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ACIFLUORFEN

Task 1: Review and Evaluation of Individual Studies

Task 2: Environmental Fate and Exposure Assessment

Contract No. 68-01-6679

Final Report

March 8, 1983

Submitted to:
Environmental Protection Agency
Arlington, Virginia 22202

Submitted by:
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ACIFLUORFEN

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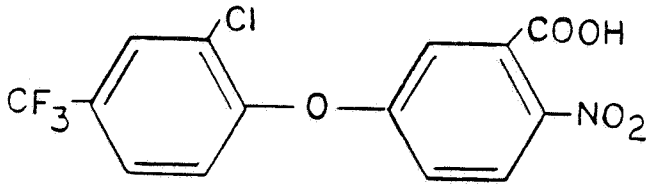
Environmental Fate and Exposure Assessment of Acifluorfen

1. INTRODUCTION

Acifluorfen is proposed for use as a selective postemergent herbicide for use in soybeans. This compound is primarily a broadleaf killer and is most effective on seedlings. It's herbicidal activity is primarily via contact action. The following data are submitted to support the environmental fate and reentry exposure data requirements for registering acifluorfen as a herbicide for use in soybeans.

2. STRUCTURE AND NOMENCLATURE

2.1	COMMON NAME	Acifluorfen
2.2	TRADE NAME	Tackle
2.3	COMPANY IDENTIFICATION	MC-10109 (acid form), MC-10978, LS-80-1213 (sodium salt)
2.4	CHEMICAL NAME	Sodium 5-(2-chloro-4-(trifluoromethyl) phenoxy-2-nitrobenzoate)
2.5	STRUCTURE	



3. USE AND APPLICATION RATES

Acifluorfen is proposed for use at 1-1.5 lb ai/A with only one application per season. Only soybeans may be planted the year acifluorfen is applied. Acifluorfen is formulated as an aqueous solution (2 lb ai/pint). Surfactants may be added when spraying, but are not included in the formulation. Refer to the proposed product label in Appendix A.

4. SCIENTIFIC STUDIES

4.1 STUDY 1

Norris, F.A., and A.E. Hassell. November, 1980. Hydrolytic stability of MC-10978 in buffered solutions. Mobil Chemical Company. Technical Memorandum TME-80.17. Acc. No. 071323.

Procedure

Acifluorfen (acid form, test substance not characterized further, purity and source unspecified) was added at 25 and 1 ppm to aqueous solutions buffered at pH 3, 6, and 9 (Table 1). Methanol was used as a cosolvent, but was ≤0.4% of the buffered solution. The solutions were maintained at 18-25°C and 40°C and were analyzed for acifluorfen at 0, 7, 14, 28, 60, and 120 days. A separate vial (containing 4 ml) was prepared for each treatment combination-sampling date. Untreated controls were included for each pH-temperature-time treatment. All vials were sealed during incubation.

Methodology

The solutions were directly analyzed for acifluorfen using liquid chromatography (280 nm). The solvent system used was acetic acid:methanol:water (1:60:39) and the stationary phase was a microbondapak C-18 (10 micron) cartridge in a radial compression module.

Results

The 120-day recoveries were the only data given. Recoveries ranged from 97 to 107%. Neither temperature nor pH had an effect on hydrolysis.

Table 1. Buffers used in acifluorfen hydrolysis study.

pH	Buffer
3	405 ml 0.1 M disodium citrate plus 595 ml 0.1 N hydrochloric acid. Add 0.05% sodium azide to inhibit microbial growth.
6	877 ml 1/15 M monopotassium phosphate plus 123 ml 1/15 M disodium phosphate.
9	500 ml 0.1 M boric acid in 0.1 M potassium chloride plus 213 ml 0.1 N sodium hydroxide. Dilute to 1 liter.

Conclusions

This study did not provide sufficient information to be evaluated. The test substance was not sufficiently characterized. The day zero recoveries of acifluorfen were not given. This study was not conducted within the appropriate temperature limits for EPA Data Requirements for Registering Pesticides, 1983. The report did not state that the study was conducted in the dark.

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4.2 STUDY 2

Somma, N., F.A. Norris, and A. Guardigli. September, 1982. Photodegradation of Tackle in aqueous solution. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/048. Acc. No. 071323.

Procedure

An aqueous solution (503.5 ml) of nitrophenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity 1 mCi/mM, ^{13}C nitro ring-labeled, 50% enriched, sodium salt, purity and source unspecified; from autoradiographs it is estimated that radiochemical purity was >95%) at 26.6 µg/ml was irradiated with artificial light to simulate exposure to sunlight. The acifluorfen solution was in a photovessel in which air was continuously bubbled through the solution and the offgases were passed through a foam plug (Identi-Plugs, Gaymor Industries). The glass in the photovessel did not filter light above 290 nm and did not admit light below 270 nm. The samples were continuously irradiated in a photochemical chamber reactor fitted with two sets of lamps that radiated UV light primarily at 285-310 nm and 320-375 nm. The most intense radiation was at 350 nm (1.46×10^4 erg/cm^2-sec) and 300 nm (1.66×10^4 erg/cm^2-sec). The intensity of the summer sun is $1 - 3 \times 10^4$ erg/cm^2-sec at 350 nm. Subsamples were removed for analysis at time zero and at several intervals up to 216 hours. The experiment was repeated three times.

Methodology

An aliquot from the photovessel was appropriately diluted with methanol and was then directly assayed for acifluorfen using HPLC (280 nm). A second aliquot was adjusted to pH 1 with HCl and then extracted three times with methylene chloride. The aqueous fraction was further extracted with ethyl acetate (35-40 hours in a liquid-liquid extractor). The ^{14}C content of all fractions was determined by using LSC. The solvent extracts were evaporated to dryness. The residues were dissolved in methanol and analyzed with TLC. The TLC plates (silica gel) were developed in two dimensions using toluene:tetrahydrofuran:acetic acid (45:30:1) followed by chloroform:acetic acid (90:10). Radioactive spots on the TLC plates were visualized with autoradiography. The ^{14}C activity of each spot was determined with one of two methods: the spots were scraped, added to water and counting cocktail, and counted (LSC) or the scraped spots were extracted with methanol and the methanol was counted. Extracts from the 192-hour sample from the first trial were subjected to HPLC analysis to identify photodegradation products.

The foam plugs used to trap volatiles were Soxhlet extracted at the end of each trial (10 hours, ethyl acetate). The total ^{14}C activity in the extract was determined by using LSC. Compounds in the extract were identified with GCMS. Presumably the ^{13}C enrichment of the test substance was intended to aid in the GCMS analyses. Interpretations of the MS data were not given.

Results

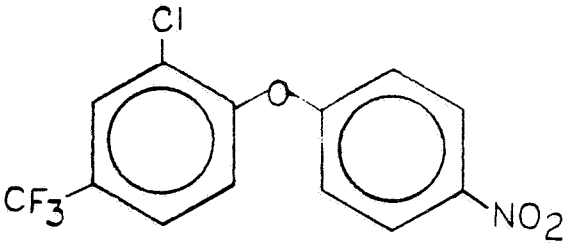
Acifluorfen recoveries are given in Table 2. The study estimated half-life val- (average half-life 120 hours). The data followed first order kinetics for all trials (r^2 = 0.963-0.990). Insufficient samples were collected from trial 1 to ues from plots of the raw data. The values obtained were rough approximations

obtain a reliable half-life value. The half-life values from Trials 2 and 3, calculated from the first order rate constant, were 89 and 95 hours, respectively. In Trial 2, the total recovery of ^{14}C in solution decreased with time. This decrease was correlated with the breakdown of acifluorfen ($r = 0.987$). After 216 hours, 68.3% of the ^{14}C activity was found in solution, and 3.7% was recovered from the foam plugs.

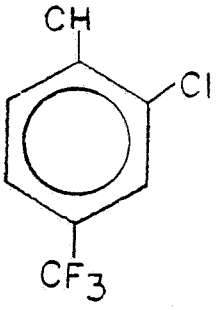
Initially, most of the radioactivity in solution from the Trial 3 samples was extracted into methylene chloride. Decreased levels of ^{14}C activity in the methylene chloride extracts were correlated to acifluorfen breakdown ($r = 0.994$). The decreased ^{14}C activity in the methylene chloride fraction was paralleled by an increase in the radioactivity in the ethyl acetate. The TLC analysis indicated that acifluorfen was almost exclusively in the methylene chloride fraction and constituted most of the ^{14}C activity in that fraction.

One major degradate (4-[2-chloro-4-(trifluoromethyl)phenoxy]-1-nitrobenzene) was found. Its highest measured concentration (6.6% of the total ^{14}C activity) occurred at 72 hours. Fifteen unidentified TLC spots were listed as containing minor amounts of ^{14}C (≤3.4%/spot). However, from the autoradiographs, it appears that at least 20 distinct TLC spots were separated out of the methylene chloride and ethyl acetate extracts.

Two volatile compounds trapped by the foam plugs were identified (2-chloro-4-trifluoromethyl phenol and 4-[2-chloro-4-(trifluoromethyl)phenoxy]-1-nitrobenzene). These compounds were not quantified.



4-[2-Chloro-4-(trifluoromethyl)phenoxy]-1-nitrobenzene (MC-10074)



2-Chloro-4-trifluoromethyl phenol

Figure 1. Photodegradation products of acifluorfen.

Table 2. Acifluorfen levels during photolysis study.

Time (hrs)	Acifluorfen (µg/ml)
Trial 1	
0	32.9
48	27.2
192	9.55
Trial 2	
0	31.5
48	23.5
96	19.45
168	10.85
192	7.85
216	5.5
Trial 3	
0	29.6
24	25.3
72	19.85
96	18.10
216	6.05

Conclusions

The study does not provide sufficient information to be evaluated. The acifluorfen solutions were not buffered and the pH of the solutions was not given. The temperature at which the study was conducted was not given. Unless a constant temperature and pH were maintained at appropriate values and this data is supplied for review, no conclusions can be made from this study. Sterile conditions were not maintained. It is assumed that the lack of nutrients in combination with the UV radiation slowed microbial activity sufficiently to prevent biologic metabolism of acifluorfen. Dark controls were not included in the study. Additional data are needed at two other pH levels to fulfill data requirements.

The observations in the following summary are contingent upon the submission of appropriate pH and temperature data to verify the conditions under which the experiment was performed. The submission of an acceptable hydrolysis study that indicates that acifluorfen is stable in water is also required. The average half-life of acifluorfen exposed to UV radiation (285-375 nm) was 92 days. The irradiation intensity was roughly equivalent to natural summer sunlight in the 320 to 360 nm range. Numerous degradates resulted from photodegradation. A primary degradate, 4-[2-chloro-4-(trifluoromethyl)phenoxy]-1-nitrobenzene, was found in solution (6.6%) and also was volatilized from the solution. Other degradates in solution did not individually exceed 3.4%. A second degradate volatilized from solution was identified as 2-chloro-4-trifluormethyl phenol. Quantitative results for volatile compounds were not given. Decreased ^{14}C activity in the acifluorfen solution, which correlated with acifluorfen degradation, suggested the loss of volatile ^{14}C compound(s) not recovered in the foam traps. The ^{14}C recovery data from Trial 3 solvent extracts did not agree with the Trial 2 results.

In addition to pH and temperature data the following information should be provided to clarify the results of this study. Carbon-14 recovery from all solutions and extracts for all trials is needed to determine if an untrapped ^{14}C compound volatilized during acifluorfen photolysis or if descrepancies in the data are an artifact of LSC. Bubbling air through the acifluorfen solution would encourage evaporation of the water resulting in concentration of acifluorfen and its nonvolatile degradates. Therefore changes in the solutions' volume in relation to time should be provided. Volatile compounds and gross ^{14}C activity recovered from foam plug traps should be reported.

4.3 STUDY 3

Gerecke, D.R., and J.P. Wargo. August, 1982. Photodegradation of Tackle (MC 10109) on a soil surface. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/045. Acc. No. 071323.

Procedure

A toluene solution of trifluoromethyl phenyl ring-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity, 37.99 µCi/mg, 98.3% radiochemical purity, acid form) was sprayed onto soil TLC plates (silt loam, 6.3% sand, 66.8% silt, 26.9% clay, 2.7% organic matter, pH 6.1, CEC 11.3 meq/100 g, 43% moisture at 1 atm) at 0.5 lb ai/A. The TLC plates were prepared by pouring a distilled water-soil slurry (screened to pass 250 µm) onto 20 x 20 cm glass plates. The plates were allowed to air dry and were divided into 4.5 x 4.5 cm squares by scoring. Each 4.5-cm square was individually treated with acifluorfen. Two treated plates (16 squares each) were hung in a photochemical reactor. An additional treated plate served as a dark control. A fourth plate was an untreated control. The plates in the reactor were continuously irradiated with UV light primarily at 285-310 nm and 320-375 nm. The light intensity was comparable to summer sunlight. Samples were collected by scraping one 4.5 cm square from each of the treated-exposed plates at 0, 1, 2, 4, and 8 hours and 1, 2, 3, 7, 14, 28, and 30 days after radiation was initiated. Samples were taken from the untreated and dark controls at 0, 1, 3, 14, and 30 days.

Methodology

All soil samples were refluxed with distilled water and methanol (1:3) for 1 hour. The extract was filtered and the soil was washed with methanol. The filtered wash and the extract were combined and brought to a constant volume with methanol. Replicate subsamples of each extract were assayed for ^{14}C activity by using LSC. A subsample of each extract was directly spotted on silica gel TLC plates and developed in a saturated atmosphere with chloroform:acetic acid (9:1) or toluene:tetrahydrofuran:acetic acid (45:30:1). Radioactive spots were located by using autoradiography and quantified by scraping and LSC. Residual ^{14}C in the extracted soil was determined by combustion and LSC.

Results

Recoveries of ^{14}C and [^{14}C]acifluorfen are summarized in Table 3. All reported data were normalized relative to day 0 recovery. Initial loss of acifluorfen was relatively rapid (average loss of 0.25%/hour during the first 72 hours). The rate of acifluorfen loss decreased with time (average loss of 0.02%/hour during the 72- to 720-hour interval. Increased levels ^{14}C bound residues coincided with acifluorfen degradation. Unaltered acifluorfen accounted for most of the extractable ^{14}C. No other ^{14}C compounds were isolated from the solvent extracts. Overall recovery of ^{14}C was between 91.4 and 117.2% and did not correspond to acifluorfen degradation. Recovery of acifluorfen from dark controls did not vary with time (95.6-102.4%). Less than 3% of the ^{14}C was not solvent extracted from the dark controls.

Table 3. Material balance of ^{14}C from soil applied [^{14}C]acifluorfen irradiated with UV light.

Exposure time (hrs)	% ^{14}C recovered[a]			
	Extractable	Acifluorfen	Bound residue	Total
0	97.8	94.9	2.2	100.0
1	96.8	94.0	2.8	99.6
2	90.0	93.6	2.8	92.8
4	87.5	92.1	3.9	91.4
8	88.9	86.0	9.6	98.4
24 (1 day)	91.1	84.1	12.4	103.5
48 (2 days)	94.7	82.5	14.7	109.4
72 (3 days)	87.7	77.6	19.1	106.8
168 (7 days)	87.3	68.8	29.9	117.2
336 (14 days)	87.1	78.7	17.2	104.3
672 (28 days)	82.8	74.0	22.2	105.0
720 (30 days)	81.5	68.4	29.4	110.9
	Dark treated controls			
24 (1 day)	96.2	95.4	2.5	98.7
72 (3 days)	95.6	94.9	2.4	98.0
336 (14 days)	99.6	94.7	2.5	102.1
720 (30 days)	102.4	94.7	2.8	105.2

[a]Recovered based on total ^{14}C found at time zero.

