11-19-01

Data Evaluation Report on the aerobic biotransformation of diflufenzopyr in soil

PMRA Submission Number {.....} EPA MRID Number 45444002

Data Requirement: PMRA DATA CODE:
EPA DP Barcode: D276313
OECD Data Point:
EPA Guideline: 162-1

Test material:
Common name: Diflufenzopyr
Chemical name
IUPAC: 2-{1-[4-(3,5-Difluorophenyl)semicarbazono]ethyl}nicotinic acid
CAS name: 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: Singh, M. 2001. Aerobic soil metabolism of ^{14}C-BAS 654 H. Unpublished study performed and sponsored by BASF Corporation, Research Triangle Park, NC. BASF Protocol No. 61198 and Registration Document No. 2001/5000085. Study initiated October 22, 1999 and completed June 19, 2001 (p. 10).

EXECUTIVE SUMMARY:

The biotransformation of [phenyl-U-^{14}C]- and [pyrdinyl-4,6-^{14}C]-labeled 2-(methyl-(((3,5-difluorophenylamine)-carbonyl)-hydrazone)-methyl)-3-pyridine carboxylic acid (diflufenzopyr) was studied in sandy loam soil (pH 7.2, organic matter 1.5%) from Madison County, Illinois, incubated in darkness for 312-327 days under aerobic conditions at 20 ± 1°C and a soil moisture content of 75% of 1/3 bar. [Phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-labeled diflufenzopyr were applied at a rate of 0.23 mg a.i./kg soil (equivalent to 0.26 kg a.i./ha) and 0.27 mg a.i./kg (0.31 kg a.i./ha), respectively. This experiment was conducted in accordance with US EPA Subdivision N Guideline §162-1 and European Council Directive 91/414/EEC and in compliance with USEPA GLP (1989) Standards. The test system consisted of petri dishes containing treated soil maintained in a flow-through apparatus with traps for the collection of CO_2 and volatile organics. [Phenyl-U-^{14}C]diflufenzopyr-treated samples were analyzed after 0, 7, 14, 30, 64, 101, 126, 218 and 316 days of incubation, and [pyrdinyl-^{14}C]diflufenzopyr-treated samples after 0, 6, 14, 34, 69, 90, 132, 196, 272 and 327 days. Soil samples were sequentially extracted with ethyl acetate, tetrahydrofuran, and acetonitrile:0.05 M ammonium carbonate (1:1, v:v), then [^{14}C]diflufenzopyr residues were analyzed by reverse-phase HPLC.

Mean material balances were 96.2% and 100.7% of the applied radioactivity in the phenyl- and pyridinyl-label studies, respectively. [Phenyl-U-^{14}C]diflufenzopyr decreased from 88.5% of the applied at time 0 to 2.5% at the end of the study period. [Pyridinyl-4,6-^{14}C]diflufenzopyr decreased from 90.9% at time 0 to 1.2% at the end of the study.

For both [phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-labeled diflufenzopyr, the major transformation product was volatilized $^{14}CO_2$ accounting for 30.13% and 45.07% of the applied, respectively, at 316-327 days posttreatment. In the [phenyl-U-^{14}C]diflufenzopyr-treated soil, the minor transformation products were 5-difluorophenyl urea (M4; maximum 5.8% at 64 days), carbamoyl phthalazinone (M5; maximum 3.9% at 30 days), and 3,5-difluoroaniline (M2), M23 and PH3 (each ≤1.5%). Four unidentified [^{14}C]compounds (PH1, PH2, PH4 and PH5) were each ≤4.02%. In the [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil, two major transformation products were 8-methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione (M9; maximum 27.3% at 272 days) and 8-methyl-5-hydroxy-pyrido-(2,3-d)-pyridazinone (M1; maximum 13.2% at 34 days). The minor transformation products were M5 (maximum 4.3% at 34 days), 2-acetyl nicotinic acid (M6; maximum 3.2% at 6 days), and M23 (≤1.05%). Five unidentified [^{14}C]compounds (PY1, PY2, PY3, PY4 and PY5) were each ≤1.5%. Extractable [^{14}C]residues decreased from 95.4% of the applied at day 0 to 7.5% at 316 days in phenyl-label study, and from 95.1% at day 0 to 31.8% at 327 days in the pyridinyl-label study. Nonextractable [^{14}C]residues in [phenyl-U-^{14}C]diflufenzopyr-treated soil increased from 5.0% of the applied at time 0 to 65.7% at 218 days and were 61.1% at 316 days; 7.7-12.6% of the applied was associated with the fulvic acid (218- and 316-day samples), 0% with humic acid, and 37.6-44.2% with humin. Nonextractable [^{14}C]residues in [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil increased from 1.6% at time 0 to 26.7% at 69 days, and were 24.4% at 272-327 days; 3.7-6.6% of the applied was associated with

the fulvic acid (132-, 196- and 327-day samples), 0% with humic acid, and 13.9-19.2% with humin.

In the biotransformation pathway proposed by the registrant, diflufenzopyr can initially degrade to several transformation products including 3,5-difluoroaniline (M2), 5-difluorophenyl urea (M4), carbamoyl phthalazinone (M5), 2-acetyl nicotinic acid (M6), and/or M23. Those transformation products can further degrade to 8-methyl-5-hydroxy-pyrido-(2,3-d)-pyridazinone (M1), 3,5-difluoroaniline (M2), 2-acetyl nicotinic acid (M6) and/or 8-methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione (M9) with eventual production of CO_2 and soil bound residues.

Results Synopsis:

Soil type:	Sandy loam.
Half-life values:	[Phenyl-U-^{14}C]diflufenzopyr: 59 days (r^2 = 0.90).
	[Pyridinyl-4,6-^{14}C]diflufenzopyr: 50 days (r^2 = 0.86).
Major transformation products:	CO_2
	8-Methyl-5-hydroxy-pyrido-(2,3-d)-pyridazinone (M1).
	8-Methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione (M9).
Minor transformation products:	3,5-Difluoroaniline (M2).
	5-Difluorophenyl urea (M4).
	Carbamoyl phthalazinone (M5).
	2-acetyl nicotinic acid (M6).
	M23 (proposed structure p.26).
	PH3 (most probably an artifact; proposed structure p. 25).

Study Acceptability: This study is acceptable and fulfills the guideline requirement for an aerobic biotransformation study in soil.

I. MATERIALS AND METHODS

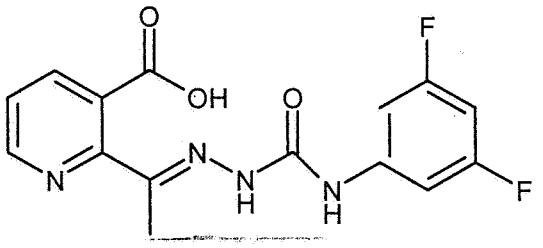
GUIDELINE FOLLOWED: This study was conducted in accordance with USEPA Subdivision N Guideline §162-1 and European Council Directive 91/414/EEC (p. 10). There were no study deviations that affected the validity of the results.

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 Materials: Two radiolabels were studied separately: **[Phenyl-U-^{14}C]**- and **[pyridinyl-4,6-^{14}C]**-labeled 2-(methyl-(((3,5-difluorophenyl amine)-carbonyl)-hydrazone)-methyl)-3-pyridine carboxylic acid.

Chemical Structure:



Description: Technical. Off-white solid (p. 12).

Purity:

[phenyl-U-^{14}C]-labeled: Radiochemical purity: >96% (pp. 12-14, 38). Inventory/Lot No. 278/980921. Specific activity: 255,137 dpm/µg (115 µCi/mg, 4.25 MBq/mg).

[pyridinyl-4,6-^{14}C]-labeled: Radiochemical purity: >97% (pp. 12-14, 39). Inventory/Lot No. 279/980921. Specific activity: 243,782 dpm/µg (110 µCi/mg, 4.06 MBq/mg).

Storage conditions of test chemicals: In darkness at a low (unspecified) temperature (p. 12).

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.	Solubilities in buffer solutions 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.	

Table 1: Physico-chemical properties of diflufenzopyr.		
Parameter	**Values**	**Comments**
Stability at room temperature:	Not reported.	

Data obtained from p. 12 of the study report, except where noted.

2. Soil Characteristics:

Table 2: Description of soil collection and storage.	
Description	**Details**
Geographic location:	Madison County, Illinois.
Collection date:	November 1, 1999. Received at BASF 11/02/99.
Storage conditions:	Under refrigeration at 7°C until use.
Soil preparation:	2 mm sieved.

Data obtained from p. 13 of the study report.

Table 3: Properties of the soil.	
Property	**Details**
Texture:	Sandy loam.
sand (%):	69
silt (%):	22
clay (%):	9
pH:	7.2
Organic matter (%):	1.5
CEC (meq/100 g): CEC (meq/100 g) - Na Acetate method:	884.7 7.1
Moisture at 1/3 Bar (%):	15.1
Bulk density - disturbed (g/cm^3):	1.42
Soil Taxonomic classification:	Not provided.
Soil Mapping Unit (for EPA):	Not provided.

