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

APR 26 1994

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Sodium Acifluorfen. Case No. 2605. Nature of Residue in Poultry, Residue Method in Plants, and Magnitude of Residue in Soybeans. MRID No. 42828201, 42825701 & 42825702. CBRS No. 12181. DP Barcode: D192899.

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Attached is a review of a poultry metabolism study, a residue analytical method for plant commodities, and soybean residue field trials 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 qualitative nature of the residue in poultry is adequately understood. No metabolites that indicate cleavage of the diphenyl ether bond have been identified. Acifluorfen, acifluorfen acetamide, and descarboxy acifluorfen have been found to be present in all matrices of poultry.

The requirement for the radiovalidation of the enforcement analytical method(s) is reserved until the HED Metabolism Committee determines the residues to be regulated.

BASF Method D9205 is adequate for data collection on the combined residues of acifluorfen, its amine metabolite, and their methyl esters in soybean grain. Before this method may be considered for tolerance enforcement, the method must be successfully validated by an independent laboratory and submitted

to EPA for Agency validation.

The combined residues of the sodium salt of acifluorfen and its metabolites (the corresponding acid, methyl ester, and amino analogues) were <0.1 ppm (<0.02 ppm for each metabolite) in/on soybeans treated at 1x the revised maximum seasonal rate. However, interim storage stability data showed that acifluorfen amine declined to 27% of the initial level after only 1.5 months (field samples were stored for 6 months). The field residue data are adequate pending receipt of the completed storage stability study and a determination that amine metabolite instability does not appreciably affect the total combined residue level.

If you need additional information, please advise.

Attachment: Dynamac review of poultry metabolism, plant residue method, and soybean field trials of sodium acifluorfen

cc(without Attachment):RF
cc(with Attachment):Circ, SF, List B File, Cheng, Dynamac
RDI:ARRathman:4/24/94:MMetzger:4/25/94:EZager:4/25/94
7509C:CBRS:LCheng:CM#2:RM804D:4/20/94:03:a\ACIFLUORFEN\POULMETA.DYN

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DYNAMAC
CORPORATION
Environmental Services

Final Report

SODIUM ACIFLUORFEN
Shaughnessy No. 114402
(DP Barcode D192899;
CBRS No. 12181; Case No. 2605)

TASK 4
Registrant's Response to Residue Chemistry Data Requirements

October 4, 1993

Contract No. 68-D2-0053

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

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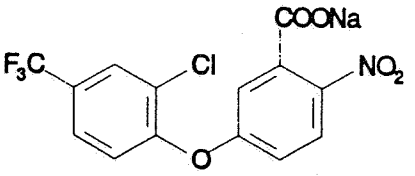
SODIUM ACIFLUORFEN



Shaughnessy No. 114402; Case 2605

(CBRS No. 12181; DP Barcode D192899)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

The Sodium Acifluorfen Phase 4 Reviews (S. Funk, 2/14/91) identified the following deficiencies: plant and animal metabolism; residue analytical methods; storage stability for peanuts and processed commodities, rice and processed commodities, soybean processed commodities, and animal commodities; magnitude of the residue in peanuts, rice, and soybeans; and magnitude of the residues in animals. In response to the data requirements, BASF Corporation (1993; MRIDs 428157-01 and -02, and 42828201) submitted data pertaining to poultry metabolism, residue analytical methods, and the magnitude of the residue in soybeans. These data are reviewed here for their adequacy in fulfilling the outstanding data requirements. The Conclusions and Recommendations stated in this document pertain only to the nature of the residue in poultry, residue analytical methods, and the magnitude of the residue in soybeans. Other data requirements stated in the Phase 4 Reviews are not addressed herein.

The qualitative nature of the residue in soybeans is not adequately understood. The Sodium Acifluorfen Phase 4 Reviews concluded that the submission of summaries of soybean metabolism studies (purchased from Rhone-Poulenc) were unacceptable for purposes of reregistration because the majority of the ^{14}C-residues were not identified.

The qualitative nature of the residue in peanuts and rice is not adequately understood for the following reasons: (i) in peanuts, the majority of ^{14}C-residues have not been identified in samples that were collected at maturity; and (ii) for rice commodities, incomplete quantitative data pertaining to metabolite identification were submitted; and (iii) radiovalidation of methods used for the peanut and rice metabolism studies remain outstanding data requirements pending determination of the residues to be regulated. The peanut and rice metabolism studies may be upgraded upon submission of the required metabolite identification data.

The qualitative nature of the residues in ruminants is not adequately understood. The Sodium Acifluorfen Phase 4 Reviews concluded that although total ^{14}C-residues in animal commodities were very low following the feeding of radiolabeled sodium acifluorfen to ruminants, the purpose of the metabolism study is to determine the nature of the residue in animals. Since the magnitude of the residue in animal commodities is not the issue to be resolved, metabolism studies pertaining to ruminants must be provided.

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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 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.

Adequate methodology is available for the enforcement of tolerances of sodium acifluorfen and its amine and methyl ester metabolites in or 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 derivatize acifluorfen residues to methyl esters prior to GC/ECD analysis.

