Long-Term Degradation and Potential Plant Uptake of Diflufenican under Field Conditions

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Diflufenican is a widely used highly effective residual herbicide used preemergence or early postemergence for the control of weeds in cereals. Diflufenican was applied at two sites presowing, each year for a four-year period. Soil samples were analyzed immediately following application, at 6 months and 12 months after application each year. In addition, at one of the sites in the last year, samples were taken over a more frequent time course to establish the pattern of decline. Each year a cereal (wheat) and maize crop were grown at the two sites as main crop and rotational crop, respectively. Analysis of the crop parts showed no residues in any of the wheat or maize plant parts in excess of 0.001 mg/kg, the limit of determination. Results of the analysis showed a consistent steady decline each year with no change (enhancement or decrease) in the rate. There was no accumulation from one year to the next, over the four-year period of the residual soil concentrations of diflufenican which were only slightly above 0.001 mg/kg the limit of determination. There was no evidence of movement of diflufenican below the surface layer in the soil. A DT_{50} value of ca. 14 days was calculated in the fourth year at one of the sites, followed by a more steady decline with a DT_{90} of 228 days.

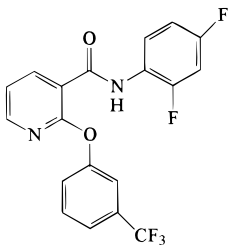
Keywords: *Long-term degradation; residues; wheat; rotational crop; diflufenican*

INTRODUCTION

Diflufenican [*N*-(2,4-difluorophenyl)-2-(3-trifluoromethylphenoxy)-3-pyridine carboxyamide C.A.] Rhône-Poulenc code M&B 38544, used extensively for weed control in cereals, was discovered in 1979 and has been marketed since the mid-1980s (Figure 1).

Diflufenican acts on carotenoid biosynthesis (Haynes and Kirkwood, 1992) and is herbicidally active on germinating broad-leaved weeds (Kyndt et al., 1985). Diflufenican is used preemergence or early postemergence (Cramp et al., 1987) for control of weeds in winter cereals, especially for "difficult" dicotyledons. It is generally mixed with trifluralin, isoproturon, chlortoluron, and other compounds to provide a wide spectrum of weed control. It is used at rates of between 50 and 250 g ha^{-1}.

Laboratory and field studies (Main et al., 1995; Rouchaud et al., 1991) have shown that diflufenican is readily adsorbed by soil with consequently very low mobility. Diflufenican is an effective herbicide because it lasts long enough to provide good weed control over the winter period. However, this persistence raises concerns for the effects on the environment and rotational crops. This is especially true for compounds such as diflufenican which can be used repeatedly over several years. Therefore, this study has been set up at two contrasting sites with different pedoclimatic conditions, but similar agronomic practices, to determine the persistence in soil of diflufenican and uptake into a main crop and a rotational crop with repeated annual applications over a four-year period.



Empirical Formula:	$C_{19}H_{11}F_5N_2O_2$
Molecular Weight:	394.3
Appearance:	White crystalline solid, without odour
Melting Point:	162.5°C
Vapour Pressure:	4.25×10^{-6} Pa at 25°C
Water Solubility:	<0.05 mg/litre
Log P:	4.5-4.9

Figure 1. Structure of diflufenican.

The two locations selected were in Central and Northern Italy, namely Rome and Bologna respectively (Figure 2). At each site the field trial was conducted on two plots: one plot with continuous wheat and one plot with a rotational crop (maize) following a wheat crop. Soil samples were collected at different depths (to a maximum of 0.9 m below the surface) at regular intervals following treatment each year. The crops (wheat and maize) were taken at harvest to determine potential uptake in the main and rotational crop parts.

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Figure 2. Selected sites.

Table 1. Soil Characteristics (SISS Methods)

site	soil type usda[a]	clay (%)	silt (%)	sand (%)	org matter (g/100 g)	pH	CEC[b] meq/100g
Rome	clay	41	23.6	35.3	2.41	7.78	29.05
Bologna	loam	17	39	44	1.81	8.06	20.9

[a] USDA, United States Department of Agriculture. [b] CEC, Cation Exchange Capacity.

