2-20-02

Data Evaluation Report on the anaerobic biotransformation of diflufenzopyr in water-sediment system

PMRA Submission Number {......} EPA MRID Number 45444001

Data Requirement: PMRA DATA CODE:
EPA DP Barcode: D276313
OECD Data Point:
EPA Guideline: 162-3

Test material:
Common name: Diflufenzopyr
Chemical name
IUPAC: 2-{1-[4-(3,5-Difluorophenyl)semicarbazono]ethyl}nicotinic acid
CAS: 2-[1[[[(3,5-Difluorophenyl)amino]carbonyl]hydrazono]ethyl]-3-pyridinecarboxylic acid
CAS No: 109293-97-2
Synonyms: 2-(Methyl-(((3,5-difluorophenylamine)-carbonyl)-hydrazone)-methyl)-3-pyridine carboxylic acid; BAS 654 H
SMILES string:

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CITATION: Guirguis, A. 2001. Anaerobic aquatic metabolism of ^{14}C-Phenyl-BAS 654 H. Unpublished study performed and sponsored by BASF Corporation, Research Triangle Park, NC. BASF Study No. 63840 and Registration Document No. 2001/5000042. Study initiated January 22, 2000 and completed April 23, 2001.

EXECUTIVE SUMMARY:

The anaerobic biotransformation of [phenyl-U-^{14}C]-labeled 2-(methyl-(((3,5-difluorophenylamine)-carbonyl)-hydrazone)-methyl)-3-pyridine carboxylic acid (diflufenzopyr) was studied in pond water-sediment (water pH 7.6, total organic carbon 13.1 mg/L; sediment texture loamy sand, pH 5.3, organic matter 3.3%) from Holly Spring, North Carolina, for 270 days in darkness at 25 ± 1°C. [Phenyl-U-^{14}C]diflufenzopyr was applied at the rate of 0.4 mg a.i./kg water. The water:sediment ratio used was 4:1 (100 mL water:25 g sediment). This experiment was conducted in accordance with US EPA Subdivision N Guideline §162-3 and Canadian Trade Memorandum T-I-255, DACO 8.2.3.5.5 and 8.2.3.5.6 and in compliance with USEPA GLP (1989) Standards. The closed test system consisted of a glass centrifuge vessel containing treated water-sediment; anaerobic conditions were established prior to treatment. Samples were analysed after 0, 2, 5, 7, 15, 33, 61, 90, 187 and 270 days of incubation. Water samples were analysed without extraction. Sediment samples were sequentially extracted with 0.05 M pH 7 Trizma buffer:acetone (2:1, v:v) and acetone. [^{14}C]Diflufenzopyr residues were analysed by reverse-phase HPLC and normal-phase one-dimensional TLC. Identifications of [^{14}C]diflufenzopyr and its transformation products were done by HPLC co-chromatography with unlabeled ~~reference standards and~~ confirmed by MRM LC/MS/MS or GC/MS.

Anaerobic conditions were maintained throughout the study. Overall material balances in the water-sediment systems averaged 96.78 ± 5.88% (range 90.44-105.61%) of the applied radioactivity. [^{14}C]diflufenzopyr in the total water-sediment system decreased from 99.3% of the applied at day 0 posttreatment to 53.84% at 1 week, 10.73% at 1 month and was ≤1.51% at 2 months. [^{14}C]Residues quickly equilibrated between water and sediment then became less soluble with time with distribution ratios for [^{14}C]residues between the water phase and sediment of 25:1 at day 0, 1:1 at 1 week, 1:3 at 1 month, 1:21 at 6 months and 1:42 at 9 months. [^{14}C]Diflufenzopyr in the water layer decreased from 99.33% of the applied at day 0 posttreatment to 49.53% at 5 days, 24.45% at 2 weeks and was last detected at 3.61% at 1 month. [^{14}C]Diflufenzopyr in the sediment increased from 16.81% at 2 days (day 0 sediment not extracted) to a maximum 18.59% at 2 weeks, then decreased to 1.50-1.51% at 2-3 months and was not detected at 6 months.

The major and sole transformation product detected in the water layer was 3,5-difluoroaniline (M2, BH-654-2) increasing from 1.50% of the applied at day 0 to a maximum 18.54% at 15 days and was last detected at 16.02% at 90 days. No major transformation products were detected in sediment extracts. Minor transformation products in sediment extracts were carbamoyl phthalazinone (M5, BH 654-5) detected at a maximum 8.07% of the applied at 90 days and M2 detected at a maximum 4.89% at 61 days. Seven unidentified [^{14}C]compounds (Unk 1-7) were detected in sediment extracts each at <5.0% of the applied. Extractable [^{14}C]residues in sediment increased from 21.34% of the applied at 2 days to 26.43% at 15 days, then decreased 4.30% at 270 days. Nonextractable [^{14}C]residues in the sediment soil increased from 8.23% of the applied at 2 days to 83.80% at 270

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days; [^{14}C]residues associated with fulvic acids, humic acid and humins were detected at maximums of 38.19% (187 days), 13.17% (187 days) and 32.19% (270 days), respectively.

Biotransformation pathway scenarios proposed by the registrant included diflufenzopyr degrading to carbamoyl phthalazinone (M5) which then degrades to 3,5-difluoroaniline (M2), degrading directly to M2, or becoming sediment bound residues.

Results Synopsis:

Test system:	Pond water-loamy sand sediment.
Half-life in water:	7.3 days ($r^2 = 0.99$)
Half-life in sediment:	16.5 days ($r^2 = 0.94$)
Half-life in entire system:	10.2 days ($r^2 = 0.99$)
Major transformation products:	3,5-Difluoroaniline (M2)
Minor transformation products:	Carbamoyl phthalazinone (M5)

Study Acceptability: This study is acceptable and fulfills the guideline requirement for an aerobic biotransformation study of phenyl-labeled diflufenzopyr in soil. Along with the pyridinyl-label study (MRID 44329608), Subdivision N Guideline §162-3 is fulfilled for diflufenzopyr.

