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

DEC 9 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Sodium Acifluorfen, Reregistration. BASF Corporation Response to Phase 4 Review. Metabolism in Peanuts and Rice (MRID Nos. 42368301, 42368302).
CBRS No. 10199. DP Barcode No. D180455.

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Attached is a review of residue chemistry data pertaining to the nature of the residue in peanuts and in rice, submitted by BASF Corporation in response to Phase 4 Review (2/14/91). This information was reviewed by Dynamac Corporation under supervision of CBRS, HED. The data assessment has undergone secondary review in the branch and has been revised to reflect branch policies.

The review identified deficiencies in both metabolism studies which are potentially upgradable. Additional work is necessary to identify metabolites.

If you need additional input please advise.

Attachment 1: Review of Sodium Acifluorfen Residue Chemistry Data
Attachment 2: Additional Guidance for Metabolism Studies
Attachment 3: Sodium Acifluorfen Unofficial Residue Chemistry Summary Through 12/9/92

cc (with Attachments 1 and 3):Abbotts, Reg. Std. File,
cc (with Attachment 1):Circ, SF, Dynamac
cc (without any Attachments):RF
RDI:FBSuhre:12/7/92:MSMetzger:12/8/92:EZager:12/8/92
H7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:12/9/92

DYNAMAC
CORPORATION
Environmental Services

Final Report

SODIUM ACIFLUORFEN
Shaughnessy No. 114402; Case 2605
(CBRS No. 10199; DP Barcode D180455)

TASK 4
Registrant's Response to Residue Chemistry Data Requirements

October 22, 1992

Contract No. 68-D2-0053

Submitted to:
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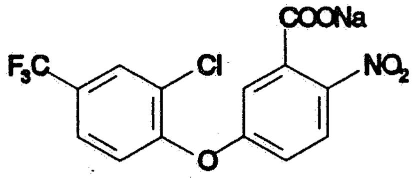
SODIUM ACIFLUORFEN



Shaughnessy No. 114402

(CBRS No. 10199; DP Barcode D180455; Case 2605)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Sodium Acifluorfen Phase 4 Reviews (S. Funk, 2/14/91) of available residue chemistry data identify the following deficiencies: plant and animal metabolism; residue analytical methods; storage stability; magnitude of the residue in peanuts, rice, and soybeans; processing studies in peanuts, rice, and soybeans; and magnitude of the residue in animals.

In response to requirements for reregistration, BASF Corporation submitted data pertaining to the metabolism of [^{14}C]sodium acifluorfen in peanuts (1992; MRID 42368301) and rice (1992; MRID 42368302). The submitted data are reviewed and evaluated in this document for adequacy in fulfilling the data requirements for plant metabolism.

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 or on plant and in animal commodities [40 CFR § 180.383]. The Pesticide Analytical Manual (PAM) Vol. II lists Method I, a GLC method with electron capture detection, as available for the enforcement of sodium acifluorfen tolerances.

The Conclusions and Recommendations stated below apply only to the nature of the residue of sodium acifluorfen in peanuts and rice. No other data requirements in the Sodium Acifluorfen Phase 4 Reviews are addressed herein.

CONCLUSIONS

1. Metabolism in peanuts: The qualitative nature of sodium acifluorfen in peanuts is not adequately understood because the study failed to identify the majority of ^{14}C-residues in samples of peanut commodities that were collected at maturity. The study may be upgraded if the registrant can address the specific issues and requirements listed below.

1a. In reporting radioactivity in each fraction, registrant should also report the initial sample TRR, allowing losses during extraction to be determined.

1b. Non-extractable residues remaining after acid hydrolysis represent barely 10% of TRR in vines, and smaller percentages in hulls and kernels. No further work is required to release the remaining non-extractable residues.

1c. Because HPLC System II was used to confirm the identity of metabolites, representative chromatograms from the confirmatory analysis should be provided.

1d. The data provided indicate that metabolism is extensive, and little acifluorfen is found, in mature peanut commodities. However, the data are insufficient to identify the metabolites present in mature commodities; less than 1% of TRR in kernels, 10% of TRR in hulls, and 16% of TRR in vines have been identified. Unidentified polar metabolites in the organic fraction from the ACN:HCl extraction account for up to 43.3% of TRR (0.370 ppm) in vines, and 39.2% of TRR (0.404 ppm) in hulls.

1e. Because of the small magnitudes of individual chromatographic peaks, further characterization of residues in peanut kernels is not required. In mature hulls and vines, each individual metabolite ≥10% of TRR should be identified. Residues extracted by either or both of the ACN:HCl method and the Bligh-Dyer method may be used for identification, but the overall tabulation of identified residues must be reconciled between the two extracts (that is, "double-counting" the same metabolite from both extracts is not allowed). Identification or characterization of residues should be consistent with Additional Guidance for Conducting Plant and Livestock Metabolism Studies, D. Edwards and E. Zager, 7/16/92, a copy of which is attached.

1f. Registrant proposed that metabolism of [^{14}C]sodium acifluorfen in peanut plants proceeds by conversion of the parent compound to form polar and acidic conjugates, reduction and cleavage of the nitro group, decarboxylation, and cleavage of the diphenyl ether bond. The registrant also believes that ^{14}C-fractions are incorporated into insoluble plant materials. Because polar metabolites and/or conjugates have not been identified, and evidence for

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incorporation into plant components has not been provided, the proposed metabolic scheme cannot be considered justified.

1g. Storage times and conditions at the analytical laboratory must be provided. If storage times were greater than 6 months between harvest and analysis, evidence should be provided that the profile of residues did not change during the period between collection and final analysis.

1h. Radiovalidation of method(s) using samples from the metabolism study remains an outstanding requirement, pending determination of the residues to be regulated.

2. Metabolism in Rice: The qualitative nature of the residue in rice is not adequately understood because complete quantitative data pertaining to metabolite identification were not submitted; the results of metabolite identification in rice straw were described by the registrant in narrative text. It may be possible to upgrade the study if the registrant can address the specific issues and requirements listed below.

2a. The registrant should resolve the discrepancies in the reported HPLC retention times for reference standards of acifluorfen and amino-acifluorfen vs. the reported retention time for acifluorfen in rice samples. This discrepancy could be resolved by the submission of HPLC profiles of reference compounds recorded on the same day as the analysis of metabolites.

2b. Samples were collected at 97 days, while samples at the minimum PHI of 50 days might have contained greater TRR. Considering that acifluorfen is still present in the 97 day samples, metabolism would be expected to be more extensive than at the minimum PHI. The samples at 97 days may therefore be acceptable, provided identification of residues is adequate.

2c. Registrant should explain in more detail how quantitative assignment of residues in rice grain was made. Assignments should be supported by calculations and data on radioactivity applied to TLC plates and recovered.

2d. Organosoluble residues from hulls should be examined for the putative acifluorfen peak; identification of acifluorfen by two methods in hull extracts could be translated to grain.

2e. For each identified metabolite (whether tentative or confirmed) in rice straw, complete quantitative data must be provided. These data should be presented in a table which must include the amount (% of TRR and ppm equivalents) for each extractable fraction of rice straw. The amount (% of TRR and ppm equivalents) of unidentified polar metabolites should also be provided. Further characterization or identification of residues should be conducted based on Additional Guidance for metabolism studies (attached). Cleavage by appropriate enzymes should facilitate identification of putative conjugates.

