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Degradation and Environmental Fate of 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl)urea

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Radiolabeled preparations of the insect growth regulator PH-6040 were evaluated for degradation in a laboratory model ecosystem and after exposure to sheep microsomes, *Pseudomonas*, soil, and ultraviolet light. The parent compound was found to be moderately stable but was not highly concentrated through food chains or by direct absorption from water. The degradative pathways were almost entirely through cleavage between the carbonyl and amide groups of the urea bridge to give 2,6-difluorobenzamide and 2,6-difluorobenzoic acid, 4-chlorophenylurea, and 4-chloroaniline.

The insect growth regulator 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea (PH-6040, OMS-1804) has novel and highly specific activity against a wide variety of insects (Neal, 1974; Wellinga et al., 1973a,b; Jakob, 1973; Van Daalen et al., 1972). The compound is very effective against mosquito larvae, inhibiting growth and development of fourth instar *Culex pipiens quinquefasciatus* larvae at concentrations of 0.0001–0.001 ppm. The biochemical lesion is suggested to result from the inhibition of chitin synthesis with a resulting defect in endocuticular deposition (Post and Vincent, 1973). As a result, the insect seems characteristically to be unable to complete development. There appear to be no discernible effects on higher animals, and the mouse oral LD_{50} is in excess of 3000 mg/kg and the LC_{50} to the guppy >100 ppm. Thus, PH-6040 is a fourth generation insecticide with many attractive properties of selectivity. As it is likely to have extensive usage, we report here on the degradation and environmental fate of PH-6040 in a laboratory model ecosystem, under photochemical stimulation, and in soil.

MATERIALS AND METHODS

Radiolabeled Compounds and Model Metabolites. ^{14}C and ^{3}H radiolabeled compounds were provided by Philips-Duphar, The Netherlands. Three preparations all with >99% radiopurity as determined by TLC using the solvent system shown in Table I were supplied. These were (A) 2,6-difluorobenzoyl (^{14}C=O label, sp act. 0.32 mCi/mmol), (B) *p*-chlorophenyl (^{14}C-ring-U, sp act. 2.35 mCi/mmol), and (C) ^{3}H-ring substituted 2,6-difluorobenzoyl (sp act. 22.9 mCi/mmol). The 2,6-difluorobenzoic acid, mp 159–160°, and 2,6-difluorobenzamide, mp 143–145°, used as model degradation products, were also supplied by Philips-Duphar. The *p*-chloroaniline, mp 67–69°, was a recrystallized commercial sample, and *p*-chloroacetanilide, mp 173–175°, *p*-chlorophenylurea, mp 210–211°, *p*-chloro-*N*-methylaniline, bp 67–68° (0.5 mmHg), *p*-chloro-*N,N*-dimethylaniline, mp 28–30°, and 4,4′-dichloroazobenzene, mp 180–185°, were prepared in this laboratory.

Model Ecosystem Technology. Three laboratory model ecosystems (Metcalf et al., 1971; Metcalf, 1974) were treated with one each of the three radiolabeled preparations of PH-6040, A, B, and C. The *Sorghum vulgare* plants on the terrestrial portion of the model system were treated with 5.0 mg of the radiolabeled compound dissolved in about 1 ml of acetone using a micropipet to cover the leaves, so that the equivalent rate of treatment was 1 lb/acre. Ten fourth instar salt marsh caterpillars, *Estigmene acrea*, were allowed to feed on the treated leaves and to disperse the radiolabeled compound into the 7-l. aquatic portion of the model ecosystem for food chain uptake by plankton, *Daphnia magna*, *Oedogonium cardiacum* (alga), *Physa* sp. (snail), *Culex pipiens quinquefasciatus* (mosquito larva), and *Gambusia affinis* (fish). The

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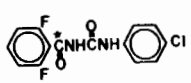
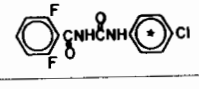
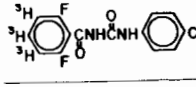
Table I. Degradation of ^{14}C-Radiolabeled 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl)urea in Model Ecosystem

