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JUN 14 1994

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Karen R. Blundell
Senior Registration Specialist
BASF Corporation
P. O. Box 13528
Research Triangle Park, NC 27709-3528

SUBJECT: Confined rotational crop data for reregistration of sodium acifluorfen, chemical code 114402

Dear Ms. Blundell:

Your May 24, 1993 submission of data for guideline requirement 165-1, confined rotational crop, for the reregistration of sodium acifluorfen has been reviewed. Our conclusions are described below.

GDLN 165-1 Confined rotational crop **MRID 427856-01**

These data, MRID 427856-01, are upgradable with the submission of additional information. The major metabolite has not been identified. This metabolite remained to be the major residue or exceeded 0.01 ppm in crops when sampled near the 12-month interval. Identification and characterization of the major metabolite is required to upgrade these data to acceptable. Quantitative data depicting the stability of the major metabolite and acifluorfen in frozen storage are required.

If you do not provide these data within 90 days from your receipt of this letter, we may pursue appropriate regulatory action to ensure your compliance with our statutory goals. If you have any questions regarding this letter, please call the Chemical Review Manager, Tom Luminello, at (703) 308-8075.

Sincerely yours,

Jay S. Ellenberger, Chief
Accelerated Reregistration Branch
Special Review and
Reregistration Division

Enclosure

cc: Joanne Miller, PM-23
Leung Cheng, HED

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 12 1994

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Sodium Acifluorfen. Case No. 2605. Confined Rotational Crop Study in Chard, Radish, Turnips, Sorghum, and Wheat. MRID No. 42785601. CBRS No. 11988. DP Barcode: D192150.

FROM: Leung Cheng, Chemist
Special Review Section II
Chemistry Branch II - Reregistration Support
Health Effects Division (7509C)

THROUGH: Andrew R. Rathman, Section Head
Chemistry Branch II - Reregistration Support
Health Effects Division (7509C)

TO: Thomas Luminello, Jr, CRM 52
Accelerated Reregistration Branch
Special Review/Reregistration Division (7508W)

Attached is a review of a confined rotational crop study for sodium acifluorfen submitted by the registrant for reregistration. This information was reviewed by Dynamac Corporation under the supervision of CBRS, HED. The data assessment has undergone secondary review in the branch and has been revised to reflect branch policies.

The submitted study in which the chlorophenyl ring is radiolabeled with carbon-14 is inadequate since the major residue (M1) has not been identified (see conclusion 3a of the attached review). M1 remained to be the major residue or exceeded 0.01 ppm in crops when sampled near the 12-month interval. Quantitative data depicting the stability of M1 and acifluorfen in frozen storage are required.

If you need additional information, please advise.

Attachment: Dynamac review of confined rotational crop study of sodium acifluorfen

cc(without Attachment):RF
cc(with Attachment):Circ, SF, List B File, Cheng, Dynamac
RDI:ARRathman:5/10/94:MSMetzger:5/11/94:EZager:5/11/94
7509C:CBRS:LCheng:CM#2:RM804D:5/4/94:03:a\ACIFLUORFEN\ROTATION

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DYNAMAC
CORPORATION
Environmental Services

Final Report

SODIUM ACIFLUORFEN
Shaughnessy No. 114402
(DP Barcode D192150;
CBRS No. 11988; Case No. 2605)

TASK 4
Registrant's Response to Residue Chemistry Data Requirements

September 22, 1993

Contract No. 68-D2-0053

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
2275 Research Boulevard
Rockville, MD 20850-3268

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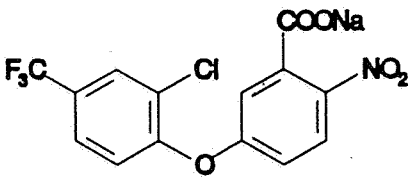
SODIUM ACIFLUORFEN



Shaughnessy No. 114402; Case 2605

(CBRS No. 11988; DP Barcode D192150)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to requirements for reregistration, BASF Corporation submitted a confined rotational crop study (under CBRS No. 11988; 1993; MRID 42785601). The submitted data are reviewed and evaluated in this document for adequacy in fulfilling the reregistration requirements for Guideline No. §165-1.

The qualitative nature of the residue in soybeans is not adequately understood. Summaries of soybean metabolism studies, submitted in response to the Phase 4 Reviews, were deemed unacceptable for purposes of registration because the majority of the ^{14}C-residues were not identified. The qualitative nature of the residue in peanuts is not adequately understood because the majority of ^{14}C-residues have not been identified in samples that were collected at maturity. The qualitative nature of the residue in rice is not adequately understood because quantitative data pertaining to metabolite identification in/on rice commodities were incomplete. Radiovalidation of methods used for the peanut and rice metabolism studies is required pending determination of the residues to be regulated.

The qualitative nature of the residues in poultry is adequately understood (L. Cheng, 4/26/94, CBRS No. 12181). A ruminant metabolism study using sodium acifluorfen labeled in the nitrophenyl ring (NPR) with carbon-14 remains outstanding (L. Cheng, 5/12/94, CBRS No. 12130).

Adequate methodology is available for the enforcement of tolerances of sodium acifluorfen and its amine and methyl ester metabolites in/on soybeans, milk, and beef liver. A GC/electron capture detection (ECD) method and a GC/MS method are listed in Pesticide Analytical Manual (PAM) Vol. II as Methods I and A, respectively (Pesticide Reg. Sec. 180.383). Similar methods have been used for the collection of data concerning residues in/on soybean, peanut, vegetable, and animal commodities. It should be noted that in Methods I and A, diazomethane is used to methylate acifluorfen residues to methyl esters prior to GC/ECD analysis.

Tolerances for residues of sodium acifluorfen are currently expressed as the combined residues of the sodium salt of acifluorfen (sodium 5-[2-chloro-4-trifluoromethyl)-phenoxy]-2-nitrobenzoic acid) and its metabolites (the corresponding acid, methyl ester, and amino analogs) in/on plant and animal

commodities [40 CFR §180.383]. No Codex MRLs have been established for sodium acifluorfen; therefore, there are no questions with respect to compatibility with the U.S. tolerances.