2f. The registrant proposed that [^{14}C]sodium acifluorfen is metabolized in rice by two pathways: (i) a route involving formation of amino acifluorfen by the reduction of nitro group; and (ii) a route involving rapid cleavage of the diphenyl ether bond. Only two metabolites, acifluorfen and amino-acifluorfen, were identified in rice commodities, and the identification of acifluorfen was confirmed only in straw. The proposed metabolic route involving cleavage of the diphenyl ether bond cannot be considered justified until putative conjugated metabolites are identified.

2g. The data presented are insufficient to validate the stability during frozen storage of organosoluble residues in grain, nor aqueous soluble residues in straw. Storage conditions between harvest and homogenization should be provided for grain and straw. Considering the very long storage times, evidence should be provided that the profiles of radioactive residues did not change during storage. If chromatographic profiles at early extraction are not available, then material balances indicating that residue identification and quantitation account for nearly all residues at early extraction will be sufficient evidence for storage stability.

2h. Radiovalidation of method(s) using samples from the metabolism study remains an outstanding data requirement, pending determination of the residues to be regulated.

RECOMMENDATIONS

Further work is necessary to resolve the deficiencies identified in Conclusions 1 and 2 above. If it proves necessary to conduct a new metabolism study in rice, the nature of the residue in grain and straw at a PHI of 50 days should be determined; exaggerated rates may be necessary to generate TRRs large enough for adequate identification. The results of the present study can be used as guidance on which compounds would be likely metabolites.

A copy of Additional Guidance for Conducting Plant and Livestock Metabolism Studies, D. Edwards and E. Zager (7/16/92), is provided as an attachment to this review. The registrant should consult this document for guidance on the extent to which further characterization/identification of unidentified metabolites should be conducted. However, the registrant should also recognize that the goal of a metabolism study is identification of the chemical components of the residue.

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The molecular structures of sodium acifluorfen along with the metabolites identified (tentatively or with confirmation) in peanut and rice commodities are presented in Table 1.

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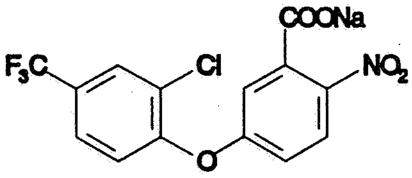
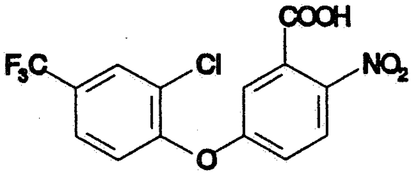
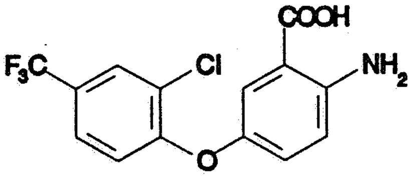
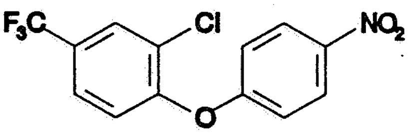
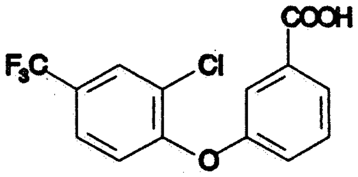
Table 1. Sodium acifluorfen and its metabolites in peanut and rice commodities.

Code	Chemical Name	Structure	Substrate	MRID	Common Name
I.	Sodium 5-(2-chloro-4-trifluoromethylphenoxy)-2-nitrobenzoate				**sodium acifluorfen**
II.	5-(2-Chloro-4-trifluoromethylphenoxy)-2-nitrobenzoic acid		peanut kernels and hulls peanut vines rice grain and forage rice straw	42368301[a] 42368301 42368302[a] 42368302	**acifluorfen**
III.	2-Amino-5-(2-chloro-4-trifluoromethylphenoxy)benzoic acid		peanut kernels and hulls peanut vines rice grain and straw	42368301[a] 42368301 42368302[a]	**amino acifluorfen**

(continued; footnotes follow)

Table 1 *(Continued)*.

Code	Chemical Name	Substrate	MRID
Structure		Common Name	
IV.	4-(2-Chloro-4-trifluoromethylphenoxy)nitrobenzene		
		peanut kernels and hulls	42368301[a]
		peanut vines	42368301
		descarboxyl-acifluorfen	
V.	3-(2-Chloro-4-trifluoromethylphenoxy)benzoic acid		
		peanut kernels and hulls	42368301[a]
		peanut vines	42368301
		desnitro-acifluorfen	

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[a] Identification not confirmed by a second method.

DETAILED CONSIDERATIONS

Reregistration Requirements

Phase 4 Review (2/14/91, S.R. Funk) found that new plant metabolism studies were required for reregistration. Sodium acifluorfen radiolabeled in a non-labile part of the molecule (such as one or perferably both of the phenyl rings) should be applied to a legume vegetable (soybeans), a cereal grain (rice), and peanuts reflecting the currently registered uses. The specific activity and/or application rates should be high enough to allow for adequate identification of the metabolites/degradates. Efforts should be made to extract the majority of the radiolabeled chemicals (≥ 90%) and all major radiolabeled extracted metabolites must be characterized. Major components (≥ 0.1 ppm) of unextractable residue should be released (acid, base, enzyme) and identified. The plant material from the metabolism studies should be tested using the data collection method(s) and enforcement analytical method(s). The present submission consisted of metabolism studies on peanuts and rice.

Metabolism of [^{14}C]sodium acifluorfen in peanuts

In-life phase

BASF Corporation submitted data (1992; MRID 42368301) pertaining to the metabolism of [^{14}C]sodium acifluorfen in peanuts. Field-grown peanuts received two foliar applications of uniformly CF_3-ring-labeled [^{14}C]sodium acifluorfen (specific activity 8.005 μCi/mg, 3.071 mCi/mMol, 1.777 x 10^4 dpm/μg; radiochemical purity 98.3%) at a rate equivalent to 0.5 lb ai/A/application (1x the maximum registered single use rate). The first application was made when the plants were in bloom stage and the second 22 days following the first, when the plants were in the early pegging stage.

Presently, a 2 lb/gal SC/L formulation (Blazer®, EPA Reg. No. 7969-79) is registered to BASF for multiple postemergence applications on peanuts at 0.25-0.5 lb ai/A/application with an established maximum seasonal rate of 2 lb ai/A and a preharvest interval of 75 days; the label restricts the feeding of sodium acifluorfen-treated feed or forage of peanuts to livestock.

Samples of vines, hulls, and kernels were collected just prior to treatment, at 0 and 22 days following the first application, and at 1, 27, 48, and 62 days following the second application. Samples were immediately frozen on dry or blue ice and transported to freezers where they were stored at -30° to -20° C. Samples were shipped on dry ice to the analytical laboratory (Hazleton Laboratories America, Inc., Madison, WI) where they were kept frozen at unspecified temperatures and intervals until processing and analysis.