Data obtained from pp. 30, 63 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None.

2. Experimental conditions:

Table 4: Experimental design.

Parameter		[Phenyl-U-^{14}C]diflufenzopyr	[Pyridinyl-4,6-^{14}C]diflufenzopyr
Duration of the test:		312 days.	328 days.
Soil condition (air dried/fresh):		Not specified.	
Soil (g/replicate):		35 g (wet/dry wt. not specified).	
Application rates:		0.23 mg a.i./kg; 0.26 kg a.i./ha.	0.27 mg a.i./kg; 0.31 kg a.i./ha.
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 samples at day 0. One to three samples at each sampling interval after day 0; it was not specified how many samples were collected at each interval.	
Test apparatus (Type/material/volume):		Soil samples (ca. 35 g) were weighed into twenty-two petri dishes. Following treatment, two samples were taken for day 0 analysis, then the remaining twenty samples were placed in two closed glass columns (ten samples per column) and maintained in an incubator. Each column was equipped with inlet/outlet ports for volatiles collection.	
Details of traps for CO_2 and organic volatiles, if any:		Humidified, CO_2-free air was drawn continuously (flow rate not specified) through each glass column then sequentially through 1 N sodium hydroxide (two traps), ethylene glycol (one trap) and 0.1 N sulfuric acid (one trap).	
If no traps were used, is the system closed/open?		Volatiles traps were used.	
Co-solvent.	Identity:	Acetone:dimethyl sulfoxide (9:1, v:v) diluted with pH 7 Trizma (Tris) buffer.	
	Final concentration:	6.4%.	3.0%.
Test material application.	Volume of test solution used/treatment:	0.2 mL of 40.65 µg a.i./mL test solution.	0.2 mL of 47.8 µg a.i./mL test solution.

Table 4: Experimental design.

Parameter		[Phenyl-U-^{14}C]diflufenzopyr	[Pyridinyl-4,6-^{14}C]diflufenzopyr
	Application method (eg: applied on surface, homogeneous mixing etc.):	Applied drop-wise to soil surface.	
	Is the co-solvent evaporated?	No.	
Microbial biomass/Microbial population of test soil (determined prior to treatment). Total:		11/09/99 - 24.2 µg/g dry wt. soil. 01/18/00 - 29.9 µg/g dry wt. soil. 02/16/00 - 36.3 µg/g dry wt. soil.	11/26/99 - 320.7 µg/g dry wt. soil. 01/26/00 - 33.1 µg/g dry wt. soil.
	Actinomycetes:	7.18 x 10^6 CFU /g dry wt. soil.	
	Fungi:	1.14 x 10^4 CFU/g dry wt. soil.	
	Bacteria	3.82 x 10^6 CFU/g dry wt. soil.	
Any indication of the test material adsorbing to the walls of the test apparatus?		Not determined.	
Experimental conditions.	Temperature (°C):	20 ± 1°C.	
	Moisture content: Moisture maintenance method:	75% of 1/3 bar. Treated samples were weighed every 2 weeks following treatment and at each sampling interval, and the weights were compared to samples weight recorded at the onset of the experiment; water was added as needed.	
	Continuous darkness (Yes/No):	Yes.	
Other details, if any:		None.	

Data obtained from pp. 13-16, 40, 64, 65, 67, 70-85, 93-109 of the study report.

3. Aerobic conditions: Humidified, CO_2-free air was drawn continuously through the glass columns containing the treated soil samples; no determinations were made, such as redox potentials, to verify that aerobic conditions were maintained.

4. Supplementary experiments: The following supplemental experiments were conducted:

To identify degradates, additional experiments were conducted at an exaggerated rate (pp. 14-16). Soil samples (ca. 35 g) were treated with [phenyl-U-^{14}C]diflufenzopyr at 1.75 µg/g (0.2 mL of 305.5 µg a.i./mL test solution; co-solvent concentration 75%, p. 14) *OR* [pyridinyl-4,6-

^{14}C]diflufenzopyr at 2.87 µg/g (0.2 mL of 502.45 µg a.i./mL test solution; co-solvent concentration 28.3%, p. 15). The treated soil samples (ten per label) were placed in glass columns and maintained in darkness at 20 ± 1°C in an incubator. The samples were kept aerated and soil moisture was maintained at 75% of 1/3 bar as described for the test samples.

Volatility of transformation product M2: To determine the volatility of M2, extracts (phenyl label study) from which [^{14}C]M2 was isolated were mixed with ethyl acetate:THF (2:1, v:v) and the resulting solution was reduced to dryness under nitrogen (p. 27). The remaining residue was redissolved in acetone:DMSO (9:1, v:v) and aliquots of the solution were analyzed for total radioactivity by LSC.

5. Sampling:

Table 5: Sampling details.

Parameters	[Phenyl-U-^{14}C]diflufenzopyr	[Pyridinyl-4,6-^{14}C]diflufenzopyr
Nominal sampling intervals:	0, 7, 14, 30, 64, 101, 102, 126, 218 and 316 days.	0, 6, 14, 34, 69, 90, 132, 196, 272 and 327 days.
Sampling method for soil samples:	One to three samples were collected at each interval. Sand (ca. 10 g) was added to the soil sample and thoroughly mixed with a spatula. The soil/sand sample was then transferred to a stainless steel container for extraction.	
Method of collection of CO_2 and volatile organic compounds:	Trapping solutions were collected and replaced at each sampling interval.	
Sampling intervals/times for: sterility check, if sterile controls are used: Moisture content: Redox potential/other:	 Sterile controls were not used. Every two weeks after treatment and at each sampling interval. Not determined.	
Sample storage before analysis:	Storage conditions were not reported; soil samples were extracted within 1 day of collection.	
Other observations, if any:	None.	

Data obtained from pp. 14, 16, 17, 70-85, 93-109 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Each soil sample (pre-mixed with 10 g sand) was extracted sequentially as follows: twice with ethyl acetate, once with tetrahydrofuran (THF), and three times with acetonitrile:0.05 M ammonium carbonate (1:1, v:v; pp. 17, 42). The organic extracts (ethyl acetate and THF) were combined, evaporated to dryness under nitrogen, and

redissolved in acetone:dimethyl sulfoxide (9:1, v:v). The aqueous extracts (Acetonitrile: ammonium carbonate) were combined, reduced to dryness under nitrogen, and the remaining residue was redissolved in Trizma buffer:dimethyl sulfoxide (9:1, v:v).

Non-extractable residue determination: Triplicate samples of post-extracted soil were air dried and analyzed by LSC following combustion. To characterize non-extractable [^{14}C]residues in the soil, selected post-extracted soil samples were further extracted three times by shaking with 0.1 N sodium hydroxide (pp. 21, 58). The extracts were centrifuged and the supernatants were analyzed for total radioactivity by LSC. The combined extract was acidified (HCl, pH 1) followed by centrifugation to precipitate humic acids. The humic acid precipitate was redissolved in 0.5 N sodium hydroxide and analyzed by LSC. The acidified extract (fulvic acid) was partitioned with ethyl acetate and the resulting organic and aqueous phases were analyzed by LSC. The organic phase was concentrated, redissolved in acetone:DMSO, and analyzed by HPLC as described for test extracts (pp. 59, 60). [^{14}C]Residues remaining in the post-extracted soil (humins) were quantified by LSC following combustion.

Total ^{14}C measurement: Triplicate aliquots (0.25-1.0 mL) of the ethyl acetate, THF and acetonitrile:ammonium carbonate soil extracts were analyzed for total radioactivity by LSC (p. 17). Extracted soil samples were air-dried, homogenized using a mortar-pestle, then aliquots were analyzed for total radioactivity by LSC following combustion.

Aliquots (volume not specified) of each trapping solution were analyzed for total radioactivity by LSC (p. 16). To quantify $^{14}CO_2$, an aliquot (ca. 6.0 mL) of the sodium hydroxide trapping solution was reacted with 5 N sulphuric acid (ca. 7 mL), then released $^{14}CO_2$ was trapped in Harvey cocktail solution contained in a vigreux column and aliquots were analyzed by LSC; the remaining neutralized NaOH:H_2SO_4 solution was also analyzed by LSC (pp. 16, 41).

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

Identification and quantification of parent compound and transformation products: Aliquots of the organic and aqueous phase extracts were analyzed by reverse-phase HPLC using the following conditions: YMC ODS AQ column (4.6 x 250 mm, 5 µm particle size), mobile phase gradient of (A) 0.05% aqueous trifluoroacetic acid to (B) acetonitrile (A:B, v:v, 98:2, 60:40, 20:80, 0:100), injection volume 75-100 µL, flow rate 1 mL/minute, UV (unspecified wavelength) and radioactive flow detection (pp. 16, 17). Column eluates were collected and analyzed for total radioactivity by LSC to determine column recoveries.