CONCLUSIONS AND RECOMMENDATIONS

Qualitative Nature of the Residue in Poultry

1. The qualitative nature of the residue in poultry is adequately understood. The study involved dosing hens separately with chlorophenyl ring (CPR) and nitrophenyl ring (NPR) labeled [^{14}C]sodium acifluorfen at 111x and 92x the maximum daily dietary burden, respectively. No metabolites were identified that indicate cleavage of the diphenyl ether bond. Acifluorfen was a major residue in egg white (up to 40% of the total radioactive residue; TRR), egg yolk (up to 22%), liver (up to 11%), and thigh muscle (up to 18%). Acifluorfen acetamide accounted for up to 20% of the residue in egg white, 15% in liver, and 20% in thigh muscle. Descarboxy acifluorfen comprised up to 11% of the residue in egg white, 30% in egg yolk, 15% in liver, and 50% in thigh muscle. Descarboxy acifluorfen was the major residue in fat accounting for 50-70% of the residue.

2. The study successfully characterized/identified the following levels of radioactivity in matrices of hens orally dosed with CPR- and NPR-labeled acifluorfen: egg white (87.2-90.7% TRR), egg yolk (64.5-74.5% TRR), fat (89.9-98.5% TRR), liver (84.3-90.8% TRR), and thigh muscle (88.3-106.2% TRR).

3. Except for descarboxy acifluorfen (0.2246 ppm) in fat of CPR-labeled treated hens, residues of acifluorfen and its metabolites, acifluorfen acetamide and descarboxy acifluorfen, were present at levels below 0.04 ppm. Combined residues of acifluorfen, acifluorfen acetamide, and descarboxy acifluorfen were: egg white (0.0060-0.0064 ppm), egg yolk (0.0139-0.0506 ppm), fat (0.0104-0.2246 ppm), liver (0.0165-0.0334 ppm), and thigh (0.0032-0.0114 ppm). Unidentified extractable and non-extractable components in all matrices each were $\leq$0.0173 ppm.

4. There were differences observed between residue levels in tissues from the CPR- and NPR-labeled treated hens. In general, total radioactive residues (TRR) were greater from dosing with the CPR-label, about 2x in muscle and liver, and up to 10x in fat. The metabolite profiles differed as well in some cases. Egg yolks for example contained acifluorfen at 8% and descarboxy acifluorfen at almost 30% of the TRR from the CPR label, whereas the NPR label resulted in about 20% acifluorfen and 3.6% descarboxy acifluorfen. As another example, CPR-labeled residues in thigh muscle consisted of ~2% acifluorfen, 2% acifluorfen acetamide, and ~50% descarboxy acifluorfen; as NPR-labeled residues, these

compounds accounted for 18%, 20%, and 5.5% of the TRR, respectively. Addressing the differences in descarboxy acifluorfen in CPR-labeled treated fat (0.2246 ppm, 74.4% TRR) and NPR-labeled treated fat (0.0092 ppm, 52.8% TRR), the registrant pointed out: (i) the higher administered dose of the CPR-labeled test substance than of the NPR-labeled test substance (11.1 ppm vs. 9.2 ppm); and (ii) a higher level of [^{14}C]descarboxy acifluorfen impurity in the CPR-labeled test substance than in the NPR-labeled test substance (2.2% vs. 0.3%.).

5. The requirement for the radiovalidation of the enforcement analytical method(s) is reserved until the HED Metabolism Committee determines the residues to be regulated.

Residue Analytical Methods

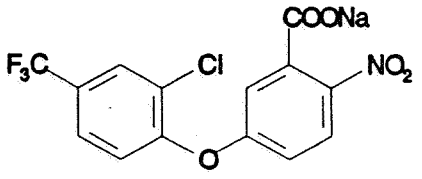
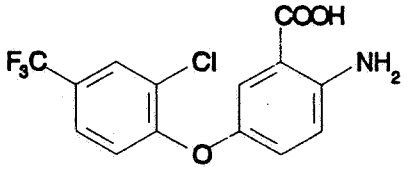
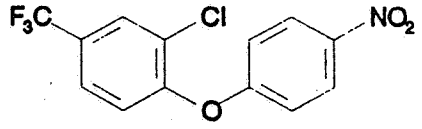
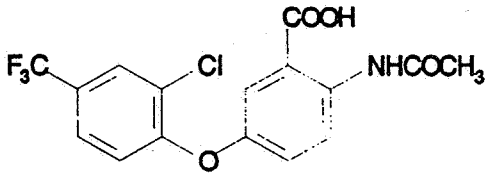
6. BASF Method D9205 is adequate for data collection on the combined residues of acifluorfen, its amine metabolite, and their methyl esters in soybean grain. Before this method may be considered for tolerance enforcement, the method must be successfully validated by an independent laboratory and submitted to EPA for Agency validation.

Magnitude of the Residue in Soybeans

7. The combined residues of the sodium salt of acifluorfen and its metabolites (the corresponding acid, methyl ester, and amino analogues) were <0.1 ppm (<0.02 ppm for each metabolite) in/on soybeans treated at 1x the revised maximum seasonal rate. However, interim storage stability data showed that acifluorfen amine declined to 27% of the initial level after only 1.5 months (field samples were stored for 6 months). The field residue data are adequate pending receipt of the completed storage stability study and a determination that amine metabolite instability does not appreciably affect the total combined residue level.

The molecular structures of sodium acifluorfen and its metabolites that were identified in poultry matrices are presented in Table 1.

Table 1. Sodium acifluorfen and its metabolites in poultry matrices (MRID 42828201).