I. MATERIALS AND METHODS

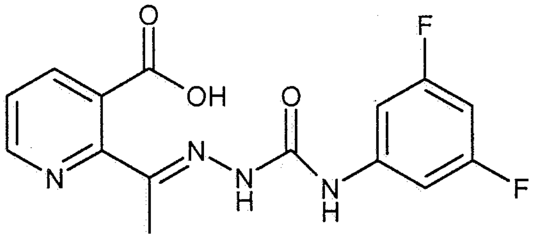
GUIDELINE FOLLOWED: This study was conducted in accordance with USEPA Subdivision N Guideline §162-3 and Canadian Trade Memorandum T-I-255, DACO 8.2.3.5.5 and 8.2.3.5.6 (p. 12). No deviations affected the validity of the study.

COMPLIANCE: This study was conducted in compliance with USEPA GLP Standards (40 CFR, Part 160; 1989; p. 3). Signed and dated GLP, Data Confidentiality, Quality Assurance and Study Certification statements were provided (pp. 2-5).

A. MATERIALS:

1. Test Material: [Phenyl-U-^{14}C]-2-(methyl-(((3,5-difluorophenylamine)-carbonyl)-hydrazone)-methyl)-3-pyridine carboxylic acid.

Chemical Structure:



Description: Technical. Off-white solid (p. 14).

Purity:

[Phenyl-U-^{14}C]-labeled: Radiochemical purity: >92% (pp. 14, 37-39). Inventory/Lot No. 278/980921. Specific activity: 255,137 dpm/µg (115 µCi/mg, 4.25 Mbq/mg).

Unlabeled: ~~Purity: 99.9% (p. 15).~~ Lot No. RS-835-052494.

Storage conditions of test chemicals: [Phenyl-U-^{14}C]diflfenzopy stored in darkness at a low (unspecified) temperature; storage conditions of unlabeled test compound not specified (p. 14).

Table 1: Physico-chemical properties of diflufenzopyr.

Parameter	Values	Comments
Water solubility:	63 mg/L in unbuffered water pH 6.5. 270 mg/L in pH 5 buffer solution. 5,850 mg/L in pH 7 buffer solution. 10,546 mg/L in pH 9 buffer solution.	Solubility in unbuffered water pH 6.5 obtained from MRID 45444002, p. 12. Solubility in pH 9 buffer solution obtained from MRID 45444004, p. 10.
Vapor pressure/volatility:	$<10^{-2}$ mPa.	Obtained from Farm Chemicals Handbook 2001, p. C 144.
UV absorption:	Not reported.	
pK_a:	Not reported.	
K_{ow}/log K_{ow}:	Not reported.	
Stability at room temperature:	Not reported.	

Data obtained from p. 14 of the study report, except where noted.

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2. Water-sediment collection, storage and properties:

Table 2: Description of water-sediment collection and storage.

Description	**Details**
Geographic location:	Pond located in Holly Springs, North Carolina.
Pesticide use history at the collection site:	Pond located at a BASF Field Test Site; pesticide use history not provided.
Collection date:	36535
Collection procedures for water: sediment:	Not provided.
Sampling depth for water: sediment:	Not provided.
Storage conditions of water: sediment:	Not provided. Under refrigeration.
Preparation of water: sediment:	Not provided. 2 mm sieved then centrifuged at 3000 rpm for 15 minutes.

Data obtained from p. 16 of the study report.

Table 3: Properties of the water.

Property	**Details**
Temperature (°C):	Not provided.
pH:	7.6
Redox potential (mV):	Not provided.
Oxygen concentration (mg/L):	Not provided.
Total dissolved solids (mg/L):	152
Total organic carbon (mg/L):	13.1
Hardness (mg equivalent $CaCO_3$/L):	20
Electrical conductivity (mmhos/cm):	0.15
Sodium Absorption Ratio:	0.28
Biomass (mg microbial C/100 g, CFU or other):	Not provided.

Data obtained from p. 61 of the study report.

Table 4: Properties of the sediment.

Property	Details
Texture:	Loamy sand.
sand (%):	81
silt (%):	12
clay (%):	7
pH:	5.3
Organic matter (%):	3.3
CEC (meq/100 g):	5.4
Moisture at 1/3 Bar (%):	12.9
Bulk density - disturbed (g/cm^3):	1.16
Redox potential at 19.0°C and pH 5.3 (mV)	393
Soluble salts (mmhos/cm)	0.08
Microbial biomass (µg/g dry wt. soil):	101.8

Data obtained from pp. 62, 63 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None.

2. Experimental conditions:

Table 5: Experimental design.

Parameter		Details
Duration of the test:		270 days.
Water	Filtered/unfiltered water: filter used, if any:	Not specified.
Water-sediment/replicate	water: sediment:	100 mL 25 g

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Table 5: Experimental design.

Parameter		**Details**
Water-sediment ratio:		4:1, v:w
Application rates:		0.4 mg a.i./kg water.
Control conditions, if used (present differences from other treatments, i.e., sterile/non-sterile, experimental conditions):		No controls were prepared.
No. of Replications.	Controls, if used:	None.
	Treatments:	Duplicate at each sampling interval.
Test apparatus (Type/material/volume):		Sediment samples (25 g) were added to 250-mL glass centrifuge vessels and flooded with pond water containing 0.5% glucose. The water-sediment systems were purged with nitrogen, then the vessels were sealed and pre-incubated (temperature not specified) in darkness for >1 month to maximize initial anaerobic conditions prior to treatment.
Details of traps for CO_2 and organic volatiles, if any:		None.
If no traps were used, is the system closed/open?		Closed. Following treatment, the headspace of each water-sediment system was purged with nitrogen and the vessels sealed.
Co-solvent.	Identity:	Acetone:dimethyl sulfoxide (1.9:0.1, v:v).
	Final concentration:	0.03% based on water volume.
Test material application.	Volume of test solution used/treatment:	0.13mL of 308 µg a.i./mL test solution.
	Application method (eg: mixed/not mixed):	Not specified.
Any indication of the test material adsorbing to the walls of the test apparatus?		Not determined.
Microbial biomass/Microbial population of treated systems:		Not determined.
Experimental conditions.	Temperature (°C):	25 ± 1°C.
	Continuous darkness (Yes/No):	Yes.