For identification of parent diflufenzopyr and transformation products from [phenyl-U-^{14}C]diflufenzopyr-treated soil, high dose soil samples were extracted by shaking three times each with ethyl acetate:THF:water (1:1:0.1, v:v) and acetonitrile:ammonium carbonate (1:1, v:v; p. 18). The soil and extract were separated by centrifugation. The ethyl aceate:THF:water exracts (organic extracts) were combined, reduced to dryness by both rotary evaporation and under a

nitrogen stream. The resulting residue was redissolved in acetone:DMSO (9:1, v:v) followed by centrifugation. Multiple aliquots (35 µL each) were injected and fractionated using the reverse-phase HPLC conditions as described for the test samplesabove with fraction collection occurring in 15-second intervals (pp. 18, 43). Fractions containing isolated [^{14}C]compounds were collected and corresponding fractions were combined and partitioned with methylene chloride. The methylene chloride phases were concentrated to dryness under nitrogen and redissolved in acetone:DMSO. Isolated [^{14}C]compounds were co-chromatographed with corresponding nonradiolabeled reference standards of parent diflufenzopyr (p. 46), M2 (p. 47) and M5 (p. 44). The identity of M5 and diflufenzopyr was confirmed by LC/MS analysis (pp. 124-125 and 129-130, respectively), and M2 by GC/MS (pp. 131-132). A proposed structure for M23 was based on MS analyses (pp. 26, 126-128). The acetonitrile:ammonium carbonate extracts (aqueous extracts) were combined, adjusted to pH 9, and partitioned with ethyl acetate (p. 19). The aqueous phase was acidified (pH 2), partitioned twice with ethyl acetate, concentrated, redissolved in Trizma:DMSO (9:1, v:v), and analyzed by HPLC as previously described. The identity of the parent was confirmed using HPLC (p. 48) and LC/MS (p. 133). The ethyl acetate (organic) phase was reduced to dryness, redissolved in Trizma buffer:DMSO, and fractionated by HPLC as previously described. Eluent fractions were collected and the degradate M4 was identified using HPLC (p. 50) and LC/MS (p. 134).

For identification of parent diflufenzopyr and transformation products from [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil, high dose soil samples were extracted with ethyl acetate:THF:water and acetonitrile:ammonium carbonate as previously described for the phanyl label (p. 19). Similarly, ethyl aceate:THF:water exracts (organic extracts) were combined, concentrated and fractionated by HPLC (p. 51). Combined fractions were partitioned with either methylene chloride, ethyl acetate or methylene chloride:ethyl acetate. The organic phases were concentrated and analyzed by HPLC. From the organic extract, M5, diflufenzopyr, M1 and M9 were identified using HPLC (pp. 52, 54, 55 and 56, respectively) and LC/MS (135, 137, 138 and 139, respectively). The degradate M23 was tentatively identified based on MS analyses (p. 26, 136). Acetonitrile:ammonium carbonate (aqueous) extracts were combined, concentrated, redissolved in Trizma:DMSO, and analyzed by HPLC as previously described. The parent, M1 and M9 were identified by HPLC co-chromatograpy (p. 57).

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: Reportedly aerobicity, moisture, temperature and other environmental conditions were maintained throughout the study; however, no supporting records were provided.

B. MATERIAL BALANCE: Overall recoveries of radiolabeled material averaged 96.19% (range 87.70-101.07%) of the applied during the 316-day incubation of [phenyl-U-^{14}C]diflufenzopyr-treated soil and 100.7% (96.70-102.28%) during the 327-day incubation of [pyridinyl-4,6-^{16}C]diflufenzopyr-treated soil (p. 37).

Table 6: Biotransformation of **[phenyl-U-^{14}C]diflufenzopyr**, expressed as percentage of applied radioactivity, in sandy loam under aerobic conditions[1]

Compound (code)	Sampling intervals (days posttreatment)[2]								
	0	7	14	30	64	101	126	218	316
Diflufenzopyr (BAS 654 H)	88.49	71.66	60.1	40.71	18.72	10.57	8.68	4.22	2.51
5-Difluorophenyl urea (M4)	2.93	4.07	4.13	4.15	5.82	3.34	3.03	2.56	1.85
Carbamoyl phthalazinone (M5)	0.26	0.25	2.29	3.89	0.8	1.45	1	0.58	0.31
3,5-Difluoroaniline (M2)	ND[3]	ND	0.15	0.43	0.12	0.12	0.24	0.09	0.07
M23	0.55	1.47	0.44	0.71	0.89	0.5	0.42	0.39	0.24
Unidentified PH1	ND	ND	0.23	0.82	1	1.16	1.41	1.34	1.54
Unidentified PH2	2.4	4.02	3.19	1.48	0.25	0.27	0.28	ND	ND
Unidentified PH3	0.91	ND	0.29	0.35	0.73	0.72	0.84	0.66	0.85
Unidentified PH4	1.12	ND	ND	ND	0.23	0.37	0.11	ND	ND
Unidentified PH5	0.25	0.16	0.76	0.92	0.49	0.28	0.28	0.09	0.06
Organic extractable residues	62.79	39.86	27.4	19.52	8.12	4.4	2.36	1.5	1.07
Aqueous extractable residues	32.63	41.78	44.4	34.11	20.87	14.39	14.18	8.45	6.38
Total extractable residues	95.42	81.64	71.8	53.63	28.98	18.79	16.53	9.95	7.45
CO_2	NA[4]	1.07	2.33	5.15	7.46	10.35	11.13	22.51	30.13
Non-extractable residues	5.04	17.07	27	41.34	54.74	59.45	60.04	65.66	61.12
Total % recovery	100.5	99.78	101	100.1	91.18	88.59	87.7	98.12	98.7

1 for parent diflufenzopyr and transformation products: n = 2 at 0- and 64-days, n = 1 at all other days; for extractable/nonextractable resides: n = 2 at 0 and 64-312 days and n = 1 at 7-30 days

2 Sampling intervals reported in study were not the actual days. Based on sampling dates reported in the raw data of the study (Appendix 2. P. 70), the reviewer calculated the days posttreatment which are reported here.

3 ND = Not detected; detection limit not reported

4 NA = Not analyzed

Data obtained from pp. 29, 31, 32, 37, 70-85, 90 of the study report.

Table 7: Biotransformation of **[pyridinyl-4,6-^{14}C]diflufenzopyr**, expressed as percentage of applied radioactivity, in sandy loam under aerobic conditions[1]

Compound (code)	Sampling times (days)[2]									
	0	6	14	34	69	90	132	196	272	327
Diflufenzopyr (BAS 654 H)	90.9	70.7	52.1	27	11.4	7.19	3.59	2.13	1.37	1.23
8-Methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione (M9)	ND[3]	0.85	2.01	5.89	13	18	21.5	25.1	27.3	26.1
8-Methyl-5-hydroxy-pyrido-(2,3-d)-pyridazinone (M1)	2.2	5.25	11.6	13.2	12.1	10.7	6.04	3.72	2.25	1.9
Carbamoyl phthalazinone (M5)	1.59	4.01	3.81	4.25	2.03	1.27	0.69	0.16	0.24	0.16
2-Acetyl nicotinic acid (M6)	0.64	3.22	1.62	ND	0.44	ND	ND	ND	ND	0
M23	0.96	0.46	0.82	1.05	0.77	0.63	0.49	0.38	0.25	0.22
Unidentified PY1	ND	ND	ND	1.46	1.45	1.01	1.09	0.78	0.77	1.11
Unidentified PY2	ND	ND	ND	ND	0.94	0.59	1.4	1.5	0.76	ND
Unidentified PY3	ND	ND	ND	0.24	0.4	0.29	0.34	0.33	0.11	0.38
Unidentified PY4	ND	ND	0.3	0.19	0.94	0.75	0.61	0.49	0.33	0.54
Unidentified PY5	0.9	1.06	1.06	0.63	0.28	0.19	0.1	0	ND	ND
Organic extractable residues	63.3	36	30.9	21.5	12.2	9.07	6.42	5.24	4.17	3.79
Aqueous extractable residues	31.8	49.6	42.4	32.3	31	31.6	29.1	29.4	29.2	28
Total extractable residues	95.1	85.6	73.3	53.8	43.2	40.6	35.5	34.7	33.4	31.8
CO_2	NA[4]	2.37	8.84	21.8	31.6	35.6	39	41.9	43.9	45.1
Non-extractable residues	1.56	12	20	26.7	26.7	25	25.1	24.8	24.4	24.4
Total % recovery	96.7	99.9	102	102	101	101	99.6	101	102	101

1 for parent diflufenzopyr and transformation products: n = 2 at 0-, 64- and 132-days; n = 1 at all other days; for extractable/nonextractable resides: n = 2 at 0, 69 and 132-328 days and n = 1 at 6-34 and 90 days

2 Sampling intervals reported in study were not the actual days. Based on sampling dates reported in the raw data of the study (Appendix 3, p. 92), the reviewer calculated the days posttreatment which are reported here.

3 ND = Not detected; detection limit not reported

4 NA = Not analyzed

Data obtained from pp. 29, 31, 33, 37, 93-109, 119 of the study report.