Total radioactive residues (TRR)

Prior to analysis, vines were separated from the roots at the first joint, just below the aerial branches. Nuts were separated into hulls and kernels before shipment to the analytical laboratory. Total radioactive residues (TRR) were determined in triplicate subsamples by combustion followed by liquid scintillation spectrometry (LSS); the detection limit was 0.00936 ppm. The TRR, determined by combustion of peanut vines, hulls, and kernels are presented in Table 2.

The TRR reached maximums of 14.848 ppm in vines, 1.108 ppm in hulls, and 1.164 ppm in kernels following the second foliar treatment (1-48 days posttreatment). The TRR decreased in mature samples (collected 62 days posttreatment); these data indicate that radioactive residues of sodium acifluorfen are translocated to some extent from the foliage to kernels.

Table 2. Total radioactive residues (TRR) in or on peanut vines, hulls, and kernels following one or two foliar applications of [^{14}C]sodium acifluorfen at 1x the maximum registered single use rate.

Matrices PTI[a] (days)	Number of Samples	TRR as [^{14}C]sodium acifluorfen equivalents (ppm)
Vines		
1st Application		
0	2	3.585, 5.374
22	3	0.304, 0.433, 0.514
2nd Application		
1	3	5.436, 6.738, 14.848
27	2	0.616, 0.688
48	2	0.510, 0.601
62	2	0.727, 0.854
Hulls		
2nd Application		
27	3	0.539, 0.571, 0.670
48	3	0.770, 0.774, 1.108
62	3	0.809, 0.979, 0.997
Kernels		
2nd Application		
27	2	0.115, 0.138
48	2	0.140, 1.164
62	2	0.136, 0.150

[a] Posttreatment interval, days after first or second application.

Extraction of residues

Two extraction methods were used. In the first method, samples of vines, hulls, and kernels from each sampling interval were extracted with acetonitrile:1 N hydrochloric acid (ACN:HCl, 7:3, v:v). In the second method, a modified Bligh-Dyer procedure was used where samples were homogenized and extracted by sequential additions of chloroform, methanol, and water. The Bligh-Dyer extraction method was used only on mature samples collected at the final sampling interval (62 days posttreatment).

Characterization of residues

The peanut tissue extracts obtained by each extraction procedure were filtered and the filtrates were partitioned into aqueous, organic, and non-extractable fractions. The ACN:HCl filtrates were further extracted with toluene. The filtrates from the Bligh-Dyer procedure were sequentially extracted with chloroform and ethyl acetate. All organosoluble fractions were pooled and concentrated by rotatory evaporation, lyophilization, or evaporation under a stream of nitrogen. The radioactivity present in organic and aqueous fractions was quantified by LSS; the non-extractable radioactivity was determined by LSS following combustion. The distribution of radioactivity in the organic, aqueous, and solid fractions is presented in Table 3.

Both extraction procedures provided adequate and comparable recovery of ^{14}C-residues. Approximately 86-116% of the TRR was recovered by the ACN:HCl method and 105-108% of TRR by the Bligh-Dyer method (Table 3). However, the distribution of TRR with each extraction method varied slightly. In mature samples of peanut vines and hulls, the ACN:HCl method partitioned the majority of the radioactivity into the organic fraction whereas the Bligh-Dyer method partitioned much of the radioactivity into the aqueous-soluble fraction. In peanut kernels, the aqueous-soluble residues accounted for 29.7-42.5% of TRR (0.041-0.059 ppm). It should be noted that the registrant used the sum of the radioactivity determined in each organosoluble, aqueous-soluble, and non-extractable fraction to calculate the % of TRR in each fraction; therefore loss of radioactivity, if any, could not be determined.

Conclusion 1a: In reporting radioactivity in each fraction, registrant should also report the initial sample TRR, allowing losses during extraction to be determined.

Table 3. Distribution of radioactive residues in organic, aqueous, and non-extractable fractions of peanut vines, hulls, and kernels following one or two foliar applications of $[^{14}C]$sodium acifluorfen at 1x the maximum registered single use rate.

Matrices and PTI[a] (days)	TRR as $[^{14}C]$sodium acifluorfen equivalents[b]						Total Recovery %
	Organic		Aqueous		Non-extractable		
	ppm	%	ppm	%	ppm	%	
Vines							
1st Application							
0	3.49	89.2	0.105	2.7	0.318	8.1	109.1
	5.20	91.2	0.120	2.1	0.382	6.7	106.1
22	0.187	59.5	0.070	22.3	0.057	18.2	103.2
	0.331	60.1	0.122	22.2	0.098	17.7	107.1
2nd Application							
1	5.330	88.0	0.201	3.3	0.524	8.7	111.3
27	0.370	53.9	0.153	22.3	0.163	23.8	111.5
48	0.267	50.4	0.145	27.4	0.118	22.2	104.0
	0.361	55.6	0.131	20.2	0.157	24.2	108.1
62	0.435	53.5	0.178	21.9	0.200	24.6	112.0
	0.437	49.9	0.238	27.1	0.202	23.0	102.7
62[c]	0.067	9.2	0.337	46.4	0.204	28.1	105.6
	0.159	21.9					
Hulls							
2nd Application							
27	0.250	36.2	0.321	46.6	0.118	17.2	108.1
48	0.417	41.0	0.425	41.8	0.175	17.2	110.9
62	0.561	49.9	0.332	29.5	0.232	20.6	109.2
62[c]	0.105	13.0	0.384	47.5	0.134	16.6	104.5
	0.222	27.4					
Kernels							
2nd Application							
27	0.023	21.4	0.041	39.1	0.041	39.5	85.8
48	0.030	17.5	0.052	29.7	0.092	52.8	111.0
62	0.027	16.2	0.057	34.5	0.082	49.3	116.1
62[c]	0.010	7.2	0.059	42.5	0.054	39.1	108.3
	0.027	19.5					

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[a] Posttreatment interval, days after first or second applications.

[b] Radioactivity extracted by ACN:HCl method, unless indicated otherwise.

[c] Radioactivity extracted by modified Bligh-Dyer method and partitioned into ethyl acetate and chloroform respectively.

Hydrolysis of aqueous-soluble and non-extractable residues

The aqueous-soluble and non-extractable fractions of peanut tissue matrices, collected 62 days after the second foliar application and extracted by ACN:HCl, were subjected to acid hydrolysis. The aqueous extracts (pH 1.0) were refluxed for 1 hour at an unspecified temperature; the solids were refluxed with 2 N HCl for 4 hours at an unspecified temperature and filtered. The aqueous-soluble and non-extractable hydrolysates were separately mixed with 2% sodium chloride and partitioned three times with ethyl acetate. The results of these procedures are presented in Table 4.

Table 4. Radioactivity released by acid hydrolysis of the aqueous and non-extractable fractions from ACN:HCl extract of peanut vines, hulls, and kernels.