		 ppm as difluorobenzoylchlorophenylurea equivalents								
	R_f[a]	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)	H_2O	*Oedogonium* (alga)	*Physa* (snail)	*Culex* (mosquito)	*Gambusia* (fish)
Total extractable ^{14}C		0.02356	1.0311	0.6670	5.2455	1.6444	0.06909	0.6548	2.2306	13.3614	6.0701
Unknown 1	0.90		0.4028		0.7201						
$(CH_3)_2NC_6H_4Cl$	0.83						0.0034	0.0778			
Unknown 2	0.77	0.00025									
$C_6H_3F_2CONHCONHC_6H_4Cl$	0.70	0.0057	0.4748	0.4891	4.4225	0.1097	0.0220	0.4019	2.0979	13.1369	0.3193
Unknown 3	0.60						Trace				
Unknown 4	0.57	Trace									
$C_6H_3F_2COOH$	0.52	0.0018				0.1644					
$H_2NC_6H_4Cl$	0.50						0.0078	0.0389			0.3193
$C_6H_3F_2CONH_2$	0.45	0.00031									
Unknown 5	0.43						0.00055				
Unknown 6	0.38	0.00020									
Unknown 7	0.36						0.00061				
$CH_3CONHC_6H_4Cl$	0.33						0.00056				0.0297
Unknown 8	0.26						0.00022				
Unknown 9	0.20						0.00015				
$H_2NCONHC_6H_4Cl$	0.12						0.0072	0.0227		0.2071	0.1263
Polar	0.0	0.0060	0.1535	0.1779	0.1028	1.3703	0.0078	0.1135	0.1327	0.0174	5.2755
Unextractable		0.0093	4.9559	1.6210	1.9340	0.8227	0.0188	0.5726	0.2713	0.3436	0.8469

[a] TLC with benzene–dioxane–acetic acid (90:30:1, by volume).

Table II. Degradation of ^{3}H-Radiolabeled 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl)urea in Model Ecosystem

		 ppm as difluorobenzoylchlorophenylurea equivalents				
	R_f[a]	H_2O	*Oedogonium*	*Physa*	*Culex*	*Gambusia*
Total extractable ^{3}H		0.00615	0.1488	0.3865	1.9320	0.7730
Unknown 1	0.90					
Unknown 2	0.77	0.00005	0.0139			
$C_6H_3F_2CONHCON-HC_6H_4Cl$	0.72	0.00168	0.1097	0.3714	1.8466	0.1350
$C_6H_3F_2COOH$	0.52	0.00037			0.0777	0.0226
$C_6H_3F_2CONH_2$	0.45	0.00008				0.0189
Polar	0.0	0.00172	0.0252	0.0151	0.0077	0.5965
Unextractable		0.00225	0.0405	0.0645	0.0251	0.1328

[a] TLC with benzene–dioxane–acetic acid (90:30:1, by volume).

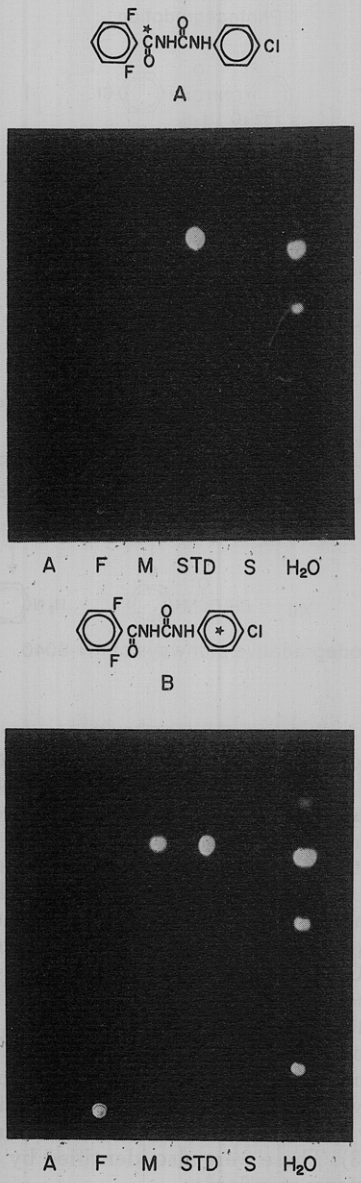


Figure 1. Radioautograms of ^{14}C-radiolabeled PH-6040 from preparations A and B after thin-layer chromatography of extracts from components of the model ecosystem: (A) alga; (F) fish; (M) mosquito larva; (S) snail; (STD) ^{14}C-radiolabeled PH-6040.

duration of the model ecosystem experiments was 33 days and the aquaria were kept in an environmental chamber at 80°F (26.7°C) with a 12-hr photoperiod of 5000 ft-c. Following completion of the experiment, the water and organisms were removed from the model systems, weighed, homogenized, and extracted with acetone. The extracts were concentrated and subjected to TLC on fluorescent silica gel (E. Merck GF-254) and radioautography on no-screen X-ray film. The residues after extraction were combusted for total unextractable radioactivity by the Schöniger oxygen flask technique (Kelly et al., 1961).