CONCLUSIONS AND RECOMMENDATIONS

1. The qualitative nature of the residue in rotational crops is not adequately understood since the major residue (M1) has not been identified (see Conclusion 3a below). M1 remained to be the major residue or exceeded 0.01 ppm in crops when sampled near the 12-month interval. Quantitative data depicting the stability of Metabolite 1 and acifluorfen in frozen storage are required.

2. Total ^{14}C-residues exceeded 0.01 ppm in/on all commodities of chard, turnips, sorghum, radish, and wheat that were planted 39, 103, 145, 313, and 370 days after acifluorfen that was labeled with [^{14}C] in the chlorophenyl ring (CPR) was applied to sandy soil outdoors at 0.5 lb ai/A (1x the revised maximum seasonal rate). Residue accumulation declined from shorter rotation intervals to longer rotation intervals in/on all rotational crop commodities.

3a. The study characterized/identified ≥66% of the total radioactive ^{14}C-residues in/on all crop commodities. The parent, acifluorfen, was identified and confirmed in all rotational crop commodities at all sampling intervals and was highest in 39 DAT sorghum fodder (0.024 ppm, 12.3% TRR) and lowest in 145 DAT wheat grain (<0.001 ppm). The principal residue extracted into methanol from all crop substrates was Metabolite 1, a polar component suggested by the registrant possibly to be a sugar conjugate of 2-chloro-4-trifluoromethylphenol. However, the registrant stated that they could not propose a structure for this metabolite when mass spectroscopic data were taken into consideration. Metabolite 1 was the major residue (36-65% TRR) in/on all tested crops except in 103 DAT radish (13-31% TRR).

3b. Three other unknown polar metabolites, designated as Metabolites 2, 3, and 4, also were detected in extracts of rotational crop commodities, but were present only at insignificant levels (<0.001-0.013 ppm). In wheat straw only, a component (designated Metabolite 2a) somewhat less polar than Metabolite 2, was present at insignificant levels (0.005 ppm). An unspecified, but minor, polar component also was detected in chard (0.002-0.005 ppm). Metabolite 3 was tentatively identified as either amino- or desnitro-acifluorfen only in 39 DAT chard (0.009 ppm, 6.5% TRR) by TLC and HPLC.

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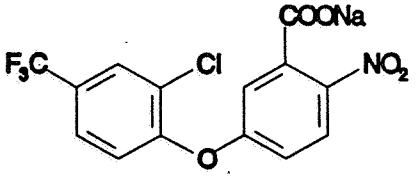
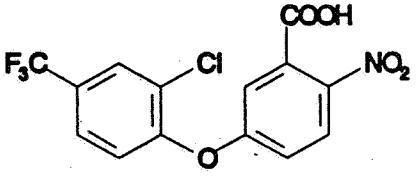
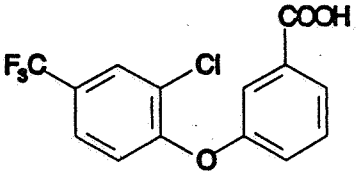
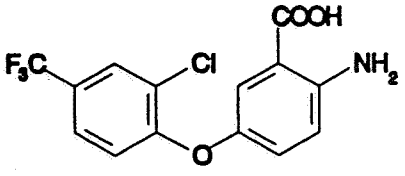
The molecular structures of sodium acifluorfen and its metabolites characterized and identified in confined rotational crop commodities are presented in Table 1.

Table 1. Sodium acifluorfen and its metabolites in rotational crops (MRID 42785601).

Code	Chemical Name Structure	Substrate Common Name
I.	sodium 5-(2-chloro-4-(trifluoromethyl)phenoxy)-2-nitrobenzoate 	**sodium acifluorfen**
II.	5-(2-chloro-4-(trifluoromethyl)phenoxy)-2-nitrobenzoic acid 	chard radish tops and roots, sorghum forage, fodder, and grain, turnips, wheat, forage, fodder, and grain **acifluorfen**
III.	3-(2-chloro-4-trifluoromethylphenoxy)benzoic acid [a] 	chard **desnitro-acifluorfen**
IV.	2-amino-5-(2-chloro-4-(trifluoromethyl)phenoxy)benzoic acid [a] 	chard **amino-acifluorfen**

[a] Identification not confirmed by a second method.

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DETAILED CONSIDERATIONS

Confined Rotational Crops

Directions for use

A search of the Reference Files System (REFS) conducted 7/26/93 identified four soluble concentrate/liquid formulations (SC/L) registered to BASF Corporation. Two 2 lb/gal SC/L formulations [EPA Reg. Nos. 7679-79 and 7679-80] are registered for: (i) postemergence use on soybeans at 0.25-0.75 lb ai/A/application (not to exceed 1 lb ai/A/season) at the 1-2 trifoliate stage; (ii) use on peanuts at 0.25-2 lb ai/A/application from preemergence up to the cracking stage, and no more than 0.5 lb ai/A postemergence (the seasonal rate should not exceed 2 lb ai/A); and (iii) postemergence use on rice at 0.25-0.5 lb ai/A/application (not to exceed two applications and 0.5 lb ai/A/season) at the tillering stage through the boot stage. Applications to rice may not be made in CA. *[Note: A proposed revision in the maximum seasonal rate for soybeans from 1.0 lb ai/A to 0.5 lb ai/A was approved by CBRS "provided this rate revision occurs on all labels reflecting this registered use of sodium acifluorfen on soybeans" (J. Smith, CBRS No. 9784, 6/9/92). In addition, CBRS had no objection to the registrant conducting field trials at the proposed revised use rate of 0.5 lb ai/A/ season.]*

A 1.33 lb/gal MAI SC/L formulation [EPA Reg. No. 7679-76] is registered for postemergence use on soybeans at the 2-3 leaf stage and on peanuts from the cracking stage to the 2-leaf stage at 0.25-0.5 lb ai/A/application (not to exceed 0.5 lb ai/A). A 0.67 lb/gal MAI SC/L formulation [EPA Reg. No. 7679-77] is registered for postemergence use on soybeans at up to 0.25 lb ai/A at the 1-2 leaf stage followed by up to 0.25 lb ai/A of an SAI formulation (for a seasonal rate of 0.5 lb ai/A).