Fractions	TRR as [^{14}C]sodium acifluorfen equivalents					
	Vines		Hulls		Kernels	
	ppm	%	ppm	%	ppm	%
Initial TRR	0.854	100.0	1.031	100.0	0.143	100.0
Organosoluble	**0.437**	**49.9**	**0.561**	**49.9**	**0.027**	**16.2**
Aqueous-soluble	**0.238**	**27.1**	**0.332**	**29.5**	**0.057**	**34.5**
Hydrolyzed/aqueous	0.045	5.1	0.022	2.0	0.016	9.6
Hydrolyzed/EtAc	0.182	20.7	0.290	25.8	0.037	22.1
Unaccounted[•]	0.011	-	0.020	1.7	0.004	2.8
Non-extractable	**0.202**	**23.0**	**0.232**	**20.6**	**0.082**	**49.3**
Hydrolyzed/aqueous	0.023	2.6	0.014	1.2	0.037	22.2
Hydrolyzed/EtAc	0.017	1.9	0.039	3.5	0.022	13.0
Unhydrolyzed	0.092	10.5	0.100	8.9	0.012	7.5
Unaccounted[•]	0.070	8.0	0.079	7.0	0.011	6.6

[•] Calculated by the study reviewer.

The acid hydrolysis of aqueous-soluble fraction released an additional 25.8% of TRR (0.227 ppm) in vines, 27.8% of TRR (0.312 ppm) in hulls, and 31.7% of TRR (0.053 ppm) in kernels. The hydrolysis of non-extractable fraction released an additional 4.5% of TRR (0.04 ppm) in vines, 4.7% of TRR (0.053 ppm) in hulls, and 35.2% of TRR (0.059 ppm) in kernels. It is estimated by the study reviewer that up to 9.4% of TRR (0.015 ppm) was unaccounted (i.e. lost due to evaporation and/or work up).

The non-extractable residues remaining after hydrolysis accounted for 10.5% of TRR (0.092 ppm) in vines, 8.9% of TRR (0.1 ppm) in hulls, and 7.5% of TRR (0.012 ppm) in kernels.

Conclusion 1b: Non-extractable residues remaining after acid hydrolysis represent barely 10% of TRR in vines, and smaller percentages in hulls and kernels. No further work is required to release the remaining non-extractable residues.

Identification of residues extracted by ACN:HCl method

Aliquots of the toluene fractions from this extraction method as well as the extractable hydrolysates were analyzed using one- or two-dimensional thin-layer chromatography (TLC) on silica gel plates developed with the following solvent systems: (i) A: chloroform:acetic acid (9:1, v:v); (ii) B: n-butanol:acetic acid:water (12:3:5, v:v:v); and (iii) C: toluene:tetrahydrofuran:acetic acid (45:30:1, v:v:v). It was not specified which solvent system was used for each peanut commodity or fraction. Radioactive areas were located by autoradiography, visualized under UV light, and identified by comparison to reference standards (acifluorfen, amino-acifluorfen, descarboxyl-acifluorfen, and desnitro-acifluorfen), which had been cochromatographed with the aliquots. Selected TLC plates were divided into zones, and the silica gel was scraped from the plates and analyzed using LSS. Representative TLC chromatograms were provided.

To confirm the nature of the metabolites tentatively identified by TLC, aliquots of the pooled organic fractions (toluene and extractable hydrolysates) of peanut vines were analyzed using one of these three high-performance liquid-chromatography (HPLC) systems: (i) System I: Licrosorb RP-18 column with a mobile phase of 100% water changing to 100% acetonitrile in a series of step and linear gradients over 75 minutes, and UV detection at 254 nm; (ii) System II: Bondapak phenyl column with a mobile phase of ACN:water:methanol (45:45:10, v:v:v), and UV detection at 215 nm; and (iii) System III: Lichrosorb Si-100 column with a mobile phase consisting of 100% chloroform:acetic acid (9:1, v:v) changing to 100% isopropanol in a series of step and linear gradients over 65 minutes, and UV detection at 254 nm. The radioactivity in the HPLC eluate fractions was determined by LSS.

Prior to HPLC analysis, the ACN:HCl extracted organosoluble fractions from the vines were pooled, concentrated, and separated by preparative 1-D TLC using solvent System A, and radioactive zones were identified by autoradiography. Four regions corresponding to reference standards of acifluorfen (Region A), amino-acifluorfen (Region B), descarboxyl-acifluorfen (Region C), and desnitro-acifluorfen (Region D) were removed from the plates by sequential elution with chloroform and methanol. The eluates from the silica gel plates were then concentrated and analyzed to confirm the presence of acifluorfen and its metabolites using HPLC

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System II. No raw data or representative chromatograms depicting analysis of organosoluble metabolites by HPLC System II were presented by the registrant.

Conclusion 1c: Because HPLC System II was used to confirm the identity of metabolites, representative chromatograms from the confirmatory analysis should be provided.

The identification and distribution of radioactive residues from the organic fraction in or on peanut commodities is presented in Table 5.

The parent compound, sodium acifluorfen, was not identified in any peanut commodities.

In <u>peanut vines</u> collected immediately after the first foliar application, 76.6% of TRR (4.110 ppm) was tentatively identified by TLC as acifluorfen; amino-acifluorfen, descarboxyl-aciflurofen, and desnitro-acifluorfen were minor metabolites, each comprising $\leq$1.3% of TRR. In mature peanut vines, all of these metabolites were identified by TLC <u>and</u> confirmed by HPLC; they collectively accounted for 6.3-15.8% of TRR (0.033-0.135 ppm). The unidentified polar metabolites accounted for up to 43.3% of TRR (0.370 ppm) in mature vine samples (collected 62-days posttreatment).

In <u>peanut hulls</u> (10% of TRR, 0.101 ppm) and <u>kernels</u> (0.8% of TRR, 0.008 ppm), the same metabolites were tentatively identified by TLC but not confirmed. In mature samples, the unidentified polar metabolites accounted for 39.2% of TRR (0.404 ppm) in hulls and 35.8% of TRR (0.051 ppm) in kernels.

The registrant stated that these polar metabolites are a complex mixture of low molecular weight components and that a large percentage of ^{14}C-residues had retention times between those of acifluorfen and its desnitro metabolite in the HPLC systems. These polar metabolites must be further characterized/identified.

Table 5. Identification (by TLC) of ^{14}C-residues from the organosoluble fractions of peanut matrices extracted with ACN:HCl. Bolding indicates that a confirmatory technique (HPLC) was used.