Conclusions

Acifluorfen applied to the surface of a dry silt loam soil at 5 ppm and irradiated with UV light (>285 nm) was slowly degraded (~32% degradation in 30 days). The initial rate of degradation was relatively high, but decreased rapidly with time. Presumably this variation in degradation rate is related to physical or chemical protection by the soil. Because of the high variation in acifluorfen recovery with time, the half-life estimate (87 days: calculated from the rate constant of a first order model) for the photodegradation of acifluorfen is very poor (r^2 = 0.551). No degradates were isolated. Carbon-14 from [^{14}C]acifluorfen breakdown was recovered as bound residue. The temperature at which this study was conducted was not given and should be reported. The actual recoveries of acifluorfen, not normalized results, must be submitted. This study tenatively fulfills data requirements contingent upon the submission of the requested data.

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4.4 STUDY 4

Wargo, J.P. July, 1982. Metabolism of carbon-14 labeled MC-10978 in Kansas, Virginia, Georgia, and New Jersey soils under aerobic and anaerobic conditions. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/040. Acc. No. 071324.

Procedure

Aliquots (1.5 kg) of four soils (sandy loam from Georgia, silt loam from New Jersey, sandy loam from Virginia, and clay loam from Kansas; see Table 4), previously sieved (2 mm), were treated with ether solutions (50 ml) of nitrophenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity 1.124 mCi/mmole, 50% ^{13}C enriched in the nitrophenyl ring, 98+% radiochemical purity, acid form, obtained from Mobil Chemical Company) and trifluoromethyl phenyl-labeled [^{14}C]acifluorofen (uniformly ring-labeled, presumed specific activity 35.2 µCi/mg, 98+% radiochemical purity, acid form, obtained from Mobil Chemical Company) at 5 ppm. The ^{13}C enrichment was included to facilitate the MS identification of degradates. It was not clear if the trifluoromethyl phenyl-labeled [^{14}C]acifluorfen was diluted with unlabeled acifluorfen. The specific activity of 35.2 µCi/mg assumes that the label was undiluted. The solvent was allowed to evaporate from the soils. Each soil aliquot was mixed and then divided into 30 subsamples (50 g). The subsamples to be incubated aerobically were watered to 30% of the soils' water capacity at 1 atm and were placed in a flow-through incubation apparatus. The soils were continuously aerated with humidified CO_2-free air. Off-gasses were scrubbed through ethylene glycol and sodium hydroxide traps. The anaerobic samples were flooded with distilled water (100 ml), purged with nitrogen for 10 minutes and then sealed to exclude air. All samples were incubated in the dark at 22° C. Duplicate samples of the aerobic trifluoromethyl phenyl-labeled [^{14}C]acifluorfen treated soils were analyzed after 0, 3, 7, 14, and 63 days of incubation and at two later dates. Other treated soils were analyzed (duplicate subsamples) at 0, 7, 14, and 63 days, and at two later dates. Unreplicated controls of aerobic soil were analyzed at 0, 3, 7, 14, and 63 days. Anaerobic controls were analyzed at 0 and 14 days after treatment. No description of the controls was given. Some variation in the sampling times was evident from the reported results.

Methodology

Each soil sample was extracted twice with each of three solvent systems [absolute methanol followed by 1% (v:v) acetic acid in methanol followed by 2% (v:v) hydrochloric acid in methanol]. The extractions were carried out at room temperature on a roller mill. The flood water from the anaerobic soils was separated from the soil before extraction. The water was acidified and partitioned with methylene chloride. The ^{14}C in all extracts (soil and water) was quantified by using LSC. The methylene chloride extract was concentrated for TLC analysis. It is assumed that the soil extracts were concentrated. All extracts were analyzed by using TLC (silica gel) with two dimensional development. Initially, plates were developed with toluene:tetrahydrofuran:acetic acid (45:30:1) and chloroform:acetic acid (9:1). For metabolite confirmation toluene:methanol (4:1) and chloroform:acetic acid (9:1) were used. A separate TLC analysis was employed to determine the dissipation rate of acifluorfen. Soil extracts with an acifluorfen standard were streaked onto silica gel plates which were then developed with chloroform:acetic

acid (9:1). The acifluorfen zone was located by using UV light. This band and area above and below this band were scraped and assayed for ^{14}C by using LSC. The results from the two dimensional TLC analysis of individual extracts were confirmed with one dimensional TLC analyses of the hydrochloric acid:methanol extracts (replicates pooled) and the combined methanol and acetic acid:methanol extracts (replicates pooled) with two or three solvent systems (toluene:tetrahydrofuran:acetic acid (45:30:1); toluene:methanol (3:1); and methylene choride:hexane (3:1)). A number of standards were chromatographed with the samples. The combined methanol and acetic acid:methanol extracts were evaporated to dryness and redissolved in methanol before TLC analysis. All TLC spots, unless otherwise stated, were scraped, mixed with water and counting cocktail, and assayed for ^{14}C activity by using LSC.

Bound residues not extracted by the three initial extractions were determined by combustion and LSC. The bound residues were further characterized by extraction of selected samples with 2% hydrochloric acid in methanol at reflux temperatures. These samples were concentrated and analyzed for acifluorfen and ^{14}C degradates with TLC, presumably the same methodology used to analyze the combined methanol and acetic acid:methanol extracts. Additional selected soils were extracted twice with 0.5 N sodium hydroxide for 1 minute. After the extraction, the soil was dried and combusted to determine ^{14}C associated with humin. The sodium hydroxide extract was acidified. The resultant precipitate (humic acid) was assayed for ^{14}C activity by using combustion and LSC. Radioactivity in the remaining supernatant (fulvic acid) was determined by using LSC.

Two acifluorfen degradates isolated by TLC were purified using HPLC and subjected to MS analysis. One of these compounds (later identified as the desnitro analog of acifluorfen) was methylated with diazomethane prior to GCMS analysis.

Radioactivity in the sodium hydroxide and ethylene glycol traps of the flow-through incubation apparatus was determined by using LSC.

Results

The rate of acifluorfen degradation was highly variable. In aerobic soils, half-life values ranged from 14-63 days to >112 days (Table 5). Variation in the rate of degradation was generally no greater among different soil types than it was between soils treated with different ^{14}C labels. Most of the ^{14}C from degraded acifluorfen was recovered in the extracts (Table 6). Recoveries of ^{14}C in the bound residue slowly increased with time but did not appear to closely correspond to acifluorfen degradation. The accumulation of bound residues was not a simple function of soil type nor ^{14}C label position. Volatile loss of ^{14}C and $^{14}CO_2$ evolution was very slow (<7.5% in 490 days). In anaerobic soils, acifluorfen half-life values varied from <7 days to >161 days. The relative rates of breakdown among soil types did not correspond to the rates observed in aerobic soils. A substantial portion of the ^{14}C from metabolized acifluorfen was recovered as bound residue (Table 7). The relative rates of acifluorfen degradation and the accumulation of ^{14}C in bound residue indicated that ^{14}C was not directly incorporated into the bound residue from the initial step in acifluorfen metabolism.

Overall recovery of ^{14}C in aerobic and anaerobic soils was near 100% except for anaerobic samples taken at 490 days and anaerobic soils treated with nitrophenyl-labeled [^{14}C]acifluorfen incubated for 161 days. Total ^{14}C recovery from these

samples ranged from 62.1 to 77.3%. The relative distribution of ^{14}C in soil organic fractions is given in Table 8. Changing the ^{14}C label position did not substantially alter the ^{14}C distribution in organic matter. Anaerobic incubation favors slight increases of ^{14}C in the humin and humic acid fractions with a decrease in fulvic acid compared to aerobic incubation. A substantial portion of the ^{14}C reported as bound residues was extracted by hot 2% hydrochloric acid in methanol (Table 9).

The only ^{14}C metabolites identified from aerobic soils were from the methanol extract of the New Jersey soil after 63 days incubation. A small quantity of the methyl ester of acifluorfen (MC-10108 Figure 2) was identified by using TLC, HPLC, and MS (≤1.7%). Trace amounts (≤0.5) of the amino analog (MC-14621) and the desnitro analog of acifluorfen (MC-10879) were identified by using TLC. These same degradates were found in the anaerobic soils. The desnitro analog of acifluorfen was purified by HPLC, methylated using diazomethane and identified using GC/MS. A substantial portion of ^{14}C did not move from the origin during TLC analysis of some samples. Utilizing reverse phase TLC as a clean-up procedure the ^{14}C at the origin was identified as the desnitro (10%) and amino (90%) derivatives of acifluorfen. Breakdown of acifluorfen in the flood water of anaerobic samples during storage before analysis resulted in the production of several ^{14}C degradates. The methylester analog of acifluorfen was identified as one of the degradates.

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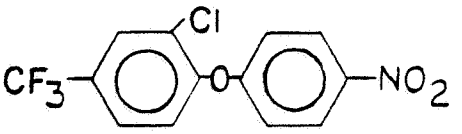
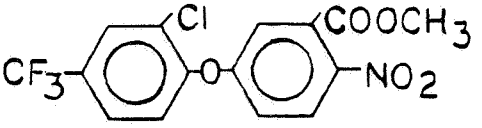
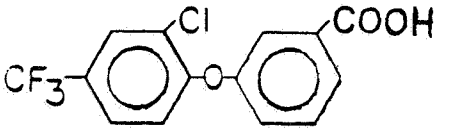
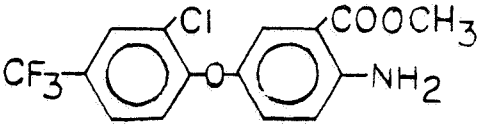
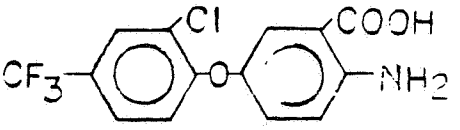
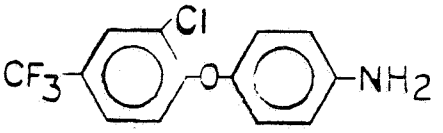
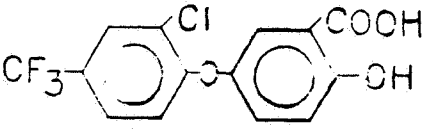
COMPOUND NO.	CHEMICAL STRUCTURE
MC-10074	
MC-10108	
MC-10879	
MC-14620	
MC-14621	
MC-15412	
MC-15598	

Figure 2. Acifluorfen degradates.

Table 4. Soil characteristics.

Soil and source	Mechanical analysis (%)			pH	Organic matter (%)	CEC (meq/100 g)	Water Capacity[a]
	Sand	Silt	Clay				
Sandy loam, Georgia	76.4	15.6	8.0	6.2	0.8	3.9	23.8
Sandy loam, Virginia	60.4	27.0	12.6	5.0	1.1	7.0	28.0
Silt loam, New Jersey	6.3	66.8	26.9	6.1	2.7	11.3	43.0
Clay loam, Kansas	24.4	47.2	28.4	6.5	1.9	12.9	38.4

[a]Percent saturation at 1 atm.

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Table 5. Total recovery of [^{14}C]acifluorfen in solvent extracts of four soils treated with 5 ppm acifluorfen[a].

Soil	Aerobic (Days)						Anaerobic (Days)							
	0	14	56	63	191	112	0	7	14	28	48	63	91	161
Trifluoromethyl phenyl label														
Sandy loam, Georgia	99.7	84.0	--[b]	68.9	--	38.5	93.8	91.8	69.8	--	37.0	--	--	--
Silt loam, New Jersey	99.3	82.2	--	75.7	--	52.2	97.2	80.8	69.7	--	39.9	--	--	--
Sandy loam, Virgina	97.5	89.9	80.5	--	69.0	--	88.7	--	--	91.0	--	--	--	65.9
Clay loam, Kansas	99.3	87.2	--	--	64.1	--	99.3	44.8	--	3.5	--	--	0.6	--
Nitrophenyl label														
Sandy loam, Georgia	99.0	82.5	--	61.6	--	--	100.7	--	76.7	--	--	51.5	--	--
Silt loam, New Jersey	97.5	76.6	--	38.6	--	--	100.5	69.1	52.3	--	--	19.3	--	--
Sandy loam, Virginia	99.5	90.4	--	73.3	--	--	101.2	99.2	--	--	--	90.2	--	--
Clay loam, Kansas	99.7	99.6	--	83.6	--	--	103.2	57.7	8.2	--	--	0.9	--	--

[a]Values expressed as percent of ^{14}C initially added.

[b]Samples not taken or not analyzed.

Table 6. Material balance of ^{14}C from four soils treated with trifluoromethyl phenyl- or nitrophenyl-labeled [^{14}C]acifluorfen incubated aerobically.

Trifluoromethyl phenyl label						Nitrophenyl label					
Time[a] (days)	% ^{14}C recovered					Time (days)	% ^{14}C recovered				
	Extractable	CO_2	Volatiles	Bound	Total		Extractable	CO_2	Volatiles	Bound	Total
	Sandy loam from Georgia										
0	99.7	<0.1	<0.1	0.3	100.0	0	99.0	<0.1	<0.1	1.0	100.0
63	94.7	0.6	<0.1	1.1	96.4	63	87.2	0.9	<0.1	2.5	90.6
112	94.9	0.8	<0.1	2.1	97.8	126	81.4	2.3	<0.1	3.9	87.6
490	77.8	1.5	1.4	10.2	90.9	392	96.3	2.6	<0.1	3.7	102.6
	Silt loam from New Jersey										
0	99.3	<0.1	<0.1	1.0	100.3	0	97.5	<0.1	<0.1	2.5	100.0
63	88.2	0.6	<0.1	4.1	92.9	63	83.3	2.2	0.1	15.8	101.4
112	90.2	0.8	<0.1	4.9	95.9	126	80.3	6.5	0.1	18.8	105.7
490	84.1	1.2	<0.1	8.8	94.1	392	74.7	7.5	0.1	16.5	98.8
	Sandy loam from Virginia										
0	97.5	<0.1	<0.1	2.6	100.1	0	99.5	<0.1	<0.1	0.5	100.0
56	96.4	0.3	<0.1	3.6	100.3	63	96.7	0.3	<0.1	2.0	99.0
91	91.3	0.4	<0.1	2.4	94.1	126	101.9	0.7	<0.1	3.0	105.6
490	81.4	0.6	0.2	7.4	89.6	382	71.5	0.9	<0.1	1.6	74.0
	Clay loam from Kansas										
0	99.3	<0.1	<0.1	0.7	100.0	0	99.7	<0.1	<0.1	0.2	99.9
42	93.9	0.6	<0.1	5.9	100.4	63	99.5	0.2	<0.1	3.7	103.4
91	97.3	0.6	<0.1	2.3	100.2	126	93.9	0.6	<0.1	4.1	98.6
490	93.3	0.7	<0.1	2.3	96.9	392	93.8	0.7	<0.1	5.2	99.7

[a]Not all sampling dates included, but longest sampling interval is given.

Table 7. Material balance of ^{14}C from four soils treated with trifluoromethyl phenyl- or nitrophenyl-labeled [^{14}C]acifluorfen incubated anaerobically.

Trifluoromethyl phenyl label					Nitrophenyl label				
Time[a] (days)	% ^{14}C recovered				Time (days)	% ^{14}C recovered			
	Water layer	Extractable	Bound	Total		Water layer	Extractable	Bound	Total
	Sandy loam from Georgia								
0	--	93.8[b]	0.8	94.6	0	88.4	12.3	0.4	101.1
49	36.8	29.7	4.4	70.9	63	47.2	31.5	5.5	84.2
181	51.2	30.7	13.6	95.5	126	40.9	32.4	11.0	84.3
490	19.4	24.6	18.1	62.1	392	27.6	28.2	7.2	63.0
	Silt loam from New Jersey								
0	--	97.2[b]	1.6	98.8	0	60.3	40.2	2.0	102.5
49	39.7	36.7	5.4	81.8	63	9.9	55.2	29.2	98.8
182	26.0	42.6	27.1	95.7	126	1.4	49.0	44.1	94.5
490	1.7	28.1	39.6	69.4	392	0.8	25.1	36.2	62.1
	Sandy loam from Virginia								
0	17.8	16.9	0.3	89.0	0	69.2	32.0	0.4	101.6
56	76.6	22.1	1.9	100.6	63	68.4	30.0	0.8	99.2
91	74.0	22.9	2.5	99.4	126	62.7	32.0	2.0	96.7
161	64.3	21.6	2.6	88.5	392	35.4	36.9	5.0	77.3
	Clay loam from Kansas								
0	--	99.3[b]	0.7	100.0	0	81.6	21.6	0.4	103.6
56	2.4	39.0	30.8	72.2	63	7.2	64.2	26.9	98.3
91	1.9	36.0	72.3	110.2	126	5.7	61.7	30.4	97.8
161	16.6	42.8	31.1	90.5	392	2.4	38.8	25.8	67.0

[a]Not all sampling dates included, but longest sampling interval is given.