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	
		hen egg white, egg yolk, fat, liver, and thigh muscle **Acifluorfen**
III.	4-(2-chloro-4-(trifluoromethyl)phenoxy)nitrobenzene acid *	
		hen egg white, egg yolk, fat, liver, and thigh muscle **Descarboxy acifluorfen**
IV.	5-(2-chloro-4-(trifluoromethyl)phenoxy)-2-acetamidobenzoic acid	
		hen egg white, egg yolk, fat, liver, and thigh muscle **Acifluorfen acetamide**

* Confirmed by HPLC/thermospray-MS in hen fat, and by HPLC and TLC in egg yolk.

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DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Poultry

BASF Corporation (1993; MRID 42828201) submitted data depicting the metabolism of sodium acifluorfen that was labeled with [^{14}C] in the chlorophenyl ring (CPR) (specific activity, 18.03 mCi/mmole, 10,4333.8 dpm/ug; 95-96% radiochemical purity) and in the nitrophenyl ring (NPR) (specific activity, 16.0 mCi/mmole, 92,586.8 dpm/ug; radiochemical purity 96%) in laying hens. The biological portion of this study was conducted by Battelle Columbus Laboratories (BCL, Columbus, OH). Two groups of five white leghorn hens were orally dosed with CPR labeled sodium acifluorfen and NPR labeled sodium acifluorfen, once daily for 5 consecutive days at 11.1 ppm and 9.2 ppm, respectively. The test substances were prepared in gel capsules containing water (~pH 5.0-7.5). A control group of five hens was fed capsules containing water only. Two-dimensional TLC analyses indicated that descarboxy acifluorfen accounted for 2.2% and 0.3% of the radioactivity in the CPR- and NPR-labeled test substances, respectively.

Based on current tolerances of 0.1 ppm each for soybeans, rice grain, and peanuts, the maximum daily dietary intake of acifluorfen residues by poultry is 0.1 ppm based on a diet consisting of 50% soybeans, 20% soybean grain dust, 20% rice grain, and 10% peanut meal. Therefore, the dose of sodium acifluorfen administered to the test hens is ~92x the maximum daily dietary burden for the CPR-labeled test substance and ~111x for the NPR-labeled test substance.

During the study, the base diet consisted of commercial poultry feed; water was provided *ad libitum*. Calculations deriving the daily dose of acifluorfen as well as information pertaining to feed consumption, egg production, and general health of the test animals were provided. There was a 3-day acclimation period during which food consumption was monitored. During the experimental period, eggs were collected from each hen twice daily and excreta were collected once daily. Whites and yolks were separated and pooled each day, and stored at ~-20 C. Hens were sacrificed 22-23 hours following the final dose. White meat (breast), dark meat (thigh), skin with adhering fat, liver, kidney, fat, GI tract, eggs present in the oviduct, partially formed eggs, the residual carcass, and blood were collected at the sacrifice period. All samples were frozen at ~ -20 C until analysis at BCL.

Total radioactive residues (TRR)

Two sets of TRR data were presented by the registrant for each tissue type. For one set of TRR data (designated "Initial Sampling"), a subsample of each tissue type from each hen was analyzed for total radioactivity. For the second set of TRR data ("designated "Bulk Sampling"), whole tissue samples of each tissue type, from each hen were pooled and then subsampled prior to analysis by combustion/LSS. The "Bulk Sampling" subsamples were used for subsequent residue extraction, characterization, and identification procedures. Subsamples of liver, fat, and muscle were homogenized prior to analysis by liquid scintillation counting with a spectrometer following combustion. Egg white and yolk subsamples were analyzed directly by LSS. All analyses were conducted in triplicate. The LSS limits of detection were 0.00272-0.00466 ppm for tissues and eggs from hens treated with NPR-labeled test substance, and 0.00252-0.00419 ppm for tissues and eggs from hens treated with CPR-labeled test substance. Individual limits of detection for each matrix are presented in Table 2. The distribution of radioactivity into eggs and tissues (Initial Sampling) is presented in Tables 3 and 4. The TRR values of the Bulk Sampling data set are presented together with the extraction, characterization, and metabolite identification data in Tables 5 (eggs), 6 (fat), 7 (liver), and 8 (thigh muscle).

The data indicate that radioactive residues were found in eggs and all tissues of the test hens. Radioactive residues were low in egg whites (<0.00371-0.0436 ppm and <0.00304-0.0240 ppm) and in NPR-labeled egg yolks (<0.00466-0.0717 ppm) throughout the experimental period. Residues were highest in CPR-labeled egg yolks at the Day 4 (0.0530-0.112 ppm) and Day 5 (0.0784-0.162 ppm) sampling intervals.

In tissues, residues were highest in NPR-labeled liver (0.0353-0.204 ppm) and kidney (0.214-0.823 ppm), and in CPR-labeled liver (0.0603-0.134 ppm), fat (0.198-0.361 ppm), skin (0.0987-0.148 ppm), and kidney (0.544-1.42 ppm). The radioactivity ranged from 0.00282-0.0579 ppm in the remainder of the NPR-labeled tissues and 0.00451-0.0196 ppm in the remainder of the CPR-labeled tissues.