Table 2. Treatment Dates

site	year 1	year 2	year 3	year 4
Rome	23 Dec 92	8 Jan 94	28 Oct 94	15 Nov 95
Bologna	23 Oct 92	25 Oct 93	21 Oct 94	20 Nov 95

MATERIALS AND METHODS

Field Studies and Diflufenican Treatment. *Site Preparation.* Neither diflufenican nor a closely related herbicide had been applied to the selected plots within at least a two-year period immediately preceding the study. At each site the application area was divided into two plots: one plot with continuous wheat production and one plot with rotational crop production, wheat−maize. A sufficient buffer zone (1.5 m) was left between plots to prevent cross-contamination.

Soil Characteristics. The soil characteristics of the trial sites are shown in Table 1. Soils were analyzed according to the Italian Society for Soil Sciences (SISS) standard methods (MiPA, 1994).

Herbicide Application. At the Rome site the application area was 90 × 120 m, while at Bologna it was 10 × 100 m. Diflufenican formulated as an emulsifiable concentrate (EC) formulation tradename BLIZZARD containing 60 g/L diflufenican, was applied at a rate of 132 g ai ha^{-1}, which corresponds to a soil concentration of 0.039 mg/kg for 0.30 m soil depth with a bulk density of 1.15 g/cm^3. Application was by tractor-mounted spraying boom (10 m), the pump being calibrated before each application and simulated spraying performed to determine the correct volume of water.

The wheat crop was treated preemergence. The dates of the diflufenican treatments are given in Table 2.

The application rate and spatial distribution were verified by surface sampling with Petridishes during the application.

Cultivations. At the Rome site, in the summer before the first sowing the plots were ploughed to 40 cm depth and harrowed before wheat sowing. Each year after wheat harvest, one plot was ploughed at 40 cm depth (to form a seed bed) and the other was harrowed and maize was seeded in the stubble. Similarly, at Bologna in the summer before the first sowing the plots were ploughed to 40 cm depth.

Each year at the two sites following maize harvest, two plots were ploughed at 25 cm depth to sow wheat. The sowing dates are given in Table 3.

Fertilizer and additional pesticides were applied to the plots according to good agricultural practice (Table 4). Rainfall was supplemented with irrigation at both sites, but irrigation was generally not applied during the winter months. Sprinkler irrigation was applied following maize seed planting and for a two-month period in the summer every 10−15 days. Rainfall and temperature data were collected for each year (Table 5).

Sampling Soil. Each year soil samples were taken at the 6 months postapplication in Rome and wheat harvest at Bologna (soil depth 0.3 m); and at 12 months postapplication before wheat sowing at both locations (soil depth 0.3 m). In the last year at Rome, soil samples were taken at 15 (0.15 m), 31 (0.15 m), 62 (0.15 m), 100 (0.15 m), 133 (0.15 m), 191 (0.3 m), 231 (0.3 m), and 388 (0.9 m) days after application to determine the rate of dissipation of diflufenican in soil.

At both sites 16 soil cores were taken from the plots at each sampling using the bucket auger technique (modified Eijkelkamp tube) and samples from the same depth were mixed to obtain either four or two composite samples for analysis. The soil sampling dates are shown in Table 6.

The samples from the Rome trial were placed in labeled polythene bags and transported to the laboratory the same day, while the Bologna samples were frozen and transported with dry ice to the Rome laboratory by car. Soil samples were dried for 24 h in a drying oven (30 °C) and sieved (2 mm). Each sample was transferred to a poly(tetrafluoroethylene) bottle and stored frozen at −20 ± 3 °C until analyzed.

Sampling Crop. At both sites each year, crop samples were harvested at maturity from different points in the plots and mixed to obtain a homogeneous sample. On harvest day the samples were placed in uniquely labeled polythene bags or cloth bags and transported under dry ice to the laboratory at Rome. The Bologna samples were transported under dry ice to Rome.

Wheat samples were separated into straw and grain. The straw was minced, and grain was dried in drying oven (30 °C) and the moisture content calculated. The mean moisture content for Rome grain was 3.32% and for Bologna 2.75%.

Undried maize samples were husked, and the grain sample was dried at 30 °C and the moisture content calculated. The average moisture content for Rome grain samples was 43.77% and for Bologna samples was 36.22%. Each crop sample was placed in a poly(tetrafluoroethylene) bottle and stored at −20 ± 3 °C until analyzed.