Matrices PTI[a] (days)	TRR as [^{14}C]sodium acifluorfen equivalents											
	Acifluorfen		Descarboxyl-acifluorfen		Amino-acifluorfen		Desnitro-acifluorfen		Total Identified Metabolites		Unidentified Polar Metabolites	
	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%
Vines												
1st Application												
0	4.110	76.6	0.070	1.3	0.043	0.8	0.021	0.4	4.244	79.1	0.328	6.1
	2.500	69.5	0.086	2.4	0.047	1.3	0.047	1.3	2.680	74.5	0.226	6.3
22	0.015	2.9	0.006	1.1	0.003	0.5	0.006	1.2	0.030	5.7	0.164	32.0
	0.012	3.8	0.002	0.8	0.009	2.9	0.003	1.0	0.026	8.5	0.095	31.2
2nd Application												
1	3.990	73.3	0.027	0.5	0.049	0.9	0.076	1.4	4.142	76.1	0.288	5.3
27	0.029	4.7	0.005	0.8	0.007	1.1	0.009	1.4	0.050	8.0	0.167	27.1
48	0.027	4.5	0.007	1.2	0.006	1.0	0.006	1.0	0.046	7.7	0.153	25.4
	0.020	4.0	0.003	0.5	0.005	0.9	0.005	0.9	0.033	6.3	0.149	29.3
62	0.073	8.6	0.012	1.4	0.023	2.7	0.014	1.6	0.122	14.3	0.228	26.7
	0.065	8.9	0.013	1.8	0.023	3.1	0.012	1.6	0.113	15.4	0.181	24.9
62-Hydrolyzed[b]	0.078	9.2	0.014	1.6	0.026	3.0	0.017	2.0	0.136	15.8	0.370	43.3
Hulls												
2nd Application												
27	0.016	2.5	0.005	0.8	0.005	0.8	0.017	2.7	0.043	6.8	0.164	25.7
48	0.039	4.3	0.005	0.5	0.017	1.8	0.004	0.4	0.065	7.0	0.271	29.5
62	0.036	3.5	0.007	0.7	0.028	2.7	0.010	1.0	0.081	7.9	0.315	30.6
62-Hydrolyzed[b]	0.044	4.3	0.009	0.9	0.031	3.0	<0.017	1.7	<0.101	9.9	0.404	39.2
Kernels												
2nd Application												
27	ND	ND	<0.001	<0.1	<0.001	0.1	ND	ND	<0.002	<0.2	0.020	16.4
48	<0.001	0.1	<0.001	0.1	<0.001	0.1	<0.001	0.2	<0.004	0.5	0.020	12.5
62	<0.001	0.2	<0.001	0.2	<0.001	0.2	<0.001	0.1	<0.004	0.7	0.015	10.5
62-Hydrolyzed[b]	<0.002	<0.2	<0.002	<0.2	<0.002	<0.2	<0.002	<0.2	<0.008	<0.8	0.051	35.8

[a] Posttreatment interval, days following the first or second foliar application.

[b] These data represent the ethyl acetate fractions of samples that were acid-hydrolyzed.

Identification of residues extracted by Bligh-Dyer Method

The aqueous and organic fractions of 62-day samples of peanut vines, hulls, and kernels were analyzed using HPLC Systems I, II, and/or III as previously described. Prior to HPLC analysis, the aqueous fractions from all the three peanut tissues were lyophilized and extracted with methanol; the methanol extract was reserved for metabolite identification. The organosoluble extracts were analyzed directly without additional sample preparation, unless otherwise indicated.

Vines: Characterization of the ethyl acetate fraction (9.2% of TRR, 0.067 ppm; see Table 3) yielded five peaks (all <0.049 ppm) using HPLC Systems I and seven peaks (all <0.023 ppm) using HPLC System III. None of these were identified. The chloroform fraction (21.9% of TRR, 0.159 ppm) was purified on a silica gel column eluted sequentially with hexane, chloroform, methanol and water. Only the radioactive residues eluted with chloroform (13.3% of TRR, 0.097 ppm) were analyzed by HPLC System III; this process separated four radioactive components (11.0% of TRR, <0.059 ppm) that were not identified. The radioactivity present in other eluates (2.6-3.3% of TRR, 0.019-0.024 ppm) was also not identified. HPLC analysis (System I) of the aqueous fraction (46.4% of TRR, 0.337 ppm; see Table 3) yielded three major peaks ranging from 14.0 to 24.4% of TRR (0.102-0.177 ppm) but none of these peaks corresponded with any of the reference standards. Six minor peaks collectively accounted for 23.9% of TRR (<0.044 ppm) but were not identified.

Hulls: Aliquots of the ethyl acetate fraction (13.0% of TRR, 0.105 ppm; see Table 3) were analyzed by HPLC Systems I and III. HPLC System I separated one large peak (10.3% of TRR, 0.083 ppm of TRR) and six minor peaks (totaling <3.7% of TRR, <0.001-0.009 ppm). HPLC System III separated seven components (totaling 11.7% of TRR, 0.001-0.064 ppm). None of these peaks corresponded with the reference standards. The chloroform fraction (27.4% of TRR, 0.222 ppm; see Table 3), when analyzed by HPLC System III, yielded one major radioactive peak (11.3% of TRR, 0.091 ppm) which eluted within the retention time span of acifluorfen and the other three derivatives, but no further characterization was carried out. The aqueous fraction (47.5% of TRR, 0.384 ppm) was analyzed by HPLC System I; acifluorfen accounted for 4.7% of TRR (0.038 ppm) and desnitro-acifluorfen accounted for 0.5% of TRR (0.004 ppm) of TRR. Six other radioactive peaks were found; peak #5 accounted for 15.1% of TRR (0.122 ppm) and the remaining five peaks collectively accounted for 25.0% of TRR (0.006-0.065 ppm).

Kernels: The aqueous fraction of peanut kernels (42.5% of TRR, 0.059 ppm; see Table 3) was analyzed by reverse-phase HPLC System I, recovering 90.8% of the radioactivity as eight peaks. Peak #4 (4.8% of TRR, 0.007 ppm) and peak #8 (<0.5% of TRR, <0.001 ppm) appeared to coelute with acifluorfen and desnitro-acifluorfen, respectively; the remaining six other radioactive peaks accounted for 0.5-5.7% of TRR (<0.001-0.008 ppm) but were not identified.

CBRS Comments, Identification of residues

Conclusion 1d: The data provided indicate that metabolism is extensive, and little acifluorfen is found, in mature peanut commodities. However, the data are insufficient to identify the metabolites present in mature commodities; less than 1% of TRR in kernels, 10% of TRR in hulls, and 16% of TRR in vines have been identified. Unidentified polar metabolites in the organic fraction from the ACN:HCl extraction account for up to 43.3% of TRR (0.370 ppm) in vines, and 39.2% of TRR (0.404 ppm) in hulls.

In kernels, even if residues were 5 times higher, the standards of Additional Guidance (attached) would only require characterization of the residues, consisting of comparing the chromatographic behavior of residues with those of standards. This has already been performed, and no further characterization of residues in kernels is required. Metabolites in hulls tentatively identified by TLC are also small enough in magnitude that only characterization, and not identification by a second method, is required. However, in the absence of useful data from kernels, the nature of the residue in hulls and vines must be determined.

Conclusion 1e: Because of the small magnitudes of individual chromatographic peaks, further characterization of residues in peanut kernels is not required. In mature hulls and vines, each individual metabolite $\geq$10% of TRR should be identified. Residues extracted by either or both of the ACN:HCl method and the Bligh-Dyer method may be used for identification, but the overall tabulation of identified residues must be reconciled between the two extracts (that is, "double-counting" the same metabolite from both extracts is not allowed). Identification or characterization of residues should be consistent with Additional Guidance for Conducting Plant and Livestock Metabolism Studies, D. Edwards and E. Zager, 7/16/92, a copy of which is attached.