C. TRANSFORMATION OF PARENT COMPOUND: [Phenyl-U-^{14}C]diflufenzopyr decreased from 88.49% of the applied at time 0 to 40.71% at 30 days, 18.72% at 64 days, 10.57% at 101 days, and was 2.51% at 316 days (final sampling interval, p. 32). [Pyridinyl-4,6-^{14}C]diflufenzopyr decreased from 90.85% at day 0 to 52.08% at 14 days, 26.96% at 34 days, 11.39% at 69 days, 3.59% at 132 days and was 1.23% at 327 days (p. 33).

REGISTRANT-CALCULATED HALF-LIFE: The registrant-calculated half-lives for [phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-labeled diflufenzopyr in aerobic sandy loam soil were 20.3 days (r^2 = 1.0) and 15.9 days (r^2 = 1.0), respectively, using the Gustafson model for nonlinear regression; DT_{75} (75% decline time) values were also determined (pp. 23, 36, 144-145).

REVIEWER-CALCULATED HALF-LIFE: Half-lives determined by the reviewer using least-squares linear regression analysis assuming first-order linear degradation were 59.2 days (r^2 = 0.90) and 49.9 days (r^2 = 0.86) for [phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-labeled diflufenzopyr, respectively.

Table 8: Half-life values of diflufenzopyr in aerobic sandy loam soil.

^{14}C-Label	First order nonlinear[1]			DT_{75} (days)	First order linear		
	half-life (days)	Regression equation	r^2		reviewer-calc		Regression equation
					half-life (days)	r^2	
Phenyl	20.3	$\ln C = \ln C_0 - \alpha \ln(1 + \beta t)$, where C_0, α and β are determined in sequence as unknowns. With α and β determined, half-lives were calculated by half-life = $[0.5^{-(1/\alpha)} - 1]/\beta$	0.998	51	59.2	0.9	Linear form y = mx + b as $\ln C = -kt + \ln C_0$; $\ln C_0$ is initial concentration (b = y intercept), lnC 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).
Pyridinyl	15.9		0.998	37.2	49.9	0.86	

1 Registrant-calculated half-lives. Equation obtained from MRID 45444001, p. 22. Data obtained from pp. 36, 144, 145 of the study report. The registrant did not use the actual sampling intervals to calculate half-lives.

TRANSFORMATION PRODUCTS:

Table 9: Chemical names for identified transformation products of diflufenzopyr in aerobic sandy loam soil.

BASF Code	Chemical Name(s)	HPLC retention time (minutes)	Molecular weight (g/mol)
M9	8-Methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione	10:10-11:20	177

Table 9: Chemical names for identified transformation products of diflufenzopyr in aerobic sandy loam soil.

BASF Code	Chemical Name(s)	HPLC retention time (minutes)	Molecular weight (g/mol)
M6	2-Acetyl nicotinic acid	12:0-13:20	165
M1	8-Methyl-5-hydroxy-pyrido-(2,3-d)-pyridazinone	13:30-14:30	161
M4	5-Difluorophenyl urea	19:00-20:00	172
M2	3,5-Difluoroaniline	22:00-22:30	129
M5	Carbamoyl phthalazinone	26.30-27:25	316

Data obtained from pp. 29, 32-33 of the study report.

For both [phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-labeled diflufenzopyr the major transformation product was volatilized $^{14}CO_2$ accounting for 30.13% and 45.07% of the applied radioactivity, respectively, at the final sampling interval 316-327 days posttreatment (p. 37). All other transformation products detected in extracts from [phenyl-U-^{14}C]diflufenzopyr-treated soil were minor with each comprising ≤6% of the applied radioactivity at any sampling interval (p. 32). Minor transformation products of [phenyl-U-^{14}C]diflufenzopyr included M4 detected at a maximum 5.82% of the applied at 64 days posttreatment, M5 a maximum 3.89% at 30 days, M2 and M23 (proposed structure p. 26) each ≤1.47%, PH3 (proposed structure p. 25) ≤0.91%, and four unidentified [^{14}C]compounds (PH1, PH2, PH4 and PH5) each ≤4.02%. In extracts from [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil, two major transformation products were M9 increasing to a maximum 27.26% at 272 days (26.10% at 327 days) and M1 increasing to 13.15% at 34 days (1.90% at 327 days; p. 33). Minor transformation products of [pyridinyl-4,6-^{14}C]diflufenzopyr included M5 detected at a maximum 4.25% of the applied at 34 days, M6 a maximum 3.22% at 6 days, M23 at ≤1.05%, and five unidentified [^{14}C]compounds (PY1, PY2, PY3, PY4 and PY5) each ≤1.50%.

NON-EXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues decreased from 95.42% of the applied at day 0 to 7.45% at 316 days in [phenyl-U-^{14}C]diflufenzopyr-treated soil and from 95.14% at day 0 to 31.75% at 327 days in [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil (p. 37). Nonextractable [^{14}C]residues in [phenyl-U-^{14}C]diflufenzopyr-treated soil increased from 5.04% of the applied at day 0 to 65.66% at 218 days and were 61.12% at 316 days; 7.69-12.63% of the applied was associated with the fulvic acid (in 218- and 316-day samples), 0% with humic acid and 37.55-44.23% with humins (p. 34). Nonextractable [^{14}C]residues in [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil increased from 1.56% at day 0 to 26.66% at 69 days, and was 24.36-24.40% at 272-327 days; 3.67-6.64% of the applied was associated with the fulvic acid (132-, 196- and 327-day samples), 0% with humic acid and 13.92-19.17% with humins. In extracts from fulvic acid, parent compound, M2, M4, M5, PH1-3 were each detected at ≤2.59% of the applied in [phenyl-U-^{14}C]diflufenzopyr-treated soil,

and parent compound, M1, M5, M6, M9, PY1, PY2 and PY4 were each detected at ≤1.12% from [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil (p. 35).

VOLATILIZATION: Volatilization of $^{14}CO_2$ was significant for both labels increasing to 30.13% and 45.07% of applied for [phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-diflufenzopyr treated soils, respectively, by the final sampling interval, 316-327 days posttreatment (p. 37).

TRANSFORMATION PATHWAY: A biotransformation pathway (p. 61) for the degradation of diflufenzopyr in aerobic soil was proposed by the registrant. Diflufenzopyr can initially degrade to several transformation products including 3,5-difluoroaniline (M2), 5-difluorophenyl urea (M4), carbamoyl phthalazinone (M5), 2-acetyl nicotinic acid (M6), and/or M23 (proposed structure p. 26). Those transformation products can further degrade to 8-methyl-5-hydroxy-pyrido-(2,3-d)-pyridazinone (M1), 3,5-difluoroaniline (M2), 2-acetyl nicotinic acid (M6) and/or 8-methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione (M9) with eventual production of CO_2 and soil bound residues.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: In an experiment to determine volatility of transformation product 3,5-difluoroaniline (M2), 42% of the applied radioactivity was lost when an ethylacetate:tetrahydrofuran solution containing [^{14}C]M2 was evaporated to dryness (p. 27).

III. STUDY DEFICIENCIES: No deficiencies were noted. This study can be used to fulfill Subdivision N Guideline §162-1 data requirements.

IV. REVIEWER'S COMMENTS:

1. The transformation product 3,5-difluoroaniline (M2) detected in [phenyl-U-^{14}C]diflufenzopyr-treated soil was found to volatilize (42% of applied radioactivity volatilized) when the solvent containing M2 was evaporated to dryness (p.27). The reported methods used in this study involved a step where soil extracts were evaporated to dryness prior to quantitation of diflufenzopyr and its transformation products (p. 17). The study author concluded that M2 would not have exceeded 5% of the applied radioactivity at any given sampling interval based on an overall average material balance of 96% of the applied (p. 27). However, individual material balances from [phenyl-U-^{14}C]diflufenzopyr-treated soil were 99.39-101.51% of the applied from 0 to 30 days posttreatment, decreased to 87.54-87.86% at 126 days, then increased to 97.98-98.26% at 218 days and was 96.86-100.57% at 316 days (pp. 70-85). It's conceivable that M2 was being formed and lost from soil extracts during the evaporation step; however, the maximum amount M2 could have accounted for during the 316-day study would have been ca. 12% of the applied radioactivity.

. The study author referenced a soil residue method for M2 that does not involve evaporation prior to quantitation (reference No. 2 below), but it does not appear that the method was used during this study because a description of the methods was not provided.

2. Compound PH3 (proposed structure p. 25) was reportedly an artifact resulting from the reaction of diflufenzopyr with a component of the pH 7 Trizma buffer used to prepare the dosing solutions (pp. 14-15). The artifact compound was also detected in an aqueous photolysis study (MRID 45444004 of this submission; compound designated P9 in that study) comprising maximums of 11.0-13.36% of the applied radioactivity at pH 7 and 30.08-33.87% at pH 9 in irradiated and dark control [phenyl-U-^{14}C]diflufenzopyr solutions, but was <1.5% in all other test solutions. It was reported in this study that the structure presented for PH3/P9 (p. 25) had been confirmed by MS in the aqueous photolysis study; however, while a structure for PH3/P9 was proposed based on MS analyses (p. 28, MRID 45444004), the isolated [^{14}C]compound was not compared to a reference standard for confirmation of identification. In this study, PH3/P9 was detected at <1% of the applied in extracts from [phenyl-U-^{14}C]diflufenzopyr-treated soil (p. 32). PH3/P9 was also detected in a soil photolysis study (MRID 45310901, previously submitted), and in that study PH3 + M4 (could not be separated chromatographically) were detected at ≤6.5% of the applied radioactivity in extracts from [phenyl-U-^{14}C]diflufenzopyr-treated irradiated and dark control soil. M Singh authored all three studies.