Table 5: Experimental design.

Parameter	Details
Other details, if any:	None.

Data obtained from pp. 18, 19, 40, 64 of the study report.

3. Anaerobic conditions: Water-sediment systems, amended with 0.5% glucose (based on water volume) and contained in sealable vessels, were purged with nitrogen, sealed and incubated >1 month (p. 18). Each water-sediment system was then treated with [phenyl-U-^{14}C]diflufenzopyr after which the headspace of each system was purged with nitrogen and the vessels resealed. The systems were analyzed for dissolved oxygen content, redox potential and pH prior to treatment and at each sampling interval.

4. Supplementary experiments: Additional high dose experiments were conducted to facilitate identification of degradates (p. 18). Water-sediment systems were treated with [phenyl-U-^{14}C]diflufenzopyr (0.26 mL of 307 µg a.i./mL test solution) plus non-radiolabeled diflufenzopyr (0.26 mL of 963 µg a.i./mL solution); final treatment rate was 3.3 mg a.i./kg water (pp. 18, 64). The number of systems treated and incubation conditions were not specified. Water-sediment systems were collected after 15, 90, 187 and 270 days (p. 30).

5. Sampling:

Table 6: Sampling details.

Parameters	**Details**
Sampling intervals:	0, 2, 5, 7, 15, 33, 61, 90, 187, and 270 days.
Sampling method:	Duplicate water-sediment systems were collected at each interval. For systems collected at 0-33 and 90 days, one replicate was taken for analysis while the second replicate was placed in frozen storage; both replicates collected at 61, 187 and 270 days were analyzed.
Method of collection of CO_2 and volatile organic compounds:	Upon sampling, the headspace gases of each vessel were purged (flow rate not specified) via nitrogen through two traps containing sodium hydroxide then directly into a biological oxidizer for combustion (4-minute cycle).
Sampling intervals/times for: sterility check, if sterile controls are used: Redox potential/other:	 Sterile controls were not used. Following removal of headspace gases, the redox potential, dissolved oxygen and pH of the water-sediment system were measured using a platinum redox electrode (Fischer Scientific), dissolved oxygen meter (Orion), and pH meter (Orion) in conjunction with an electrometer.
Sample storage before analysis:	Analysis was initiated upon sampling. HPLC analysis of water layers was accomplished within 1 day. HPLC of sediment extracts was done within four weeks; storage conditions of samples during this interval was not reported.
Other observations, if any:	None.

Data obtained from pp. 19, 21, 22, 40 of the study report.

C. ANALYTICAL METHODS:

Separation of water and sediment: Water-sediment systems were centrifuged at 2,500 rpm for 20 minutes, after which the water layer was decanted into a graduated cylinder (pp. 21, 41).

Extraction/clean up/concentration methods: Each water layer was centrifuged (speed and interval not specified), decanted and filtered (filter type or pore size not specified), then either analyzed directly by HPLC or first concentrated by rotary evaporation (temperature not specified.

Sediment samples collected after day 0 were extracted three times each with 0.05 M pH 7 Trizma buffer:acetone (2:1, v:v) then acetone; all volumes were 25 mL (p. 21). For each extraction, the

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sediment:extract was shaken for 20 minutes then separated by centrifugation. Trizma:acetone and acetone extracts were combined, concentrated by rotary evaporation and centrifuged.

Non-extractable residue determination: Previously extracted sediment samples were further extracted with 0.5 N sodium hydroxide (25 mL) for ca. 24 hours with shaking followed by one extraction for 30 minutes with sonication (p. 21). Extracts were separated from sediment by centrifugation, combined, acidified with concentrated hydrochloric acid to ca. pH 1 with the resulting precipitate (humic acid) was removed by centrifugation. The humic acid precipitate was redissolved in 0.5 N sodium hydroxide (5 mL). The remaining acidified extract (fulvic acid) was partitioned with ethyl acetate (2 x 50-60 mL). The organic phase was concentrated by rotary evaporation and analyzed by HPLC as described below.

Total ^{14}C measurement: For all sampling intervals, triplicate aliquots (0.25 mL) of the water layer were analyzed for total radioactivity by LSC prior to centrifugation (p. 21, 65). Prior to extraction, subsamples of 0- to 7-day sediment were air-dried, then aliquots were analyzed for total radioactivity by LSC following combustion.

Triplicate aliquots (0.5 mL) of the Trizma:acetone and acetone sediment extracts were separately analyzed by LSC (pp. 66-77). For quantitation of non-extractable sediment residues, aliquots of the NaOH extract, redissolved humic acid, fulvic acid fraction prior to partitioning, plus organic and aqueous phases of the fulvic acid fraction after partitioning were analyzed by LSC. [^{14}C]Residues remaining in the extracted sediment (humins) were quantified by LSC following combustion.

Aliquots (volume not specified) of the NaOH trapping solutions were analyzed for total radioactivity by LSC following combustion (pp. 66-77).

Derivatization method, if used: A derivatization method was not employed.