[b]Total of ^{14}C activity extracted from soil and in water layer.

Table 8. Distribution of ^{14}C in bound residue[a].

Fraction	Condition	Distribution (%) Kansas soil	Virginia soil	Georgia soil	New Jersey soil
	Nitrophenyl label				
Humin	Aerobic	45.3	45.0	33.9	34.2
	Anaerobic	50.8	52.5	38.3	35.6
Fulvic acid	Aerobic	24.0	19.1	32.6	16.7
	Anaerobic	15.3	9.2	21.8	12.9
Humic acid	Aerobic	30.7	35.9	33.5	49.1
	Anaerobic	33.9	38.3	40.0	51.6
	Trifluoromethyl phenyl label				
Humin	Aerobic	--	--	37.4	46.3
Fulvic acid	Aerobic	--	--	31.2	17.4
Humic acid	Aerobic	--	--	31.4	36.3

[a]Day-63 samples.

Table 9. Percent ^{14}C recovered in hot hydrochloric acid in methanol (2%) extracts of soil treated with 5 ppm [^{14}C]acifluorfen.

Soil	^{14}C-label position	Incubation environment	Incubation time (weeks)	% ^{14}C recovered
Georgia	CF_3-Ø[a]	aerobic	70	4.5
New Jersey	CF_3-Ø	aerobic	70	3.2
New Jersey	NO_2-Ø[b]	aerobic	9	6.0
			18	6.7
			56	6.6
Kansas	CF_3-Ø	anaerobic	4	12.4
			8	11.8
Kansas	NO_2-Ø	anaerobic	2	15.9
			9	15.5
New Jersey	NO_2-Ø	anaerobic	1	3.2
			2	3.1
			9	11.9
			18	14.6
			56	11.4

a^{14}C in trifluoromethyl phenyl ring.

b^{14}C in nitrophenyl ring.

Conclusions:

Acifluorfen was found to degrade at varying rates in four soils under both aerobic and anaerobic conditions. Three degradates of acifluorfen breakdown were identified: the methylester, desnitro, and amino analogs of acifluorfen. The identification of the methylester and desnitro analogs was confirmed (MS analysis), but the amino analog was only tentatively identified.

Additional conclusions cannot be made because the techniques used were unsubstantiated and much of the data were not given in the report. The extraction procedures used are questionable. After six extractions using three solvent systems, additional ^{14}C, which was identified as the parent acifluorfen, the desnitro and/or the amino analogs of acifluorfen, was extracted using hot methanol acidified with HCl. Tests demonstrating the extraction efficiency of the procedures used is needed. Analysis of the flood water from the anaerobic soils at time zero revealed substantial quantities of radioactive degradates. It is presumed that these degradates are an artifact of improper storage procedures or analytical methodologies. Although a number of TLC solvent systems were used, none appeared adequate to clearly separate the amino and desnitro analogs of acifluorfen. Overall, the TLC analyses of unpurified extracts was poor. This was evident by the poor resolution seen in some of the autoradiographs. The identification of the amino and desnitro analogs in the origin of TLC analyses quantitatively demonstrated the inadequacy of chromatographing uncleaned extracts. The procedure used to extract organic matter was inadequate because of the short extraction time.

Except for a few isolated recoveries from individual samples, no quantitative ^{14}C recoveries from the TLC analyses were given. Acifluorfen recoveries were not reported in over half of the samples collected. From the sample autoradiographs in the report it is apparent that at least some of these analyses for which no data were given were performed. The results given indicate that the rate of acifluorfen degradation is highly variable. In order to fully evaluate this variability, the data from each replicate should be given and should be accompanied with a statistical analysis to provide estimates of half-life values with appropriate confidence limits. The acifluorfen recoveries that were reported are in error because the acifluorfen extracted by the hot acidified methanol was not included in these data.

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4.5 STUDY 5

Piznik, M., and J.P. Wargo. September 1982. Abbreviated aerobic/anaerobic soil metabolism study with radiolabeled Tackle (MC-10978). Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/047. Acc. No. 071324.

Procedure

A New Jersey loamy sand soil (83.6% sand, 7.2% silt, 9.2% clay, 1.9% organic matter, pH 5.6, CEC 5.6 meq/100 g, 45% moisture at 1 atm) was sieved (4 mm), air-dried, and treated with an acetone solution of nitrophenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity, 10.13 mCi/mM, acid form, purity and source unspecified) at 4.82 ppm. Soil samples (50 g) were individually treated with the test substance. After the acetone was allowed to evaporate, sufficient water was added to each sample to obtain a soil moisture level of 27%. The samples were allowed to stand for 1 hour and then were attached to an air manifold that brought humidified air to the soil and scrubbed off gases from the soil through ethylene glycol and sodium hydroxide traps. The samples were incubated in the dark at 22°C and were periodically checked and adjusted for moisture loss. After 28 days, two samples were removed from the manifold, fortified with dried alfalfa meal (2 g/sample), flooded with deionized water (100 ml), purged with nitrogen, and sealed. Aerobic samples were collected for analysis at 0, 1, 3, 7, 14, 28, and 56 days, and 3, 4, 6, 9, and 12 months. The anaerobic samples were analyzed 28 and 56 days after flooding. The sodium hydroxide and ethylene glycol traps were sampled periodically throughout the study. The study was not replicated.

Methodology

Three subsamples of each sample were assayed for total ^{14}C activity by combustion and LSC. The remaining soil was sequentially extracted by shaking with dichloromethane:methanol (1:1) for 1 hour, refluxing with methanol:0.1 N hydrochloric acid (3:1) for 1 hour, and refluxing with 2% hydrochloric acid in methanol for 1 hour. After each extraction the extract was filtered, the soil washed with methanol, and the methanol combined with the corresponding extract. All extracts were assayed for ^{14}C activity by LSC, and then were evaporated to dryness. The residue from the 2% hydrochloric acid in methanol extract was dissolved in deionized water and extracted three times with ethyl acetate. The ^{14}C activity in the aqueous and ethyl acetate fractions was determined by LSC. These extracts were then evaporated to dryness. Prior to extracting the anaerobic soil samples, the flood water was removed, filtered, and counted for ^{14}C activity by LSC. After extraction, all soils were combusted and ^{14}C activity was determined by LSC.

The dried extracts were taken up in acetone, and analyzed with TLC. Silica gel TLC plates were spotted with extracts along with authentic standards of acifluorfen and suspected metabolites of acifluorfen, and then were developed in an atmosphere saturated with one of three solvent systems: chloroform:acetic acid (9:1), toluene:tetrahydrofuran:acetic acid (45:30:1), and toluene:methanol (3:1). Standards were visualized with UV light. Radioactive spots were located by using autoradiography, and then were scraped, and either directly counted or extracted with methanol and the methanol was counted (LSC) to quantify the ^{14}C activity.

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Results

The recovery data reported was normalized such that total ^{14}C recovered for each sample was set equal to 100%. Therefore, all percent data was recalculated by this reviewer, basing recovery on the quantity of ^{14}C initially added to each sample (14,985,862 dpm/sample). Acifluorfen disappearance from aerobic soil was rapid (61.5% loss in 7 days, Table 10). The recovery data approximated first order kinetics ($r^2 = 0.867$) and the half-life was calculated to be 17 days. The customary regression of ln (100/% recovery) on time was utilized to calculate the half-life ($t_{1/2}$ = ln 2/-slope) instead of the method used in the report [100-(% recovery) plotted against time; $t_{1/2}$ = 1/(slope) (recovery at time zero)]. No reference or reason was given in the report to support the use of this unorthodox regression; the formula used to calculate the half-life was incorrect.

The primary metabolite found in the aerobic soil was the amino analog of acifluorfen (2-chloro-4-trifluoromethyl)phenoxy-2-aminobenzoic acid; MC-14621, Figure 2, pg 13). The concentration of this product increased as acifluorfen decreased during the first 14 days of incubation. After reaching a maximum concentration of 17.7% of the total ^{14}C, the levels of the amino analog decreased. The levels of ^{14}C remaining at the origin of the TLC plates increased with time up to day 56 of the incubation (28.7%) and then decreased to 20.4%. A portion of the ^{14}C from acifluorfen degradation was retained as bound residue, reaching 19.0% after 84 days incubation (Table 11). Recovery of $^{14}CO_2$ was low (4.1% after 84 days) and ^{14}C detected in the ethylene glycol trap was inconsequential. Evidently all analyses were discontinued with the day 84 samples.

Acifluorfen degradation in anaerobic soil was also rapid. Only a small fraction of acifluorfen remained in the soil when anaerobic conditions were initiated (9.4%), but pesticide levels were reduced over 60% within 28 days of anaerobic incubation. Levels of the amino analog of acifluorfen in anaerobic soil were similar to those found in aerobic soil, however, somewhat greater recoveries of ^{14}C at the TLC origin and lower recoveries of ^{14}C in bound residues were found in the anaerobic soil.

All the ^{14}C recovery data from the TLC analyses of the soil extracts was not given. It is apparent from the sutoradiographs that radioactive spots with R_f values similar to MC-15598, MC-10108 and/or MC-10074, MC-15412, and MC-10879 were present as well as several unidentified spots (Figure 2, pg 13). Generally the ^{14}C activity in these spots appeared to be low, although several spots may have moderate quantities of ^{14}C associated with them.

Overall recoveries of ^{14}C were quantitative at day zero, but decreased as acifluorfen recovery decreased (65-67% recovery at day 84). No explanation is given for these losses. Poor recovery of bound residues by inefficient combustion techniques may partially explain the low recoveries. Loss of a volatile not trapped in sodium hydroxide or ethylene glycol is also possible.

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Table 10. Recovery of acifluorfen and its degradates.

Incubation time (days)	% ^{14}C recovered[a]		
	Acifluorfen	MC-14621	TLC origin
Aerobic			
0	98.1	0.1	0.3
1	81.1	1.4	1.8
3	59.8	9.3	6.0
7	38.5	16.0	13.0
14	17.2	17.1	19.4
28	9.4	5.9	22.9
56	3.7	6.5	28.7
84	3.0	9.0	20.4
Anaerobic[b]			
28	3.5	7.1	30.6
56	2.8	8.4	25.0

[a]Data calculated basing recovery on 14,985,862 dpm initially added to each sample.

[b]Anaerobic conditions were implemented after 28 days of incubation under aerobic conditions.

Table 11. Distribution of ^{14}C from [^{14}C]acifluorfen-treated soil incubated under aerobic and anaerobic conditions[a].

Incubation time (days)	% ^{14}C recovered[b]				
	$^{14}CO_2$	Combustion[c]	Extractable	Bound residue	Total[d]
Aerobic					
0	--	--	100.9	0.1	101.0
1	<0.1	79.4	89.6	0.6	91.2
3	<0.1	80.3	84.3	2.0	86.3
7	0.1	80.8	82.8	3.4	86.3
14	0.4	80.5	76.4	5.2	82.0
28	0.7	62.7	62.0	8.9	71.6
56	1.3	97.4	58.2	12.8	72.3
84	4.1	74.9	42.0	65.1	65.1
Anaerobic[e]					
28	--	--	63.3	4.7	68.0
56	--	--	54.1	12.5	66.6

[a]Carbon-14 from ethylene glycol traps was <0.1% after 84 days aerobic incubation and therefore is not listed.

[b]Data calculated basing recovery on 14,985,862 dpm initially added to each sample.

[c]Total ^{14}C in soil determined by combustion.

[d]Total ^{14}C recovered as $^{14}CO_2$, solvent extractable, and bound residue.

[e]Anaerobic conditions were implemented after 28 days of incubation under aerobic

Conclusions

In a loamy sand soil from New Jersey [^{14}C]acifluorfen was rapidly degraded (half-life 6 days under aerobic, <28 days under anaerobic conditions). The loss of ^{14}C as CO_2 was very low in aerobic soil and was not measured in anaerobic soil. A major degradate was identified as (2-chloro-4-trifluoromethyl)phenoxy-2-aminobenzoic acid. Concentrations of this compound increased as acifluorfen concentrations decreased up to day 14. Other ^{14}C degradates were isolated using TLC but quantitative results were not reported. Most of the ^{14}C activity from degraded acifluorfen was recovered as bound residue or as presumably unidentified polar materials (^{14}C at the TLC origin) in the solvent extract. The slow accumulation of ^{14}C in the bound residues suggested that the secondary metabolism of acifluorfen degradates contributed ^{14}C to this fraction rather than the primary metabolism of acifluorfen.

The efficiency of the extraction procedure was not given. The difficulty in removing all the acifluorfen and closely related degradates was made apparent in Study 4. Additionally, it was shown in Study 4 that the TLC solvent systems were not necessarily capable of moving the amino and denitro analogs of acifluorfen off of the origin. The inclusion of metabolites that are closely related to acifluorfen, in the TLC origin or in bound residues is a false indication of the degree to which the test substance has degraded. It also presents an inaccurate distribution of degradates. Therefore, the efficiency of the extraction procedure and the efficiency of the TLC separations must be provided to evaluate this study. A large portion of the quantitative ^{14}C data from the TLC analyses was not given. These data should be provided. The material balance showed decreased recovery with time. A explanation of where ^{14}C was lost is necessary. Because of these deficiencies, this study does not meet data requirements.

4.6 STUDY 6

Gemma, A.A., and J.P. Wargo. October, 1982. The metabolic fate of ^{14}C-MC-10978 in New Jersey loamy sand soil. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/051. Acc. No. 071324.

Procedure

Soil columns (56 cm in diameter, 61 cm long) containing a New Jersey loamy sand (83.6% sand, 7.2% silt, 9.2% clay, 1.9% organic matter, pH 5.6, CEC 5.6 meq/100 g) in which nine to ten soybean plants per column were growing were sprayed with [^{14}C]acifluorfen (trifluormethyl phenyl uniformly ring-labeled, specific activity 2.85 mCi/mM, sodium salt, 98.1% radiochemical purity, 50% ^{13}C enrichment of trifluormethyl phenyl ring, mixed with analytical grade unlabeled acifluorfen, source unspecified) at 5 lb ai/A. The soybeans had three or four trifoliate leaves when sprayed. The test substance was sprayed in an aqueous solution containing 0.125% X-77 surfactant. Twelve columns were sprayed. Drainage from the columns was controlled and the drainage water was collected. The columns were placed in a field in New Jersey. Six soil cores were taken at random from the columns at 0, 1, 2, 4, 7, 14, 28, and 60 days. The cores were divided into 0- to 3- and 3- to 6-inch segments and replicate samples were pooled. Samples were frozen (0°F) until analyzed.

Methodology

A subsample of each sample was refluxed (1 hour) with dichloromethane:methanol (1:1). After extraction, the soil was washed with methanol and water. Following filtration, the extract and washes were combined and assayed for ^{14}C activity by LSC. A portion of the extracted soil was reextracted by refluxing (1 hour) with 2% hydrochloric acid in methanol. The extract was filtered and then assayed for ^{14}C activity by LSC. The organic solvents were evaporated both from the dichloromethane:methanol and the hydrochloric acid:methanol extracts. A 1% aqueous solution of sodium sulfate was mixed with the remaining aqueous portions of the extracts. The samples were acidified with hydrochloric acid and then were partitioned three times with ethyl acetate. The combined ethyl acetate was dried over sodium sulfate and then evaporated to dryness. The residue was taken up in methanol, and then assayed for total ^{14}C activity and analyzed by TLC. Radioactivity in the remaining aqueous fractions was quantified by LSC.