3. Sampling intervals for [phenyl-U-^{14}C]diflufenzopyr-treated soil were reported as 0, 7, 14, 30, 64, 97, 122, 215 and 312 days posttreatment; however, actual sampling intervals were 0, 7, 14, 30, 64, 101, 102 (diflufenzopyr and transformation products quantitation results from this sample were not reported), 126, 218 and 316 days (pp. 70-85, 121. For [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil the 132-, 273- and 328-day sampling intervals actually occurred at 132, 272 and 327 days, respectively (pp. 93-109, 122).

4. Except for samples collected the day of treatment (day 0), it was not reported and could not be determined how many treated samples were collected at each sampling interval. It was reported that twenty-two soil samples were each separately treated with [phenyl-U-^{14}C]-labeled or [pyridinyl-4,6-^{14}C]-labeled diflufenzopyr, and two treated soil samples of each label were taken as day 0 samples (p. 15). The remaining twenty treated soil samples of each label were placed in the glass columns and incubated. Of the twenty [phenyl-U-^{14}C]diflufenzopyr-treated soil samples that were incubated, thirteen soil samples were extracted and analyzed (not including day 0 samples); single soil samples collected at 7, 14, 30, 101 and 102 days (diflufenzopyr and transformation products quantitation results from 102-day sample were not reported), duplicate samples collected at 64, 126 and 218 days, and three samples collected at 316 days were extracted and analyzed (p. 70-85). Of the twenty [pyridinyl-4,6-^{14}C]diflufenzopyr-treated soil samples that were incubated, fifteen soil samples were extracted and analyzed (not including day 0 samples); single soil samples collected at 6, 14, 34 and 90 days, duplicate samples collected at 69, 132, 196 and 272 days, and three samples collected at

327 days were extracted and analyzed (p. 93-109). It is unclear why the remaining samples were either not analyzed and/or results from analyses of those samples were not reported.

5. Diflufenzopyr transformation products M5, M2, M4, M1 and M9 were identified in soil extracts using reverse-phase HPLC and co-chromatography with nonradiolabeled reference standards (pp. 44/52, 47, 50, 55/57, 56/57, respectively) with identifications confirmed by LC/MS (pp. 124-125/135, 131-132, 134, 138 and 139, respectively). The study author reported that M23 was positively identified (p. 11); however, supporting results were not provided. A proposed structure for M23 was based on MS analyses (pp. 126-128, 136), but the isolated [^{14}C]compound was not compared to a reference standard; however, M23 comprised ≤1.47% of the applied radioactivity in extracts from [phenyl-U-^{14}C]- and [pyridinyl-4,6-^{14}C]-diflufenzopyr-treated soil.

6. The initial soil CEC value of 381.7 meq/100 g was unusually high which typically indicates a saline soil. The saturated salinity of the test soil was measured at 5.63 mmhos/cm (p. 66) and is considered a moderate level of salinity. Reanalysis of the CEC value using the NaAcetate method determined a soil CEC value of 7.1 meq/100g.

7. Column eluates were reportedly collected for each HPLC run and analyzed for total radioactivity by LSC to determine column recoveries; however, HPLC recoveries were not provided for review.
8. 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). The target application rate for this study was selected based on the field application rate which would correspond to ca. 0.21-0.27 ppm assuming a soil layer of 5 cm.

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

1. Guirguis, A. and C. Yu. 1989. Determination of the water solubility for SAN-835 H. Sandoz Agro Inc. Project No. 414055-4. BASF Reg. Doc. No. 1989/5244.

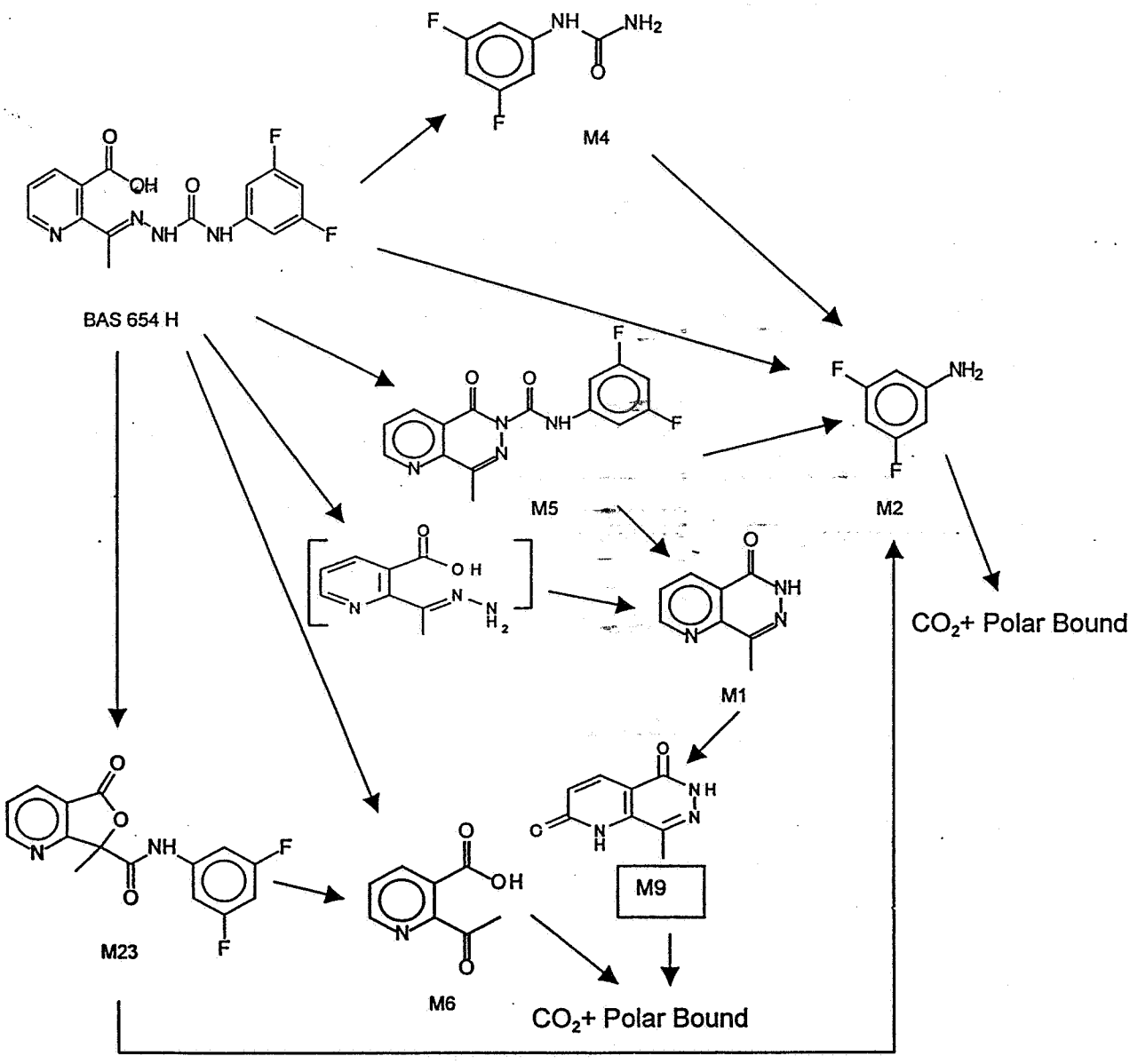
2. Panek, M. 2001. Validation of BASF method number D0005, analytical method for the LC/MS/MS determination of BAS 654 H and metabolites M1, M5, M6 and M9 and GC/MS determination of M2 in soil (method includes analysis for dicamba and dicamba metabolite DCSA). Study No. 61200. BAS Reg. Doc. No. 2001/5001491.

3. Singh, M. 2001. Photolysis of ^{14}C-BAS 654 H in aqueous media. Study No. 61360. BAS Reg. Doc. No. 2001/5000872.

4. Singh, M. 2000. Photolysis of ^{14}C-BAS 654 H on soil. Study No. 61359. BAS Reg. Doc. No. 2000/5267.

5. Tong, R. 1996. Aerobic soil metabolism of SAN-836 H. Sandoz Agro Inc. Project No. 414215. Report No. 5. BASF Reg. Doc. No. 1996/5380.