Each formulation may be applied in 20 gal/A finished spray using ground equipment or 5-10 gal/A using aerial equipment. Each formulation may be tank mixed with adjuvants and other herbicides. A 50-day PHI has been established for soybeans and rice, and a 75-day PHI has been established for peanuts. An 18-month rotational interval has been established for root crops (such as carrots, turnips, sweet potatoes, etc.). Treated plants may not be used for feed or forage. *[These use patterns were obtained from sodium acifluorfen end-use products currently registered to BASF Corporation.]*

Sodium acifluorfen that was labeled with [^{14}C] in the chlorophenyl ring (CPR) (97.8% radiochemical purity; specific activity 18.03 mCi/mM, 0.0498 mCi/mg) was dissolved in 0.1 N sodium hydroxide, then combined with 0.025 M sodium phosphate buffer and 40 uL of non-ionic surfactant to prepare the test substance. The test substance was applied at an equivalent field rate of 0.5 lb ai/A to portions of a sandy soil (90% sand, 5% silt, 5% clay, 0.5% organic matter, pH 6.5, cation exchange capacity 2.3) that had been placed in three galvanized steel containers (2-ft x 3-ft x 2-ft deep). The test substance was applied using a plastic pump spray bottle. No cover crop was planted. Each of the three tanks was divided into a grain section, a leafy crop section, and a root crop section. Immediately prior to planting of the rotational crops, the soil was tilled to a depth of two inches and fertilizer was applied. The containers were planted with rotational crops at the following posttreatment intervals: (i) chard, sorghum, and turnips, 39 days after treatment (DAT); (ii) chard and radishes, 103, 298, and 313 DAT; (iii) wheat, 145 DAT; and (iv) sorghum, 370 DAT. The 39 DAT chard and turnip crops had poor germination rates due to the presence of sodium acifluorfen. The 103 DAT chard and radish crops suffered some phytotoxic leaf damage approximately two weeks following planting. The 298 DAT chard and radish crops were destroyed by cutworms (March 23, 1992) and were replanted two weeks later (313 DAT, April 7, 1992).

The rotational crops were harvested at the following postplanting intervals: 55-84 days for chard, 63 days for turnips, 61-63 days for sorghum forage, 104-105 days for sorghum fodder and grain, 34-71 days for radishes, 153 days for wheat forage, and 224 days for wheat hay and grain (at maturity). Leafy vegetable and root crops were harvested when the majority of plants reached maturity. Chard was harvested by cutting the plant at ground level. Turnips and radishes were harvested by hand-pulling the plants from the soil, and radishes were separated into tops and roots; turnips were not separated into tops and roots because few plants grew to maturity. Forage samples were taken for each grain crop at a time previous to maturity according to standard agricultural practices. The remainder of the grain crop was harvested when the plants reached maturity with the plant cut off at or slightly above ground level; the grain was separated from the stem in the field and was stored separately. All crop samples were stored 21-232 days at -30 C to 0 C until analysis.

Total radioactive residues (TRR)

Two methods were used to determine the TRR in/on rotational crops. In one method, the TRR in all matrices were determined directly by Liquid Scintillation Spectrometry (LSS) following combustion. In the second method, the TRR in rotational crop samples were determined after extraction with aqueous methanol, by summing the radioactivity found in the extract and the non-extractable residues (determined by combustion/LSS), except for 39 DAT chard and turnips which were analyzed by combustion/LSS only. The TRR values obtained using the two methods were similar. There were three replicates for each crop commodity and each replicate was analyzed in triplicate; TRR values are expressed in [^{14}C]acifluorfen equivalents. Radioactivity in the extracts was determined by LSS, and radioactivity in the non-extractable residues was determined by LSS following combustion. The limit of detection was not specified. The TRR in/on plant commodities determined using extracted matrices, are listed in Table 2. The TRR data determined by combustion/LSS are not presented here because the registrant used the TRR data obtained for the extracted matrices for all subsequent metabolite TRR calculations.

The data presented in Table 2 indicate that ^{14}C-residues >0.01 ppm accumulated in/on all rotational crop commodities of chard, sorghum, wheat, and radish tops that were planted 39, 103, 145, 313, and/or 370 days after [^{14}C]sodium acifluorfen was applied to sandy soil at 1x the maximum registered rate. Residue accumulation declined from the shorter rotation intervals to the longer rotation intervals. Accumulation was greatest in/on 39 DAT chard (0.413 ppm), sorghum fodder (0.198 ppm), and turnips (0.122 ppm), and in/on 103 DAT chard (0.140 ppm). *[Note: The present submission also contains data (TRR, fractionation, characterization, identification, and storage stability) on soils as a result of application of the test substance. These soil data are not addressed herein.]*

Table 2. Total radioactive residues (TRR) found in/on rotational crops grown in aged sandy soil treated with [^{14}C] sodium acifluorfen at 0.5 lb ai/A.

Substrate	TRR (ppm) [a]				
	39 DAT	103 DAT	145 DAT	313 DAT	370 DAT
Chard	0.413 [b]	0.140	--	0.032	--
Turnips	0.122 [b]	--	--	--	--
Sorghum forage	0.062	--	--	--	0.014
Sorghum fodder	0.198	--	--	--	0.030
Sorghum grain	0.070	--	--	--	0.017
Radish tops	--	0.067	--	0.027	--
Radish roots	--	0.018	--	0.008	--
Wheat forage	--	--	0.035	--	--
Wheat fodder	--	--	0.082	--	--
Wheat grain	--	--	0.040	--	--

[a] Average of three replicate analyses; each replicate was the average of triplicate sample analyses.

[b] TRR values determined by combustion/LSS of unextracted matrices; all other TRR values determined by summing the radioactivity in the aqueous methanol extract and the non-extractable residues.