Metabolism by salt marsh caterpillar was investigated by applying 50 μg of each radiolabeled preparation to 200 mg of Vail moth medium (Vail et al., 1967), allowing fourth instar larvae to feed, and collecting feces for 24 hr. Both feces and body homogenate were extracted with anhydrous sodium sulfate before analysis by TLC and radioautography.

Soil persistence was investigated by application of 100 μg of each of A and B radiolabeled preparations in 1 ml of acetone to 10 g of air-dried Drummer soil (17.4% moisture) (silty clay loam) and thorough mixing. The soil samples were kept in petri dishes in an incubator at 80°F (26.7°C) for 1, 2, or 4 weeks, before extraction with acetone, and assay by TLC and radioautography.

Degradation by *Pseudomonas*. A cell suspension of 0.04 g of *Pseudomonas putida* in 2 ml of 0.05 *M* phosphate buffer (pH 7.0) was incubated with 10 μg of ^{14}C-radiolabeled PH-6040 preparations A and B. After 6 hr at approximately 30°, the solutions were extracted with diethyl ether, concentrated, and analyzed by TLC and radioautography.

Sheep Liver Microsomal Metabolism. A standard preparation of sheep liver microsomes was used to study degradation of ^{14}C-radiolabeled PH-6040 with A and B labels. Approximately 33 μg of the radiolabeled compounds was applied to glass beads and exposed to 1.6 mg of microsomal protein suspended in 0.05 *M* Tris-HCl buffer (pH 7.4) with NADPH (5.0×10^{-4} *M*), glucose 6-phosphate (G-6-P) (2.5×10^{-3} *M*), $MgCl_2$ (7.5×10^{-3} *M*), and G-6-P dehydrogenase (1 unit). After 60 min incubation at 39°, the solution was extracted with diethyl ether and the concentrated extract evaluated by TLC on silica gel and radioautography.

Photodegradation. Suspensions of PH-6040 in methanol at 1 mg/ml and in aqueous dioxane solution were irradiated in quartz flasks in a Srinivasan-Griffin reactor equipped with 16 GE G8T5 bulbs with a total irradiating power of 20.8 W at 254 nm. The nature of the photolysates was evaluated by ir spectra in chloroform and by mass spectrometry at 70 eV.

RESULTS AND DISCUSSION

The 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea, mp 239°, has a water solubility of about 1.0 ppm at 25° and an octanol/water partition coefficient of approximately 3500. From studies of the environmental degradation of related compounds, e.g. the herbicide monuron or *N*-(4-chlorophenyl)-*N'*,*N'*-dimethylurea and dichlorbenil or 2,6-dichlorobenzonitrile (Menzie, 1969), it appears that ring halogenation of phenyl groups suppresses ring hydroxylation as a degradative pathway (Lu and Metcalf, 1975). The environmental degradability of PH-6040 would likely depend largely upon the stability of the –C(O)NHC(O)NH– bridging moiety.

Degradation in Model Ecosystem. The results of the model ecosystem evaluations with the ^{14}C-radiolabeled preparations A and B are shown in the radioautograms of TLC plates (Figure 1) and in Table I. The intact parent compound (R_f 0.70) identified by cochromatography and its characteristic mass spectrum [*m/e* 310, 312 (P^+), 153, 155 (ClC_6H_4NCO), 141 ($C_6H_3F_2CO$), 127, 129 ($ClC_6H_4NH_2$), and 113 ($C_6H_3F_2$)] was present in all the organisms of the model ecosystems and comprised the following percentages of total extractable ^{14}C for labels A and B: alga, 46 and 61; snail, 73 and 90; mosquito, 84 and 98; and fish, 6.7 and 5.2. Thus, PH-6040 is clearly persistent in the organisms of the model ecosystem. However, it does not show a high degree of ecological magnification as demonstrated by the lowest concentrations in the fish at the top of the food chain, and the ecological magnification values (E.M. or parts per million of parent compound in fish/parts per million in water) from label A (19) and from label B (14). The concentration of the parent compound in the mosquito larva *Culex* is, however, relatively high: EM from label A is 779 and that from label B is 596. This demonstrates the remarkable affinity of PH-6040 for the insect cuticle and probably reflects its site of action in preventing endocuticle formation (Post and Vincent, 1973).