Table 2. Limits of detection (LSS) for ^{14}C-residues in tissues and eggs of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Specimen Type	[^{14}C] Sodium Acifluorfen Equivalents (ppm)	
	NPR-Labeled	CPR-Labeled
Egg White	0.00371	0.00304
Egg Yolk	0.00466	0.00419
Liver	0.00304	0.00270
Kidneys	0.00331	0.00295
Fat	0.00329	0.00301
Breast	0.00272	0.00252
Skin	0.00310	0.00273
Thigh	0.00291	0.00258

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Table 3. Total radioactive residues (TRR) in egg whites and egg yolks of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Sample Interval	[^{14}C]Sodium Acifluorfen Equivalents [a] (ppm)	
	Egg White	Egg Yolk
NPR Label (Number of Samples)		
Predose	<0.00371 (3)	<0.00466 (3)
Day 1	<0.00371-0.00448 (3)	<0.00466 (3)
Day 2	<0.00371-0.0389 (4)	<0.00466-0.0104 (4) [b]
Day 3	<0.00371-0.0436 (5)	<0.00686-0.0125 (5) [c]
Day 4	<0.00371-0.0346 (5)	<0.0186-0.0488 (5)
Day 5	<0.00371-0.0229 (5)	0.0275-0.0717 (5)
CPR Label (Number of Samples)		
Predose	<0.00304 (3)	<0.00419 (3)
Day 1	<0.00304-0.0154 (3)	<0.00419 (3)
Day 2	0.00359-0.00979 (4)	0.0698-0.0257 (4)
Day 3	0.00312-0.0187 (5)	0.0237-0.0644 (5)
Day 4	0.00721-0.0236 (5)	0.0530-0.112 (5)
Day 5	0.00413-0.0240 (4)	0.0784-0.162 (4)

[a] All values are the average of triplicate analyses.
[b] White and yolk accidentally mixed in one sample, but white TRR was below detection limit.
[c] One yolk sample was contaminated with water.

Table 4. Total radioactive residues (TRR) in tissues of hens orally dosed once daily with NPR-labeled acifluorfen and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively; range of five samples for each matrix.

Matrix	ppm [^{14}C]Sodium Acifluorfen Equivalents *	
	NPR Label	CPR Label
Breast muscle	0.00282-0.00894	0.00451-0.0108
Thigh muscle	0.00327-0.0157	0.00830-0.0196
Liver	0.0363-0.204	0.0603-0.134
Fat	0.0101-0.0342	0.198-0.361
Skin	0.0125-0.0579	0.0987-0.148
Kidney	0.214-0.823	0.544-1.42

* All values are the average of triplicate analyses.

Extraction and hydrolysis of residues

The registrant provided descriptions and flow charts of the fractionation schemes for hen matrices. In egg yolks, the registrant's tabular data presentation indicated that two unknown metabolites were observed in a hexane extract. A more detailed description of the egg yolk fractionation procedure in the text and in the submitted flowchart shows that the two unknowns were detected by TLC analysis (described below under "Characterization/identification of residues") in an aqueous methanol phase following partitioning of the extract. For all matrices, the radioactivity in fractions designated as "pellets", following centrifugation of various extracts, was not reported.

Except for CPR-labeled thigh muscle (0.0083-0.0196 ppm), residues in breast and thigh muscle samples were <0.01 ppm; therefore, only the residues in thigh muscle samples were subjected to characterization/identification procedures and were considered representative of all muscle tissue. Similarly, only day-5 eggs were subjected to residue characterization/identification procedures because the highest egg residue levels were observed at this sampling interval. Subsamples of egg whites, egg yolks, and tissues were extracted as described below.

Egg white: Residues in egg white were extracted by blending with acetonitrile (ACN), followed by centrifugation of the homogenate and filtering; this procedure was repeated twice and the ACN extracts were combined. An aliquot of the combined ACN extracts was concentrated by evaporation, redissolved in methanol (MeOH), and centrifuged. The supernatant was then analyzed by LSS and HPLC. Non-extractable residues remaining after the initial extraction were analyzed by combustion/LSS.

Egg yolk: The extraction procedure for egg yolk residues was similar to the procedures described above for egg white, except that the extractable residues obtained from CPR-labeled egg yolks were also analyzed by 2-D TLC. Also, non-extractable residues remaining after the initial extraction were extracted with hexane. An aliquot of the combined extracts was concentrated by evaporation and extracted three times with MeOH:water (9:1, v:v). The aqueous MeOH phase was analyzed by LSS and TLC, and the hexane fraction was analyzed by LSS; non-extractable residues remaining after the hexane extraction were analyzed by combustion/LSS.

Fat: Residues in fat were blended three times with ACN:water (9:1, v:v) followed by filtration. An aliquot of the combined extracts was concentrated by evaporation, redissolved in ACN, and centrifuged. The supernatant was then analyzed by LSS and HPLC and by 2D-TLC. Non-extractable residues were extracted twice with hexane, and the combined hexane extracts were analyzed by LSS; the remaining non-extractable residues were analyzed by combustion/LSS.

Liver: Residues in liver were extracted using the same procedures described for egg whites, except for the fractionation of the non-extractable residues remaining after the initial extraction. Non-extractable residues were extracted twice by blending with MeOH and centrifuged. The supernatants were filtered and the MeOH extract was analyzed by LSS. The remaining solids were then extracted twice by blending with hexane, and centrifuged. The combined hexane extracts were analyzed by LSS and non-extractable residues were analyzed by combustion/LSS.

Thigh muscle: The extraction procedure for muscle was similar to the procedure described for liver, except that the concentrated ACN:water extract was redissolved in ACN and the non-extractable residues were not subjected to additional fractionation procedures.