Identification and quantification of parent compound and transformation products: Aliquots of the water layers, sediment extracts and organic fulvic acid extracts were analyzed by reverse-phase HPLC under the following conditions: YMC ODS AQ column (5 µm particle size; length and i.d. not specified), gradient mobile phase combining (A) 0.1% aqueous trifluoroacetic acid and (B) acetonitrile [percent A:B at 0 min. 95:5 (v:v), 5 min. 95:5, 15 min. 70:30, 24 min. 20:80, 28 min. 20:80, 30 min. 90:10, 35 min. 95:5, 40 min. 90:10], injection volume not specified, flow rate 1 mL/minute, UV (254 nm) and radioactivity detection (pp. 19, 20, 43-49). For column recoveries, 40-second fractions were collected and analyzed for total radioactivity by LSC.

[^{14}C]Diflufenzopyr and [^{14}C]M2 (BH 654-2) were identified by co-chromatography with unlabeled reference standards of parent diflufenzopyr and 3,5-difluoroaniline, respectively (p. 45). Identification of [^{14}C]diflufenzopyr was confirmed by multiple reaction monitoring (MRM) reverse-phase HPLC/MS/MS under the following conditions: Keystone Scientific Aquasil C18 column (2 x 100 mm, 5 µm particle size), gradient mobile phase combining (A) 4 mM ammonium formate:0.1% aqueous formic acid (ratio not specified) and (B) 4 mM ammonium formate:0.1% methanolic formic acid [percent A:B at 0 min. 30:70 (v:v), 1 min. 30:70, 6 min. 5:95, 7 min. 30:70], injection volume not specified, flow rate 0.3 mL/minute, PE Sciex API 300 MS with electrospray ionization (no additional MS parameters provided; pp. 20, 52, 53). Identification of [^{14}C]M5 (BH 654-5) as carbamoyl phthalazinone was also confirmed by MRM LC/MS/MS (p. 54). Identification of M2 as 3,5-difluoroaniline was confirmed by GC/MS under the following conditions: DB5MS fused capillary column (30 m x 0.34 mm, 0.5 µ film thickness), splitless, 30-second vent delay, column temperature 50°C held for 2 minutes then to 250°C at 8°C/minute, ion source temperature 180°C, electron energy 35 eV (p. 20, 55-59).

Aliquots of organic fulvic acid extracts were also analyzed using normal-phase one-dimensional TLC on silica gel plates developed with either ethyl acetate:methanol:ammonium hydroxide (70:25:5, v:v), ethyl acetate:acetic acid:water (92:4:4, v:v), or n-butanol:acetic acid:water (50:20:30, v:v; pp. 20). Unlabeled reference compounds were co-chromatographed with the extracts (p. 50, 51). Following development, areas of radioactivity were detected and quantified using an AMBIS RadioAnalytical Imager. Unlabeled reference compounds were detected under UV light (wavelength not specified).

Detection limits (LOD, LOQ) for the parent compound: Not reported.

Detection limits (LOD, LOQ) for the transformation products: Not reported.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: Anaerobicity was maintained throughout the study. Following treatment, redox potentials decreased from -79.3 mV at day 0 to -232.4 mV at 90 days and ranged from -202.6 to -223.5 mV at 187-270 days (p. 27). During the 270-day study, dissolved oxygen ranged from 0.03-0.09 mg/L and pH values gradually increased from 4.38 at day 0 to 5.29-5.37 at 270 days. Reportedly the incubation temperature was maintained throughout the study; however, no supporting records were provided.

B. MATERIAL BALANCE: Overall recoveries of radiolabeled material in the water-sediment systems averaged 96.78 ± 5.88% of the applied during the 270-day incubation (p. 29). Material balances steadily decreased from 104.87-105.61% of the applied at 0-2 days posttreatment to 91.00% at 33 days, were 101.39% at 61 days, then again decreased to 90.44% at 270 days. Recovery of total radioactivity in the water layer decreased from 100.83% at day 0 to 2.12% at 270 days and in the sediment and increased from 4.04% at day 0 to 89.23% at 187 days and was 88.10% at 270 days in the sediment layer (Attachment).

Table 7: Biotransformation of [phenyl-U-^{14}C]diflufenzopyr, expressed as percentage of applied radioactivity (n = 1 at 0- to 33- and 90-day intervals and n = 2 at 61-, 187- and 270-day intervals), in water-loamy sand sediment systems under anaerobic conditions.

Compound (code)		**Sampling times (days)**									
		0	2	5	7	15	33	61	90	187	270
Diflufenzopyr (BAS 654 H)	water	99.33	68.4	49.53	36.56	24.45	3.61	ND[1]	ND	ND	NA[2]
	sediment	NA	16.81	16.37	17.28	18.59	7.12	1.51	1.5	ND	NA
3,5-Difluoroaniline (M2, BH 654-2)	water	1.5	7.56	12.32	12.8	18.54	17.39	17.04	16.02	ND	NA
	sediment	NA	2.31	3.12	3.81	2.81	2.58	4.89	1.76	1.1	NA
Carbamoyl phthalazinone (M5, BH 654-5)	water	ND	ND	ND	ND	ND	ND	ND	ND	ND	NA
	sediment	NA	0.77	2.62	1.14	3.91	7.71	2.43	8.07	0.63	NA
Unidentified Unk 1[3]	sediment	NA	ND	ND	ND	ND	ND	ND	ND	2.09	NA
Unidentified Unk 2[3]	sediment	NA	ND	ND	ND	ND	ND	ND	ND	1.17	NA
Unidentified Unk 3[3]	sediment	NA	ND	ND	ND	ND	ND	0.54	0.29	1.29	NA
Unidentified Unk 4[3]	sediment	NA	ND	ND	ND	ND	ND	4.97	2.53	ND	NA
Unidentified Unk 5[3]	sediment	NA	1.45	1.68	3.46	1.11	3.96	0.9	0.65	0.67	NA
Unidentified Unk 6[3]	sediment	NA	ND	ND	ND	ND	ND	1.18	0.63	1.15	NA
Unidentified Unk 7[3]	sediment	NA	ND	ND	ND	ND	ND	ND	ND	0.35	NA
CO_2	entire system	ND	0.07	0.12	0.07	0.04	0.06	0.04 0.09	0.05	0.05 0.05	0.19 0.23
Total organic volatiles	entire system	ND	0.01	0.02	0.01	0.01	0.01	0.02 0.03	0.04	0.01 0.01	0.01 0.01
Non-extractable residues	sediment	4	8.3	14.5	18.5	24.2	48.5	67.7	61.9	80.7	83.8
Total % recovery	water	100.8	75.96	61.85	49.36	42.99	21	16.80 17.27	16.02	3.89 4.43	2.35 1.89
	sediment[4]	4.04	29.57	38.25	44.21	50.67	69.93	84.26	77.35	89.23	88.1

