accumulated for accurate analysis by GLC. Most of the PH_3 was desorbed in the first 2–3 days but small amounts continued to desorb for many weeks following treatment. After 220 days of aeration, PH_3 was still present in the grain and desorbing at the rate of ppt (10^{-12} g). For a wide range of concentrations, about 10% of the PH_3 applied was found to be adsorbed. The amount of physically sorbed PH_3 increased with the fumigant concentration applied and with the length of exposure. Because of reduced reactivity even when the temperature was increased to 85 °C for several days, unreacted PH_3 still desorbed slowly from wheat. Corn aerated 26 days desorbed 0.004 ng/g in 2 days.

Hydrogen phosphide (PH_3), also known as phosphine, is toxic at very low concentrations and it is used in fumigation of grains and other stored products. Because of its wide use as a fumigant it was necessary to investigate the behavior of PH_3 insofar as sorption and desorption could occur during and after fumigation. In previous publications Berck (1968) and Berck and Gunther (1970) attributed to chemisorption the PH_3 portion which could not be recovered after fumigation. On the other hand, Rauscher and Mayr (1968) found complete recovery when they fumigated wheat with PH_3 for short periods of time. Robinson and Bond (1970) after fumigation with radioactive $^{32}PH_3$ showed that small amounts of residual radioactive ^{32}P remained for days following the treatment. To investigate this apparent difference in results, low levels of PH_3 desorbing from treated materials were determined using an extremely sensitive method that could measure amounts down to ppt (10^{-12} g).

MATERIALS AND METHODS

Soft winter wheat of 13.1% and field corn of 8.8% moisture content were fumigated with 0.5 and 5 mg of PH_3/kg of commodity at 25 °C. The fumigation of wheat was made in 240-mL gas-adsorbing bottles by injecting with a syringe 1–100 μg of PH_3 gas. The PH_3 gas was generated from Phostoxin tablets by the method described by Kashi and Bond (1975). Because very small quantities of fumigant were involved in these experiments and because the concentration in air was always well below the lower explosive limit of 1.79% by volume in air, no hazards were created by handling this fumigant. After treatment the materials were left to aerate with laboratory environment for a period of several weeks, and the rate of desorption was determined at selected time intervals. This was done by placing wheat or corn samples in a glass desorption chamber consisting of a 240-mL gas adsorption bottle fitted with stopcocks. The desorbed PH_3 was collected in a cold trap (Dumas, 1978) and analyzed by gas chromatography. A sample size of 1–100 g of fumigated commodity was used depending on the level of PH_3 absorbed. The rate of desorption for a range of concentrations and exposure times was determined mainly at 25 °C, but it was also investigated at 45 and 85 °C to determine if desorption or reaction could be accelerated at higher temperatures. The sorption on the empty desorption chambers was determined and found to be less than 3% in 24 h for 50 μg of PH_3 applied.

Phosphine retention in wheat was determined by treating wheat with a dosage of 5 mg/kg of PH_3 using Phostoxin pellets which were placed at the bottom center of a 25-L container containing 13.7 kg of wheat and

Agriculture Canada, Research Institute, University Sub Post Office, London, Ontario, Canada N6A 5B7.

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