Using the procedures described above, extractable residues accounted for 74.5-90.7% TRR in eggs and tissues of CPR-labeled treated hens, and 64.5-106.2% of TRR in eggs and tissues of NPR-labeled treated hens. Non-extractable residues, accounting for 0.0003-0.0173 ppm in eggs and tissues of CPR-labeled hens, and 0.0001-0.0114 ppm in eggs and tissues of NPR-labeled hens, were not further analyzed. Total recoveries of radioactive residues from edible matrices were 87.1-106.2% for CPR-labeled treated hens, and 87.7-120.2% for NPR-labeled treated hens.

Characterization/Identification of residues

All egg and tissue matrices: Residues in all ACN and/or ACN:water extracts (except for liver) were analyzed by HPLC on a Partisphere C-18 column using a mobile phase consisting of 0.25% aqueous glacial acetic acid (Solvent A) and 0.25% glacial acetic acid in ACN (Solvent B), changing from A:B (80:20, v:v) to A:B (5:95, v:v) in a series of step and linear gradients over an interval of 55 minutes (HPLC System I). Metabolites were detected by UV absorbance (254 nm) and a radioflow detector; eluate fractions were collected and quantified by LSS. Liver extracts were analyzed by HPLC using similar operating conditions except that the mobile phase changed from A:B (80:20, v:v) to A:B (5:95, v:v) in a series of step and linear gradients over an interval of 90 minutes (HPLC System II). Metabolites were detected and quantified as previously described above. In both systems, metabolites were identified by co-chromatography and comparison of the retention times of the radioactive components with those of the following reference standards: acifluorfen, descarboxy acifluorfen, desnitro-acifluorfen, amino-acifluorfen, acifluorfen methyl ester, acifluorfen acetamide, amino-acifluorfen methyl ester, acifluorfen acetamide methyl ester, 2-chloro-4-trifluoromethyl phenol, 2-amino-5-hydrobenzoic acid, and 2-nitro-5-hydroxybenzoic acid.

Egg yolks: Residues in the aqueous-MeOH extracts of egg yolks were analyzed by 1D-TLC on silica gel using a mobile phase consisting of dichloromethane:acetic acid (90:10, v:v). Radioactive residues were detected and quantified by radioscan TLC; non-labeled reference standards were visualized by UV light; metabolite identifications were made by comparison of the R_fs with the R_fs of the reference standards.

Yolks of CPR-labeled treated hens only (>0.05 ppm) were additionally analyzed by 2D-TLC on silica gel using hexane:ethyl acetate:glacial acetic acid (10:4:1, v:v:v) in the first dimension, and methylene chloride:hexane (5:1, v:v) in the second dimension. Residues were detected and quantified as described above for 1D-TLC.

Fat: ACN:water extracts from the fat of CPR-labeled hens only (>0.05 ppm) were analyzed by 2D-TLC as previously described above for egg yolks..

Residues in fat from CPR-labeled hens were also subjected to analysis by HPLC/thermospray-MS Two aliquots of hen fat were each extracted with ACN:water (9:1, v:v) three times, followed by centrifugation and filtration of the supernatants. The supernatants were combined, concentrated by rotary evaporation, and microcentrifuged. This procedure yielded two phases which were mixed with MeOH:water (1:1, v:v) to yield a single phase mixture. The mixture was purified on C-18 SPE columns preconditioned with one volume of HPLC Solvents B or A. Residues were eluted sequentially with A:B (50:50, v:v; 25:75, v:v; and 5:95, v:v). Duplicate aliquots were taken for analysis by LSS and all fractions containing ~6% of the applied radioactivity were neutralized with 6 M ammonium hydroxide, pooled, and concentrated by rotary evaporation. Concentrated eluates were then applied to a C-18 column preconditioned sequentially with 100% B and A:B (50:50, v:v). The residues were eluted with two volumes of MeOH, pooled, concentrated, and purified by HPLC (system not specified). An unspecified metabolite isolated from hen fat extracts was then analyzed by HPLC/thermospray-MS operating in the positive ion, electron impact (EI) mode. The HPLC isocratic mobile phase consisted of aqueous 50 mM ammonium acetate:aqueous 90% MeOH (20:80, v:v).

The study successfully characterized/identified the following levels of radioactivity in matrices from hens orally dosed with CPR- acifluorfen: egg white (90.7% TRR), egg yolk (74.5% TRR), fat (89.9% TRR), liver (90.8% TRR), and thigh muscle (88.3% TRR). The study also successfully characterized/identified the following levels of radioactivity from NPR-labeled acifluorfen: egg white (87.2% TRR), egg yolk (64.5% TRR), fat (98.5% TRR), liver (84.3% TRR), and thigh muscle (106.2% TRR).

The parent, acifluorfen (0.0004-0.0113 ppm), and two metabolites, acifluorfen acetamide (0.0003-0.0142 ppm) and descarboxy acifluorfen (0.0004-0.2246 ppm) were present in all matrices. Except for descarboxy acifluorfen (0.2246 ppm, 74.4% TRR) in fat of CPR-labeled hens, residues of acifluorfen and its metabolites were present at insignificant levels (≤0.0393 ppm). No other acifluorfen metabolites were identified in fat of CPR-labeled hens. Combined residues of acifluorfen, acifluorfen acetamide, and descarboxy acifluorfen were: egg white (0.0060-0.0064 ppm), egg yolk (0.0139-0.0506 ppm), fat (0.0104-0.2246 ppm), liver (0.0165-0.0334 ppm), and thigh (0.0032-0.0104 ppm). Unidentified extractable and non-extractable components in all matrices each were ≤0.0173 ppm.