The data from the ^{3}H-radiolabeled preparation C were very similar to those from ^{14}C label A as shown in Table II. However, unknown 1 was not found suggesting it may contain a C=O group. The intact parent compound (R_f 0.72) comprised the following percentages of total extract-

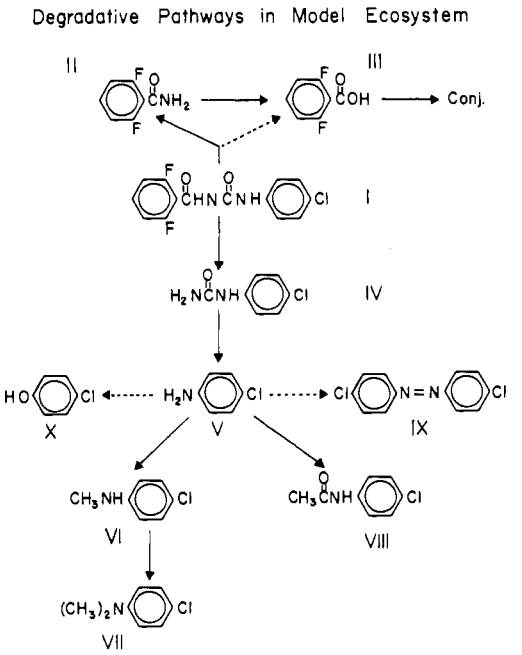


Figure 2. Degradative pathways of PH-6040 in the model ecosystem.

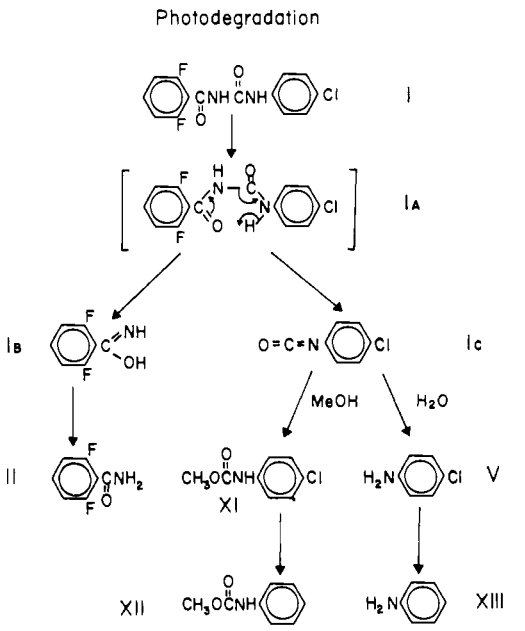


Figure 3. Photodegradative pathways of PH-6040.

able ^{3}H: alga, 74%; snail, 96%; mosquito, 95%; and fish, 17.5%, in good correspondence with labels A and B (above). The EM values for the parent compound were fish, 80, and mosquito larva, 1099. The unextractable ^{3}H was substantially lower than the ^{14}C values for labels A and B (see Tables I and II).

Comparison of the parameters of ecological magnification (EM), biodegradability index (BI or parts per million of polar radioactivity/parts per million of nonpolar radioactivity) for the three radiolabeled preparations is both reassuring about replicatability and informative because of the position of the radiolabel. EM values for the parent compound were: snail, A, 86; B, 95; and C, 221; fish, A, 19; B, 14; and C, 80; and mosquito, A, 779; B, 596; and C, 1099. The BI values for the series were: snail, A, 0.182; B, 0.063; and C, 0.040; fish, A, 4.99; B, 6.64; and C, 3.38; and mosquito, A, 0.035; B, 0.005; and C, 0.004.

The data in Table I suggest the degradation pathways of PH-6040 (I) as indicated by radiolabeling in both halves of the molecule. The key degradation products found from label A ($C_6H_3F_2{}^{14}C{=}O$) were 2,6-difluorobenzamide (II) (R_f 0.45) and 2,6-difluorobenzoic acid (III) (R_f 0.52) which were identified by cochromatography of the respective radioactive areas (Figure 1) with known standards. Only minute traces of other radiolabeled degradation products were found (R_f 0.77 and 0.38) from this label. Data from the ring-^{3}H-labeled compound did not show any other ring-labeled unknowns suggesting that decarboxylation of the benzoic acid moiety does not readily occur. The stability was also confirmed in $^{14}CO_2$ trapping experiments using the same organisms: alga, snail, mosquito, and fish, in a closed model aquatic ecosystem (Lu and Metcalf, 1975). No $^{14}CO_2$ was detected from I, A label over a 3-day period. The high level of polar radioactivity from A label in the fish (50%) is presumably due to conjugates of 2,6-difluorobenzoic acid.