All TLC separations were accomplished with saturated development of silica gel plates with toluene:tetrahydrofuran:acetic acid (45:30:1). Spots were identified by comparison with authentic standards (from Mobil Chemical Company) spotted adjacent to the samples. The standards were visualized by using UV light. Radioactive spots were located by using autoradiographs and were quantified by scraping and LSC. Subsamples of unextracted and extracted soil were combusted and the ^{14}C released was quantified by using LSC to determine total ^{14}C activity and bound residue, respectively.

Results

Recovery of ^{14}C from the treated soil was near 100% on day zero but was reduced to <50% for all subsequent sampling dates (Table 12). Most of the radioactivity recovered was in the surface 3 inches of soil. Essentially all of the recoverable ^{14}C (≥94%) was extractable through day 14 of the study (Table 13). Increasing

amounts of radioactivity were shifted from the extractable to the bound residue fractions in the 28 and 60 day samples. At time zero, 91% of the recoverable ^{14}C was unaltered acifluorfen. By day 60, <11% of the recovered ^{14}C was present as the parent compound. Recovery of (2-chloro-4-trifluoromethyl)phenyl-2-aminobenzoic acid (MC-14621) ranged from <0.1 to 3.4%. Nonpolar degradates (MC-10879, MC-10074, MC-14620, and MC-10108; See Figure 2, pg 13) collectively accounted for 3.4 to 13.7% of the recovered activity. Most of the extractable ^{14}C from degraded acifluorfen was at the TLC origin or was relatively polar (i.e., had a lower R_f value than acifluorfen). Carbon-14 recoveries from TLC analyses appeared to be highly variable.

Table 12. Total ^{14}C in soil treated with acifluorfen at 0.5 lb ai/A.

Sampling interval	Soil depth (in)	Total radioactivity ^{14}C acifluorfen equiv. (ppm)[a]
0 day	0-3	0.89
	3-6	0.07
1 day	0-3	0.40
	3-6	0.03
2 days	0-3	0.28
	3-6	0.13
4 days	0-3	0.32
	3-6	0.03
7 days	0-3	0.41
	3-6	0.02
14 days	0-3	0.26
	3-6	0.02
28 days	0-3	0.28
	3-6	0.02
2 months	0-3	0.34
	3-6	0.09

[a]Determined by combustion.

Table 13. Distribution of ^{14}C in soil treated with [^{14}C]acifluorfen[a].

Length of incubation (days)	Distribution of recovered ^{14}C (%)		Distribution of extractable ^{14}C (%)					
	Extractable	Bound residue	Origin[b]	Acifluorfen	Polar[c]	Nonpolar[d]	MC-14621[e]	Total
0	98.0	2.0	1.0	91.0	3.1	3.4	--	98.5
1	94.0	6.0	1.1	85.6	3.3	3.5	--	93.5
2	99.0	1.0	16.9	49.6	12.8	6.4	3.4	89.1
4	94.0	6.0	15.7	49.1	12.1	7.8	3.4	88.1
7	95.0	5.0	12.0	59.1	10.6	6.0	1.9	89.6
14	98.0	2.0	20.5	44.1	18.6	7.6	1.9	92.7
28	75.0	25.0	26.1	15.3	19.6	7.7	1.0	69.7
60	39.7	60.3	10.8	10.8	12.2	13.7	1.9	49.9

[a]Data normalized. Percent recovery based on total ^{14}C recovered.

[b]Carbon-14 recovered at the TLC origin.

[c]Compounds with R_f values lower than the R_f of acifluorfen, including 5-(2-chloro-4-(trifluoromethyl)phenoxy)-2-hydroxybenzoic acid (MC-15598).

[d]MC-10879 3-(2-chloro-4-(trifluoromethyl)phenoxy)-benzoic acid
MC-10074 4-(2-chloro-4-(trifluoromethyl)phenoxy)-1-nitrobenzene
MC-14620 5-(2-chloro-4-(trifluoromethyl)phenoxy)-1-amino (methylbenzoate)
MC-10108 5-(2-chloro-4-(trifluoromethyl)phenoxy)methylbenzoate

[e]5-(2-chloro-4-(trifluormethyl)phenoxy)-2-aminobenzoic acid

Conclusions

No conclusions can be made from this study. The temperature and moisture levels of the soil were not given. It is not known if the soil was leached, and if so if any ^{14}C was recovered at depths below 6 inches or in the soil leachate. Over 50% of the ^{14}C added as acifluorfen could not be recovered by day 1 of the incubation. No explanation of this loss was attempted. The quantification of ^{14}C associated with the parent compound, individual metabolites or groups of metabolites from the TLC analysis was highly variable. The total ^{14}C recovery from TLC plates did not necessarily match the total extractable ^{14}C recovery, suggesting errors in computation. The individual recovery of most metabolites was not given. At least a range of recoveries for each metabolite should be reported. Extractable ^{14}C was partitioned between an aqueous and an organic phase. The radioactivity in each phase should be reported.

4.7 STUDY 7

Gemma, A.A., and J.P. Wargo. October, 1982. Tackle soil metabolism: Metabolic fate of ^{14}C-MC-10978 in Maryland silt loam soil. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/053. Acc. No. 071324.

Procedure

Fourteen plastic cylinders (8-inch diameter, 18-inch length) were sunk into a silt loam soil [12.8% sand, 73.2% silt, 14.0% clay, 2.7% organic matter, pH 7.6, CEC 11.21 meq/100 g, 12.39% moisture at 0.33 bar (field capacity)] to a depth of 13 inches in a field on the eastern shore of Maryland. On June 23, 1981 the soil in the cylinders was treated with [^{14}C]acifluorfen at 2.5 lb ai/A. Seven cylinders were treated with 23.7/µCi of nitrophenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity 2.6 µCi/mg, purity and source unspecified). The remaining seven cylinders were treated wth 23.40 µCi of trifluoromethyl phenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity 2.6 µCi/mg, purity and source unspecified). Controls were maintained adjacent to the treated cylinders but no description was given. A single cylinder from each ^{14}C label treatment was removed 1 hour, and 3, 7, 14, 28, 56, and 365 days after treatment. The samples were sealed and frozen (0°C) until analysis. The day 0 and possibly the 3- and 7-day samples were stored at room temperature for an undetermined period of time. Weather data were obtained from Royal Oak, Maryland, located ~2 miles from the test site.

Methodology

The entire 0- to 2-inch layer and core samples from depths of 2-6 and 6-12 inches were mixed and subsampled for extraction and combustion. All extractions, combustion, TLC, and LSC methods are the same as used in Study 6 of this report.

Results

The recovery of ^{14}C in the soil cylinders is given in Table 14. Recovery of the nitrophenyl ^{14}C label was much higher than theoretically possible for the application rate reported. Generally most of the ^{14}C from either label was found in the surface 2-inch layer of soil. Relatively high concentrations were sporatically observed in the subsurface samples. However, no logical pattern of ^{14}C movement through the soil was apparent. Significant losses of ^{14}C with time were observed with both labels. The nitrophenyl label ^{14}C decreased 86% after 1 year; the trifluoromethyl phenyl ^{14}C decreased 64% in the same time period. No explanation of how ^{14}C losses occurred was given.

The distribution of ^{14}C between extractable and bound residues as well as TLC degradate characterization data given in Table 15. It is obvious that extensive degradation of acifluorfen occurred in the time zero samples due to improper sample storage. It is likely that degradation during storage occurred in other samples as well. Therefore, the rate of degradation cannot be accurately determined. Several ^{14}C degradates were identified including MC-14621, MC-15598, MC-10879, MC-10074, and MC-14620 (Figure 2, pg. 13). The amino analog of acifluorfen was found in all samples incubated 0 to 56 days but was not detected in the 365 day samples. Collectively the concentrations of other ^{14}C metabolites was relatively constant throughout the study. After 1 year, more than 50% of the recoverable ^{14}C remained in the soil as bound residues. A substantial portion of the extractable ^{14}C was associated with materials too polar to move from the TLC origin.

Table 14. Total ^{14}C residues in Maryland silt loam soil treated with [^{14}C]acifluorfen.

Sampling interval (days)	Soil depth (in)	Total ^{14}C acifluorfen equivalent (ppm)[a]		Rainfall (in)	
		NO_2	CF_3	Per interval	Cumulative
0	0-2	6.80	3.64	0	0
	2-6	<0.02	<0.02		
	6-12	<0.02	<0.02		
3	0-2	6.52	2.27	0.23	0.23
	2-6	2.15	<0.02		
	6-12	1.93	0.03		
7	0-2	2.34	2.76	0	0.23
	2-6	0.11	0.12		
	6-12	<0.02	<0.02		
14	0-2	1.37	2.78	2.36	2.59
	2-6	0.02	0.06		
	6-12	<0.02	0.06		
28	0-2	2.06	1.39	1.07	3.66
	2-6	0.03	1.38		
	6-12	0.09	0.04		
56	0-2	1.79	1.81	4.17	7.83
	2-6	0.52	0.03		
	6-12	0.08	<0.02		
365	0-2	0.97	1.31	35.88	43.71

[a] NO_2 - nitrophenyl ^{14}C label; CF_3 - trifluoromethyl phenyl ^{14}C label

Table 15. Distribution of ^{14}C in soil treated with [^{14}C]acifluorfen[a].

Length of incubation (days)	Distribution of recovered ^{14}C (%)		Distribution of extractable ^{14}C (%)				
	Extractable	Bound residue	Origin[b]	Acifluorfen	MC-14621[c]	Polar[d]	Nonpolar[e]
Trifluoromethyl phenyl label							
0	74.5	25.5	27	16	7	12	6
3	86.5	13.5	22	18	13	14	6
7	84.6	15.4	31	12	8	15	8
14	79.3	20.7	28	6	9	15	7
28	78.8	21.2	38	7	3	16	4
56	78.2	21.8	38	10	9	14	10
360	46.2	53.8	20	2	--	7	8
Nitrophenyl label							
0	95.6	4.4	37	6	12	12	8
3	96.4	3.6	37	5	8	14	7
7	73.3	26.7	37	4	7	13	6
14	82.0	18.0	41	3	5	13	6
28	70.0	30.0	42	5	4	13	4
56	76.5	23.5	25	18	3	16	5
360	45.7	54.3	18	3	--	13	9

[a]Data normalized. Percent recovery based on total ^{14}C recovered.

[b]Carbon-14 recovered at the TLC origin.

[c]5-(2-chloro-4-(trifluormethyl)phenoxy)benzoic acid

[d]Compounds with R_f values lower than the R_f of acifluorfen, including 5-(2-chloro-4-(trifluoromethyl)-phenoxy)2-hydroxybenzoic acid (MC-15598).

[e]MC-10879 3-(2-chloro-4-(trifluoromethyl)phenoxy)benzoic acid
MC-10074 4-(2-chloro-4-(trifluoromethyl)phenoxy)-1-nitrobenzene
MC-14620 5-(2-chloro-4-(trifluoromethyl)phenoxy)-1-amino (methylbenzoate)
MC-10108 5-(2-chloro-4-(trifluoromethyl)phenoxy)methylbenzoate

Conclusions

Conclusions cannot be made from this study because a large portion of the ^{14}C applied to soil columns in the field as [^{14}C]acifluorfen could not be recovered. Samples from three of seven sampling intervals were not stored properly and continued to metabolize acifluorfen during storage. This included the day 0 samples. Sporadic high recoveries of ^{14}C suggested large errors in sample analyses and possibly in soil treatment. Quantitative recovery of ^{14}C from TLC analyses was not sufficiently detailed. The distribution between aqueous and ethyl acetate fractions during the extraction and cleanup procedures was not given.

4.8 STUDY 8

Norris, F.A, and K.M. Miller. December, 1980. Mobility of MC 10978 in four soil types. Mobil Chemical Company. Technical Memorandum TME-80.24. Acc. No. 071325.

Procedure

Soil columns (5-cm inner diameter, 30-cm length) were tightly packed with four sieved (2 mm) soils: a sandy loam from Georgia, a sandy loam from Virginia, a silt loam from New Jersey, and a clay loam from Kansas (Table 4, pg 14). Six columns were prepared for each soil and an additional 150 g of each soil was treated with an ether solution of trifluormethyl phenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity 1 mCi/mM, from Mobil Chemical Company, purity unspecified) at an unspecified rate. After solvent evaporation and mixing, 30-g aliquots of each treated soil were added to the top of each of four columns containing the corresponding soil. A fifth 30-g aliquot of each treated soil was frozen for later extraction and analysis. Duplicate treated columns and a single untreated column for each soil were immediately eluted with 500 ml of water. This was reported as being equivalent to 20 acre-inches of water but was calculated by the reviewer to be equivalent to 10 acre-inches of water. The leachate was collected and the columns, after being allowed to drain overnight, were divided into 5-cm segments. The columns that were not immediately eluted were moistened with 10 ml of water, covered with aluminum foil, and incubated (temperature unspecified) in the dark for 1 month. The columns were further incubated for 45 days, with 15 ml of water added every working day. Leachate from the columns was collected. At the completion of this incubation, the columns were segmented in 5-cm sections for analysis.

Soil TLC plates of the test soils were prepared from a soil (sieved through 250 µm mesh) slurried with water. The soil was ~2 mm thick on the plates. Every plate was spotted with 10,000 dpm each of [^{14}C]DDT, [^{14}C]2,4-D, [^{14}C]bifenox, and [^{14}C]-acifluorfen, and was developed with water.

Methodology

Soil from the leaching study was allowed to air dry sufficiently to allow homogenization. Triplicate subsamples of each segment were combusted and the ^{14}C released was quantitated by LSC. Aliquots of the column leachates were analyzed with two dimensional TLC (solvent systems not specified). The total ^{14}C activity in the eluates was determined by LSC. Radioactive spots on the soil TLC plates were visualized by autoradiography. A microbial assay of the soils prior to use was performed by Biospherics Inc., Rockville, Maryland.

Results

The ^{14}C distribution in the unaged columns is summarized in Table 16. Most of the ^{14}C was eluted from the columns for all soils. The mobility of ^{14}C in the aged columns was much more variable. A large percentage of ^{14}C was eluted from columns of Georgia sandy loam and Virginia sandy loam. Relatively small quantities of ^{14}C were eluted from the heavier textured soils. In all soils, a substantial portion of radioactivity was found at all depths, but a greater percentage of ^{14}C activity

was in the surface 5 cm than any other segment. Acifluorfen was the only ^{14}C labeled compound detected in the leachate.

Results of acifluorfen mobility on soil TLC is given in Table 17. Acifluorfen mobility was similar to 2,4-D. It would be classified as an intermediate to mobile compound.

The microbial assay demonstrated that the soils were biologically active, containing 10^5 - 10^6 bacteria, 10^5 - 10^7 actinomycetes, and 10^4 -10^5 fungi per gram.

Table 16. Distribution of ^{14}C in soil columns leached immediately and 30 days after treatment with [^{14}C]acifluorfen[a].

Column segment (cm)	Georgia sandy loam	Virginia sandy loam	New Jersey silt loam	Kansas clay loam
Immediately leached				
0-5	10.40	3.72	5.68	1.79
5-10	0.14	0.68	1.09	0.30
10-15	0.20	1.15	1.74	0.59
15-20	0.21	1.71	2.32	0.89
20-25	0.33	2.33	3.64	1.48
25-30	0.37	2.67	6.05	2.55
Leachate	88.74	87.74	79.48	92.42
Aged 30 days				
0-5	29.65	11.97	44.45	31.19
5-10	6.26	3.53	15.85	10.53
10-15	6.21	6.30	12.22	12.19
15-20	5.27	4.34	10.18	15.59
20-25	6.85	5.61	9.57	14.76
25-30	6.36	6.34	6.99	13.65
Leachate	38.51	61.93	0.77	2.10

[a]Expressed as % of ^{14}C recovered.

Table 17. R_f values from soil TLC of [^{14}C]acifluorfen and other pesticide standards.