The distribution of TRR in hen matrices is presented in Tables 5 (eggs), 6 (fat), 7 (liver), and 8 (thigh muscle). Summaries of the metabolites characterized/identified in hen matrices are presented in Tables 9 (egg white), 10 (egg yolk), 11 (fat), 12 (liver), and 13 (muscle).

Table 5. Distribution of total radioactive residues (TRR) in egg white and yolks of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Fraction	%TRR	ppm *	Characterization/Identification
CPR-labeled egg white (Day 5, 0.0113 ppm)			
ACN	90.7	0.0103	HPLC: Acifluorfen (38.7% TRR, 0.0044 ppm), acifluorfen acetamide (3.1% TRR, 0.0003 ppm), descarboxy acifluorfen (11.2% TRR, 0.0013 ppm), six unknowns: CF3EWA1 (3.4% TRR, 0.0004 ppm), CF3EWA2 (2.2% TRR, 0.0003 ppm), CF3EWA3 (4.6% TRR, 0.0005 ppm), CF3EWA6 (8.9% TRR, 0.0010 ppm), CF3EWA7 (2.8% TRR, 0.0003 ppm), CF3EWA9 (0.8% TRR, 0.0001 ppm), and ≤26 unspecified peaks, each <2% TRR (collectively 15.0% TRR, 0.0017 ppm). Total Identified/Characterized = 90.7% TRR, 0.0103 ppm.
Pellet	N/R (not reported)	N/R	N/A (not further analyzed).
Non-extractable	11.6	0.00103	N/A.
CPR-labeled egg yolk (Day 5, 0.137 ppm)			
ACN	63.1	0.0863	HPLC/TLC: Acifluorfen (8.3% TRR, 0.0113 ppm), descarboxy acifluorfen (28.7% TRR, 0.0393 ppm), five unknowns: CF3YA1 (1.9% TRR, 0.0026 ppm), CF3YA2 (4.8% TRR, 0.0066 ppm) CF3YA4 (4.5% TRR, 0.0061 ppm), CF3YA5 (2.8% TRR, 0.0038 ppm), CF3YA7 (1.4% TRR, 0.0020 ppm), and ≤23 unspecified peaks, each <2% TRR (collectively 10.7% TRR, 0.0146 ppm). Total Identified/Characterized = 63.1% TRR, 0.0863 ppm.
Pellet	N/R	N/R	N/A.
Non-extractable	12.6	0.0173	N/A.
Hexane	N/R	N/R	N/A.
MeOH	11.4	0.0156	TLC: Two unknowns: CF3YH1 (5.6% TRR, 0.0076 ppm) and CF3YH2 (5.8% TRR, 0.0080 ppm).
Hexane	N/R	N/R	N/A.
Pellet	N/R	N/R	N/A.
Pellet	N/R	N/R	N/A.

(continued; footnotes follow)

Table 5 *(continued)*.

Fraction	%TRR	ppm *	Characterization/Identification
NPR-labeled egg white (Day 5, 0.0126 ppm)			
ACN	87.2	0.0109	HPLC: Acifluorfen (29.8% TRR, 0.0038 ppm), acifluorfen acetamide (20.7% TRR, 0.0026 ppm), five unknowns: NO2EWA1 (1.6% TRR, 0.0002 ppm), NO2EWA4 (7.5% TRR, 0.0010 ppm), NO2EWA5 (5.5% TRR, 0.0007 ppm), NO2EWA6 (1.2% TRR, 0.0002 ppm), NO2EWA7 (1.4% TRR, 0.0002 ppm), and ≤30 unspecified peaks, each <2% TRR (collectively 19.5% TRR, 0.0022 ppm). Total Identified/Characterized = 87.2% TRR, 0.0109 ppm.
Pellet	N/R	N/R	N/A.
Non-extractable	19.2	0.0024	N/A; <0.05 ppm.
NPR-labeled egg yolk (Day 5, 0.0492 ppm)			
ACN	40.8	0.0201	HPLC: Acifluorfen (21.8% TRR, 0.0108 ppm), acifluorfen acetamide (2.6% TRR, 0.0013 ppm), descarboxy acifluorfen (3.6% TRR, 0.0018 ppm), one unknown: NO2YA1 (2.0% TRR, 0.0010 ppm), and ≤29 unspecified peaks, each <2% TRR (collectively 10.8% TRR, 0.0052 ppm). Total Identified/Characterized = 40.8% TRR, 0.0201 ppm.
Pellet	N/R	N/R	N/A.
Non-extractable	23.2	0.0114	N/A.
Hexane	N/R	N/R	N/A.
MeOH	23.7	0.0117	TLC: Two unknowns: NO2YH1 (6.1% TRR, 0.0030 ppm) and NO2YH2 (17.6% TRR, 0.0087 ppm).
Hexane	N/R	N/R	N/A.
~~Pellet~~	N/R	N/R	N/A.
Pellet	N/R	N/R	N/A.

* All values are mean of triplicate analyses for "Bulk Samples".