Table 7: Biotransformation of [phenyl-U-^{14}C]diflufenzopyr, expressed as percentage of applied radioactivity (n = 1 at 0- to 33- and 90-day intervals and n = 2 at 61-, 187- and 270-day intervals), in water-loamy sand sediment systems under anaerobic conditions.

Compound (code)		**Sampling times (days)**									
		0	2	5	7	15	33	61	90	187	270
	entire system	104.9	105.6	100.2	93.65	93.71	91	101.4	93.46	93.45	90.44

[1]Not detected; detection limit not specified. [2]Not analyzed. [3]Not detected in water layer. [4] Total % recovery in sediment = "ERR Total" + total "RRR I" from Table 3, p. 29. Data obtained from p. 29, 32, 66-77 of the study report.

C. TRANSFORMATION OF PARENT COMPOUND: [Phenyl-U-^{14}C]diflufenzopyr in the total water-sediment system decreased from 99.3% of the applied at day 0 posttreatment to 53.84% at 7 days, 10.73% at 33 days and was ≤1.51% at 61 days (p. 33). [^{14}C]Diflufenzopyr in the water layer decreased from 99.33% at day 0 to 49.53% at 5 days, 24.45% at 15 days, 3.61% at 33 days and was not detected (detection limit not specified) thereafter (p. 32). [^{14}C]Diflufenzopyr in the sediment increased from 16.81% at 2 days to a maximum 18.59% at 15 days, then decreased to 1.50-1.51% at 61-90 days and was not detected at 187 days (day 0 sediment was not extracted). Distribution ratios for [^{14}C]residues between the water phase and sediment of 25:1 at day 0, 3:1 at 2 days, 1:1 at 7 days, 1:3 at 33 days, 1:5 at 61 days, 1:21 at 187 days and 1:42 at 270 days (Attachment).

HALF-LIFE: Half-life values of [phenyl-U-^{14}C]diflufenzopyr in the water-sediment system were determined by the registrant using the Gustafson and Holden model for nonlinear regression; DT_{75} (75% decline time) values were also determined (pp. 22, 25, 35, 79, 80). Half-life values of [^{14}C]diflufenzopyr in water-sediment were also determined by the Dynamac reviewer using least squares linear regression analysis assuming degradation followed first order kinetics calculated by Corel Quattro Pro 8 software Corel Quattro Pro 8 software.

Table 8: Half-life values of diflufenzopyr in anaerobic water-loamy sand sediment.

System	First order nonlinear[1]			DT_{75} (days)	First order linear[2]		
	half-life (days)	Regression equation	r^2		half-life (days)	Regression equation	r^2
water	5.76	$\ln C = \ln C_0 - \alpha \ln(1 + \beta t)$, where C_0, α and β are determined in sequence as unknowns. With α and β determined, half-lives were calculated by half-life = $[0.5^{-(1/\alpha)} - 1]/\beta$	0.98	12.11	7.3	Linear form $y = mx + b$ as $\ln C = -kt + \ln C_0$; $\ln C_0$ is initial concentration (b = y intercept), $\ln C$ is concentration at time t (y), k is the slope (m), t is time (x) or $kt = \ln C_0 - \ln C$. Half-life (t ½) = -(ln/k).	0.99
sediment	7.87		0.9	18.64	16.5		0.94
entire system	7.48		0.97	16.49	10.2		1

[1]Equation and data obtained from pp. 22, 35, 79, 80 of the study report.

[2]Values determined by Dynamac reviewer using Corel Quattro Pro 8 software.

TRANSFORMATION PRODUCTS:

Table 9: Chemical names for identified transformation products of diflufenzopyr in anaerobic water-loamy sand sediment.

BASF Code	Chemical Name(s)	HPLC retention time (minutes)	Molecular weight (g/mol)
M2, BH 654-2	3,5-Difluoroaniline	24:06-24:26	129
M5, BH 654-5	6-(3,5-difluorophenyl-carbamoyl-8-methyl-pyrido-(2,3-d)-5-pyridazinone, Carbamoyl phthalazinone[1]	27:31-28:52	316

[1]Chemical name obtained from p. 29 of MRID 45444002 of this submission.

Data obtained from pp. 15, 16, 43, 44, 46-49 of the study report.

The major and sole transformation product detected in the water layer was 3,5-difluoroaniline (M2, BH-654-2) increasing from 1.50% of the applied at day 0 posttreatment to a maximum 18.54% at 15 days then decreasing to 16.02% at 90 days and was not detected (detection limit not specified) at 187 days (p. 32).

No major transformation products were detected in sediment extracts. Minor transformation products in sediment extracts were carbamoyl phthalazinone (M5, BH 654-5) detected at a maximum 8.07% of the applied at 90 days and 3,5-difluoroaniline (M2, BH 654-2) detected at a maximum 4.89% at 61 days (p. 32). Seven unidentified [^{14}C]compounds (Unk 1-7) in sediment extracts were each detected at <5.0% of the applied.

Half-life values (nonlinear regression) and DT_{75} values of [^{14}C]M2 in the water-sediment system were also determined by the registrant (pp. 25, 35).

Table 10: Half-life values of difluoroaniline (M2) in anaerobic water-loamy sand sediment.