Pesticide	Georgia sandy loam	Virginia sandy loam	New Jersey silt loam	Kansas clay loam
Acifluorfen	0.82	0.50	0.57	0.68
2,4-D	0.78	0.67	0.70	0.69
DDT	0.00	0.00	0.00	0.00
Bifenox	0.00	0.00	0.00	0.00

Conclusions

Approximately 30% of the ^{14}C residues in [^{14}C]acifluorfen treated soil aged 30 days was recovered in the aqueous fraction when flooded with water. The concentration of ^{14}C in the water increased over a 14-day interval but the decrease in the soil upon adding the flood water occurred within 1 day. It is presumed that this discrepancy resulted from the slow diffusion of solubilized ^{14}C from the soil solution to the flood water. The average concentration in the water after obtaining equilibrium was 24 ppm (acid form acifluorfen equivalent). Carbon-14 concentrations in the fish were near or below the detection limit in a large number of samples; therefore, rates of accumulation and depuration cannot be accurately determined. Average bioaccumulation factors after obtaining steady state conditions in the uptake study were 2.9 in the whole fish and viscera and 2.1 in the fillet. The extent of depuration could not be determined because of the inability to accurately measure residues at the levels present.

The methods efficiency reported includes combustion efficiency and counting efficiency (quench). All treated samples were corrected for counting efficiency based on the control samples and the combustion efficiency was not compensated for. All samples were counted in two channels and this data presumably could be used to construct a quench curve based upon the channels ratio. All treated samples should be individually corrected for counting efficiency and for combustion efficiency. The errors in the data presented are assumed to be relatively small (<10%). No characterization of the metabolites was given.

4.9 STUDY 9

Norris, F.A, and A. Guardigli. May, 1982. Adsorption-desorption of acifluorfen sodium (LS-80-1213, MC-10978) from a silt loam soil. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. PDD Report No. 82/030. Acc. No. 071325.

Procedure

Five grams of a silt loam soil from New Jersey (6.3% sand, 66.8% silt, 26.9% clay, 2.7% organic matter, pH 6.1, CEC 11.3 meq/100 g, moisture content 21%) was mixed with 50 ml of an aqueous solution of nitrophenyl ring-labeled [^{14}C]-acifluorfen (uniformly ring-labeled specific activity 29.12 µCi/mg, sodium salt, purity and source unspecified) for 4 hours. Prior to use the soil was sieved (2 mm). Acifluorfen concentration in the solutions were ~0, 0.047, 0.094, and 0.94 µg/ml. All solutions contained a small amount of sodium hydroxide (~0.0004 N NaOH) except for the zero concentration which was distilled water. One or two replicates of each treatment were prepared. After mixing (roller mill) the samples were centrifuged (~2000 g for 10 minutes). The supernatant was removed and brought to a volume of 50 ml with deionized water. Fifty ml of clean deionized water was then mixed (roller mill) with the soil for 2 hours. The suspension was centrifuged and the supernatant was removed and brought to a volume of 50 ml. This desorption procedure was repeated twice. The soil was then mixed with 50 ml of methanol for 2 hours. The methanol was removed from the soil by filtration.

Methodology

The ^{14}C activity of each supernatant or filtrate was determined using LSC. The data were fitted to the Freundlich adsorption model and adsorption/desorption coefficients and 1/n values were calculated with computer assistance. Data from the third desorption cycle was not fitted to the desorption model because of the low recoveries of ^{14}C desorbed.

Results

The results are summarized in Table 18. Freundlich K values are low indicating that acifluorfen is not strongly adsorbed. The K values for desorption and adsorption were not statistically different (probability not given). The values for 1/n were significantly lower for desorption than for adsorption. The methanol desorption removed 28-73% of the remaining sorbed acifluorfen.

Table 18. Adsorption/desorption coefficients (K) and 1/n values from acifluorfen adsorption and desorption in silt loam soil determined using the Freundlich adsorption isotherm.

Cycle	K	1/n
Adsorption	1.02	0.94
Desorption 1	0.71	0.45
Desorption 2	1.51	0.47

Conclusions

Quantitative conclusions regarding the mobility of acifluorfen in soil cannot be made from this study because the procedures used were improper. The volume of supernatant removed from the adsorption and desorption cycles was diluted rather than being measured. The temperature was not held constant during this study and the pH of the solutions was not measured. Distilled/deionized water was used instead of the required calcium solution. Adsorption and desorption equilibration times were not determined. The study generally demonstrated that acifluorfen was very mobile in the silt loam tested, but the results are approximations and do not meet data requirements. This resulted in an overestimation of the initially adsorbed acifluorfen. The acifluorfen in solution that was not decanted from the soil was not corrected for and resulted in an overestimation of the desorption of acifluorfen.

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4.10 STUDY 10

Norris, F.A., and C.C. Ku. April, 1981. Field dissipation and leaching studies. Mobil Chemical Company. Progress Memorandum PME-81.48. Acc. No. 071325.

Procedure

Small field plots in New Jersey, Virginia, Georgia, and Kansas were treated with acifluorfen (test substance not characterized) at 1 lb/A. A characterization of the soils in the plots is given in Table 19. Soil cores were collected ~0, 1, 3, 7, 14, 28, 90, and 180 days after treatment. The cores were segmented (~7.5 cm/segment) and frozen until analyzed. Depth and dates of sampling and number of cores taken (1 or 2) varied with plot site.

Methodology

Samples were analyzed using Mobil Chemical Method 157-81. This method is described in Study 11 of this review. The concentration of acifluorfen was determined in each soil segment on a wet weight basis. From this the total number of µg of acifluorfen was calculated. This value was divided by the cross sectional area of the core sample to give µg/cm^2, then converted to lb/A.

Results

Recovery of acifluorfen was calculated in an unorthodox manner which is not stictly incorrect but can be misleading. Therefore, all data were recalculated to µg of acifluorfen recovered per segment of soil. Recovery of acifluorfen with depth was quite variable, with large quantities found in subsurface samples at time zero in some soils (Table 20). No movement of acifluorfen from the surface down through the soil profile was observed. Rainfall during the experiment varied from 0 to 9.8 inches (Table 21). The total recovery of acifluorfen tended to decrease with time (Table 22). The recoveries at time zero were extremely high compared to the application rate. Total acifluorfen found in the profile at time zero suggested that 2-29 lb/A had been incorporated into the soil.

Table 19. Soil characteristics.

Soil and source	Mechanical analysis (%)			Organic matter (%)	pH	CEC (meq/100 g)	Field capacity[a]
	Sand	Silt	Clay				
Sand, Georgia	95.8	3.2	1.0	0.7	6.0	7.57	4.27
Loamy sand, Virginia	80.0	15.2	4.8	0.9	4.6	11.57	6.98
Silt loam, New Jersey	6.3	66.8	26.9	2.7	6.1	11.3	--
Silt loam, Kansas	39.2	53.0	7.8	1.9	6.9	36.02	15.86

[a]Percent moisture at 0.33 bar (field capacity).

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Table 20. Acifluorfen recovery from field plots treated at 1 lb/A.

Time after application (days)	Sampling depth (cm)	Acifluorfen recovered (µg)[a] Georgia sand	Virginia loamy sand	New Jersey silt loam	Kansas silt loam
0	0-7.5	137	234	195	57
	7.5-15	37	319	10	7
	15-22.5	28	717	4	4
	22.5-30	70	--	111	10
1	0-7.5	193	106	214	53
	7.5-15	88	97	12	ND[b]
	15-22.5	18	544	4	ND
	22.5-30	71	--	19	ND
3	0-7.5	46	116	237	117
	7.5-15	53	90	10	ND
	15-22.5	31	100	8	ND
	22.5-30	24	--	10	ND
7	0-7.5	46	89	104	25
	7.5-15	15	0	ND	ND
	15-22.5	ND	0	ND	ND
	22.5-30	ND	--	ND	ND
14	0-7.5	48	96	102	68
	7.5-15	21	29	ND	ND
	15-22.5	ND	15	ND	ND
	22.5-30	8	--	ND	ND
28-31	0-7.5	70	58	21	21
	7.5-15	24	22	ND	ND
	15-22.5	16	ND	ND	ND
	22.5-30	12	--	ND	ND
84-90	0-7.5	ND	--	46	ND
	7.5-15	21	--	ND	ND
	15-22.5	ND	--	ND	ND
	22.5-30	ND	--	ND	ND
109	0-22.5	--	ND	--	--

[a]All values for New Jersey and time zero Kansas are an average from duplicate samples (or subsamples?). All other values are based on a single determination.

[b]ND = nondetectable; limit of detection not given.

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Table 21. Cumulative rainfall at four sites where acifluorfen dissipation studies were conducted.

Days after application	Cumulative rainfall (inches)[a]			
	Kansas silt loam	Georgia sand	Virginia loamy sand	New Jersey silt loam
0	0	0	0	0
1	0	0	0	0
3	0	0	t	t
7	0	0	t	0.88
14	0	0	0.6	1.09
28	0	0.4	5.2	1.93
90	--	4.9	9.8	8.69

[a]t = trace, not quantitated.

Table 22. Total recovery of acifluorfen from 22.5 or 30 cm core samples[a].

Time after application (days)	Georgia[b] sand	Virginia[c] loamy sand	New Jersey[b] silt loam	Kansas[c] silt loam
0	273	1270	320	78
1	370	748	250	52
3	154	307	265	116
7	62	89	105	25
14	78	140	102	68
28-31	124	79	21	21
84-90	21	--	46	ND
109	--	109	--	--

[a]Total µg of acifluorfen recovered from 22 mm (diameter) core.

[b]Core depth 30 cm.

[c]Core depth 22.5 cm.

Conclusions

Conclusions cannot be made from the data generated in this study. The concentration of acifluorfen in soil was determined on a wet weight basis (i.e. µg acifluorfen/g wet soil). Therefore, actual concentrations of acifluorfen in the soil are not given. The reported results were highly variable. Total recoveries at time zero suggested that acifluorfen was incorporated into the soil at concentrations from 2-29 times the given application rate. Acifluorfen was found at all sampling depths at time zero although the herbicide was sprayed onto the soil surface and was not incorporated, indicating that the soils were previously contaminated with acifluorfen. Sampling was inadequate. Only the analysis of a single sample was reported for most treatment combinations. Two replicate analyses were reported for a portion of the samples. It is not clear if two cores were removed from a single plot, one core removed from duplicate plots, two subsamples removed from each segment of a single core or if two HPLC analyses were conducted on an extract from a single sample. The replication between these "replicates" was poor. A substantially larger number of samples should have be analyzed to compensate for the high variability. The plot areas were not characterized, the temperature or time of the year when the study was conducted was not given, and appropriate controls were not included. The test substance was not characterized.

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4.11 STUDY 11

Ku, C.C., and K.M. Miller. November, 1980. Determination of Mobil 10978 and Mobil 10109 residues in soils - Mobil Chemical Method 157-80. Mobil Chemical Company. Acc. No. 071325.

Ku, C.C., and F.A. Norris. May, 1981. Validation of Mobil Chemical Method 157-81 "Determination of Mobil 10978 and Mobil 10109 residues in soil". Mobil Chemical Company. Progress Memorandum PME-81.57. Acc. No. 071325.

Procedure

Four soils from Georgia, Virginia, New Jersey, and Kansas were fortified with acifluorfen (sodium salt, test substance uncharacterized, purity and source unspecified) at 0, 0.05, 0.10, 0.50, and 1.00 µg/g and sent to Biospherics Inc., Rockville, Maryland for analysis. Refer to Table 19, pg 40 for soil charteristics.

Methodology

Soil was extracted with a methanol:water mixture (proportions unspecified). The extract was acidified to pH 1 and then partitioned with methylene chloride. The methylene chloride was evaporated to dryness and residue was taken up in methanol: water:acetic acid (60:39:1). Acifluorfen, acid and salt forms, were quantified using HPLC analysis. The mobile phase was the same mixture of methanol, water, and acetic acid as used to dissolve the extracted residue. Detection was at 280 nm.

Results

Acifluorfen recoveries ranged from 71 to 121% (Table 23). Overall recovery in all soils at all fortification levels was 92.1% with a standard deviation of 12.5.

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Table 23. Percent recovery of acifluorfen in soil determined using Mobil Chemical Method 157-81.

Fortification level, ppm	Georgia sand	Virginia loamy sand	New Jersey silt loam		Kansas silt loam	Average (std. dev.)[a]
0.05	72.0	--	--	--	106.0	89.3 (13.9)
0.05	Void	--	--	--	90.0	--
0.10	--	92.0	121.0	71.0	--	87.3 (17.2)
0.10	--	91.0	75.0	74.0	--	--
0.50	--	98.4	102.0	101.0	--	95.6 (6.0)
0.50	--	84.0	90.8	99.0	--	--
1.00	105.0	--	--	94.0	98.9	95.0 (7.7)
1.00	91.6	--	--	--	84.6	--
Average (std. dev.)[a]	89.5 (13.6)	91.4 (5.1)	91.9 (15.4)		94.9 (8.2)	Overall = 92.1 (12.5)

[a]Standard deviation calculated for sample statistics (N degrees of freedom), not as an estimate of population statistics (N-1 degrees of freedom).

Conclusions

Mobil Chemical Method 157-80/81 recovered an average of 92.1% of the acifluorfen added to four soils with a standard deviation of 12.5[1]. A detection limit was not established. It is not clear if method 157-81 is identical to method 157-80 with the modifications listed in the Biospherics report. This should be clarified.

[1]Standard deviation calculated for sample statistics not as an estimation of population statistics.

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4.12 STUDY 12

Gemma, A.A., J.P. Wargo, Jr., and G. Heinzelmann. September, 1982. Tackle greenhouse rotational crop study: The potential uptake of ^{14}C MC 10978 in various crops from soil treated with ^{14}C MC 10978. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/046. Acc. No. 071326.

Procedure

Plastic tubs (1 x 1 x 2 feet) were filled with 10 inches of previously sieved (2 mm) silt loam soil from New Jersey (6.3% sand, 66.8% silt, 26.9% clay, 2.7% organic matter, pH 6.1, CEC 11.3 meq/100 g, 43% moisture at 1 atm). Six tubs were prepared. Three tubs were treated with an ethanol solution of trifluoromethyl phenyl-labeled [^{14}C]acifluorfen (uniformly ring-labeled, specific activity 0.994 mCi/mM, purity and source unspecified). The reported rate of application was 0.16 lb ai/A. The 0.16 lb ai/A rate was selected for this study on the basis of other laboratory studies where acifluorfen fell to the soil at 0.16 lb/A when sprayed onto soybeans at 0.5 lb ai/A. The actual rate of application is questioned. If only 0.16 lb ai/A were applied, the test substance would have had to been <50% pure. This is an extremely low purity for radiolabeled material and does not conform to data requirements. Three untreated tubs served as controls. All tubs were aged in a greenhouse. The greenhouse environment was controlled to provide 16 hours of daylight (6×10^4 - 8×10^4 lumens/m^2) at 30°C. The temperature was set at 20°C during the dark interval. The soil was moistened just prior to pesticide treatment.

After aging 1 month, 'Gage' winter wheat, 'Viroflag 99MR' spinach, and 'French Breakfast' radish seeds were each planted in separate control and treated tubs. The soil was watered as needed for good plant growth. Plants were sampled 1 month after seeding and at harvest. Two more identical plantings were made at 4 months and 1 year after pesticide treatment. The plant tissue sampling schedule is given in Tables 1 and 2. Entire plants were removed from the soil when sampled. Roots were removed from the wheat and spinach and were discarded. The mature wheat was separated into grain, hulls, and stems. The spinach and immature wheat tops were washed with water to remove adhering soil. The radish plants were washed and then divided into roots and tops. All samples were weighed and then frozen until analyzed.