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Table 6. Distribution of total radioactive residues (TRR) in fat of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Fraction	%TRR	ppm *	Characterization/Identification
CPR-labeled (0.302 ppm)			
ACN/water	87.0	0.2627	HPLC/TLC: Descarboxy acifluorfen (74.4% TRR, 0.2246 ppm) and ≤37 unspecified peaks, each <2% TRR (collectively 12.6% TRR, 0.0381 ppm). Total Identified/Characterized = 87.0% TRR, 0.2627 ppm.
Pellet	N/R (not reported)	N/R	N/A (not further analyzed).
Non-extractable	0.1	0.0003	N/A.
Hexane	2.9	0.0089	N/A.
Pellet	N/R	N/R	N/A.
NPR-labeled (0.0174 ppm)			
ACN/water	87.9	0.0153	HPLC/TLC: Acifluorfen (3.9% TRR, 0.0007 ppm), acifluorfen acetamide (2.8% TRR, 0.0005 ppm), descarboxy acifluorfen (52.8% TRR, 0.0092 ppm), five unknowns: NO2FAW1 (1.1% TRR, 0.0002 ppm), NO2FAW2 (2.5% TRR, 0.0004 ppm), NO2FAW5 (6.0% TRR, 0.0011 ppm), NO2FAW6 (2.0% TRR, 0.0004 ppm), NO2FAW8 (1.4% TRR, 0.0003 ppm), and ≤29 unspecified peaks, each <2% TRR (collectively 15.4% TRR, 0.0025 ppm). Total Identified/Characterized = 87.9% TRR, 0.0153 ppm.
Pellet	N/R	N/R	N/A.
Non-extractable	0.8	0.0001	N/A.
Hexane	10.6	0.0018	N/A.
Pellet	N/R	N/R	N/A.

* All values are mean of triplicate analyses for "Bulk Samples".

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Table 7. Distribution of total radioactive residues (TRR) in liver of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Fraction	%TRR	ppm *	Characterization/Identification
CPR-labeled liver (0.0917 ppm)			
ACN/water	81.3	0.0746	HPLC: Acifluorfen (5.2% TRR, 0.0048 ppm), acifluorfen acetamide (15.4% TRR, 0.0142 ppm), descarboxy acifluorfen (15.7% TRR, 0.0144 ppm), eight unknowns: CF3LAW1 (1.4% TRR, 0.0013 ppm), CF3LAW2 (5.3% TRR, 0.0049 ppm), CF3LAW3 (6.9% TRR, 0.0064 ppm), CF3LAW5 (0.8% TRR, 0.0007 ppm), CF3LAW7 (1.9% TRR, 0.0017 ppm), CF3LAW8 (1.5% TRR, 0.0014 ppm), CF3LAW9 (9.7% TRR, 0.0089 ppm), CF3LAW10 (3.0% TRR, 0.0028 ppm), and ≤38 unspecified peaks, each <2% TRR (collectively 14.5% TRR, 0.0131 ppm). Total Identified/Characterized = 81.3% TRR, 0.0746 ppm.
Pellet	N/R (not reported)	N/R	N/A (not further analyzed).
Non-extractable	15.4	0.0141	N/A.
MeOH	8.7	0.0080	N/A.
Pellet	N/R	N/R	N/A.
Hexane	0.8	0.0007	N/A.
Non-extractable	N/R	N/R	N/A.
NPR-labeled liver (0.0789 ppm)			
ACN/water	74.5	0.0601	HPLC: Acifluorfen (11.4% TRR, 0.0092 ppm), acifluorfen acetamide (8.9% TRR, 0.0073 ppm), ten unknowns: NO2LAW1 (1.9% TRR, 0.0015 ppm), NO2LAW2 (1.8% TRR, 0.0014 ppm), NO2LAW3 (8.0% TRR, 0.0065 ppm), NO2LAW5 (3.6% TRR, 0.0029 ppm), NO2LAW7 (1.0% TRR, 0.0009 ppm), NO2LAW8 (2.6% TRR, 0.0021 ppm), NO2LAW9 (1.5% TRR, 0.0013 ppm), NO2LAW10 (10.9% TRR, 0.0088 ppm), NO2LAW11 (1.0% TRR, 0.0008 ppm), NO2LAW12 (6.1% TRR, 0.0049 ppm), and ≤36 unspecified peaks, each <2% TRR (collectively 15.8% TRR, 0.0125 ppm). Total Identified/Characterized = 74.5% TRR, 0.0601 ppm.
Pellet	N/R	N/R	N/A.

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Fraction	%TRR	ppm *	Characterization/Identification
Non-extractable	11.6	0.0094	N/A.
MeOH	6.3	0.0050	N/A.
Pellet	N/R	N/R	N/A.
Hexane	3.5	0.0028	N/A.
Non-extractable	N/R	N/R	N/A.

* All values are mean of triplicate analyses for "Bulk Samples".