In model aquatic ecosystem studies with benzoic acid, Lu and Metcalf (1975) found that hippuric acid (R_f 0.05), the glycine conjugate of benzoic acid, was one of the principal metabolic products. The present study with I did not indicate any compound with these chromatographic properties. It appears that the ortho fluoro groups may deactivate the benzoic acid to glycine conjugation. The EM value for 2,6-difluorobenzoic acid in fish was 91.

The key degradation products found with I, B label (ClC_6H_4-^{14}C-ring), were 4-chlorophenylurea (IV) (R_f 0.12) and 4-chloroaniline (V) (R_f 0.50) which were present in water and in tissues of snail and fish in very similar quantities suggesting that cleavage of the parent compound occurs at both C(O)–N bonds (Figure 1). The EM values for 4-chlorophenylurea, 18, identified by cochromatography and mass spectrometry [m/e 170 and 172 (P^+) and 127 and 129 ($ClC_6H_4NH_2$)] and 4-chloroaniline, 41, do not indicate appreciably greater biomagnification than that of the parent substance.

The 4-chlorophenylurea (IV) is doubtless the precursor of the 4-chlorophenylaniline (V) and the latter is further detoxified by acetylation to 4-chloroacetanilide (VIII) (R_f 0.33) and by methylation to 4-chloro-*N,N*-dimethylaniline (VII) (R_f 0.83). These were also identified by cochromatography with known standards.

These metabolic pathways for 4-chloroaniline are similar to those found for aniline in a model aquatic ecosystem study (Lu and Metcalf, 1975), where acetanilide and *N,N*-dimethylaniline are the principal metabolic products formed from [^{14}C]aniline in the fish *Gambusia*.

Although herbicides producing 4-chloroaniline as a degradation product, e.g. *N*-(4-chlorophenyl)-*N',N'*-dimethylurea or monuron, have been shown to produce 4,4'-dichloroazobenzene in soil (Bartha et al., 1968), this compound (R_f 0.77) was not found in the model ecosystem study from B label. Neither was further degradation of 4-chloroaniline to 4-chlorophenol (R_f 0.63) observed in the model ecosystem (Menzie, 1969). The degradative pathways concluded from the model ecosystem study are summarized in Figure 2.

Photodegradation. The pattern of degradation in water shown for PH-6040 A and B labels in Figure 1, without extensive degradation products in the organisms of the model ecosystem, suggests that photodegradation is an important process with this molecule. Photodegradation studies of suspensions of I in methanol at 254 nm produced colored products after 9 hr of irradiation. The solution analyzed by TLC showed 2,6-difluorobenzamide (II), isolated in 68% yield with identity confirmed by melting point and ir and mass spectra [m/e 157 (P^+), 141 ($F_2C_6H_3CO$), 113 ($C_6H_3F_2$)]. Methyl phenylcarbamate (XII), identified by cochromatography and mass spectrometry [m/e 151 (P^+), 119 (C_6H_5NCO), 106, 92

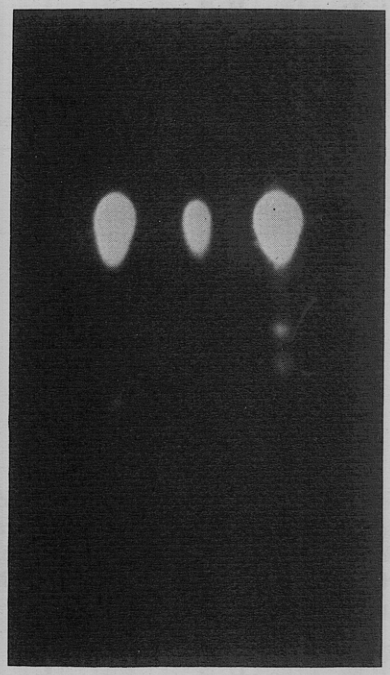


Figure 4. Radioautogram of ^{14}C-radiolabeled PH-6040 after thin-layer chromatography of extracts from sheep microsomal study: (A) benzoyl $^{14}C{=}O$; (B) [^{14}C]chlorophenyl ring; (STD) ^{14}C-radiolabeled PH-6040.