Methodology

All plant parts were mixed with dry ice and ground in a blender. The wheat grain, hulls, and straw from the 1-month plantings were extracted three times with 80% methanol in a Polytron homogenizer. The methanol was evaporated off of the filtered extracts and the remaining aqueous phase was partitioned three times with ethyl acetate. The ethyl acetate was evaporated to dryness and the residue taken up in acetonitrile. The acetonitrile was partitioned three times with hexane. Both the hexane and acetonitrile fractions were assayed for ^{14}C activity by LSC. The acetonitrile fraction was concentrated for possible TLC analysis. The aqueous fraction remaining after ethyl acetate extraction was acidified to pH <3 with hydrochloric acid, allowed to sit at room temperature overnight, and then partitioned three times with ethyl acetate. The aqueous fraction was then adjusted to pH >10 with sodium hydroxide, allowed to sit overnight, and partitioned three times with ethyl acetate. The aqueous and ethyl acetate fractions were assayed for ^{14}C activity by LSC. The ethyl acetate extracts were concentrated for possible TLC analysis.

The wheat plant parts from the 4-month and 1-year plantings were extracted with 80% methanol as were the samples from the 1-month plantings. The methanol was evaporated from the extracts and the aqueous phase was acidified (pH <3) with hydrochloric acid and extracted three times with ethyl acetate. Radioactivity was quantified in the extracts by LSC.

The ethyl acetate extracts of the acidified extracts (all plantings) were cleaned up via silica gel column chromatography. The column was prewashed with hexane. The extracts were evaporated to dryness and then redissolved in 10 ml of hexane. This was added to the column and the column eluted with: hexane, dichloromethane: hexane (3:1), toluene, methanol:toluene (1:19), methanol:toluene (1:9), methanol: toluene (1:1), and methanol. Each eluate was evaporated to dryness, redissolved in a small volume of methanol, assayed for ^{14}C activity by LSC and characterized by TLC.

One dimensional TLC plates (silica gel) were developed in one of two solvent systems: toluene:tetrahydrofuran:acetic acid (45:30:1) or chloroform:methanol:acetic acid (85:10:5). Two dimensional TLC plates (silica gel) were developed sequentially with chloroform:acetic acid (90:10) and toluene:tetrahydrofuran:acetic acid (45:30:1). All development was in a saturated atmosphere. Unlabeled standards were spotted on all plates to compare to the samples. Selected standards were overspotted on the plant extracts on the two dimensional plates. Standards were located with UV. Radioactive spots were visualized with autoradiography. Selected samples were cleaned via TLC using chloroform:methanol:acetic acid (85:10:5). Radioactive spots from the cleanup plates were scraped and eluted with methanol. The eluates were combined and analyzed with one or more of the TLC methods described above. Radioactive spots from the TLC plates of selected samples were quantified by LSC.

Unextracted ^{14}C in the wheat samples was quantified by combustion and LSC. Total ^{14}C in plant samples was determined by adding the extractable and unextractable residues. The total ^{14}C activity in spinach and radish roots and tops was quantified by combustion and LSC.

Results

Uptake of ^{14}C was inversely proportional to the length of soil aging before the crops were planted (Tables 24 and 25). The highest residue recovery (1.05 ppm acifluorfen equivalent) was in the straw from wheat planted 1 month after soil treatment. All other recoveries were <0.5 ppm acifluorfen equivalent. Residues in plants seeded 4 months or longer after soil treatment were <0.2 ppm. The distribution of ^{14}C extracted from mature wheat is given in Tables 26 and 27. Most of the radioactivity in the plants was extracted with methanol:water. Distribution of radioactivity among the extracts or column eluates was varied.

Most of the radioactivity extracted into neutral ethyl acetate from the 1-month plantings of wheat straw was identified as unaltered acifluorfen (24% of the total ^{14}C in the straw). Acifluorfen accounted for 8 and 27% of the radioactivity in the grain and straw, respectively, from the 4-month plantings. The desnitro and decarboxy degradates of acifluorfen (MC-10879 and MC-10074, Figure 2, pg 13) comprised 16 and 7%, respectively, of the total residue in the straw and 7 and ^{14}C in the straw). Acifluorfen accounted for 8 and 27% of the radioactivity in

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the grain and straw, respectively, from the 4-month plantings. The desnitro and decarboxy degradates of acifluorfen (MC-10879 and MC-10074, Figure 2, pg 13) comprised 16 and 7%, respectively, of the total residue in the straw and 7 and 25%, respectively, in the grain. Other samples were not quantitatively analyzed for degradates. Trace amounts of acifluorfen, desnitro (MC-10879) and/or amino (MC-14621) analogs of acifluorfen and decarboxy (MC-10074) and/or methylcarboxy (MC-10108) analogs of acifluorfen were identified in the pH <3 ethyl acetate extract of wheat straw from the 4-month planting. Additional work was performed to verify the identity of these degradates. Neither the methodology nor the results of this additional work were given.

Table 24. Total ^{14}C residues in wheat planted as a rotational crop.

Plant part	Soil aging period (months)	Sampling interval (days)[a]	Total ^{14}C, acifluorfen equivalent (ppm)
Forage	1	27	0.16
Grain		86	0.09
Hulls		86	0.38
Straw		86	1.05
Forage	4	63	0.15
Grain		129	0.04
Hulls		129	0.13
Straw		129	0.25
Forage	12	17	0.03
Grain		81	<0.02
Hulls		81	0.05
Straw		81	0.04

[a]Days after planting.

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Table 25. Total ^{14}C residues in spinach and radishes planted as rotational crops.

Crop	Soil aging period (months)	Sampling interval (days)[a]	Total ^{14}C, acifluorfen equivalent (ppm)
Radish			
leaves	1	27	0.02
roots		27	<0.02
leaves		--	0.24
roots		--	0.03
leaves	4	63	0.05
roots		63	0.02
leaves		87	0.09
roots		87	<0.02
leaves	12	32	<0.02
roots		32	<0.02
Spinach leaves	1	27	0.61
		86	0.47
	4	63	0.09
		87	0.09
	12	49	0.04

[a]Days after planting.

Table 26. Distribution of ^{14}C residues in wheat.

Fraction	% of ^{14}C recovered		
	Straw	Grain	Hulls
Soil aged 1 month			
MeOH/H_2O	77.2	70.0	79.3
Bound residue	22.8	30.0	20.7
EtOAc (neutral)	46.5	12.7	38.0
Aqueous	37.8	19.9	38.4
Acetonitrile	36.4	28.1	35.7
Hexane	0.5	21.4	0.4
EtOAc (pH <3)	28.1	6.6	24.7
EtOAc (pH <12)	1.8	3.3	21.1
Aqueous	6.2	7.4	1.1
Soil aged 4 and 12 months[a]			
MeOH/H_2O	81.4	69.8	--
Bound residue	18.6	30.2	--
EtOAc (pH <3)	64.2	64.6	--
Aqueous	15.8	5.5	--

[a]Presumed to be the average of the second and third plantings.

Table 27. Radioactivity in fractions from silica gel column from ethyl-acetate extract of wheat.

Fraction	% of total ^{14}C residue		
	One-month plantings	Two-month plantings	
	Straw	Straw	Grain
Hexane	ND[a]	0.2	0.5
Dichloromethane:hexane (3:1)	ND	4.2	8.4
Toluene	ND	0.1	3.2
Toluene:methanol (95:5)	34	26.5	47.8
Toluene:methanol (90:10)	42	12.4	5.1
Toluene:methanol (50:50)	30	9.7	ND
Methanol	14	0.8	ND
TOTAL	120	53.9	65.0

[a]ND = nondetectable.

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Conclusions

Accumulation of ^{14}C in rotational crops (wheat, spinach, and radishes) planted 1 month after soil treatment varied from <0.02 to 1.05 ppm (acifluorfen equivalent). Radioactive residues declined substantially in crops seeded 4 and 12 months after soil treatment (<0.05 ppm in 12-month plantings). Additional information is needed to make final conclusions on the uptake of acifluorfen residues by rotational crops. Data used to select the 0.16 lb ai/A application rate must be provided. The actual application rate used in this study must be verified. The watering schedule for the entire experiment, as well as volumes of leachate and the ^{14}C activity of the leachate from each tub of soil must be provided. Radioassays of the soil, when treated and when planting and sampling the crops, should be submitted. The rationale for varying the sampling and harvest dates for wheat should be explained. The TLC analyses used in the study were judged in previous studies to be inadequate in separating all the acifluorfen metabolites extracted from soils. Insufficient information is given in this study to evaluate these methods for analysis of plant extracts. Therefore, it must be assumed that the TLC methodology used in this study is not adequate. The additional work performed to verify the identity of some degradates must be described. Overall, the data given were insufficient to evaluate the accumulation of acifluorfen degradates in rotational crops.

Either some of the data reported were in error or a large error in ^{14}C assayed occurred with some fractions of the extract. The worst example of this is 12.7% of the ^{14}C was found in the neutral ethyl acetate extract of grain from the 1 month plantings of wheat (Table 26). This was evaporated to dryness, redissolved in acetonitrile and partitioned with hexane. Recovery of ^{14}C in the acetonitrile and hexane fractions was 28.1 and 21.4%, respectively. Such discrepancies in the data must be explained.

4.13 STUDY 13

Gemma, A.A., J.P. Wargo, and G. Heinzelmann. September, 1982. Tackle field rotational crop study: The potential uptake of ^{14}C-MC 10978 in various crops grown under field conditions in soil treated with ^{14}C-MC 10978. Rhone-Poulenc Inc., Monmouth Junction, New Jersey. ASD Report No. 82/042. Acc. No. 071326.

Spare, W.C., F. Dillon, and C. Hutchinson. October, 1982. Field metabolism studies with ^{14}C-MC-10978. Prepared for Rhone-Poulenc, Inc. by Biospherics, Inc., Rockville, Maryland. Report No. 349. Acc. No. 071326.

Procedure

The Rhone-Poulenc report did not necessarily coincide with the Biospherics report. All field work other than the actual application of acifluorfen was performed by Biospherics. Therefore, the procedures listed are primarily based on the Biospherics report. Two 4- by 8-foot plots were set out in St. Michael's, Maryland. The plots contained a silt loam soil (12.8% sand, 73.2% silt, 14% clay, 2.7% organic matter, pH 7.6, CEC 11.2 meq/100 g, bulk density 1.2 g/cm^3, and 12.39% moisture at 0.33 bar). Each plot was bordered by an aluminum sheet, extending 1 foot above and below the soil surface. Shortly before seeding, the plots were tilled and fertilized (1361 lb/A of 5-10-5). Two rows of 'Bragg' soybeans were seeded lengthwise on 20-inch centers in each plot on May 27, 1981. The initial seeding was too light and soybeans were seeded again on June 7, 1982. One of the two plots was treated with [$^{13}C/^{14}C$]acifluorfen (nitrophenyl uniformly ring-labeled ($^{13}C/^{14}C$), specific activity 2.6 µCi/mg, purity and source unspecified) in a 0.2% X-77 aqueous solution at 0.18 lb ai/A on June 23, 1981. The soybeans had at least three trifoliate leaves at the time of spraying. The plot was sealed with a plastic canopy when treated to prevent spray drift. The untreated plot served as a control. Soybeans were watered if necessary and were weeded two to three times per week. Weeds pulled from the treated plots were returned to the plot.

Soybeans were removed from the plots 28 days after treatment. The plots were cultivated, divided into three equal sections and seeded with 'Red Coat' wheat, 'Detroit Dark Red' beets, and 'Black Seeded Simpson' lettuce. These crops were watered and weeded as were the soybeans. The crops were sampled 14, 21, 35, 45, 63, and 91 days after seeding. One row of wheat was not harvested until 365 days after planting. The plots were cultivated (except for one half of each wheat plot) and seeded with the same crop 119 days after acifluorfen treatment. Wheat and lettuce were sampled 16, 23, 35, and 63 days after planting. Beets were sampled 63 days after planting. The germination and/or growth was poor for lettuce and beets because of the cold weather (crops were planted October, 1981). A final harvest of wheat was made 246 days after planting. Wheat from both the first and second planting had to undergo vernalization to produce grain. On June 23, 1982 (392 days after acifluorfen treatment) the plots were cultivated and seeded. The same cultivars of lettuce and beet were seeded but 'ERA' spring wheat was planted in place of winter wheat. Crops were sampled at midgrowth (28 days after planting for lettuce and 49 days for wheat and beets) and at maturity (49 days after planting for lettuce and 91 days for wheat and beets).

Crops sampled were packed in dry ice for shipment from Biospherics to Rhone-Poulenc. The roots were removed from the wheat and lettuce and the lettuce was washed with water to remove adhering soil particles. Mature wheat was separated

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into straw, grain, and hulls. The beet plants were rinsed with water and the tops separated from the roots.

Methodology

Each sample was ground with dry ice in a blender. Subsamples of the ground samples were combusted and the radioactivity released was quantified by using LSC. All recoveries were reported as ppm of acifluorfen equivalent. The detection limit was 0.02 ppm. It is assumed that the incorporation of ^{13}C in the test substance was to aid in the MS identification of degradates; however such analyses were not performed.

Results

Recovery of ^{14}C in the rotational crops is given in Table 28. Radioactivity ranged from 0.08 to 0.19 ppm acifluorfen equivalent in the 1-month planting for the first three weeks after seeding. All other recoveries were $\leq$0.03 ppm.

Table 28. Total ^{14}C residues in rotational crops.

Crop	Soil aging period (days)	Sampling interval (days)[a]	Total ^{14}C acifluorfen equiv. (ppm)
Lettuce	28	14	0.19
		21	0.10
		35	<0.02
		45	<0.02
		63	<0.02
		Harvest (91)	<0.02
	119	16	0.03
		23	0.02
		35	<0.02
		63	<0.02
	392	28	<0.02
		Harvest (49)	<0.02
Beets	28	14	0.14
		21	0.08
		35	<0.02
		45	<0.02
		63	<0.02
Leaf-		Harvest (91)	<0.02
Root-			<0.02
	193	63	0.03
	392	49	<0.02
Leaf-		Harvest (91)	<0.02
Root-			<0.02
Wheat	28	14	0.15
		21	0.08
		35	<0.02
		45	<0.02
		63	<0.02
Straw-		Harvest (337)	<0.02
Hulls-			<0.02
Grain-			<0.02
	119	16	0.02
		23	<0.02
		35	<0.02
		63	<0.02
Straw-		Harvest (245)	<0.02
Hulls-			<0.02
Grain-			<0.02
	392	49	<0.02
Straw-		Harvest (91)	<0.02
Hulls-			<0.02
Grain-			<0.02

aSampling intervals are not necessarily as given in report but were calculated from the planting and sampling dates given.

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Conclusions

Accumulation of ^{14}C from [^{14}C]acifluorfen treated silt loam was low in rotational crops of wheat, lettuce, and beets. The rate of application, however, was much too low (0.18 lb ai/A) to meet EPA data requirements. Biospherics changed the protocol for the row spacings of soybeans and the plot size for the rotational crops. This was clearly stated in the Biospherics reports, but Rhone-Poulenc did not appear to be aware of this change and applied acifluorfen as called for in the original protocol, resulting in the low rate of application. It was noted that the final Rhone-Poulenc report is based on the Biospherics report but is dated 1 month earlier than the Biospherics report. Additional deficiencies in this study are: soil was not analyzed for ^{14}C residues at sampling times, the ^{14}C residues were not characterized (identification of parent and degradates), the study was conducted at only one site and the weather data were not summarized.

4.14 STUDY 14

Thompson, C.M., and W. Cranor. January, 1981. Uptake, depuration and bioconcentration of ^{14}C-MC 10978 by bluegill sunfish (Lepomis macrochirus). Submitted to Mobil Oil Corporation by Analytical BioChemistry Laboratories, Incorporated, Columbia, Missouri. ABC Report No. 26610. Acc. No. 071327.

Procedure

Bluegill sunfish (Lepomis macrochirus) obtained from Osage Catfisheries, Inc., Osage Beach, Missouri, were held in culture tanks (16-hour photoperiod) for at least 14 days before use. The fish were inspected for disease and parasites. Parasites were identified but were judged not to be a serious problem. Therefore, the fish were treated with formalin (167 ppm) for 1 hour, 16 days prior to initiating the experiment. Fish were fed a standard commercial fish food at a daily amount equivalent to 3% of their body weight during the holding period. The average weight and length of the fish at the initiation of testing, determined from a representative sampling of the fish, was 4.7 g and 54 mm, respectively.