System	First order nonlinear			DT_{75} (days)
	half-life (days)	Regression equation	r^2	
water	25.4	$\ln C = \ln C_0 - \alpha \ln(1 + \beta t)$, where C_0, α and β are determined in sequence as unknowns. With α and β determined, half-lives were calculated by half-life = $[0.5^{-(1/\alpha)} - 1]/\beta$	0.831	51.4
sediment	9.2		1	97.3
entire system	27.4		0.973	55.1

Data obtained from pp. 81, 82 of the study.

NON-EXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues in sediment increased from 21.34% of the applied at 2 days (day 0 sediment not extracted) to 26.43% at 15 days, then decreased 4.30% at 270 days (p. 29). Nonextractable [^{14}C]residues in the sediment soil increased from 8.23% of the applied at 2 days to 83.80% at 270 days; [^{14}C]residues associated with fulvic acids, humic acid and humins were detected at maximums of 38.19% (187 days), 13.17% (187 days) and 32.19% (270 days), respectively (pp. 66-77). Following TLC separation of organic extracts from fulvic acids (270-day), one unidentified [^{14}C]compound (spot #7) was detected at 15.75% of the applied, with parent compound, M5 and four unknowns each detected at ≤3.05% (pp. 34, 50). Following isolation and additional TLC separation of spot #7, yielded five separate [^{14}C]compounds each detected at ≤3.99% of the applied (pp. 34, 51).

VOLATILIZATION: Volatilization of $^{14}CO_2$ was insignificant increasing to 0.19-0.23% of applied at 270 days; [^{14}C]organic volatiles were ≤0.04% at any sampling interval (pp. 66-77).

TRANSFORMATION PATHWAY: A biotransformation pathway (p. 60) for the degradation of diflufenzopyr in anaerobic water-sediment was proposed by the registrant. Scenarios included diflufenzopyr degrading to carbamoyl phthalazinone (M5) which then degrades to 3,5-difluoroaniline (M2), degrading directly to M2, or becoming sediment bound residues.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: Not applicable.

III. STUDY DEFICIENCIES: None

IV. REVIEWER'S COMMENTS:

1. The reported concentrations of the transformation product 3,5-difluoroaniline (M2), detected from [phenyl-U-^{14}C]-labeled diflufenzopyr, may have been underestimated. M2 was a major transformation product in the water layer detected at a maximum 18.54% of the applied at 15 days posttreatment, and a minor transformation product in sediment extracts detected at a maximum 4.89% at 61 days (p. 32). In an aerobic soil metabolism study, M2 was found to volatilize (42% of applied radioactivity volatilized) when solvent containing M2 was evaporated to dryness (p.27, MRID 45444002). The reported methods used in this study involved a step where water layers and sediment extracts were concentrated by rotary evaporation prior to quantitation of diflufenzopyr and its transformation products (p. 21). It was specified that sediment extracts were concentrated to a "small volume", but concentration of water layers was not further described. Material balances ranged from 90.44-105.61% of the applied during the study (p. 29). It's conceivable that M2 was ~~being lost from water~~ layers and/or sediment extracts during the concentration step. Adjusting concentrations of M2 for unaccounted for radioactivity, theoretical maximum concentrations of [^{14}C]M2 would be 26.39% of applied in the water layer and 11.58% in sediment extracts both at 33 days posttreatment.

2. It was reported that water layers were analyzed by HPLC within 1 day of collection (p. 22). Analyses of sediment samples and extracts was accomplished within 4 weeks of collection; storage conditions for samples and extracts during this period were not reported.

3. The treated water-sediment systems were reportedly incubated at 25 ± 1°C during the study, but actual recorded temperatures were not provided.

4. HPLC parameter/conditions were incomplete; length and internal diameter of the HPLC column and injection volume were not specified.

5. LC/MS/MS parameter conditions were incomplete; ratios of ammonium formate: formic acid for the mobile phase components (A) 4 mM ammonium formate:0.1% aqueous formic acid (ratio not specified) and (B) 4 mM ammonium formate:0.1% methanolic formic acid were not specified, injection volume not specified, and the MS system was described as a PE Sciex API 300 MS with electrospray ionization, but no additional operating parameters were provided.

6. Detection limits for the LSC and HPLC analytical methods used for quantitation of diflufenzopyr and its transformation products were not reported.

7. Reportedly, fractions from column eluates were collected during HPLC runs and analyzed for total radioactivity by LSC to determine column recoveries (p. 20). Column recovery from a purity check to the [^{14}C]diflufenzopyr test compound was provided (p. 38). Column recoveries from HPLC analysis of the water layers and sediment extracts should have been provided.

8. Analysis of the sodium hydroxide trapping solutions was not described in the methods section of the study report. Results presented in data spreadsheets indicate that aliquots of the NaOH trapping solutions were analyzed for total radioactivity by LSC following combustion (pp. 66-77).

9. Temperature during the initial 1-month pre-incubation phase of induce anaerobic conditions in the water-sediment systems was not specified. The water-sediment systems were then further incubated to maximize anaerobic conditions; however, that incubation interval was not reported.

10. 6-(3,5-Difluorophenyl-carbamoyl-8-methyl-pyrido-(2,3-d)-5-pyridazinone is the chemical name provided for M5 in this study (p. 16), whereas, carbamoyl phthalazinone is the name provided in an aerobic soil metabolism study (p. 16, MRID 45444002 of this submission). Chemical structures presented for M5 in both studies are identical, so the carbamoyl phthalazinone chemical name was used in this study.

11. The application rate of 0.4 mg/L used in this study was based on an application rate of 0.2 lb a.i./A (p. 22), The current recommended seasonal application rate for diflufenzopyr was reported as 0.14-0.18 lb a.i./A (0.16-0.20 kg a.i./ha; p. 15, MRID 45444002).