Extraction of ^{14}C-residues in/on plant commodities

All samples were homogenized with dry ice prior to extraction. Low moisture substrates (sorghum commodities, wheat grain, and wheat straw) were soaked in water overnight prior to extraction. Samples of the soaked commodities and the remainder of the rotational crop samples were then Soxhlet extracted with methanol for 18-22 hours. The aqueous methanol extracts for each replicate of each crop commodity were combined and analyzed by LSS. The 313 DAT radish root extracts were not further characterized because ^{14}C-residues were <0.01 ppm. Combined extracts were concentrated by centrifugal evaporation and/or a stream of nitrogen. Methanol-insoluble residues precipitated during concentration of the extracts were removed by decanting the supernatant. The insoluble residues were rinsed with aqueous methanol and the rinses were added to the supernatants. The remaining insoluble residues were designated as the concentration pellet and analyzed for radioactivity by combustion/LSS. If >90% of the radioactivity remained in the extract after concentration, the radioactivity was considered equivalent to the pre-concentration level. If <90% of the radioactivity remained in the extract after concentration, ^{14}C-residues in the concentration pellet were considered a separate fraction.

The following amounts of radioactive residues in/on plant matrices were recovered by methanol extraction: chard (86.7-96.0% TRR), sorghum forage (75.5-90.7% TRR), sorghum fodder (86.9-92.6% TRR), sorghum grain (82.2-83.5% TRR), radish top (85.3-86.6% TRR), radish root (77.6-77.8% TRR), wheat forage (96.0% TRR), wheat fodder (74.1% TRR), and wheat grain (91.1% TRR).

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Metabolite characterization and identification

Concentrated extracts were analyzed by normal-phase 1-D TLC on a silica gel plate developed with chloroform:methanol:water (60:30:4, v:v:v). Radioactive residues were detected and quantified by radio-scan chromatography using an Ambis Beta Scanning System; non-radiolabeled reference standards were visualized by UV light (254 nm). Metabolites were identified by comparison with the R_f values of the following non-radiolabeled reference standards: acifluorfen, desnitro-acifluorfen, amino-acifluorfen, descarboxy-acifluorfen, acifluorfen-methyl ester, acetamide, and 2-chloro-4-trifluoromethylphenol.

Extracts containing ^{14}C-residues >0.05 ppm were analyzed by reverse-phase HPLC on a Partisil Magnum 10 ODS-3 column using a mobile phase consisting of 0.25% acetic acid (Solvent A) and 0.25% acetonitrile (Solvent B), changing from 100% A to 10% A and 90% B in a series of step and linear gradients over a period of 55-85 minutes. Radioactive residues were detected by UV absorption at 254 nm and by flow-through radiometric detection. Metabolites were identified by comparison with the retention times of the appropriate reference standards.

The quantitative results of the characterization/identification procedures in rotational crop commodities are presented in Table 3. A summary of the metabolites identified and confirmed in each rotational crop is presented in Tables 4 through 7.

The study successfully characterized/identified ≥66% of the total radioactive ^{14}C-residues in/on all crop commodities. The magnitude of the ^{14}C-residues identified was: 75.6-84.1% TRR in chard, 73.4-73.7% TRR in sorghum forage, 64.4-74.3% TRR in sorghum fodder, 66.4-78.2% TRR in sorghum grain, 74.5-79.5% TRR in radish top, 66.3% TRR in radish root, 92.1% TRR in wheat forage, 66.7% TRR in wheat straw, and 89.8% TRR in wheat grain.

The parent, acifluorfen, was identified and confirmed in/on all rotational crop commodities at all sampling intervals and was highest in 39 DAT sorghum fodder (0.024 ppm, 12.3% TRR) and lowest in 145 DAT wheat grain (<0.001 ppm). The major radioactive component observed in all analyzed extracts from rotational crop commodities was designated as Metabolite 1. Metabolite 1 levels were highest in 39 DAT sorghum fodder (0.110 ppm, 55.7% TRR), but was only a minor component in all other rotational crop commodities (0.002-0.05 ppm, 13-54.9% TRR) regardless of sampling interval. Analyses by TLC also detected four other metabolites, designated as Metabolites 2, 2a (wheat straw only), 3, and 4, in extracts of rotational crop commodities. Metabolites 1, 2, and 2a were more polar than all reference standards of acifluorfen and its metabolites used in the study; aside from a statement in the text concerning the presence of Metabolite 2a in wheat straw, no other information concerning this metabolite was provided. Metabolites 3 and 4 were less polar than the reference standards. Analyses of chard extracts by TLC indicated that Metabolite 3 had an R_f value close to that of the amino analogue of acifluorfen. Analysis of Metabolite 3 by HPLC was inconclusive. Metabolite 4 eluted with the solvent front. Since Metabolites 2-4 were only minor residues (<0.001-0.013 ppm), no further characterization was conducted on these metabolites.

Characterization of Metabolite 1

The 39 DAT sorghum fodder extract was used to further characterize Metabolite 1; the characterization procedures are described below.

To verify that Metabolite 1 was the same component in all analyzed matrices, a comparison of samples analyzed on different TLC plates were scraped, eluted from the silica with methanol, and re-analyzed by TLC on one set of plates. A sample of Metabolite 2 and the acifluorfen reference standard were developed concurrently, with the Metabolite 1 samples. The TLC re-analyses

confirmed that Metabolite 1 residues isolated from each rotational crop extract had the same R_f value and were distinct from Metabolite 2 and acifluorfen. The registrant provided representative TLC chromatograms. Citing a previously submitted peanut metabolism study (1992; MRID 42368301) and two technical journal articles (Frear et al., Pesticide Biochemistry and Physiology 20(1973):299-310; and Shimabukuro et al., Pesticide Biochemistry and Physiology 3(1973):483-494), the registrant conducted additional characterization procedures based on the premise that Metabolite 1 was an acidic polar metabolite consisting of a 6-O-malonyl-β-glucoside containing the chlorophenyl group or homoglutathione and/or cysteine conjugates containing the nitrophenyl group.

Metabolite 1 was isolated from concentrated extracts by adjusting the extract to pH 5 with phosphate buffer and applying it to a preconditioned quaternary amine column; ~15% of the radioactivity was not retained. The retained residues were eluted with three 10 mL volumes of 0.5 M sodium chloride. The three elutions respectively removed 42.8%, 28.2%, and 2.8% of the radioactivity from the column; the first and second eluates were combined and concentrated. The supernatant was removed from the precipitated salt, the salt was rinsed with methanol, and the rinse was combined with the supernatant. The extract was then applied to two preparative silica TLC plates and residues on the first plate were separated and detected as previously described; residues applied to the second plate were developed using chloroform:methanol:water:acetic acid (60:30:4:4, v:v:v:v). Radioactive zones that corresponded to Metabolite 1 were scraped from the plates, eluted from the silica with methanol, and filtered. The sample was further purified to remove excess salt by preparative HPLC on a YMC AQ-303 column using an isocratic mobile phase consisting of 0.25% acetic acid in water. The eluate was adjusted to pH 2 and partitioned with ethyl acetate. The ethyl acetate fractions were dried and redissolved in methanol:water (1:1, v:v). Analyses of purified Metabolite 1 extracts were conducted with a mass spectrometer using an IonSpray interface; samples were analyzed in both the negative and positive ion detection modes; no other details were provided. The registrant indicated that no Metabolite 1 structure could be proposed based on the observed data.