Table III. Degradation of 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl)urea by Sheep Microsomes

		% total ^{14}C after 1 hr	
	R_f[a]	Benzoyl $^{14}C{=}O$ (A)	[^{14}C] Chlorophenyl ring (B)
$(CH_3)_2NC_6H_4Cl$	0.85		0.21
$C_6H_3F_2CONHCONHC_6H_4Cl$	0.67	99.09	98.85
Unknown 4	0.55		0.09
$C_6H_3F_2COOH$	0.51	0.19	
$H_2NC_6H_4Cl$	0.50		0.28
Unknown 5	0.43		0.28
$C_6H_3F_2CONH_2$	0.38	0.70	
$CH_3CONHC_6H_4Cl$	0.33		0.03
Unknown 9	0.22		0.05
$H_2NCONHC_6H_4Cl$	0.15		0.09
Unknown 10	0.08		0.03
Polar	0.0	0.52	0.09

[a] TLC with benzene–dioxane–acetic acid (90:30:1, by volume).

(C_6H_5NH)], was recovered in 18% yield by evaporation of the mother liquor from the crystallization of I. The ir spectrum of methyl phenylcarbamate was identical with an authentic sample but the mass spectrum showed the presence of a small amount of methyl *p*-chlorophenylcarbamate (XI) as evidenced by the characteristic halide doublet at *m/e* 185 and 187 (calculated for C_8H_8ClNO, P^+ 185). In addition to these materials a considerable quantity of polar substances was found, from which identifiable products could not be isolated.

Photodegradation studies of I in aqueous dioxane produced a dark brown solution after 4 hr irradiation. Analysis by TLC showed the presence of 2,6-difluorobenzamide (II) together with small quantities of 4-chloroaniline (V) and aniline (XIII) identified by cochromatography with authentic samples and characteristic colors, yellow and orange, respectively, obtained by reaction with *p*-nitrobenzenediazonium fluoroborate. The bulk of the photoproducts was a dark brown, chromatographically immobile material which was not characterized.

From the experiments described, the putative pathways for the photochemical degradation of I are shown in Figure 3. Photoexcited I appears to undergo a formal Norrish type II elimination at the N-1–C-2 bond to give the imidate (Ib) followed by tautomerization to form 2,6-difluorobenzamide (II) and 4-chlorophenyl isocyanate (Ic). The latter is readily solvolyzed to methyl 4-chlorophenylcarbamate (XI) in methanol or to 4-chloroaniline (V) upon decarboxylation of the carbamic acid formed in aqueous media. In the absence of evidence suggesting the competitive dechlorination of I, it is suggested that the dechlorinated methyl phenylcarbamate (XII) and aniline (XIII) are formed directly from methyl 4-chlorophenylcarbamate (XI) and 4-chloroaniline (V). These results are in general agreement with the photolysis study of Ruzo et al. (1974), published after this article was submitted.

Degradation by Sheep Liver Microsomes. Incubation of ^{14}C-radiolabeled A and B preparations of PH-6040 for 1 hr with sheep microsomes, followed by extraction of the radiolabeled products with diethyl ether, TLC, and radioautography produced the data shown in Figure 4 and Table III. The parent compound was very recalcitrant to degradation and represented 99.09% recovery with A label and 98.85% recovery with B label. With A label the 2,6-difluorobenzamide (R_f 0.38) was the principal product, indicating major cleavage between N-1 and C-2 of the bridge. With label B, the principal product was 4-chloroaniline (R_f 0.50) together with unknown 5 (R_f 0.43). The 2,6-difluorobenzoic acid (R_f 0.51), label A, was formed at about 0.25 the rate for 2,6-difluorobenzamide and corresponded to cleavage between C-1 and N-1 of the bridge. The expected product from label B, 4-chlorophenylurea (R_f 0.15), was also present in smaller quantities.