Analysis. *Soil Extraction.* Soil samples (50 g) were extracted with acetonitrile (100 mL) for 45 min at 240 rpm with a rotary shaker. The mixture was centrifuged for 10 min at 3000 rpm, the supernatant was filtered through a glass filter funnel with anhydrous sodium sulfate, and the filtrate was collected. An aliquot was evaporated to dryness and taken up in 2 mL of dichloromethane, and 1 mL of this solution was transferred onto Waters Silica Cartridges (2 g) previously conditioned with dichloromethane. The cartridges were eluted with dichloromethane which was evaporated to dryness and then taken up with 1 mL of acetone and analyzed for diflufenican by gas chromatography.

Crop Extraction. Wheat grain (20 g), maize grain (20 g), and wheat straw (5 g) samples were extracted with acetonitrile (50 mL) for 45 min at 240 rpm with a rotary shaker. The

Table 3. Seeding Dates

	year 1		year 2		year 3		year 4	
site	wheat	maize	wheat	maize	wheat	maize	wheat	maize
Rome	22 Dec 92	20 Jul 93	7 Jan 94	7 Jul 94	28 Oct 94	13 Jul 95	15 Nov 95	4 Jul 96
Bologna	23 Oct 92	16 Jul 93	25 Oct 93	4 Jul 94	21 Oct 94	3 Jul 95	20 Nov 95	8 Jul 96

Table 4. Fertilizer and Additional Pesticides

	ISPV–Tormancina Farm (Rome)				RPA–Corticella Farm (Bologna)			
	year 1	year 2	year 3	year 4	year 1	year 2	year 3	year 4
				Wheat				
fertilization	18-46 (N-P), 5 qt/ha; urea 2 qt/ha	18-46 (N-P), 5 qt/ha; urea 2 qt/ha; ammonium nitrate 2 qt/ha	12-52 (N-P), 5 qt/ha; urea, 2.5 qt/ha	10-46 (N-P), 5 qt/ha	11-22-16 (N-P-K), 5 qt/ha; urea, 1 qt/ha	11-22-16 (N-P-K), 5 qt/ha; urea, 1 qt/ha	11-22-16 (N-P-K), 5 qt/ha; urea, 1.5 qt/ha	18-0-16 (N-P-K), 3.5 qt/ha; ammonium sulfate 20%, 4 qt/ha; urea, 1.5 qt/ha
additional pesticides		Illoxan, 2.5 L/ha; U46, 1 L/ha; Effix, 3.5 L/ha			Athlet, 3.5 L/ha	Athlet, 3.5 L/ha	Modown 1.87 L/ha	Modown 2 L/ha
				Maize				
fertilization	11-26-16 (N-P-K), 5 qt/ha	11-22-16 (N-P-K), 5 qt/ha; urea, 2 qt/ha; ammonium nitrate, 2 qt/ha	11-22-16 (N-P-K), 5 qt/ha	11-22-16 (N-P-K), 5 qt/ha	18-46 (N-P), 3 qt/ha	18-46 (N-P), 4 qt/ha	22-10 (N-P), 3 qt/ha; urea, 1.5 qt/ha	22-10-0 (N-P-K), 3 qt/ha
additional pesticides	Lassomicromix, 7 L/ha; Furagon 5G, 10 kg/ha	Lassomicromix, 7 L/ha; Furagon 5G, 10 kg/ha	Lassomicromix, 7 L/ha; Furagon 5G, 10 kg/ha	Lassomicromix, 7 L/ha; Furagon 5G, 10 kg/ha; Geodinfos G8, 14 kg/ha				

Table 5. Cumulative Rainfall and Average of Temperature

	Rome		Bologna	
	rainfall total (mm)	*T* (°C)	rainfall total (mm)	*T* (°C)
		1992–1993		
Dec–Mar	201.8	6.8	193.4	4.1
Apr–July	110.4	18.2	194.2	18.8
Aug–Nov	474.5	16.3	288.0	16.2
		1993–1994		
Dec–Mar	222.2	7.9	165.4	6.4
Apr–July	257.7	18.5	330.2	18.8
Aug–Nov	258.9	18.0	362.8	17.1
		1994–1995		
Dec–Mar	266.1	6.2	179.5	4.9
Apr–July	259.9	18.0	380.0	18.0
Aug–Nov	204.5	15.3	252.2	15.4
		1995–1996		
Dec–Mar	243.3	7.9	292.9	3.8
Apr–July	281.4	18.9	250.8	18.3
Aug–Nov	566.3	6.2	371.0	17.3