Metabolite 1 was further characterized by reverse-phase HPLC using an ion-pairing reagent. An aliquot of concentrated sorghum fodder extract was analyzed by preparative HPLC with a mobile phase consisting of the ion-pairing reagent tetrabutylammonium phosphate (0.005 M, Solvent A) and methanol (Solvent B), changing from 100% A to 40% A in a series of step and linear gradients over a period of 120-180 minutes. Eluate fractions were collected and analyzed by LSS. The methanol was removed from an aliquot of the eluate containing Metabolite 1 and the residue was mixed with water. Concentrated hydrochloric acid was added to bring the final concentration to 1.5 N and the solution was incubated at 100 C for 48 hours. After acid hydrolysis, the solution was neutralized with 1 N sodium hydroxide and analyzed by preparative HPLC with a mobile phase consisting of water (Solvent A) and methanol (Solvent B), changing from 100% A to 20% A over a period of 40-110 minutes. The eluate fractions were collected and analyzed by LSS.

In another characterization method, Metabolite 1 residues were isolated from methanol extracts of sorghum fodder by preparative TLC. One aliquot of methanol solution was dried under a nitrogen stream, redissolved in acetate buffer (pH 5), and then hydrolyzed with β-glucosidase at 37 C for 24 hours. The hydrolysate was then analyzed by normal phase TLC (previously described) and by reverse-phase HPLC with a mobile phase consisting of 0.25% acetic acid in water (Solvent A) and 0.25% acetic acid in acetonitrile (Solvent B), changing from 54% A to 0% A over a period of 71-130 minutes. A second aliquot of methanol was dried and redissolved in 0.1 M acetate buffer (pH 4.6) and hydrolyzed with hesperinidase (containing some β-glucosidase) at 37 C for 24 hours. The hydrolysate was then analyzed by LSS and HPLC (as previously described for the acid hydrolysate). The data obtained from these procedures were inconclusive, but indicated that Metabolite 1 was not a β-glucoside conjugate.

Another aliquot of TLC-isolated Metabolite 1 was hydrolyzed in sodium hydroxide at 37 C for 2.5 hours. The hydrolysate was adjusted to pH 4 with hydrochloric acid and extracted three times with ether. The organic and aqueous fractions were analyzed by LSS, and the aqueous fraction was analyzed by normal phase TLC. The data obtained from these procedures were inconclusive, but indicated that Metabolite 1 was not a glutathione conjugate.

An additional aliquot of TLC-isolated Metabolite 1 was dried, redissolved in 2 N methanolic hydrochloric acid, and hydrolyzed at 78 C for 3 hours followed by hydrolysis at 70 C for 2 hours. Analysis by LSS of the neutralized hydrolysate indicated the loss of 85-90% of the radioactivity via volatilization.

A final aliquot of TLC-isolated Metabolite 1 was dried, redissolved in methanol and analyzed by LSS to verify the presence of Metabolite 1. The methanol extract was then mixed with additional methanol, ether, and diazomethane. The ether and methanol were removed under a nitrogen stream and the solution was mixed with additional methanol. The registrant indicated that the recovery of radioactivity was ~12%; the solution was further analyzed by reverse-phase HPLC. Concurrently, a solution of isolated Metabolite 1 and [^{14}C]acifluorfen containing an equivalent dpm level was derivatized under identical conditions; after removal of the solvent and the addition of methanol:water (1:1, v:v), LSS analysis indicated that the recovery of radioactivity was ~56%. The solution was further analyzed by reverse-phase HPLC using an isocratic mobile phase of 0.25% acetic acid in water:0.25% acetic acid in acetonitrile (54:46, v:v). The HPLC and LSS analyses indicated that the methyl ester derivative of acifluorfen remained in solution, whereas Metabolite 1 residues were again lost via volatilization. Derivatized Metabolite 1 residues were analyzed by mass spectrometry. The registrant indicated that no structure could be proposed for Metabolite 1, based on mass spectrometry analysis of the remaining Metabolite 1 residues.

Citing a previously submitted metabolism study for peanuts (1992; MRID 42368301) and a technical journal article for soybean metabolism (Frear et al., Pesticide Biochemistry and Physiology 20(1973):299-310), the registrant stated that the metabolism of acifluorfen in rotational crops seemed to be similar to that observed for field-grown peanuts (hulls) and soybeans (leaf), i.e. that acifluorfen is metabolized to aqueous-soluble polar components, probably via cleavage of the ether linkage followed by conjugation of the aromatic rings with glucose and/or amino acid moieties.

Table 3. Distribution of total radioactive residues (TRR) in rotational crops grown in aged sandy soil treated with [^{14}C]sodium acifluorfen at 0.5 lb ai/A.