Degradation in Soil. The ^{14}C-radiolabeled preparations A and B of PH-6040 were added to fresh air-dried Drummer soil (17.4% moisture) at 10 ppm and incubated at 80°F (26.7°) for periods of 1, 2, and 4 weeks. The radioactive material, extracted in acetone and subjected to TLC on silica gel and radioautography, gave the results shown in Table IV. It is evident that this compound is very stable in the soil under the conditions of the experiment. Traces of radiolabeled degradation products from B label appeared after 1, 2, and 4 weeks at R_f 0.12, cochromatographing with 4-chlorophenylurea (IV), and at R_f 0.49, cochromatographing with 4-chloroaniline (V) (Table IV), together with a trace of polar (R_f 0.0) radioactivity. Even after 4 weeks these degradation products comprised only 0.7% of the total extracted radioactivity.

A ^{14}C-radiolabeled degradation product from A label appeared after 1 week at R_f 0.52, cochromatographing with 2,6-difluorobenzoic acid. This comprised about 1.5% of the total extracted radioactivity after 4 weeks. Only a trace of polar (R_f 0.0) radioactivity was found with A label. Thus, in this study which was repeated twice, the PH-6040 was highly stable in Drummer soil, under the conditions of the experiment.

Degradation in Salt Marsh Caterpillar. As the first step in the model ecosystem degradation chain, the salt marsh caterpillar larva, *Estigmene acrea,* is a voracious insect capable of degrading many organic compounds (Metcalf et al., 1973). When fed 50-μg quantities of ^{14}C-radiolabeled A and B preparations of PH-6040, the parent compound was recovered as 99+% of the radioactivity in both feces and body homogenates with both A

Table IV. Persistency of 1-(2,6-Difluorobenzoyl)-3-(4-chlorophenyl)urea in Drummer Soil

		% ^{14}C as difluorobenzoylchlorophenylurea equivalents					
		1 week		2 weeks		4 weeks	
	R_f[a]	$^{14}C{=}O$ (A)	^{14}C-Ring (B)	$^{14}C{=}O$ (A)	^{14}C-Ring (B)	$^{14}C{=}O$ (A)	^{14}C-Ring (B)
$C_6H_3F_2CONHCONHC_6H_4Cl$	0.70	99.44	99.29	99.42	99.09	98.46	99.30
$C_6H_3F_2COOH$	0.52	0.56		0.55		1.49	
$H_2NC_6H_4Cl$	0.45		0.44		0.70		0.61
$H_2NCONHC_6H_4Cl$	0.12		0.25		0.15		0.16
Polar	0.0	Trace	0.02	0.03	0.06	0.05	0.03

[a] TLC with benzene–dioxane–acetic acid (90:30:1, by volume).

and B radiolabels. With A label 75.67% of the total ^{14}C was present in feces and the remainder in homogenate, and with B label the percentage in feces was 61.72%. Thus PH-6040 was nearly impervious to degradation by this insect.

Degradation by *Pseudomonas putida.* This soil microorganism is effective in the degradation of a variety of organic compounds. Exposure of ^{14}C radiolabeled PH-6040 with both A and B labels to a culture of *P. putida* produced no evidence of degradation upon extraction, TLC, and radioautography after 6 hr exposure at 30°. Both labeled preparations were recovered intact as 99.9+% of total ^{14}C.

SUMMARY AND CONCLUSIONS

The variety of evidence summarized above indicates that 1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea is moderately persistent biochemically in some organisms such as alga, snail, caterpillar, and mosquito larva. However, the fish *Gambusia* is able to degrade it more efficiently. Thus, the ecological magnification is about 40-fold greater in mosquito larva than in fish. From these data it seems clear that this insect growth regulator does not bioconcentrate in the fish through food chain transfer as do DDT and DDE, for example (Metcalf et al., 1971). This difference is partly a reflection of the lower lipid solubility and partition coefficient of PH-6040 as compared to DDT, PCB's, and other environmentally difficult compounds. The photodegradation of PH-6040 is an important factor in its eventual environmental degradability. A point of environmental concern is the possible effect of this inhibitor of chitin formation upon other nontarget arthropods, especially shrimp, crayfish, lobsters, and crabs in estuarine habitats.

Degradation both by microsomal enzymes and by photolysis is apparently initiated by cleavage at both the N-1 and C-2 bond to give 2,6-difluorobenzamide and 4-chloroformanilide and at the N-1 and C-1 bond to give 2,6-difluorobenzoic acid and 4-chlorophenylurea. The 2,6-difluorobenzamide is ultimately hydrolyzed to 2,6-difluorobenzoic acid and conjugated into predominately water-soluble products. Both 4-chloroformanilide and 4-chlorophenylurea are further degraded to 4-chloroaniline which is both acetylated and methylated biologically.

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