Conclusion 1f: Registrant proposed that metabolism of [^{14}C]sodium acifluorfen in peanut plants proceeds by conversion of the parent compound to form polar and acidic conjugates, reduction and cleavage of the nitro group, decarboxylation, and cleavage of the diphenyl ether bond. The registrant also believes that ^{14}C-fractions are incorporated into insoluble plant materials. Because polar metabolites and/or conjugates have not been identified, and evidence for incorporation into plant components has not been provided, the proposed metabolic scheme cannot be considered justified.

Storage stability

Vine, hull, and kernel samples were stored for 28-71 days at -29 to -17 C before shipment to the analytical laboratory. Samples were stored frozen at unspecified temperatures upon arrival at the analytical laboratory, but the time intervals between receipt, sample extraction, and analysis of the samples were not reported.

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Conclusion 1g: Storage times and conditions at the analytical laboratory must be provided. If storage times were greater than 6 months between harvest and analysis, evidence should be provided that the profile of residues did not change during the period between collection and final analysis.

Residue method validation

Samples from this metabolism study were not analyzed using the current enforcement methods. According to the Pesticide Analytical Manual (PAM) Vol. II, Method I, a GLC method with electron capture detection is available for the enforcement of sodium acifluorfen tolerances.

Conclusion 1h: Radiovalidation of method(s) using samples from the metabolism study remains an outstanding requirement, pending determination of the residues to be regulated.

Metabolism of [^{14}C]sodium acifluorfen in rice

In-life phase

BASF Corporation submitted data (1992; MRID 42368302) pertaining to the metabolism of sodium acifluorfen in rice. Greenhouse-grown rice plants that had been planted in three 55-gallon barrels received one "over-the-top" foliar application of uniformly CF_3-ring-labeled [^{14}C]sodium acifluorfen (specific activity 3.071 mCi/mMol, radiochemical purity 99.4%) when the plants were in the early boot stage of growth at a rate equivalent to 0.43 lb ai/A (3.4x the maximum registered single use rate or 1.7x the maximum seasonal use rate).

Presently, a 2 lb/gal SC/L formulation (Blazer®, EPA Reg. No. 7969-79) is registered to BASF for two postemergence applications to rice at 0.125 lb ai/A/application. A 50-day preharvest interval is in effect. The label restricts the feeding of sodium acifluorfen-treated rice plants to livestock. (It should be noted that label restrictions on feeding rice commodities to livestock are presently considered ineffective.)

Samples of forage and immature straw were collected 2 hours (day 0), and 7, 40, days posttreatment; samples of dry straw and grain were collected 97 days posttreatment. The grain heads were separated into florets and the rachis. The registrant stated that the florets were predominantly unfertilized and meticulously picked through to find mature grain; very few grains (ca. 1 g) were harvested from the test barrels. All samples were frozen (temperature unspecified) for an unspecified interval prior to laboratory workup. The analytical laboratory work was conducted by ABC Laboratories (Columbia, MO).

Total radioactive residues

Total radioactive residues (TRR) in or on forage, straw, and grain were determined by liquid scintillation spectrometry (LSS) following combustion. The limit of detection was calculated by the study reviewer to be 0.0014 ppm. The registrant reported that triplicate subsamples of each matrix were analyzed; however, the TRR results of all these analyses were not provided. The TRR in the harvested commodities of rice from this metabolism study are presented in Table 6.

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Table 6. Total radioactive residues (TRR) in or on rice forage, straw, hull, and grain following one foliar application of [^{14}C]sodium acifluorfen at 3.4x maximum registered single use rate.

Posttreatment Intervals (Days)	No. of samples	TRR as [^{14}C]sodium acifluorfen equivalents (ppm)			
		Forage	Straw	Hull	Grain
0	1	5.51	ND[a]	ND	ND
7	3	3.06	ND	ND	ND
40	1	2.59	ND	ND	ND
97	2	-	1.90, 2.0	0.157	0.027

[a] Not determined.

Extraction of residues

Subsamples of forage and straw were extracted three times with acetone. The acetone extracts were pooled, concentrated, and partitioned in ethyl acetate and water; the partitioning yielded ethyl acetate, aqueous, and solid fractions. The solid fraction of straw was re-extracted with water yielding a second aqueous extract. The radioactivity in the organic and aqueous extracts was determined by LSS; the radioactivity in the non-extractable solids was determined by LSS following combustion.

A second batch of straw samples was extracted three times with acetone:water (50:50, v:v), filtered, and partitioned in ethyl acetate yielding ethyl acetate, aqueous and solid fractions.

The remaining grain sample (ca. 0.405 g) was sequentially extracted with ethyl acetate and methanol. The extracts were pooled and concentrated. The organosoluble extract was reserved for metabolite identification without further characterization because of limited grain sample. The hulls were extracted four times with ethyl acetate; the solid residues were extracted with 50% sodium hydroxide, the pH of the extract was adjusted to 2, and partitioned with ethyl acetate. The extracts were then radioassayed by LSS. The distribution of radioactivity in the organosoluble, aqueous-soluble, and non-extractable fractions of forage, straw, hull, and grain is given in Table 7.

To test the efficiency of the extraction schemes, subsamples of untreated rice commodities were fortified with [^{14}C]sodium acifluorfen. Samples were then extracted using procedures described above and radioassayed. Recoveries of >95% were reported.

Table 7. Distribution of radioactive residues in organic, aqueous, and non-extractable fractions of rice forage, straw, hull, and grain following one foliar application of [^{14}C]sodium acifluorfen at 3.4x maximum registered single use rate.

Matrices	TRR as [^{14}C]sodium acifluorfen equivalents						Total Recovery %
PTI[a] in Days, (Replicates)	Organosoluble		Aqueous-soluble		Non-extractable		
	ppm	%	ppm	%	ppm	%	
Forage							
0 (1)	5.51	100.0	0.06	1.0	0.59	9.6	111.8
7 (1)	2.20	71.6	0.03	0.9	0.84	27.5	100.4
(2)	2.26	73.6	0.17	5.5	0.64	20.9	100.4
(3)	2.83	87.4	0.10	3.0	0.31	9.6	106.0
40 (1)	1.43	73.3	0.06	3.3	0.46	23.5	75.6
Straw							
97 (1)	1.53	77.1	0.22	11.1	0.23	11.8	104.3
(2)	1.47	76.0	0.20	10.3	0.27	13.7	102.0
(3)	1.53	72.5	0.19	8.8	0.39	18.7	105.4
Hull							
97 (1)	0.03	18.8	0.06	47.2	0.05	34.0	83.9
Grain							
97 (1)	0.017	61.6	-	-	0.01	38.4	100.0

[a] Posttreatment interval.

Hydrolysis of non-extractable residues

The non-extractable residues in rice straw (18.7% of TRR, 0.39 ppm; see Table 7) remaining after extraction with acetone:water were boiled in 2% sodium chloride for two hours, then filtered. The salt water extract was partitioned with ethyl acetate. The organosoluble residues were radioassayed by LSS and reserved for metabolite identification by HPLC; the aqueous-soluble residues were only radioassayed. The non-extractable residues remaining after salt water hydrolysis were boiled in 0.5% EDTA for 1 hour and filtered; the aqueous-EDTA-soluble residues were radioassayed. The remaining non-extractable residues were further hydrolyzed in 5% sodium hydroxide at room temperature for six hours, then filtered. The aqueous-sodium hydroxide-soluble fraction was adjusted to pH 2, then partitioned with ethyl acetate. The organosoluble residues were radioassayed and reserved for metabolite identification by HPLC. The combined treatment of the non-extractable residues in straw with sodium chloride, EDTA, and sodium hydroxide released 13.6% of TRR, leaving 5.1% still unextracted.