The test substance was prepared by mixing 0.56 mCi of trifluoromethyl phenyl-labeled [^{14}C]acifluorfen (ring-labeled, specific activity 13.74 mCi/mM, acid from 98.4% radiochemical purity, supplied by Mobil Oil Corporation, Ref. No. GF 266049-1), and 0.56 mCi of nitrophenyl-labeled [^{14}C]acifluorfen (ring-labeled, specific activity 11.17 mCi/mM, acid form, 98.3% radiochemical purity, supplied by Mobil Oil Corporation, Ref. No. GF 266051-1) to 124 g ai (reported as 120 g ai) of unlabeled acifluorfen (sodium salt, technical grade, 78.5% pure, supplied by Mobil Oil Corporation). The sodium salt of the unlabeled acifluorfen was prepared by reacting 297 g of acifluorfen (acid form) with 32.9 g of sodium hydroxide and adjusting the pH to 8.0 ± 0.3. It was presumed that the radiolabeled acifluorfen would be converted to the sodium salt when combined with the solution of unlabeled acifluorfen. The specific activity of the final acifluorfen solution was 9.07 µCi/g (reported as 9.4 µCi/g).

Test chambers consisted of 100-liter glass aquaria immersed in a circulating water bath held at 22 ± 1° C. Each aquarium (duplicate treated and control) held 70 liters of water which was intermittantly replaced at 500 ml/min from water from the diluter. The diluter was calibrated to deliver a nominal concentration of 3.0 mg/l of acifluorfen to the treated aquaria. Aerated well water was used throughout the study. The water was tested for oxygen, pH, hardness, alkalinity, conductivity, nitrogen, phosphate, and a number of metals and pesticides.

Before initiating the uptake study, the test solutions were allowed to flow through the aquaria for 24 hours. The study was initiated by adding 110 fish to each aquarium. The fish were observed daily for abnormal behavior and mortality. The temperature, dissolved oxygen, pH, and ammonia levels were measured every few days. On day 26 of the uptake study, a water pump malfunctioned, interrupting the water supply for 12 hours. Water from a reserve well was used during the period. No changes in water quality or delivery of acifluorfen into the aquaria resulted. After 30 days, water was siphoned from each tank until ~3 inches remained. The tanks were refilled with clean well water to a volume of 70 liters. This procedure was repeated once again and then water in the aquaria was replaced with clean well water, presumably flowing intermit-

tently at 500 ml/min. Fish and water samples were removed from each aquaria on days 1, 3, 7, 10, 14, 22, 26, and 30 of the uptake study and days 1, 3, 7, 10, and 14 of the depuration study. Water samples were also collected on day 0 of the uptake study. Either five or ten fish were removed from each aquarium at each sampling. Two of these were pooled by treatment and were used for the whole fish analysis. Three fish from each aquarium were dissected into fillets (body, muscle, skin, skeleton) and viscera (fins, head, blood, internal organs). Any remaining fish were frozen for later ^{14}C metabolite analysis.

Methodology

The temperature, dissolved oxygen, pH, and ammonia concentration was measured on each water sample, using specific ion electrodes and a thermometer. Subsamples of the water were pooled by treatment and assayed for ^{14}C activity by LSC. The pooled control samples were split. One portion was assayed by LSC to determine background. The remaining portion was spiked with a known quantity of a ^{14}C standard and assayed by LSC to determine counting efficiency. Treated samples were corrected for quench using this counting efficiency.

Fish samples were apparently pooled by treatment and homogenized with dry ice in a grinder. Duplicate subsamples were combusted and the ^{14}C released was quantified by using LSC. Portions of the control samples were spiked with a known quantity of [^{14}C]benzoic acid or [^{14}C]acifluorfen before combustion to determine method efficiency. Counting efficiencies were determined by adding a known amount of ^{14}C to original control samples after counting and then recounting the sample (LSC). The minimum limit of detection of ^{14}C was set at twice the standard deviation of the control samples above the mean backround. Carbon-14 recoveries from treated samples were corrected for quench using the counting efficiencies from corresponding controls. Values were not corrected for combustion efficiencies.

Results

Chemical and physical parameters in the aquaria were maintained to provide a healthful environment for the fish throughout the study. The temperature, dissolved oxygen, pH, and ammonia in the treated aquaria ranged from 21 to 23 C, 7.2 to 8.4 mg/l, 7.6 to 8.0, and 0.22 to 0.80 mg/l, respectively (Table 29). The values for these parameters in the control aquaria were very nearly the same. Neither the treated nor control fish displayed any abnormal behavior during the study. The mortality rate of the treated fish was 1%, but this was considered an aberrant value and was not the result of acifluorfen toxicity.

Acifluorfen concentration averaged 3.4 mg/l (acid form of acifluorfen equivalent) in the treated water (Table 30). The fish accumulated ^{14}C up to day 14 of the exposure study. Subsequent ^{14}C levels in the fish were relatively constant during the exposure interval. Average accumulation after 30 days exposure was 3.5 mg/kg (acid form of acifluorfen equivalent) in the whole fish. Concentrations in the edible portions of the fish were much lower than in the viscera. The bioconcentration factors [(mg/kg in fish)/(mg/l in water)] at the end of the exposure study were 1.1, 0.3, and 1.9 for the whole fish, fillet, and viscera, respectively during the 14-day depuration period. Uptake and depuration constants were calculated from the regression of ^{14}C concentration (acid form of acifluorfen equivalent) on time. The values were 1.9 ppm/ppm/day for uptake and

1.7 days $^{-1}$ for depuration. This model was based on a linear uptake and loss of ^{14}C, but rates of accumulation and loss were not linear. Coefficients of determination (r^2) for uptake and loss were 0.4887 and 0.6149, respectively.

Method efficiencies were averaged 82, 80, and 82% for recovery of ^{14}C in whole fish, fillets, and viscera, respectively. These values include both the combustion efficiencies and counting efficiencies. Detection limits were based on background LSC readings and therefore vary with the material being counted. Minimum detection limits were given as: 0.07 mg/l for water, 0.60 mg/kg for whole fish, 0.80 mg/kg for fillets, and 0.95 mg/kg for viscera.

Table 29. Chemical characteristics of well water used for fish studies.

Parameter	Concentration	Parameter	Concentration
BHC	----	Vapona	<0.03 ppb
αBHC	<2.0 ppt[a]	Disyston	<0.06 ppb
βBHC	<2.0 ppt[a]	Diazinon	<0.03 ppb
γBHC	<2.0 ppt[a]	Methyl Parathion	<0.08 ppb
δBHC	<3.0 ppt[a]	Malathion	<0.16 ppb
Chlordane	----	Ethyl Parathion	<0.09 ppb
Heptachlor	<4.0 ppt[a]	Dissolved oxygen	9.3 ppm
Heptachlor Epoxide	<7.0 ppt[a]	pH	8.2
αChlordane	<6.0 ppt[a]	Hardness ($CaCO_3$)	255 ppm
βChlordane	<6.0 ppt[a]	Alkalinity ($CaCO_3$)	368 ppm
Tech Chlordane	<6.0 ppt[a]	Conductivity	50 μmhos/cm
Dieldrin + Aldrin	----	Total ammonia	<0.05 ppm
Aldrin	<4.0 ppt[a]	Nitrate nitrogen	0.15 ppm
Dieldrin	<8.0 ppt[a]	Orthophosphate	0.10 ppm
DDT + Metabolites	----	Aluminum	<0.01 ppm
o,p'-DDE	<9.0 ppt[a]	Arsenic	<0.001 ppm
p,p'-DDE'	<9.0 ppt[a]	Cadmium	<0.001 ppm
o,p'-DDT	<14.0 ppt[a]	Chromium	0.001 ppm
p,p'-DDD	<15.0 ppt[a]	Cobalt	<0.001 ppm
p,p'-DDT	<16.0 ppt[a]	Copper	<0.01 ppm
Endrin	<11.0 ppt[a]	Iron	0.012 ppm
Heptachlor	<4.0 ppt[a]	Lead	0.009 ppm
Heptachlor Epoxide	<7.0 ppt[a]	Mercury	<0.0001 ppm
Lindane	<3.0 ppt[a]	Nickel	0.0157 ppm
Methoxychlor	<30.0 ppt[a]	Zinc	<0.01 ppm
Toxaphene	<60.0 ppt[a]		
PCB	----		
Aroclor 1242	<40.0 ppt[a]		
Aroclor 1016	<43.0 ppt[a]		
Aroclor 1254	<69.0 ppt[a]		
Aroclor 1260	<50.0 ppt[a]		
HCB	<2.0 ppt[a]		
Mirex	<15.0 ppt[a]		

[a]Parts per trillion.

Table 30. Recovery of ^{14}C in test water and fish tissue during flowthrough accumulation study.

Day		^{14}C Concentration (acifluorfen equivalents[a])			
		Water (mg/l)	Fillet (mg/kg)	Whole fish (mg/kg)	Viscera (mg/kg)
Uptake	0	3.2	--	--	--
	1	3.5	0.8	0.4[a]	1.6
	3	3.6	c	2.3	c
	7	3.5	c	2.7	2.5
	10	3.3	0.8	2.5	5.5
	14	3.7	1.1	4.3	6.6
	22	3.2	1.2	5.4	5.9
	26	3.1	d	3.5	5.2
	30	3.1	0.9	3.5	5.9
Depuration	1	b	1.1	2.9	4.3
	3	b	0.1[b]	0.9	1.5
	7	b	0.4[b]	0.3[b]	1.3
	10	b	0.4[b]	0.6[b]	1.8
	14	b	0.2[b]	0.1[b]	0.3[b]

[a]Acid form of acifluorfen.

[b]Semi-quantitative values, below minimum quantifiable limits.

[c]CPM below background.

[d]Sample contaminated.

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Conclusions

Conclusions on the accumulation and depuration of [^{14}C]acifluorfen must be viewed in light of the error associated with this study. The specific activity of the acifluorfen was very low. Recoveries of ^{14}C during the early uptake and late depuration intervals were at or near the detection limit and thus have a high error associated with them. The 2σ error was as high as 30%. The efficiencies of the treated samples were not directly determined. All treated samples were corrected for counting efficiency based on the control samples and the combustion efficiency was not compensated for. All samples were counted in two channels and this data presumably could be used to construct a quench curve based upon the channels ratio. All treated samples should be individually corrected for counting efficiency and for combustion efficiency.

Radiolabeled acifluorfen accumulation in bluegill sunfish plateaued at day 14 (average concentration of 4.2 mg/kg from day 14 through day 30). Concentrations were relatively low in the edible portions of the fish ($\leq$1.2 mg/kg) and high in the viscera (up to 6.6 mg/kg detected). The ^{14}C uptake was reversible. Depuration removed 97, 72, and 95% of the accumulated ^{14}C in the whole fish, fillet, and viscera, respectively, in 14 days. Fish samples were frozen and saved for ^{14}C metabolite analysis but no results were given. Characterization of the ^{14}C residues in fish is required. Fish samples were pooled for analysis and then two subsamples were analyzed. The distribution of variance in the data indicated that a single analysis of each replicate would yield more information on the variability of acifluorfen accumulation in fish.

4.15 STUDY 15

Forbis, A.D., and P. Boudreau. March, 1981. Uptake, depuration, and bioconcentration of MC-10978 by channel catfish (Ictalurus punctatus) in a static system with soil. Submitted to Mobil Oil Company by Analytical BioChemistry Laboratories, Incorporated, Columbia, Missouri. ABC Report No. 26611. Acc. No. 071-327.

Procedure

Radiolabeled acifluorfen was prepared by mixing together 22.5 µCi of trifluoromethyl phenyl-labeled [^{14}C]acifluorfen (ring-labeled, acid form, specific activity 13.74 mCi/mM, 98.4% purity, supplied by Mobil Oil Corporation, Ref. No. GF 266049-1), 22.5 µCi of nitrophenyl-labeled [^{14}C]acifluorfen (ring-labeled, acid form, specific activity 11.17 mCi/mM, 98.3% purity, supplied by Mobil Oil Corporation, Ref. No. GF 266051-1), and 67 ai mg (reported as 64.8 ai mg) of unlabeled acifluorfen (sodium salt, technical grade, 78.5% purity, supplied by Mobil Oil Corporation). The sodium salt of the unlabeled acifluorfen was prepared by reacting 297 g of acifluorfen (acid form) with 32.9 g of sodium hydroxide and adjusting the pH to 8.0 ± 0.3. The specific activity of the final acifluorfen solution was 0.66 µCi/mg (reported as 0.68 µCi/mg).

A sandy loam soil (73% sand, 23% silt, 4% clay, 0.1% organic matter, pH 8.2, CEC ~9.5 meq/100 g, 12.1% moisture at 0.33 bar) was sieved (2 mm) and air dried. A 1000-g subsample of the soil was sprayed with a solution of the combined [^{14}C]acifluorfen (solution diluted in acetone). After acetone evaporation the treated soil was mixed with 64 kg of untreated soil. The soil was subsampled for ^{14}C determination then spread across the bottom of a stainless steel aquarium (70 x 101 x 304 cm). Untreated soil (65 kg) was placed in a second aquarium as a control. The soil was maintained at ~10% moisture for 30 days (16-hour photoperiod). After aging, 1000 liters of well water was added to each aquarium and the aquaria were allowed to equilibrate for 3 days. All water used in this study was the well water characterized in Table 29, (pg 61) Table 1.

The test fish were channel catfish (Ictalurus punctatus) obtained from Northrup Hatchery (Lot 2580), Columbia, Missouri. The fish were held for a minimum of 14 days prior to use. During this period they were inspected for disease and parasites, and observed for abnormal behavior or mortality. The fish were severely infected with several parasites and were, therefore, treated with 167 ppm formalin for 1 hour. Fish were fed a standard commercial fish food. The daily feeding was equivalent to 3% of their body weight. The estimated average weight of the fish was 9.7 g and the average length was 82 mm. The fish were alternately placed into the control and treated aquaria (150 fish/aquarium) via stratified random assignment. After 30 days exposure, the remaining fish in each aquarium were placed in glass depuration aquaria (100 l tanks containing 75 liters of aerated well water). Water in the depuration aquaria was continuously replaced at the rate of 200 ml/min/aquarium. The water temperature was 15-19°C throughout the study (exposure and depuration aquaria).

Soil samples were removed from both the treated and control aquaria on days 0, 1, 15, and 30 of the aging period, days 1 and 2 of the equilibration period,

and days 1, 3, 7, 10, 14, 22, and 30 of the exposure period. Each sampling consisted of removing six to eight cores (~100 g total) using a 15 ml beaker as a sampling devise. The soil was pooled and vacuum filtered. The water removed was returned to the aquarium. The soil was frozen until analyzed. Water and fish samples were collected on days 1, 3, 7, 10, 14, 22, and 30 of the exposure phase and days 1, 3, 7, 10, and 14 during the depuration interval. Water samples were also collected on day 0 of the exposure phase. Additional water samples were collected on day 0, 7, 14, 22, and 30 of the exposure phase and day 1 of the depuration for metabolite characterization. Fish samples for metabolite characterization were collected on day 30 of the exposure period. Water samples were collected by inserting a 20-mm diameter tube into the aquaria, sealing the top of the tube and withdrawing it from the water at six-eight locations in the tank. The water was pooled and subsampled for analyses. Five fish per sampling were removed from each aquarium. Two of these were used for whole fish analysis. The remaining three fish were dissected into fillet (body, muscle, skin, skeleton) and viscera (fins, head, blood, internal organs). Water and fish samples collected for ^{14}C metabolite characterization were frozen.