Fraction	%TRR	ppm	Characterization/Identification
Substrate = 103 DAT Chard (0.140 ppm)			
Methanol	86.7	0.121	Acifluorfen (14.3% TRR, 0.020 ppm) was identified and Metabolite 1 (35.5% TRR, 0.050 ppm), Metabolite 2 (9.4% TRR, 0.013 ppm), Metabolite 3 (6.5% TRR, 0.009 ppm), Metabolite 4 (6.1% TRR, 0.009 ppm), and the polar origin (3.8% TRR, 0.005 ppm) were resolved. Total Characterized/Identified = 75.6% TRR, 0.106 ppm.
Non-extractable	13.3	0.019	Not further analyzed (N/A).
Substrate = 313 DAT Chard (0.032 ppm)			
Methanol	96.0	0.031	Acifluorfen (9.2% TRR, 0.003 ppm) was identified and Metabolite 1 (65.0% TRR, 0.021 ppm), Metabolite 3 (3.1% TRR, 0.001 ppm), and the polar origin (6.0% TRR, 0.002 ppm) were resolved. Total Characterized/Identified = 83.3% TRR, 0.027 ppm.
Non-extractable	4.0	0.001	N/A
Substrate = 39 DAT Sorghum Forage (0.062 ppm)			
Methanol	85.8	0.053	Acifluorfen (18.3% TRR, 0.011 ppm) was identified and Metabolite 1 (52.8% TRR, 0.03 ppm), Metabolite 3 (1.2% TRR, 0.001 ppm), and Metabolite 4 (1.1% TRR, 0.001 ppm) were resolved. Total Characterized/Identified = 73.4% TRR, 0.046 ppm.
Pellet	10.3	0.006	N/A
Non-extractable	14.2	0.009	N/A
Substrate = 370 DAT Sorghum Forage (0.014 ppm)			
Methanol	90.7	0.013	Acifluorfen (12.9% TRR, 0.002 ppm) was identified and Metabolite 1 (52.7% TRR, 0.008 ppm), Metabolite 2 (6.3% TRR, <0.001 ppm), and Metabolite 3 (1.8% TRR, <0.001 ppm) were resolved. Total Characterized/Identified = 73.7% TRR, <0.012 ppm.
Non-extractable	9.3	0.001	N/A
Substrate = 39 DAT Sorghum Fodder (0.198 ppm)			
Methanol	86.9	0.172	Acifluorfen (12.3% TRR, 0.024 ppm) was identified and Metabolite 1 (55.7% TRR, 0.110 ppm), Metabolite 3 (3.1% TRR, 0.006 ppm), and Metabolite 4 (5.6% TRR, 0.011 ppm) were resolved. Total Characterized/Identified = 76.7% TRR, 0.151 ppm.

(continued; footnotes follow)

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Table 3 (continued).

Fraction	%TRR	ppm	Characterization/Identification
Non-extractable	13.1	0.026	N/A
Substrate = 370 DAT Sorghum Fodder (0.030 ppm)			
Methanol	92.6	0.028	Acifluorfen (4.5 % TRR, 0.001 ppm) was identified and Metabolite 1 (74.3% TRR, 0.011 ppm) was resolved. Total Characterized/Identified = 78.8% TRR, 0.0022 ppm.
Non-extractable	7.4	0.002	N/A
Substrate = 39 DAT Sorghum Grain (0.070 ppm)			
Methanol	83.5	0.058	Acifluorfen (20.5% TRR, 0.014 ppm) was identified and Metabolite 1 (54.9% TRR, 0.038 ppm) and Metabolite 4 (2.8% TRR, 0.002 ppm) were resolved. Total Characterized/Identified = 78.2% TRR, 0.054 ppm.
Non-extractable	16.5	0.012	N/A
Substrate = 370 DAT Sorghum Grain (0.017 ppm)			
Methanol	82.2	0.014	Acifluorfen (6.3% TRR, 0.001 ppm) was identified and Metabolite 1 (60.1% TRR, 0.010 ppm) was resolved. Total Characterized/Identified = 66.4% TRR, 0.011 ppm.
Non-extractable	17.8	0.003	N/A
Substrate = 103 DAT Radish Top (0.067 ppm)			
Methanol	86.6	0.058	Acifluorfen (34.6% TRR, 0.023 ppm) was identified and Metabolite 1 (30.7% TRR, 0.021 ppm), Metabolite 2 (8.4% TRR, 0.006 ppm), Metabolite 3 (4.2% TRR, 0.003 ppm), and Metabolite 4 (1.6% TRR, 0.001 ppm) were resolved. Total Characterized/Identified = 79.5% TRR, 0.054 ppm.
Non-extractable	13.4	0.009	N/A
Substrate = 313 DAT Radish Top (0.027 ppm)			
Methanol	85.3	0.023	Acifluorfen (16.6% TRR, 0.004 ppm) was identified and Metabolite 1 (54.1% TRR, 0.015 ppm) and Metabolite 3 (3.8% TRR, 0.001 ppm) were resolved. Total Characterized/Identified = 74.5% TRR, 0.020 ppm.
Non-extractable	14.7	0.004	N/A
Substrate = 103 DAT Radish Root (0.018 ppm)			

(continued; footnotes follow)

Table 3 (continued).

Fraction	%TRR	ppm	Characterization/Identification
Methanol	77.6	0.014	Acifluorfen (44.6% TRR, 0.008 ppm) was identified and Metabolite 1 (13.0% TRR, 0.002 ppm), Metabolite 2 (3.4% TRR, <0.001 ppm), Metabolite 3 (2.3% TRR, <0.001 ppm), and Metabolite 4 (3.0% TRR, <0.001 ppm) were resolved. Total Characterized/Identified = 66.3% TRR, <0.0013 ppm.
Non-extractable	22.4	0.004	N/A
Substrate = 313 DAT Radish Root (0.008 ppm)			
Methanol	77.8	0.006	N/A
Non-extractable	22.2	0.002	N/A
Substrate = 146 DAT Wheat Forage (0.035 ppm)			
Methanol	96.1	0.034	Acifluorfen (9.0% TRR, 0.003 ppm) was identified and Metabolite 1 (64.2% TRR, 0.023 ppm), Metabolite 2 (14.4% TRR, 0.005 ppm), and Metabolite 3 (4.5% TRR, 0.002 ppm) were resolved. Total Characterized/Identified = 92.1% TRR, 0.033 ppm.
Non-extractable	3.9	0.001	N/A
Substrate = 146 DAT Wheat Straw (0.082 ppm)			
Methanol	92.4	0.076	Acifluorfen (2.7% TRR, 0.002 ppm) was identified and Metabolite 1 (53.1% TRR, 0.044 ppm), Metabolite 2a (5.9% TRR, 0.005 ppm), Metabolite 3 (3.6% TRR, 0.003 ppm), and Metabolite 4 (1.4% TRR, 0.001 ppm) were resolved. Total Characterized/Identified = 66.7% TRR, 0.055 ppm.
Pellet	18.3	0.05	N/A
Non-extractable	7.6	0.006	N/A
Substrate = 146 DAT Wheat Grain (0.040 ppm)			
Methanol	91.1	0.036	Acifluorfen (1.8% TRR, <0.001 ppm) was identified and Metabolite 1 (64.3% TRR, 0.025 ppm), Metabolite 2 (23.5% TRR, 0.009 ppm) and Metabolite 3 (0.2% TRR, <0.001 ppm) were resolved. Total Characterized/Identified = 89.8% TRR, 0.036 ppm.
Non-extractable	8.9	0.004	N/A

• Metabolite 3 = Amino- and/or desnitro-acifluorfen.