Residues in grain remaining after organic solvent extraction, representing 38.4% of TRR, were treated with 50% NaOH for 6 h, and then partitioned with ethyl ether. Registrant reported that "all" radioactivity was in the ether phase, but did not state if any residues remained unextracted or lost. The non-extractable residues in forage (up to 27.5% of TRR, 0.84 ppm) and hulls (34.0% of TRR, 0.05 ppm) were not subjected to hydrolysis procedures.

Descriptions of methods used for metabolite identification

For metabolite identification, a combination of TLC, HPLC, and GC/MS was used.

The extractable and hydrolyzable fractions of rice matrices were analyzed using one- and two-dimensional TLC on silica gel plates developed with toluene:methanol (2:1, v:v, System A) and chloroform:acetic acid (9:1, v:v, System B). Metabolites were identified by comparison with reference standards having known R_f values. Radiolabeled metabolites were detected by radioscan-TLC. The quantitation of metabolites identified by TLC was not described, except for grain. The registrant stated that due to the small amount of rice grain obtained from this study, further confirmatory analyses using a different method could not conducted.

The extractable and hydrolyzable fractions of rice matrices were also analyzed using HPLC equipped with a C-18 column eluted with solvent systems A and B (described above); detection was by UV (254 nm) and a radioactivity flow detector. Identification of metabolites was achieved by comparison with reference standards having known retention times. Eluates were collected with a fraction collector and radioactive metabolites were quantified by LSS.

An HPLC/MS method was used to confirm the identities of organosoluble metabolites in straw. Aliquots of the organic fraction were analyzed on a reversed-phase HPLC equipped with a Partisil 5 ODS-3 column, thermospray interface, and a mass detector operated in the positive ion mode, scanning in the m/z 600-105 range. It should be noted that the retention times reported (Appendix 4 of the study report) for the reference standards acifluorfen (7.5 minutes) and amino-acifluorfen (8.6 minutes) do not agree with the retention times of a representative HPLC chromatogram (Figure 40 of the study report). The submitted chromatogram indicates that the retention time of acifluorfen in rice matrices is ca. 8.5 minutes which is the reported retention time for the amino-acifluorfen reference standard.

Conclusion 2a: The registrant should resolve the discrepancies in the reported HPLC retention times for reference standards of acifluorfen and amino-acifluorfen vs. the reported retention time for acifluorfen in rice samples. This discrepancy could be resolved by the submission of HPLC profiles of reference compounds recorded on the same day as the analysis of metabolites.

A GC/MS method was used to confirm the identities of organosoluble metabolites in straw. Prior to analysis by GC/MS, the organosoluble residues were derivatized with diazomethane to methyl ester analogs. Metabolites were separated on a DB-

17 column equipped with an MS detector set at a mass scan range of 450-50 daltons.

Results of metabolite identification

The organosoluble residues from the 0 day forage sample were analyzed by TLC in Solvent System A. The results indicated that essentially all the organosoluble residue, representing 89% of the total residues, was acifluorfen. The organosoluble residues from the 7 day forage sample were analyzed by one-dimensional (1D) TLC, two-dimensional (2D) TLC, and HPLC. Registrant concluded from the chromatographic profiles that nearly all the organosoluble residue was acifluorfen, representing approximately 75% of TRR. TLC indicated a minor peak, which was not further characterized. On the basis of 2D TLC analysis of the organosoluble residues from the 40 day forage sample, registrant concluded that nearly all the organosoluble residue was acifluorfen, representing approximately 73% of TRR.

The performing laboratory encountered considerable difficulty in analyzing the 97-day rice grain sample. Initial analysis of the organosoluble extract by TLC in System A produced two peaks, of $R_f=0.34$ and 0.67 (Scan 1). Because of possible matrix interference, these peaks were scraped, reextracted, and reanalyzed by TLC in System A. Reanalysis of the peak at $R_f=0.34$ yielded peaks at $R_f=0.43$ and 0.54 (Scan 2). The broad peak at 0.43, representing 0.008 ppm, was assigned to aciflurofen; its identity was not confirmed. Reanalysis of the peak at $R_f=0.67$ from Scan 1 yielded peaks at $R_f=0.54$ and 0.86 (Scan 3), with the latter peak considered a contaminant. The two peaks at $R_f=0.54$, consistent with the mobility of amino-acifluorfen, from Scans 2 and 3 were scraped, reextracted, pooled, and analyzed by TLC in System B. Two peaks resulted, one at $R_f=0.70$ (Scan 4), consistent with the mobility of amino-aciflurofen, representing approximately 0.01 ppm. A second peak at $R_f=0.93$ represented about 5 times the radioactivity applied to the TLC plate, and was attributed to a contaminant. The peak at $R_f=0.70$ was scraped, reextracted, and reanalyzed by TLC in System B. A strong peak was observed at $R_f=0.72$, a minor peak at 0.42, and another peak at 0.93 (Scan 5). Subsequent work assigned the peaks at $R_f=0.86$ in System A and 0.93 in System B to contamination from oxadiazon, a product which was being investigated in another study at the same time. The peak at $R_f=0.72$ from Scan 5 was scraped and combined with the hull fraction, and as a result could not be directly quantitated. Registrant assigned 0.008 ppm in the organosoluble fraction to amino-acifluorfen without further explanation.

Residues in grain released by NaOH hydrolysis were analyzed by TLC in System A; two peaks were identified, at $R_f=0.52$ and 0.86 (Scan 6). The peak at 0.52 was scraped, reextracted, and reanalyzed by TLC in System B; two peaks were identified, at $R_f=0.71$ and 0.93. The peak at 0.71, representing 0.0105 ppm (essentially all the unextracted residue), was assigned to amino-acifluorfen. On the basis of further work, the peaks at 0.86 and 0.93 were assigned to oxadiazon as a contaminant. Overall, registrant assigned 30.8% of the TRR in grain to acifluorfen, and 69.2% of TRR to amino-acifluorfen.

Registrant noted that hulls are not a raw agricultural commodity, and further characterization of residues in hulls was not described in detail.

In the straw samples collected 97 days posttreatment, acifluorfen (75.2% of TRR) was identified by TLC and HPLC as the only residue in the ethyl acetate fraction. Acifluorfen was derivatized to form methyl ester, and confirmed by GC/MS.

HPLC analysis of the aqueous extracts indicated the presence of one polar metabolite (amount unspecified). After alkali hydrolysis and extraction with ethyl acetate, followed by analysis by TLC, HPLC and MS, registrant "speculated" that the polar metabolite was "similar" to the malonyl glucoside of 2-chloro-4-triflurophenol; this polar metabolite was not identified, however. HPLC analysis of the remaining (alkali hydrolysed and ethyl acetate partitioned) aqueous fraction showed the presence of one major peak of a polar nature (amount unspecified) and a smaller peak with the retention time of acifluorfen. Acifluorfen, a conjugate of the polar unknown, and at least one other organosoluble component, apparently were released by base hydrolysis of non-extractable residues of straw.