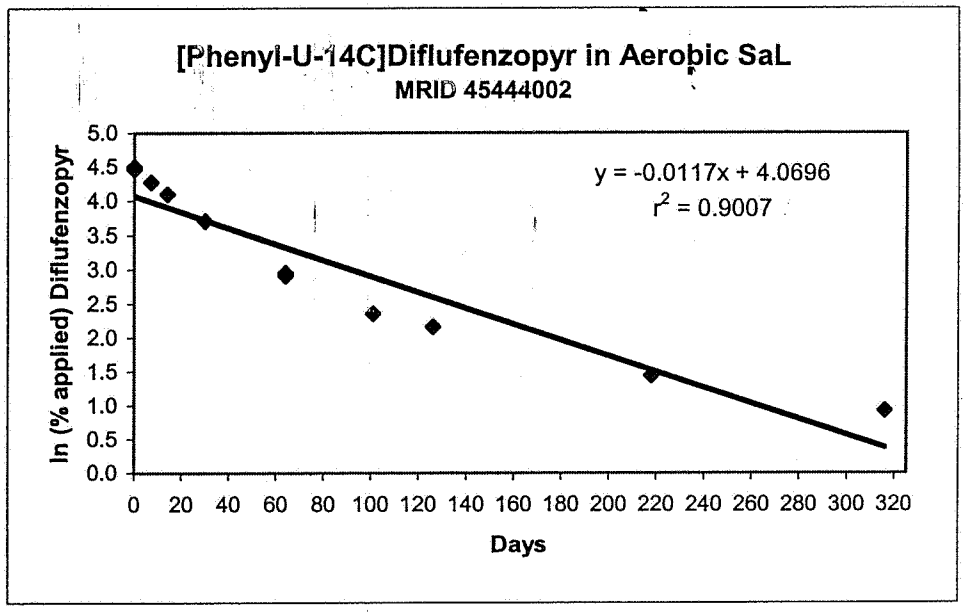
Figure 24. Aerobic Degradation Pathway.



Aerobic Metabolism of Diflufenzopyr in Sandy Loam Soil
MRID 45444002

[Phenyl-U-14C]label
Half-life Determination

Day	Diflufenzopyr %AR	Ln(%AR)
0	90.31	4.503248
0	86.66	4.461992
7	71.66	4.271933
14	60.11	4.096176
30	40.71	3.706474
64	19.1	2.949688
64	18.32	2.907993
101	10.57	2.35802
126	8.68	2.161022
218	4.22	1.439835
316	2.51	0.920283



Regression Output:

Constant	4.07
Std Err of Y Est	0.414
R Squared	0.901
No. of Observations	11
Degrees of Freedom	9
X Coefficient(s)	-0.011691
Std Err of Coef.	0.001294

half-life 59.27869 days

half-life = 59.24 days

20

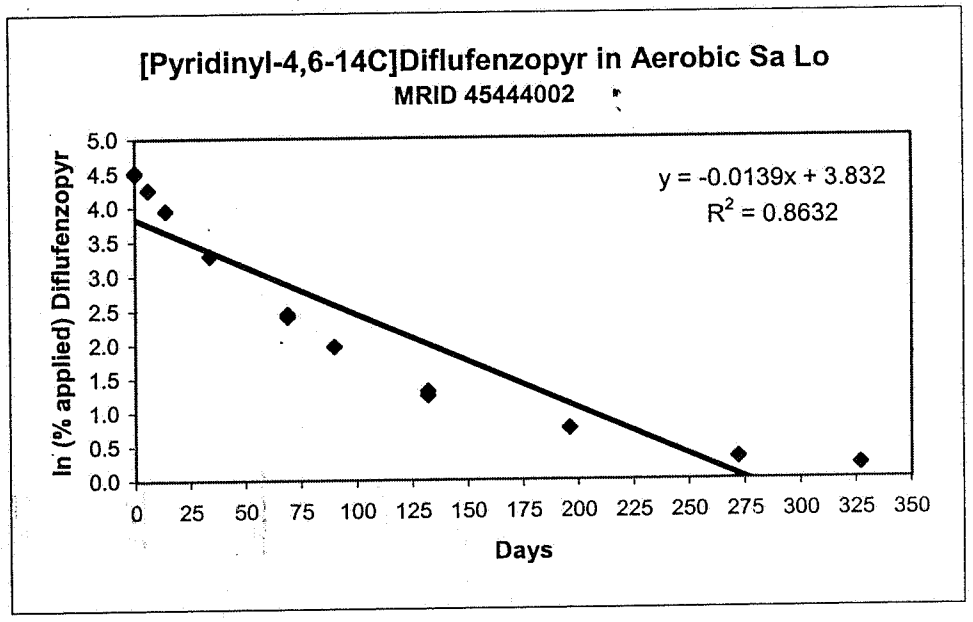
[Pyridinyl-4,6-14C]label
Half-life Determination

Day	Diflufenzopyr %AR	Ln(%AR)
0	89.91	4.498809
0	91.78	4.519394
6	70.72	4.258728
14	52.08	3.952781
34	26.96	3.294354
69	11.57	2.448416
69	11.2	2.415914
90	7.2	1.972691
132	3.7	1.308333
132	3.47	1.244155
196	2.13	0.756122
272	1.37	0.314811
327	1.23	0.207014

Regression Output:

Constant	3.832
Std Err of Y Est	0.612
R Squared	0.863
No. of Observations	13
Degrees of Freedom	11
X Coefficient(s)	-0.013889
Std Err of Coef.	0.001667

half-life 49.89676 days



half-life = 49.87 days

Data Evaluation Report on the aerobic biotransformation of M9 a.k.a. 2-keto-M1 (metabolite of diflufenzopyr) in soil

PMRA Submission Number {.....} EPA MRID Number 45444003

Data Requirement: PMRA DATA CODE:
EPA DP Barcode: D276313
OECD Data Point:
EPA Guideline: 162-1

Test material:
Common name: M9
Chemical name
IUPAC: Not provided.
CAS name: Not applicable.
CAS No: Not applicable.
Synonyms: 8-Methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione
2-Keto-M1
SMILES string:

Primary Reviewer: Lynne Binari
Dynamac Corporation
Signature: Lynne Binari
Date: 8/17/01

QC Reviewer: Kathleen Ferguson
Dynamac Corporation
Signature: Kathleen Ferguson
Date: 9/18/01

Secondary Reviewer: ~~Sid Abel~~ Michele Mahoney
EPA
Signature: Michele K. Mahoney
Date: 12/3/01

Company Code: [for PMRA]
Active Code: [for PMRA]
Use Site Category: [for PMRA]
EPA PC Code: 005108

CITATION: Singh, M. 2001. Aerobic soil metabolism of ^{14}C-M9 (metabolite of BAS 654 H). Unpublished study performed and sponsored by BASF Corporation, Research Triangle Park, NC. BASF Protocol No. 64648 and Registration Document No. 2001/5001533. Study initiated December 18, 2000 and completed June 27, 2001 (p. 9).

Data Evaluation Report on the aerobic biotransformation of M9 a.k.a. 2-keto-M1 (metabolite of diflufenzopyr) in soil

PMRA Submission Number {.....} EPA MRID Number 45444003

EXECUTIVE SUMMARY:

The biotransformation of [pyridine ring keto 2-^{14}C]-labeled 8-methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione (M9, degradate of diflufenzopyr) was studied in sandy loam soil (pH 7.5, organic matter 1.9%) from Fredolin Schwade tract, Iowa, incubated in darkness for 159 days under aerobic conditions at 27 ± 1°C and a soil moisture content of 75% of 1/3 bar. [^{14}C]M9 was applied at the rate of 0.17 mg a.i./kg soil (equivalent to 0.19 kg a.i./ha). This experiment was conducted in compliance with US EPA GLP (1989) Standards. The test system consisted of petri dishes containing treated soil maintained in a flow-through apparatus with traps for the collection of CO_2 and volatile organics. Samples were analyzed after 0, 34, 96 and 159 days of incubation. Soil samples were sequentially extracted with acetone:0.05 M ammonium carbonate (1:1, v:v), then [^{14}C]M9 residues were analyzed by reverse-phase HPLC.

The material balance was 96.9-99.1% of the applied radioactivity. [^{14}C]M9 decreased from 97.0% of the applied at time 0 to 70.0% at 159 days. Two unidentified [^{14}C]compounds (Unk-1, Unk-2) were each detected at ≤2.7% of the applied. Extractable [^{14}C]residues decreased from 97.0% of the applied at time 0 to 70.8% at 159 days. Nonextractable [^{14}C]residues increased from 1.1% of the applied at time 0 to 23.5% at 159 days; 14.1% of the applied was associated with the fulvic acid, 3.3% with humic acid, and 7.3% with the humin (96-day sample). At study termination, evolved $^{14}CO_2$ comprised 3.9% of the applied radioactivity.

The degradate of diflufenzopyr, M9, degraded slowly in aerobic sandy loam soil that was incubated for 159 days ($t_{1/2}$ ≥ 1 year).

Results Synopsis:

Soil type:	Sandy loam.
Half-life value:	1 year (r^2 = 0.85), estimated beyond the scope of the observed data
Major transformation products:	None
Minor transformation products:	CO_2

Study Acceptability: This study is acceptable and fulfills the guideline requirement for the biotransformation of M9, degradate of diflufenzopyr, in sandy loam soil under aerobic conditions.

Data Evaluation Report on the aerobic biotransformation of M9 a.k.a. 2-keto-M1 (metabolite of diflufenzopyr) in soil

PMRA Submission Number {.....} EPA MRID Number 45444003

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with USEPA Subdivision N Guideline §162-1. There were no study deviations that affected the validity of the results.