(continued; footnotes follow)

Table 4. Summary of characterized/identified residues in methanol extracts of chard grown in aged sandy soil treated with [^{14}C]sodium acifluorfen at 0.5 lb ai/A.

Metabolite	103 DAT		313 DAT	
	%TRR	ppm	%TRR	ppm
Identified				
Acifluorfen	14.3	0.020	9.2	0.003
Amino- and/or desnitro-acifluorfen (Metabolite 3) [a]	6.5	0.009	3.1	0.001
Total Identified	**20.8**	**0.029**	**12.3**	**0.004**
Characterized				
Metabolite 1 [b]	35.5	0.050	65.0	0.021
Metabolite 2	9.4	0.013	--	--
Metabolite 4	6.1	0.009	--	--
Polar origin [c]	3.8	0.005	6.0	0.002
Total Characterized	**54.8**	**0.077**	**71.0**	**0.023**
Total Characterized/Identified	**75.6**	**0.106**	**84.1**	**0.027**
Organosoluble	86.7	0.121	96.0	0.031
Non-extractable	13.3	0.019	4.0	0.001

[a] Identified by TLC, but not confirmed; amino- and desnitro-acifluorfen reference standards were not adequately resolved by HPLC.
[b] The registrant stated that the structure was unknown.
[c] Radioactive component(s) at or near TLC origin.

Table 5. Summary of characterized/identified residues in methanol extracts of sorghum grown in aged sandy soil treated with [^{14}C]sodium acifluorfen at 0.5 lb ai/A.

Metabolite	39 DAT		370 DAT	
	%TRR	ppm	%TRR	ppm
Sorghum Forage				
Identified				
Acifluorfen	**18.3**	**0.011**	**12.9**	**0.002**
Characterized				
Metabolite 1 [a]	52.8	0.033	52.7	0.008
Metabolite 2	--	--	6.3	<0.001
Metabolite 3	1.2	0.001	1.8	<0.001
Metabolite 4	1.1	.001	--	--
Polar origin [b]	--	--	--	--
Total Characterized	**55.1**	**0.035**	**60.8**	**<0.010**
Total Characterized/Identified	**73.4**	**0.046**	**73.7**	**<0.012**
Organosoluble	75.5	0.047	90.7	0.013
Non-extractable	24.5 [c]	0.015	9.3	0.001
Sorghum Fodder				
Identified				
Acifluorfen	**12.3**	**0.024**	**4.5**	**0.001**
Characterized				
Metabolite 1 [a]	55.7	0.110	74.3	0.011
Metabolite 3	3.1	0.006	--	--
Metabolite 4	5.6	0.011	--	--
Total Identified	**64.4**	**0.127**	**74.3**	**0.011**
Total Characterized/Identified	**76.7**	**0.151**	**78.8**	**0.012**
Organosoluble	86.9	0.172	92.6	0.028
Non-extractable	13.1	0.026	7.4	0.002
Sorghum Grain				
Identified				
Acifluorfen	**20.5**	**0.014**	**6.3**	**0.001**
Characterized				
Metabolite 1 [a]	54.9	0.038	60.1	0.010
Metabolite 4	2.8	0.002	--	--
Total Identified	**57.7**	**0.040**	**60.1**	**0.010**
Total Characterized/Identified	**78.2**	**0.054**	**66.4**	**0.011**
Organosoluble	83.5	0.058	82.2	0.014
Non-extractable	16.5	0.012	17.8	0.003

[a] The registrant stated that the structure was unknown.
[b] Radioactive component(s) at or near TLC origin.
[c] Value includes radioactivity observed in a "pellet" that formed upon concentration of the extract.

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Table 6. Summary of characterized/identified residues in methanol extracts of radish grown in aged sandy soil treated with [^{14}C]sodium acifluorfen at 1x.

Metabolite	103 DAT		313 DAT	
	%TRR	ppm	%TRR	ppm
Radish Top				
Identified				
Acifluorfen	**34.6**	**0.023**	**16.6**	**0.004**
Characterized				
Metabolite 1 [a]	30.7	0.021	54.1	0.015
Metabolite 2	8.4	0.006	--	--
Metabolite 3	4.2	0.003	3.8	0.001
Metabolite 4	1.6	0.001	--	--
Total Identified	**44.9**	**0.031**	**57.9**	**0.016**
Total Characterized/Identified	**79.5**	**0.054**	**74.5**	**0.020**
Organosoluble	86.6	0.058	85.3	0.023
Non-extractable	13.4	0.009	14.7	0.004
Radish Root				
Identified				
Acifluorfen	**44.6**	**0.008**	**NA [b]**	**NA**
Characterized				
Metabolite 1 [a]	13.0	0.002	NA	NA
Metabolite 2	3.4	<0.001	NA	NA
Metabolite 3	2.3	<0.001	NA	NA
Metabolite 4	3.0	<0.001	NA	NA
Total Identified	**21.7**	**<0.005**	**NA**	**NA**
Total Characterized/Identified	**66.3**	**<0.013**	**NA**	**NA**
Organosoluble	77.6	0.014	77.8	0.002
Non-extractable	22.4	0.004	22.2	0.006

[a] The registrant stated that the structure was unknown.
[b] NA = Not analyzed; TRR <0.01 ppm.

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Table 7. Summary of characterized/identified residues in methanol extracts of wheat grown in aged sandy soil treated with [^{14}C]sodium acifluorfen at 1x.