Table 6. Soil Sampling Dates

site	year 1	year 2	year 3	year 4
Rome	15 Jun 93 7 Jan 94	17 Jul 94 27 Oct 94	6 May 95 6 Nov 95	29 Nov 95 15 Dec 95 15 Jan 96 22 Feb 96 26 Mar 96 23 May 96 2 July 96 6 Dec 96
Bologna	30 Jun 93 21 Oct 93	30 Jun 94 19 Oct 94	7 Jul 95 10 Nov 95	2 Jul 96 4 Nov 96

mixture was centrifuged for 10 min at 3000 rpm and filtered (Millex). An aliquot of the filtrate was evaporated to dryness and taken up in 2 mL dichloromethane, and 1 mL of this solution was transferred onto Waters Silica Cartridges (2 g), previously conditioned with dichloromethane. The cartridges were eluted with dichloromethane/hexane (60:40), evaporated to dryness, and then taken up with 1 mL of acetone and analyzed for diflufenican by gas chromatography.

Gas Chromatographic Conditions: GC, Hewlett-Packard Model 5890; detector, 63Ni electron capture detector; column, OV 101 (Carlo Erba 30m × 0.53 mm i.d.); detector temperature, 300 °C; carrier gas, helium at a flow rate of 4 mL min^{-1}; make up, nitrogen at a flow rate of 37 mL min^{-1}; injection technique, H.O.T. cold O.C. (high oven temperature for cold column injector); injection volume, 1; column temperature: for soil 230 °C; for crops (with precolumn OV 101 Carlo Erba 1 m × 0.53 mm i.d.) wheat grain, 250 °C; wheat straw, 220 °C; maize, 230 °C; integrator, Chromjet Spectra-Physics.

The methods were validated with untreated samples to which known amounts of diflufenican were added ranging from 0.002 to 0.008 mg/kg and the average recoveries over the four-year period were for soil 92 ± 5%, for wheat grain 96 ± 3%, for wheat straw 73 ± 5%, and for maize 87 ± 6%. Extraction and analysis of untreated control samples indicated that there were no interfering substances with the same gas chromatographic retention time as diflufenican. The limit of determination for both soil and crops was 0.001 mg/kg.

RESULTS AND DISCUSSION

The results of the soil analysis for diflufenican for each year are shown in Tables 7 and 8.

Results of the crop residues show that in each of the four years no residues above the limit of determination (0.001 mg/kg) were detected in both the main crop in the following year and the rotational crop. Residue values below 0.001 mg/kg in main and rotational crops

Table 7. Residues in Soil from Rome

year	soil depth (cm)	days from treatment	residues (mg kg^{-1}) by sample	x^a	SD^b	ν %^{c}
1992/93						
		0	0.039[d]	0.039[d]	0.026	66.66
	0−15	174	0.015, 0.029	0.022	0.010	45.00
	0−30	379[e]	0.004, 0.005, 0.006, 0.004	0.005	0.001	19.15
1993/94						
		0	0.046[d]	0.046[d]	0.014	31.14
	0−30	159	0.026, 0.009	0.017	0.012	70.71
	0−30	291[e]	0.008, 0.004, 0.005, 0.005	0.005	0.002	34.64
1994/95						
		0	0.035[d]	0.035[d]	0.013	37.39
	0−30	190	0.019, 0.021	0.020	0.001	7.07
	0−30	373[e]	0.004, 0.006, 0.005, 0.004	0.005	0.001	19.14
1995/96						
		0	0.072[d,f]	0.072[d]	0.026	35.14
	0−15	15	0.024, 0.023, 0.033, 0.027	0.027	0.004	14.43
	0−15	31	0.017, 0.022, 0.013, 0.033	0.021	0.007	35.69
	0−15	62	0.013, 0.014 , 0.017, 0.022	0.016	0.004	23.12
	0−15	100	0.016, 0.027, 0.014, 0.016	0.018	0.005	28.43
	0−15	133	0.007, 0.006, 0.042, 0.030	0.021	0.015	71.19
	0−30	191	0.011, 0.010, 0.005, 0.006	0.008	0.003	33.71
	0−30	231	0.010, 0.014, 0.004, 0.005	0.008	0.004	50.04
	0−30	388	0.005, 0.005, 0.007, 0.007	0.006	0.001	18.80
	30−60	388	0.002, 0.002, <0.001, <0.001			
	60−90	388	<0.001, <0.001, <0.001, <0.001			

[a] x, mean. [b] SD, standard deviation. [c] ν %, coefficient variation percentile. [d] Average of Petri dishes results. [e] Sampling immediately before wheat sowing. [f] On 0−15 cm depth.