V. REFERENCES: The following references were cited in the study:

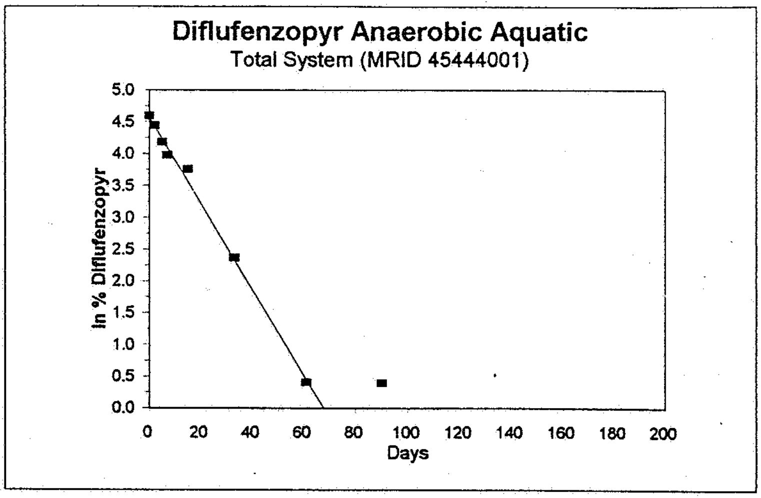
1. Gustafson, D. and L. Holden. 1990. Nonlinear pesticide dissipation in soil: a new model based on spatial varability. Environ. Sci. Technol. Vol. 24, No. 7: 1032-1038.

2. Microcal Software, Inc. Origin 6.0. One Roundhouse Plaza, Northampton, MA 01060.

Diflufenzopyr Anaerobic Aquatic Metabolism in Water:Loamy Sand Sediment
MRID 45444001

Half-life Determination Water:Sediment

Diflufenzopyr		
Day	**as % of AR***	**Ln (% AR)**
0	99.33	4.59844764
2	85.21	4.4451188
5	65.90	4.18813844
7	53.84	3.98601669
15	43.04	3.76212992
33	10.73	2.37304356
61	1.51	0.41210965
90	1.50	0.40546511
187	0.00	ERR



Calculated with 0- to 61-day data

Regression Output:

Constant		4.585
Std Err of Y Est		0.108
R Squared		0.996
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-0.0677474	
Std Err of Coef.	0.00199445	

half-life **10.2 days**

[^{14}C]Residue Water phase:Sediment ratios

Day	Water (%AR)	Sediment1 (%AR)	Ratio W:S	Ratio S:W
0	100.83	4.04	25.0	0.0
2	75.96	29.57	2.6	0.4
5	61.85	38.25	1.6	0.6
7	49.36	44.21	1.1	0.9
15	42.99	50.67	0.8	1.2
33	21.00	69.93	0.3	3.3
61	17.04	84.26	0.2	4.9
90	16.02	77.35	0.2	4.8
187	4.16	89.23	0.0	21.4
270	2.12	88.10	0.0	41.6

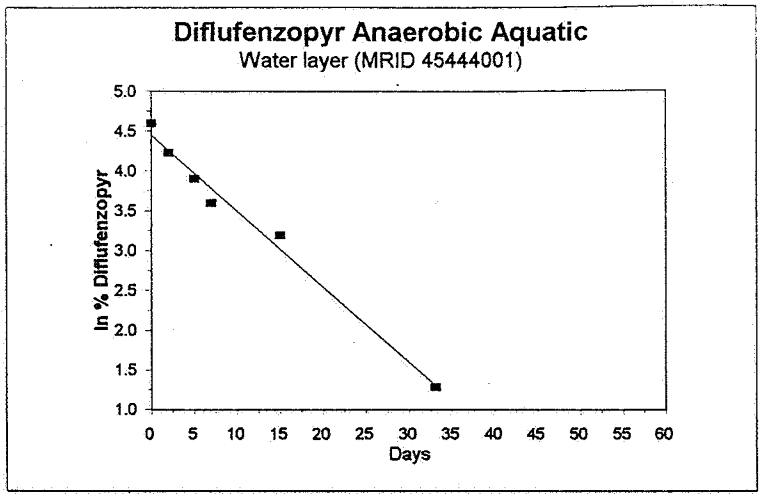
[1] Sediment = "ERR Total" + total "RRR I" in Table 3, p. 29 of study report.

*AR = Applied Radioactivity
Linear regression analysis performed using Corel Quattro Pro 8
Results from pp. 29, 33 of study report.

Diflufenzopyr Anaerobic Aquatic Metabolism in Water:Loamy Sand Sediment
MRID 45444001

Half-life Determination Water

	Diflufenzopyr	
Day	as % of AR*	Ln (% AR)
0	99.33	4.59844764
2	68.40	4.22537282
5	49.53	3.90257855
7	36.56	3.59895475
15	24.45	3.19663022
33	3.61	1.28370777
61	0.00	ERR
90	0.00	ERR
187	0.00	ERR



Calculated with 0- to 33-day data

Regression Output:

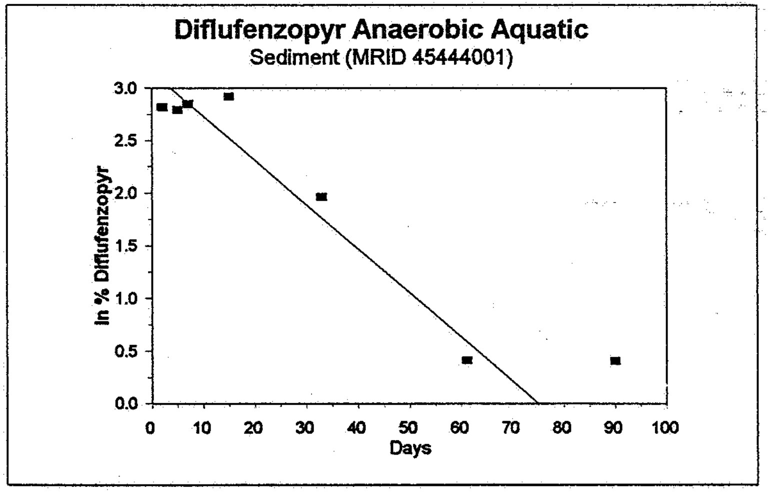
Constant	4.451
Std Err of Y Est	0.153
R Squared	0.986
No. of Observations	6
Degrees of Freedom	4