For each identified metabolite (whether tentative or confirmed) in rice straw, the % of TRR and ppm equivalents were not provided. The study reviewer could not determine the material balance of ^{14}C-residues in rice straw from these descriptions.

CBRS comments, metabolite identification

Raw agricultural commodities for rice are grain and straw; the commodity grain includes hulls, but hulls alone are not a raw agricultural commodity. Samples of grain and straw were collected at a PHI of 97 days, nearly twice the minimum PHI of 50 days. TRR in grain was small, and may have been greater at a shorter PHI. However, because most of the residue in 40 day forage was acifluorfen, the samples at 97 days may represent more extensive metabolism than would be expected at the minimum PHI. The samples at 97 days may therefore be acceptable, provided identification of residues is adequate.

Conclusion 2b: Samples were collected at 97 days, while samples at the minimum PHI of 50 days might have contained greater TRR. Considering that acifluorfen is still present in the 97 day samples, metabolism would be expected to be more extensive than at the minimum PHI. The samples at 97 days may therefore be acceptable, provided identification of residues is adequate.

The identification of residues in grain is dissatisfying. It should be noted that under the standards of Additional Guidance on metabolism studies (attached), analysis by two different TLC systems is not considered adequate for identification. However,

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CBRS recognizes that residue extraction for this study was conducted several years ago. Because the data indicate only moderate metabolism of acifluorfen, CBRS will accept the identification of amino-acifluorfen in the organosoluble and non-extractable residues, subject to further explanation. Registrant noted that putative amino-acifluorfen residues were combined with extract from hulls, but did not explain the quantitative assignment of residues. The putative identification of residues in the organosoluble fractions is complicated by the failure to confirm the identity of acifluorfen and the frequent appearance of contaminants. Registrant reported that confirmation of acifluorfen residues in grain was not possible because of limited sample size. The organosoluble residues in hulls should be examined for the putative acifluorfen peak; if the chromatographic profile is similar to that in grain, then identification of acifluorfen in hull extracts by two methods could be translated to grains. The majority of the TRR in hulls is aqueous soluble, in contrast to the situation in grain and straw (see Table 7); further work on aqueous extracts from hulls is therefore not expected to be useful.

Conclusion 2c: Registrant should explain in more detail how quantitative assignment of residues in rice grain was made. Assignments should be supported by calculations and data on radioactivity applied to TLC plates and recovered.

Conclusion 2d: Organosoluble residues from hulls should be examined for the putative acifluorfen peak; identification of acifluorfen by two methods in hull extracts could be translated to grain.

In straw, 75.2% of the residue was identified as acifluorfen. Some acifluorfen was released by hydrolysis, but was not quantitated. The submitted TLC and HPLC chromatograms of extractable fractions detected unidentified peaks which the registrant claimed were polar metabolites. However, the amounts (% of TRR <u>and</u> ppm equivalents) of these polar metabolites were not fully provided, and the polar metabolites were not identified. For the metabolite suspected of being a glucoside conjugate, or other putative conjugates, cleavage by an appropriate enzyme should facilitate identification.

Conclusion 2e: For each identified metabolite (whether tentative or confirmed) in rice straw, complete quantitative data must be provided. These data should be presented in a table which must include the amount (% of TRR <u>and</u> ppm equivalents) for each extractable fraction of rice straw. The amount (% of TRR <u>and</u> ppm equivalents) of unidentified polar metabolites should also be provided. Further characterization or identification of residues should be conducted based on Additional Guidance for metabolism studies (attached). Cleavage by appropriate enzymes should facilitate identification of putative conjugates.

The registrant proposed that [^{14}C]sodium acifluorfen is metabolized in rice by two pathways: (i) a route involving formation of amino acifluorfen by the reduction of nitro group; and (ii) a route involving rapid cleavage of the diphenyl ether bond.

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Conclusion 2f: Only two metabolites, acifluorfen and amino-acifluorfen, were identified in rice commodities, and the identification of acifluorfen was confirmed only in straw. The proposed metabolic route involving cleavage of the diphenyl ether bond cannot be considered justified until putative conjugated metabolites are identified.

Recommendation: Further work is necessary to resolve the deficiencies identified above. If it proves necessary to conduct a new metabolism study, the nature of the residue in grain and straw at a PHI of 50 days should be determined; exaggerated rates may be necessary to generate TRRs large enough for adequate identification. The results of the present study can be used as guidance on which compounds would be likely metabolites.

Storage stability

Forage and straw samples collected 7 and 97 days posttreatment, respectively, were used in a storage stability study. Samples were frozen at an unspecified temperature upon collection. Forage and straw samples were homogenized in acetone 24 days and 27 days, respectively, following collection and the homogenates were stored at ca. -20°C. Each homogenate was analyzed by TLC for the presence of acifluorfen, then refrozen. Forage and straw homogenates were stored at -20°C for 42 months and 50 months, respectively, then reanalyzed by TLC and HPLC for acifluorfen; chromatographic profiles were provided. Registrant concluded that acifluorfen comprised >87.0% of the TRR in forage at the end of the storage period.

The data presented are adequate to indicate the stability of acifluorfen in organic solvent during storage, but provide little information on the stability of aqueous soluble residues in straw, nor on the stability of residues in grain. Initial extractions of grain and straw were performed within 4 months of harvest.

Conclusion 2g: The data presented are insufficient to validate the stability during frozen storage of organosoluble residues in grain, nor aqueous soluble residues in straw. Storage conditions between harvest and homogenization should be provided for grain and straw. Considering the very long storage times, evidence should be provided that the profiles of radioactive residues did not change during storage. If chromatographic profiles at early extraction are not available, then material balances indicating that residue identification and quantitation account for nearly all residues at early extraction will be sufficient evidence for storage stability.

Residue method validation

According to the Pesticide Analytical Manual (PAM) Vol. II, Method I, a GLC method with electron capture detection is available for the enforcement of present sodium acifluorfen tolerances.

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Conclusion 2h: Radiovalidation of method(s) using samples from the metabolism study remains an outstanding data requirement, pending determination of the residues to be regulated.

EPA MEMORANDA CITED IN THIS REVIEW

CBRS No.: None
Subject: Sodium Acifluorfen: CBRS Transmittal Sheet for Phase 4 Reviews, Chemical No. 114402, Case No. 2605.
From: S.R. Funk
To: T. Luminello
Dated: 2/4/91
MRID: None

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

MASTER RECORD IDENTIFICATION NUMBERS

References (used):

42368301 Larson, J.D. (1992). Nature of ^{14}C Sodium Acifluorfen Residue in Peanuts. Laboratory Project ID: HLA 6237-100. Unpublished Study performed by Hazleton Laboratories America, Inc., 131 p.

42368302 Premkumar, N.D. (1992). Metabolism of ^{14}C Acifluorfen-Sodium in Rice. Laboratory Project ID: 35986. Unpublished Study performed by ABC Laboratories, Inc., 120 p.