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: [pyridine ring keto 2-^{14}C]-labeled 8-methylpyrido-(2,3-d)-pyridazine-2,5-(1H,6H)-dione.

Chemical Structure: See Attachment

Description: Technical. Off-white solid (p. 10).

Purity:

[^{14}C]M9: Radiochemical purity: >98% (pp. 10, 25). Inventory/Lot No. 128/960304. Specific activity: 7 x 10^5 dpm/µg (55.6 mCi/mmol, 11.67 Mbq/mg).

Unlabeled M9: Purity: 99.3% (p. 11). Lot No. 01748-022. Reg. No. 395207 (p. 12).

Storage conditions of test chemicals: In darkness at a low (unspecified) temperature (p. 10).

Physico-chemical properties: Not provided

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2. Soil Characteristics:

Table 1: Description of soil collection and storage.	
Description	**Details**
Geographic location:	Fredolin Schwade tract, Iowa.
Collection date:	November 13, 2000. Received at BASF 11/16/00
Storage conditions:	Under refrigeration at 7°C until use.
Soil preparation:	2 mm sieved.

Data obtained from p. 11 of the study report.

Table 2: Properties of the soil.	
Property	**Details**
Texture:	Sandy loam.
sand (%):	63
silt (%):	28
clay (%):	9
pH (in saturated paste):	7.5
Organic matter (%):	1.9
CEC (meq/100 g):	10.8
Moisture at 1/3 Bar (%):	15
Bulk density - disturbed (g/cm^3):	Not provided.
Soil Taxonomic classification:	Not provided.
Soil Mapping Unit (for EPA):	Not provided.

Data obtained from pp. 20, 33 of the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None.

2. Experimental conditions:

Table 3: Experimental design.

Parameter		Details
Duration of the test:		159 days.
Soil condition (air dried/fresh):		Not specified.
Soil (g/replicate):		35 g (wet/dry wt. not specified)
Application rates:		0.17 ppm (0.17 mg a.i./kg; 0.19 kg a.i./ha)
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 samples at time 0. One to two samples at each sampling interval after day 0; it was not specified how many samples were collected at each interval.
Test apparatus (Type/material/volume):		Soil samples (ca. 35 g) were weighed into twenty petri dishes. Following treatment, two samples were taken for day 0 analysis, then remaining eighteen samples were placed in two closed glass columns and maintained in an incubator. Each column was equipped with inlet/outlet ports for volatiles collection.
Details of traps for CO_2 and organic volatiles, if any:		Humidified, CO_2 air was drawn continuously (flow rate not specified) through each glass column then sequentially through 1 N sodium hydroxide (two traps) and 0.1 N sulfuric acid (one trap).
If no traps were used, is the system closed/open?		Volatiles traps were used.
Co-solvent.	Identity:	Acetone:dimethyl sulfoxide (9:1, v:v) concentrated to an unspecified volume (most of acetone removed), then diluted with pH 7 Tris buffer.
	Final concentration:	Could not be determined.
Test material application.	Volume of test solution used/treatment:	0.2 mL of 30.6 µg a.i./mL test solution.
	Application method (eg: applied on surface, homogeneous mixing etc.):	Applied drop-wise to soil surface.

Table 3: Experimental design.		
Parameter		**Details**
	Is the co-solvent evaporated?	No.
Microbial biomass/Microbial population of test soil (determined prior to treatment). Total:		43.0 µg/g dry wt. soil.
	Actinomycetes:	1.89 x 10^5 CFU /g dry wt. soil
	Fungi:	1.34 x 10^4 CFU/g dry wt. soil
	Bacteria	8.59 x 10^5 CFU/g dry wt. soil
Any indication of the test material adsorbing to the walls of the test apparatus?		Not determined.
Experimental conditions.	Temperature (°C):	27 ± ~~1~~°C.
	Moisture content: Moisture maintenance method:	75% of 1/3 ~~bar~~. Treated soil samples were weighed every 2 weeks following treatment and at each sampling interval, and the weights were compared to sample weights recorded at the onset of the experiment; water was added as needed.
	Continuous darkness (Yes/No):	Yes.
Other details, if any:		None.

Data obtained from pp. 12, 13, 26, 34, 35 of the study report.

3. Aerobic conditions: Humidified, CO_2-free air was drawn continuously through the glass columns containing the treated soil samples; no determinations were made, such as redox potentials, to verify that aerobic conditions were maintained.

4. Supplementary experiments: To identify degradates, additional experiments were conducted at an exaggerated rate (p. 13). Soil samples (ca. 35 g) were treated with [^{14}C]M9 (0.4 mL of 30.6 µg a.i./mL test solution) plus nonradiolabeled M9 (0.3 mL of 266.7 µg a.i./mL solution) at 2.62 µg/g (pp. 12, 13). The treated soil samples (three) were placed in glass columns and maintained in darkness at 27 ± 1°C in an incubator. The samples were kept aerated and soil moisture was maintained at 75% of 1/3 bar as described for the test samples.

5. Sampling:

Table 4: Sampling details.

Parameters	Details
Sampling intervals:	0, 34, 96 and 159 days posttreatment
Sampling method for soil samples:	One to two samples were collected at each interval. Sand (ca. 10 g) was added to the soil sample and thoroughly mixed with a spatula. The soil/sand sample was then transferred to a stainless steel container for extraction.
Method of collection of CO_2 and volatile organic compounds:	Trapping solutions were collected and replaced at each sampling interval and at intervals of moisture adjustment.
Sampling intervals/times for: sterility check, if sterile controls are used: Moisture content: Redox potential/other:	 Sterile controls were not used. Every two weeks after treatment and at each sampling interval. Not determined.
Sample storage before analysis:	Soil samples were extracted the day of collection.
Other observations, if any:	None.

Data obtained from pp. 13, 14, 39-44 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Each soil sample (pre-mixed with 10 g sand) was extracted (Automated Solvent Extractor at 500 psi) three times with acetone:0.05 M ammonium carbonate (1:1, v:v; pp. 14, 28). The extracts were combined, reduced to dryness under nitrogen, and the remaining residue was redissolved in Tris buffer:dimethyl sulfoxide (9:1, v:v).

Non-extractable residue determination: Post-extracted soil samples were air dried and analyzed by LSC following combustion. To characterize non-extractable [^{14}C]residues in the soil, selected post-extracted soil samples (day 96) were further extracted three times by shaking with 0.1 N sodium hydroxide (pp. 15, 29). The extracts were centrifuged, and the supernatants were combined and analyzed for total radioactivity by LSC. The combined extract was acidified (HCl, pH 1) followed by centrifugation to precipitate humic acids. The humic acid precipitate was redissolved in 0.5 N sodium hydroxide and analyzed by LSC. The acidified extract (fulvic acids) was partitioned with ethyl acetate and the resulting organic and aqueous phases were analyzed by LSC. The organic phase was concentrated by rotary evaporation, reduced to dryness under nitrogen, redissolved in Tris:DMSO, and analyzed by HPLC as described for test extracts. [^{14}C]Residues remaining in the post-extracted soil (humins) were quantified by LSC following combustion.

Total ^{14}C measurement: Triplicate aliquots (0.25-1.0 mL) of the acetone:ammonium carbonate extracts were analyzed for total radioactivity by LSC (p. 14). Extracted soil samples were air-dried, homogenized using a mortar-pestle, then aliquots were analyzed for total radioactivity by LSC following combustion.

Aliquots (volume not specified) of each trapping solution were analyzed for total radioactivity by LSC (p. 13). To quantify $^{14}CO_2$, an aliquot (ca. 1.0 mL) of the sodium hydroxide trapping solution was reacted with 5 N sulphuric acid (ca. 5 mL), then released $^{14}CO_2$ was trapped in Harvey cocktail solution contained in a vigreux column and aliquots were analyzed by LSC; the remaining neutralized $NaOH:H_2SO_4$ solution was also analyzed by LSC (pp. 14, 27).

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

Identification and quantification of parent compound and transformation products: Aliquots of each extract were analyzed by reverse-phase HPLC using the following conditions: YMC ODS AQ column (4.6 x 250 mm, 5 µm particle size), mobile phase gradient of (A) 0.05% aqueous trifluoroacetic acid to (B) acetonitrile (A:B, 98:2, 80:20, 0:100, v:v), injection volume 0.45 mL, flow rate 1 mL/minute, UV (254 nm) and radioactivity detection (pp. 14). Column eluates were collected and analyzed for total radioactivity by LSC to determine column recoveries. Samples were co-chromatographed with a non-radiolabeled reference standard of M9 (pp. 18, 31). Identification was confirmed by LC/MS (negative ion mode, p. 54) and LC/MS with multiple reaction monitoring (additional MS parameters were 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: Reportedly aerobicity, moisture, temperature and other environmental conditions were maintained throughout the study; however, no supporting records were provided.

B. MATERIAL BALANCE: Recoveries of radiolabeled material were 96.9-99.1% of the applied during the 159-day incubation (p. 21).