Methodology

Subsamples of the pooled soil cores were combusted and the ^{14}C released was quantified by LSC. A portion of the control samples were spiked with known amounts of [^{14}C]acifluorfen before combustion to determine method efficiencies. Soil moisture was determined on all samples. Water samples were pooled by treatment and were allowed to settle for at least 15 minutes. Duplicate subsamples were directly assayed for ^{14}C activity by LSC. Additional subsamples were analyzed for pH, dissolved oxygen and ammonia using specific ion electrodes. Duplicate fish samples were pooled, frozen, mixed with dry ice and ground. Duplicate subsamples of each sample were combusted and the ^{14}C released was quantified by LSC. A portion of the control samples were spiked with a known amount of either a [^{14}C]benzoic acid standard or [^{14}C]acifluorfen before combustion to determine method efficiencies.

All LSC was for 10 min/sample in a system with extra lead shielding. All samples were counted in two channels. Counting efficiency was determined by spiking the control samples after counting and then recounting (LSC). The efficiency determined from this procedure was used to correct the cpm of corresponding treated samples. Recoveries were not corrected for method efficiencies. Detection limits were set at twice the standard deviation above the mean background for the controls.

Carbon-14 recoveries for each parameter (soil, water, whole fish, and fish sections) were regressed on time. The statistical significance of the regression was determined using the T-test at the 0.05% level of probability.

Results

Radioactive residues in the soil remained relatively constant (0.89 mg/kg acid form acifluorfen equivalent) during the 30-day aging period (Table 31). Carbon-14 associated with the soil decreased ~30% when the aquaria water was added. The concentration of ^{14}C in the water increased during the first 6 days (3 days equilibration; 3 day exposure study) and then remained nearly constant with an average of 24 µg/l. Residue levels in the fish varied up and down with time. The average

levels from days 14-30 were 0.07, 0.05, and 0.07 mg/kg in the whole fish, fillets and viscera, respectively. The data did not conform to a linear model and regression analyses were meaningless ($r^2 \leq 0.64$). Fish residues were below minimum quantifiable levels throughout the depuration. The total ^{14}C in the water and soil on day 30 of the uptake study was 100% of the ^{14}C found in the soil on day 0 of the aging interval.

The detection limits in water, soil, whole fish, fillet and viscera were 9.0 µg/l, 0.40 mg/kg, 0.048 mg/kg, 0.055 mg/kg, and 0.068 mg/kg. The average ^{14}C recovery for the combustion technique was 77% for soil and 81% for fish. These values reflect the combination of combustion and counting efficiencies.

The environmental parameters in the aquaria were in an acceptable range throughout the study. Dissolved oxygen ranged from 7.5 to 9.22 mg/l; pH from 7.6 to 7.9, and ammonia from 0.15 to 0.64 mg/l. No abnormal behavior or mortality was observed.

Table 31. Recovery of ^{14}C in test water, fish tissue and soil during static fish accumulation study.

		^{14}C Concentration (acifluorfen equivalents[a])				
Day		Water (µg/l)	Soil (mg/kg)	Fillet (mg/kg)	Whole fish (mg/kg)	Viscera (mg/kg)
Aging	0	--	0.89	--	--	--
	1	--	0.95	--	--	--
	15	--	0.87	--	--	--
	30	--	0.84	--	--	--
Equilibration	1	2.6	0.59	--	--	--
Uptake[b]	0	9.6	0.80	--	--	--
	1	14	0.69	0.010[c]	0.020[c]	0.021[c]
	3	21	0.46	0.028[c]	0.041[c]	0.041[c]
	7	24	0.51	0.033[c]	0.046[c]	0.071[c]
	10	24	0.79	0.014[c]	0.010[c]	0.041[c]
	14	23	0.56	0.032[c]	0.063	0.098
	22	25	0.59	0.057	0.097	0.077
	30	26	0.49	0.071	0.062	0.045[c]
Depuration	1	c	--[d]	0.008[c]	c	0.025[c]
	3	c	--	0.013[c]	c	c
	7	c	--	0.002[c]	c	c
	10	c	--	c	c	c
	14	c	--	c	c	c

[a]Acid form of acifluorfen.

[b]Uptake day 0 is also day 3 of equilibration.

[c]Semi-quantitative values, below minimum quantifiable limits.

[d]Soil not present during depuration.

Aerobic soil metabolism studies: Four studies were submitted and reviewed. One study (Gemma and Wargo, October, 1982, ASD Report No. 82/053, Acc. No. 071324) is scientifically invalid because a portion of the samples awaiting analysis were not properly stored. The other studies (Piznik and Wargo, September, 1982, Acc. No. 071324, Gemma and Wargo, October, 1982, ASD Report No. 82/051, Acc. No. 071324 and Wargo, July, 1982, Acc. No. 071324) are scientifically invalid because analytical techniques were not proven to be adequate. All data are required.

Anaerobic soil metabolism studies: Two studies (Wargo, July, 1982, Acc. No. 071324 and Piznik and Wargo, September, 1982, Acc. No. 071324) were submitted and reviewed. Both studies are scientifically invalid because analytical methodologies were not proven to be adequate. All data are required.

Anaerobic aquatic metabolism studies: No data were submitted, but these studies are not required because acifluorfen does not have a forestry, aquatic, or aquatic impact use.

Aerobic aquatic metabolism studies: No data were submitted, but these studies are not required because acifluorfen does not have an aquatic or aquatic impact use.

Leaching and adsorption/desorption studies: Two studies were submitted and reviewed. One study (Norris and Guardigli, May, 1982, Acc. No. 071325) is scientifically invalid because procedures used were incorrect. The second study (Norris and Miller, December, 1980, Acc. No. 071325) is scientifically valid and partially fulfills data requirements by providing data on the mobility of acifluorfen in four soils. Data on the mobility of aged acifluorfen in soil are required.

Laboratory and field volatility studies: No data were submitted. Requirements for these data depend upon toxicity data, product chemistry data, soil adsorption data, and methods of application.

Terrestrial field dissipation studies: One study was submitted (Norris and Ku, April, 1981, Acc. No. 071325) and reviewed. The study is scientifically invalid because the fields used appeared to be contaminated with acifluorfen and the concentrations of acifluorfen in the field were not determined on a dry soil basis. All data are required.

Aquatic field dissipation studies: No data were submitted, but no data are required because acifluorfen does not have an aquatic or an aquatic impact use.

Forestry dissipation studies: No data were submitted, but no data are required because acifluorfen does not have a forestry use.

Long-term field dissipation studies: No data were submitted. Requirements for these data depend upon the results from the terrestrial field dissipation data; however, it is anticipated that these data will not be required.

dues were more concentrated in the viscera than in the edible portions of the fish. The bioaccumulation factors for whole fish, viscera, and fillet were <3.9, <4.3, <2.7, respectively. Concentrations in all tissues decreased immediately when fish were transferred to uncontaminated water.

Dermal and ocular exposure can occur during mixing and loading operations from spills and splashing. The use of protective clothing, especially gloves and glasses, will minimize this exposure. Inhalation exposure can occur if airborn mists result from the spraying operation. Proper use of spraying equipment will minimize this hazard. Air masks may be required in some situations.

In summary, acifluorfen is tenatively assumed to be stable to hydrolysis, but is subject to photolysis. Several degradates from photolysis in water were isolated; decarboxylated acifluorfen was the most prevalent (6.6%). Photodegradation of acifluorfen on soil reduced levels substantially (18%) during the first 3 days of exposure. Further photolysis proceeded very slowly. The metabolism of acifluorfen in soil under aerobic and anaerobic conditions was variable. Although the data were subject to question, long-term persistence of acifluorfen in soil was not very probable. A number of degradates were isolated but the amino analog of acifluorfen was the only major degradate identified. Degradation of the primary metabolites was apparent. Soil TLC analysis identified acifluorfen as an intermediate to mobile compound. Aged acifluorfen residues were substantially less mobile in soil than the parent compound. Fish accumulated acifluorfen in response to the levels in the water. Depuration did occur.

6. RECOMMENDATIONS

Available data are insufficient to fully assess the environmental fate of acifluorfen and the exposure of humans and nontarget organisms to acifluorfen. The submission of data to fulfill registration requirements (Subparts N and K) is summarized in the following:

Hydrolysis studies: One study (Norris and Hassell, November, 1980, Acc. No. 071323) was submitted and reviewed. The study is scientifically valid, but does not satisfy data requirements because most of the data were not given, the test substance was not sufficiently characterized, and the study was not conducted at an appropriate temperature. All data are required.

Photodegradation studies in water: One study (Somma, et al., September, 1982, Acc. No. 071323) was submitted and reviewed. The study is scientifically valid, but does not fulfill data requirements because the environmental conditions of the study (pH, temperature, sterility) were not given and appropriate controls were not included in the study. All data are required.

Photodegradation studies on soil: One study was submitted (Gerecke and Wargo, August, 1982, Acc. No. 071323) and reviewed. The study is scientifically valid and is in compliance with data requirements. The temperature at which the study was conducted and a complete listing on unnormalized data are required.

Photodegradation studies in air: No studies were submitted; all data are required.

Conclusions

Approximately 30% of the ^{14}C residues in [^{14}C]acifluorfen treated soil aged 30 days was recovered in the aqueous fraction when flooded with water. The concentration of ^{14}C in the water increased over a 14-day interval but the decrease in the soil upon adding the flood water occurred within 1 day. It is presumed that this discrepancy resulted from the slow diffusion of solubilized ^{14}C from the soil solution to the flood water. The average concentration in the water after obtaining equilibrium was 24 ppm (acid form acifluorfen equivalent). Carbon-14 concentrations in the fish were near or below the detection limit in a large number of samples; therefore, rates of accumulation and depuration cannot be accurately determined. Average bioaccumulation factors after obtaining steady state conditions in the uptake study were 2.9 in the whole fish and viscera and 2.1 in the fillet. The extent of depuration could not be determined because of the inability to accurately measure residues at the levels present.

The methods efficiency reported includes combustion efficiency and counting efficiency (quench). All treated samples were corrected for counting efficiency based on the control samples and the combustion efficiency was not compensated for. All samples were counted in two channels and this data presumably could be used to construct a quench curve based upon the channels ratio. All treated samples should be individually corrected for counting efficiency and for combustion efficiency. The errors in the data presented are assumed to be relatively small (<10%). No characterization of the metabolites was given.

5. EXECUTIVE SUMMARY

Acifluorfen appeared to be stable to hydrolysis in water for up to 120 days. However, the hydrolysis study submitted was not adequate to meet data requirements and therefore any conclusions regarding hydrolysis are tenative. Photolysis of [^{14}C]acifluorfen in water was demonstrated but no conclusion on the rate of photodegradation could be made because of the inadequacy of the study. A number of degradates were isolated and identified. The highest recovery of ^{14}C other than the parent compound was associated with the decarboxy derivative of acifluorfen (6.6%). Loss of volatile degradates was apparent but not quantified. Photodegradation of acifluorfen on soil was slow. Initial breakdown rates were the highest with an average loss of 0.25%/hr of acifluorfen for the first 72 hours. Thereafter, the rate of degradation decreased and after 30 days, ~72% of the applied acifluorfen remained unaltered. An estimate of the half-life was not feasible because of the nature of the data.

The soil metabolism data submitted did not meet data requirements, but tenative conclusions can be made. Both aerobic and anaerobic soil metabolism of acifluorfen was measured in several soils. The rates of breakdown were variable, with estimated half-life values ranging from a few days up to >6 months. The data were not adequate to determine whether this variation resulted from the conditions of the experiment or accurately reflected the variability of acifluorfen metabolism. Recovery of radiolabeled tracers from the ring structure of acifluorfen indicated that cleavage of either of the benzene rings, with subsequent evolution of $^{14}CO_2$, in aerobic soils was negligible. Several mechanisms in the initial alteration of acifluorfen were evident from the recovery of primary degradates. These included decarboxylation, reduction of the nitro group, and methylation of the acid moiety. Reduction of the nitro group was the primary mechanism; the amino analog of acifluorfen (2-chloro-4-trifluoromethyl)phenoxy-2-aminobenzoic acid) was the only major degradate identified. Primary degradates did not accumulate, but were degraded to relatively polar extractable products and bound residue.

Acifluorfen mobility was intermediate to mobile as determined by using soil TLC with four soils. Column leaching studies indicated that soil degradates of acifluorfen are less mobile than the parent compound. Quantitative mobility or adsorption data on acifluorfen degradates (aged acifluorfen) were not provided. Acifluorfen mobility did not correlate to soil parameters, but the mobility of ^{14}C from [^{14}C]acifluorfen treated-soil aged 30 days was inversely proportional to soil CEC ($r = -0.849$).

Uptake of acifluorfen residues by wheat, lettuce, and beets planted as rotational crops cannot be fully evaluated because the data submitted were not adequate to meet data requirements. Accumulation in crops planted 1 year after pesticide treatment was low; however, these results may be misleading because of the low rates of acifluorfen application.

The fish accumulation data submitted were inadequate to meet data requirements. It was apparent, however, that in both flow through and static systems fish accumulated acifluorfen for ~2 weeks before coming to equilibrium. The total accumulation was a function of acifluorfen levels in the water. Acifluorfen resi-

Confined accumulation studies on rotational crops: Two studies were submitted. Both were reviewed and found to be scientifically valid. One study (Gemma et al., September, 1982, ASD Report No. 82/042, Acc. No. 071326 and Spare et al., October; 1982, Acc. No. 071326) does not fulfill data requirements because the rate of application was too low. The second study (Gemma et al., September, 1982, ASD Report No. 82/046, Acc. No. 071326) does not comply with data requirements because the acceptability of the application rate could not be evaluated, soil analyses were not included, and the extraction and analytical methods were not proven to be acceptable. All data are required.

Field accumulation studies on rotational crops: No data were submitted. Data requirements are dependant upon confined accumulation studies on rotational crops.

Accumulation studies on irrigated crops: No data were submitted; however, data are not required because acifluorfen has no aquatic food crop or aquatic noncrop use, is not used in and around holding ponds used for irrigation purposes, and has no uses involving effluents or discharges to water used for crop irrigation.

Laboratory studies of accumulation in fish: One study (Thompson and Cranor, January, 1981, Acc. No. 071327) was submitted and reviewed. The study is scientifically valid and partially fulfills data requirements by providing data on the quantity of acifluorfen residues accumulated in fish. Characterization of the residues in the fish are required.

Field accumulation studies on nontarget organisms: No data were submitted; however requirements for these studies depend upon the results from laboratory studies of accumulation in fish and toxicological data.

Reentry studies: No data were submitted. All data are required.

Ancillary studies reviewed.

Static studies of accumulation in fish (Forbis and Boudreau, March, 1981, Acc. No. 071327).

Methods evaluation studies (Ku and Miller, November, 1980. Acc. No. 071325 and Ku and Norris, May, 1981, Acc. No. 071325).

7. REFERENCES

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Norris, F.A., and K.M. Miller. December, 1980. Mobility of MC 10978 in four soil types. Technical Memorandum TME-80.24. Acc. No. 071325.

Piznik, M., and J.P. Wargo. September, 1982. Abbreviated aerobic/anaerobic soil metabolism study with radiolabeled Tackle (MC-10978). ASD Report No. 82/047. Acc. No. 071324.

Somma, N., F.A. Norris, and A. Guardigli. September, 1982. Photodegradation of Tackle in aqueous solution. Rhone-Poulenc, Inc., Monmouth Junction, New Jersey. ASD Report No. 82/048. Acc. No. 071323.

Spare, W.C., F. Dillion, and C. Hutchinson. October, 1982. Field metabolism studies with ^{14}C-MC-10978. Report No. 349. Acc. No. 071326.

Thompson, C.M., and W. Cranor. January, 1981. Uptake, depuration and bioconcentration of ^{14}C-MC 10978 by bluegill sunfish (Lepomis macrochirus) ABC Report No. 26610. Acc. No. 071327.

Wargo, J.P. July, 1982. Metabolism of carbon-14 labeled MC-10978 in Kansas, Virginia, Georgia and New Jersey soils under aerobic and anaerobic conditions. ASD Report No. 82/040. Acc. No. 071324.

APPENDIX A

Proposed label for Tackle (acifluorfen)

CONFIDENTIAL

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Acifluorfen

Page _____ is not included in this copy.

Pages _77_ through _80_ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

__X__ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action

_____ FIFRA registration data.

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