Metabolite	145 DAT	
	%TRR	ppm
Wheat Forage		
Identified		
Acifluorfen	**9.0**	**0.003**
Characterized		
Metabolite 1 [a]	64.2	0.023
Metabolite 2	14.4	0.005
Metabolite 3	4.5	0.002
Total Characterized	**83.1**	**0.030**
Total Characterized/Identified	**92.1**	**0.033**
Organosoluble	96.0	0.034
Non-extractable	4.0	0.001
Wheat Straw		
Identified		
Acifluorfen	**2.7**	**0.002**
Characterized		
Metabolite 1 [a]	53.1	0.044
Metabolite 2a	5.9	0.005
Metabolite 3	3.6	0.003
Metabolite 4	1.4	0.001
Total Characterized	**64.0**	**0.053**
Total Characterized/Identified	**66.7**	**0.055**
Organosoluble	74.1	0.061
Non-extractable	25.9 [b]	0.021 [b]
Wheat Grain		
Identified		
Acifluorfen	**1.8**	**<0.001**
Characterized		
Metabolite 1 [a]	64.3	0.025
Metabolite 2	23.5	0.009
Metabolite 3	0.2	<0.001
Total Characterized	**88.0**	**<0.035**
Total Characterized/Identified	**89.8**	**0.036**
Organosoluble	91.1	0.036
Non-extractable	8.9	0.004

[a] The registrant stated that the structure was unknown.
[b] Value includes radioactivity observed in a "pellet" that formed upon concentration of the extract.

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Storage stability data

No concurrent storage stability data were presented. The following commodities were analyzed by TLC within ~1-1.5 months of harvest: (i) 145 DAT wheat straw and grain; (iii) 313 DAT radish top and root, and chard; and (iii) 370 DAT sorghum commodities. All other commodities (including 39 DAT sorghum fodder) were analyzed within ~9 months of harvest. The registrant stated that Metabolite 1 was stable in aqueous methanol extracts over the duration of the study, but no quantitative supporting data were presented. Quantitative data depicting the stability of Metabolite 1 in frozen storage are required. Also required are quantitative data depicting the stability of acifluorfen residues in frozen storage. The study is not supported by adequate storage stability data.

Study summary

The submitted confined rotational crop study is adequate to satisfy the 165-1 guideline requirements. The qualitative nature of the residues in rotational crops is adequately understood pending submission of adequate storage stability data. Total ^{14}C-residues exceeded 0.01 ppm in/on all commodities of chard, turnips, sorghum, radish, and wheat that were planted 39, 103, 145, 313, and 370 days after CPR labeled-[^{14}C] sodium acifluorfen was applied to sandy soil outdoors at 0.5 lb ai/A (1x the maximum seasonal rate). Residue accumulation declined from shorter rotation intervals to longer rotation intervals in/on all rotational crop commodities. Accumulation was lowest in/on radish roots from the 313-day rotation and greatest in/on chard (0.413 ppm) from the 29-day rotation.

The study characterized/identified ≥66.4% of the total radioactive ^{14}C-residues in/on all crop commodities. The parent, acifluorfen, was identified and confirmed in all rotational crop commodities at all sampling intervals and was highest in 39 DAT sorghum fodder (0.024 ppm, 12.3% TRR) and lowest in 145 DAT wheat grain (<0.001 ppm). The principal residue extracted into methanol from all crop substrates was Metabolite 1, an unknown polar component. Metabolite 1 accounted for 13-65% TRR in/on rotational crops. Metabolite 1 levels were highest in 39 DAT sorghum fodder (0.110 ppm, 55.7% TRR), and it was a component in all other rotational crop commodities (0.002-0.05 ppm) regardless of sampling interval. Three other unknown polar metabolites, designated as Metabolites 2, 3, and 4, also were detected in extracts of rotational crop commodities, but were present only at insignificant levels (<0.001-0.013 ppm). In wheat straw only, a component (designated Metabolite 2a) somewhat less polar than Metabolite 2, was present at insignificant levels (0.005 ppm). An unspecified, but minor, polar component also was detected in chard (0.002-0.005 ppm). Metabolite 3 was tentatively identified as either amino- or desnitro-acifluorfen only in 39 DAT chard (0.009 ppm, 6.5% TRR) by TLC and HPLC, but not confirmed. Metabolites 2, 2a, and 4 were not identified.

Exhaustive characterization procedures were conducted on methanol extracts of sorghum fodder in an attempt to identify Metabolite 1. The registrant suggested that metabolite M1 might be a sugar conjugate of 2-chloro-4-trifluoromethylphenol but was unable to propose a structure after mass spectrospic data had been analyzed.

EPA MEMORANDA CITED IN THIS REVIEW

CBRS No.: None
Subject: CBRS Transmittal Sheet for Phase 4 Reviews.
From: S. Funk
To: A. Rathman, E. Zager
Dated: 2/14/91
MRID: None

CBRS No.: 8741
Subject: Reregistration of Sodium Acifluorfen. BASF Corp. 90 Day Response to Phase 4 DCI. DP Barcode D169747
From: S. Funk
To: T. Luminello
Dated: 12/5/91
MRID: None

CBRS No.: 9784
Subject: Sodium Acifluorfen. Soybeans. Blazer Herbicide (EPA Reg. No. 7969-79) Label Revision Reducing Use Rate and Impact on DCI dated 6/7/91.
From: J. Smith
To: T. Luminello
Dated: 6/9/92
MRID: None

CBRS No.: 10199
Subject: Sodium Acifluorfen, Reregistration. BASF Corporation Response to Phase 4 Review. Metabolism in Peanuts and Rice. DP Barcode No. D180455.
From: J. Abbotts
To: J. Ellenberger
Dated: 12/9/92
MRIDs: 423683-01 and -02

MASTER RECORD IDENTIFICATION NUMBERS

Citations for the MRID documents referred to in this review are presented below.

Used:

4785601 Panek, M. G., Geiger, D. S., and Reese, C. E. (1993) ^{14}C-Sodium Acifluorfen Confined Rotational Crop. BASF Protocol No. 91058, BASF Report No. M9307. Unpublished study conducted by BASF Corporation, 13 p.

Not used:

4368301 Larson, J. D. (199) Metabolism of ^{14}C-Sodium Acifluorfen in Peanuts. Laboratory Project ID: HLA 637-100. Unpublished study performed by Hazelton Laboratories America, Inc. and sponsored by BASF Corporation. 131 p.