Table 5: Biotransformation of [pyridine ring keto 2-^{14}C]M9, expressed as percentage of applied radioactivity, in sandy loam under aerobic conditions[1]

Compound (code)	Sampling times (days) [2,3]			
	0	34	96	159
8-Methylpyrido-(2,3-d)-pyridazine-2,5(1H,6H)-dione (M9)	97	80.78	73.32	69.97
Unidentified Unk-1	ND	ND	2.7	ND
Unidentified Unk-2	ND	ND	0.5	0.81
Extractable residues	97	80.78	75.17	70.78
CO_2	NA[2]	1.62	2.5	3.87
Non-extractable residues	1.14	14.46	19.41	23.52
Total % recovery	98.14	96.86	98.58	98.17

1 n = 2 at 0- and 96-days, n = 1 at 34- and 159-days
2 Not detected; detection limit not reported
3 Not analyzed.
Data obtained from pp. 21, 22 of the study report.

C. TRANSFORMATION OF PARENT COMPOUND: [^{14}C]M9 decreased from 97.0% of the applied at day 0 posttreatment to 69.97% at 159 days (final sampling interval, p. 22).

HALF-LIFE: M9, a degradate of diflufenzopyr, degraded slowly in aerobic sandy loam soil (registrant-calculated half-life value of 366 days, $r^2 = 0.85$; pp. 17, 52).

Table 6: Registrant-calculated half-life value for [^{14}C]M9 in aerobic sandy loam soil.

Soil type	Half-life (days)	r^2	Regression equation
sandy loam	366	0.85	Linear form y = mx + b as lnC= -kt + lnC_0; lnC_0 is initial concentration (b = y intercept), lnC is concentration at time t (y), k is the slope (m), t is time (x) or kt = lnC_0 - lnC. Half-life (t ½) = -(ln/k).

Data obtained from p. 52 of the study report.

TRANSFORMATION PRODUCTS: The test compound, [^{14}C]M9, was present at 70.0% of the applied radioactivity at the end of the study period. Two unidentified [^{14}C]compounds (Unk-1, Unk-2) were each detected at ≤2.7% of the applied (p. 22).

NON-EXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues decreased from 96.5-97.5% of the applied at time 0 to 70.8% at 159 days (p. 21). Nonextractable [^{14}C]residues increased from 1.0-1.2% of the applied at day 0 to 23.5% at 159 days; at day 96, 14.1% of the applied was associated with fulvic acid, 3.3% with humic acid, and 7.3% with humins (p. 23). Organic extractable radioactivity from fulvic acid comprised an average 2.3% of the applied with parent M9 accounting for most of that radioactivity (2.0% of applied).

VOLATILIZATION: Volatilized $^{14}CO_2$ totaled 3.9% at 159 days posttreatment (p. 21).

TRANSFORMATION PATHWAY: Not applicable.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: Not applicable. The additional high dose experiment was performed only to yield sufficient material to facilitate identification of possible transformation products.

III. STUDY DEFICIENCIES: No deficiencies were noted. This study can be used to provide supplemental information on the aerobic soil metabolism of diflufenzopyr.

IV. REVIEWER'S COMMENTS:

1. Except for samples collected the day of treatment (day 0), it was not reported and could not be determined how many treated sampled were collected at each sampling interval. It was reported that twenty soil samples were each treated with [^{14}C]M9, and two treated soil samples were taken as day 0 samples (p. 13). The remaining eighteen treated soil samples were placed in glass columns and incubated. Of the eighteen treated soil samples that were incubated, only four soil samples were extracted and analyzed (not including day 0 samples); single soil samples collected at 34 and 159 days and duplicate samples collected at 96 days (p. 41-44). It is unclear why the remaining samples were either not analyzed and/or results from analyses of those samples were not reported.

2. The registrant-calculated half-life for M9 in aerobic sandy loam soil was 1 year (366 days; least squares linear regression analysis assuming degradation followed first order kinetics; pp. 17, 52). The half-life value was verified by the Dynamac reviewer using. The calculated

half-life is of limited value because the data have been extrapolated far beyond the duration of the experiment.

3. Column eluates were reportedly collected for each HPLC run and analyzed for total radioactivity by LSC to determine column recoveries; however, HPLC recoveries were not provided for review.

4. The HPLC run time was reported as 47 minutes while the mobile phase gradient composition was only reported to 37 minutes (p. 14); this appears to be a typographical error.

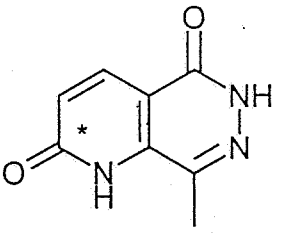
5. The current recommended maximum field application rate for diflufenzopyr was reported as 0.14-0.18 lb a.i./A (0.16-0.20 kg a.i./ha) which would correspond to concentration of in the soil of ca. 0.21-0.27 ppm assuming a soil layer of 5 cm (p. 13). 2-Keto-M1 (M9) was the most prevalent transformation product of diflufenzopyr in soil extracts in an aerobic soil metabolism study (MRID 45444002), therefore, a target application rate of the diflufenzopyr maximum field application rate was selected for this study.

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

1. Singh, M. 1996. 2001. Aerobic soil metabolism of ^{14}C-BAS 654 H. Study No. 61198. BAS Reg. Doc. No. 2001/5000085.

2. Tong, R. 1996. Aerobic soil metabolism of SAN-836 H. Sandoz Agro Inc. Project No. 414215. Report No. 5. BASF Reg. Doc. No. 1996/5380.

Chemical Structure with Radiolabel Position (*)



The radiopurity of the test substance was determined by reversed-phase high performance liquid chromatography (HPLC) before application to the test system. The radiopurity was found to be 99.43% (Figure 1).

B. Reference Substance

Analytical standard 2-keto-M1 (M9, Lot No.01748-022, 99.3%) was used in the study.

C. Soil

The soil used in this study was obtained from Fredolin Schwade tract, Iowa. The soil was collected on November 13, 2000 and received at BASF on November 16, 2000. The soil was stored in a refrigerator at 7 °C until use. Before use, the soil was sieved through a screen with2 mm openings. The soil analysis was done at Agvise Laboratories, Northwood, ND 58267. According to the USDA textural classification, the soil was characterized to be a Sandy Loam soil. Soil analysis data are summarized in Table 1 and the report is given in Appendix 1.

D. Chemical Reagent and Solvent

ACS certified reagent grade chemicals and HPLC grade solvents were used in this study.

E. Equipment

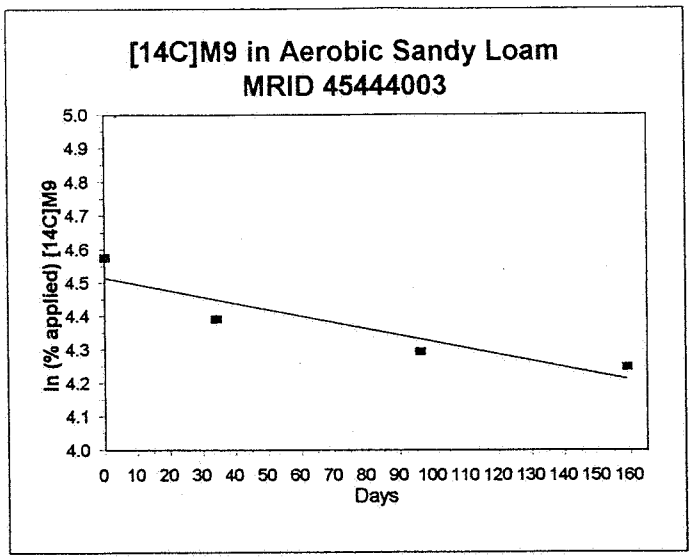
- LSC – Beckman LS 6000 11 Series Liquid Scintillation Systems
- HPLC – (Hewlett Packard): BASF System (1050 pump, 1050 UV DAD, IN/US Betaram radiomatic detector, degasser, column heater, autosampler, column: YMC ODS AQ, 250 mm x 4.6 mm, 5 µm)
- Balances – Sartorius & Mettler analytical and Sartorius & Mettler top loading
- Centrifuge – Beckman Model GS-6KR
- Shaker – IKA Model KS501
- Oxidizer - Harvey Model OX300
- Mass Spectrometers. – PE Sciex API 3000, PE Series 200 Micro Pump System and autosampler; Finnigan LCQ, Michrom MAGIC 2002 HPLC, ThermoSeparations AS 3000 autosampler, IN/US ^{14}C-detector; Hewlett Packard GC/MSD

MRID 45444003

Aerobic Metabolism of [^{14}C]M9 in Sandy Loam Soil
MRID 45444003

Half-life Determination

M9		
Day	%AR	Ln(%AR)
0	97.00	4.574711
34	80.78	4.391729
96	73.32	4.294833
159	69.97	4.248067



Regression Output:

Constant	4.514
Std Err of Y Est	0.07
R Squared	0.846
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-0.00189
Std Err of Coef.	0.000572

half-life 365.8 days

*AR = Applied Radioactivity
Linear regression analysis performed using Corel Quattro Pro 8
Results from p. 22 of study report.