Table 8. Residues in Soil from Bologna

year	soil depth (cm)	days from treatment	residues (mg kg^{-1}) by sample	x^a	SD^b	ν %^{c}
1992/93						
		0	0.024[d]	0.024[d]	0.006	25.41
	0−15	250	0.009, 0.009	0.009	0.000	
	0−30	363[e]	0.002, 0.004, 0.006, 0.002	0.003	0.002	54.71
1993/94						
		0	0.027[d]	0.027[d]	0.003	10.18
	0−30	248	0.024, 0.019	0.021	0.003	16.83
	0−30	359[e]	0.002, 0.005, 0.002, 0.005	0.003	0.002	49.49
1994/95						
		0	0.027[d]	0.027[d]	0.003	9.96
	0−30	259	0.024, 0.005	0.004	0.001	35.35
	0−30	359[e]	0.002, 0.005, 0.002, 0.004	0.003	0.001	31.91
1995/96						
		0	0.030[d]	0.030[d]	0.003	9.90
	0−30	225	0.005, 0.003, 0.019, 0.010	0.009	0.007	80.16
	0−30	350	0.003, 0.003, 0.003, 0.003	0.003	0.000	13.17
	30−60	350	<0.001, <0.001, <0.001, <0.001			
	60−90	350	<0.001, <0.001, <0.001, <0.001			

[a] x, mean. [b] SD, standard deviation. [c] ν %, coefficient variation percentile. [d] Average of Petri dishes results. [e] Sampling immediately before wheat sowing.

agree with the results of other authors for barley, oat, potato, beans, pea, turnip, savoy cabbage, scorzonera, lettuce, carrot, spinach, chicory, and onion for winter and spring applications (Rouchard et al., 1991). These results from Rome and Bologna confirm these findings even when diflufenican is used continuously for four years.

The rate of decline of diflufenican in soil in each of the four years is shown in Table 7 (Rome) and Table 8 (Bologna). In general the application of diflufenican was within an acceptable range of the target of 0.039 mg/kg. The variability of these results is probably due to spacial variation in application, and sampling and analysis; this is common in field experiments as found by other authors both in carefully prepared small-scale experiments and in large-plot experiments (Vischetti et al., 1997).

At both sites the results show comparable rates of decline each year for four years showing that there is no evidence for enhanced degradation due to microbial adaption nor any reduction in the rate. The quantity of diflufenican present in the soil at the end of each year was identical to the previous year, declining to 0.005 mg/kg at Rome site and 0.003 mg/kg at Bologna site. Thus, there was no increase in soil residue concentrations from one year to the next and therefore no accumulation occurred at either site.

The rate of decline was monitored more intensively in the fourth year at the Rome site and showed an initial rapid decline (DT_{50} ~14 days) followed by a more steady decline (DT_{90} 228 days). These values were obtained using the general power rate equation $dc/dt = KC^n$ where K is the rate constant, C is the concentration, and n is the order. For $n = 2$ a fit criteria of −0.9698 was obtained and DT_{50} and DT_{90} were calculated. A plot of the data using first-order kinetics gave a poor correlation coefficient (0.7281) and was therefore considered less appropriate. The DT_{50} value of ~14 days is somewhat shorter than other authors' results (Cramp et al., 1987; Delen *et al.*, 1987; Rouchaud et al., 1991) but this is probably due to the influences of the various physical, chemical, and biological processes as found for

trials in different places with the same products (Vischetti et al., 1997). Moreover laboratory and field studies (Rouchard *et al.,* 1991) have shown the dependence of degradation predominantly on microbial and enzymatic activity related to soil temperature. It is, therefore, probable that this is the reason for the more rapid degradation of diflufenican.

These results also confirm the low mobility of diflufenican with all of the residues remaining in the surface samples and no residues in the 30−60 cm or 60−90 cm depths after four consecutive years of application at the Rome site. The low solubility of diflufenican in water of <0.05 mg/kg (Worthing, 1994; Bic et al., 1986) and its high lipophilicity log *P* 4.9 (Knight et al., 1991) leads to the prediction that diflufenican will remain in the top 0−10 cm surface layers of soil (Rouchaud et al., 1991).

These two trial conditions gave virtually identical results showing that diflufenican is consistently readily degraded year-on-year, remains in the surface soil horizons and does not move to deeper soil layers, does not accumulate in the soil from one year to the next, and is not taken up in main and rotational crops.

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