X Coefficient(s)	-0.0951961
Std Err of Coef.	0.00557768

half-life **7.3 days**

Half-life Determination Sediment

	Diflufenzopyr	
Day	as % of AR*	Ln (% AR)
0	NA	ERR
2	16.81	2.82197395
5	16.37	2.79545039
7	17.28	2.84954976
15	18.59	2.9226238
33	7.12	1.96290773
61	1.51	0.41210965
90	1.50	0.40546511
187	0.00	ERR



Calculated with 2- to 61-day data

Regression Output:

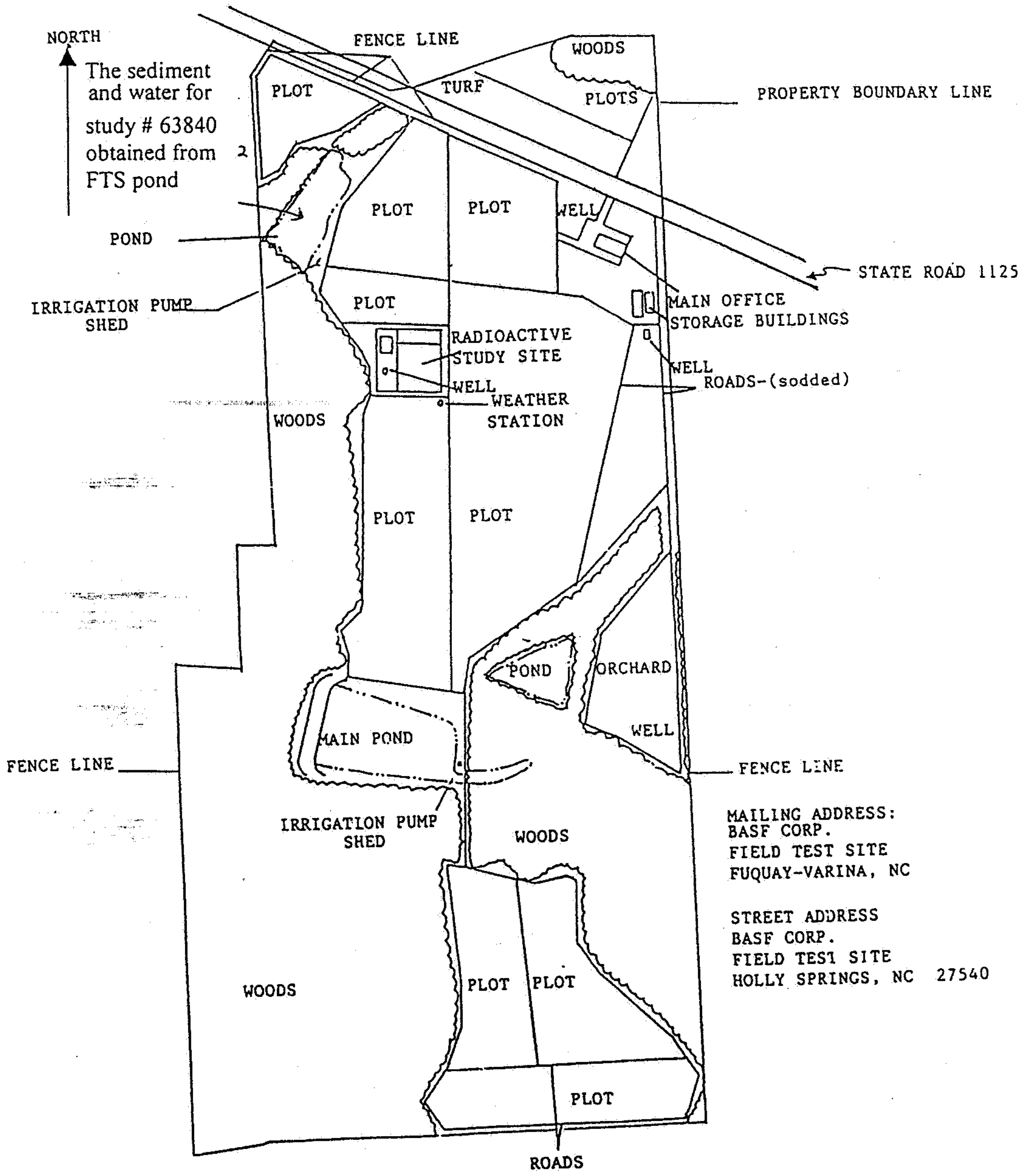
Constant	3.155
Std Err of Y Est	0.28
R Squared	0.936
No. of Observations	6
Degrees of Freedom	4

X Coefficient(s)	-0.0420037
Std Err of Coef.	0.00549443

half-life **16.5 days**

*AR = Applied Radioactivity
Linear regression analysis performed using Corel Quattro Pro 8
Results from pp. 32 of study report.

Figure 1. Map Showing the Location of the Pond on the BASF Field Test Site (FTS) Where the Sediment and Water Sample Were Obtained



The sediment and water for study # 63840 obtained from FTS pond

MAILING ADDRESS:
BASF CORP.
FIELD TEST SITE
FUQUAY-VARINA, NC

STREET ADDRESS
BASF CORP.
FIELD TEST SITE
HOLLY SPRINGS, NC 27540

All areas surrounding site are agricultural fields and rural dwellings.

:.F. 8/86

1" = Approx. 420'

Figure 25. Proposed Metabolic Pathway of ^{14}C-Phenyl BAS 654 H Under Anaerobic Aquatic Conditions