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Table 8. Distribution of total radioactive residues (TRR) in thigh muscle of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Fraction	%TRR	ppm *	Characterization/Identification
CPR-labeled thigh muscle (0.0192 ppm)			
ACN/water	88.3	0.0169	HPLC: Acifluorfen (1.9% TRR, 0.0004 ppm), acifluorfen acetamide (2.1% TRR, 0.0004 ppm), descarboxy acifluorfen (49.7% TRR, 0.0096 ppm), four unknowns: CF3TAW1 (3.2% TRR, 0.0006 ppm), CF3TAW2 (2.3% TRR, 0.0004 ppm), CF3TAW5 (1.5% TRR, 0.0003 ppm), CF3TAW6 (7.7% TRR, 0.0015 ppm), and ≤33 unspecified peaks, each <2% TRR (collectively 19.9% TRR, 0.0037 ppm). Total Identified/Characterized = 88.3% TRR, 0.0169 ppm.
Pellet	N/R (not reported)	N/R	N/A (not further analyzed).
Non-extractable	7.3	0.0014	N/A.
NPR-labeled thigh muscle (0.0072 ppm)			
ACN/water	106.2	0.0076	HPLC: Acifluorfen (17.8% TRR, 0.0013 ppm), acifluorfen acetamide (20.6% TRR, 0.0015 ppm), descarboxy acifluorfen (5.5% TRR, 0.0004 ppm), eight unknowns: NO2TAW1 (5.0% TRR, 0.0004 ppm), NO2TAW2 (2.6% TRR, 0.0002 ppm), NO2TAW3 (4.7% TRR, 0.0004 ppm), NO2TAW6 (3.8% TRR, 0.0003 ppm), NO2TAW7 (21.4% TRR, 0.0016 ppm), NO2TAW8 (10.9% TRR, 0.0008 ppm), NO2TAW10 (1.3% TRR, 0.0001 ppm), NO2TAW11 (1.6% TRR, 0.0001 ppm), and ≤24 unspecified peaks, each <2% TRR (collectively 11.0% TRR, 0.0005 ppm.) Total Identified/Characterized = 106.2% TRR, 0.0076 ppm.
Pellet	N/R	N/R	N/A.
Non-extractable	14.0	0.0010	N/A.

* All values are mean of triplicate analyses for "Bulk Samples".

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Table 9. Summary of metabolites characterized/identified in egg whites of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Metabolite	[^{14}C]Sodium Acifluorfen Equivalents	
	%TRR	ppm
CPR-label		
Identified		
Acifluorfen	38.2	0.0044
Acifluorfen acetamide	3.1	0.0003
Descarboxy acifluorfen	11.2	0.0013
Total Identified	**52.5**	**0.0060**
Characterized		
CF3EWA1	3.4	0.0004
CF3EWA2	2.2	0.0003
CF3EWA3	4.6	0.0005
CF3EWA6	8.9	0.0010
CF3EWA7	2.8	0.0003
CF3EWA9	0.8	0.0001
Other Peaks	15.0	0.0017
Total Characterized/Identified	**90.7**	**0.0103**
Non-extractable	11.6	0.0013
NPR-label		
Identified		
Acifluorfen	29.8	0.0038
Acifluorfen acetamide	20.7	0.0026
Total Identified	**50.5**	**0.0064**
Characterized		
NO2EWA1	1.6	0.0002
NO2EWA4	7.5	0.0010
NO2EWA5	5.5	0.0007
NO2EWA6	1.2	0.0002
NO2EWA7	1.4	0.0002
Other Peaks	19.5	0.0022
Total Characterized/Identified	**87.2**	**0.0109**
Non-extractable	19.2	0.0024

Table 10. Summary of metabolites characterized/identified in egg yolks of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Metabolite	[^{14}C]Sodium Acifluorfen Equivalents	
	%TRR	ppm
CPR-label		
Identified		
Acifluorfen	8.3	0.0113
Descarboxy acifluorfen	28.7	0.0393
Total Identified	**37.0**	**0.0506**
Characterized		
CF3YA1	1.9	0.0026
CF3YA2	4.8	0.0066
CF3YA4	4.5	0.0061
CF3YA5	2.8	0.0038
Other HPLC peaks (ACN)	10.7	0.0146
CF3YA7	1.4	0.0020
CF3YH2	5.8	0.0080
Total Characterized/Identified	**74.5**	**0.1019**
Non-extractable	12.6	0.0173
NPR-label		
Identified		
Acifluorfen	21.8	0.0108
Acifluorfen acetamide	2.6	0.0013
Descarboxy acifluorfen	3.6	0.0018
Total Identified	**28.0**	**0.0139**
Characterized		
NO2YA1	2.0	0.0010
Other HPLC peaks	10.8	0.0052
NO2YH1	6.1	0.0030
NO2YH2	17.6	0.0087
Total Characterized/Identified	**64.5**	**0.0318**
Non-extractable	23.2	0.0114

Table 11. Summary of metabolites characterized/identified in fat of hens orally dosed once daily with NPR-labeled and CPR-label acifluorfen for five consecutive days, at 111x and 92x, respectively.

Metabolite	[^{14}C]Sodium Acifluorfen Equivalents	
	%TRR	ppm
CPR-label		
Identified		
Descarboxy acifluorfen	74.4	0.2246
Total Identified	**74.4**	**0.2246**
Characterized		
Other Peaks	12.6	0.0381
Hexane soluble	**2.9**	**0.0089**
Total Characterized/Identified	**89.9**	**0.2716**
Non-extractable	0.1	0.0003
NPR-label		
Identified		
Acifluorfen	3.9	0.0007
Acifluorfen acetamide	2.8	0.0005
Descarboxy acifluorfen	52.8	0.0092
Total Identified	**59.5**	**0.0104**
Characterized		
NO2FAW1	1.1	0.0002
NO2FAW2	2.5	0.0004
NO2FAW5	6.0	0.0011
NO2FAW6	2.0	0.0004
NO2FAW8	1.4	0.0003
Other peaks	15.4	0.0025
Hexane soluble	10.6	0.0018
Total Characterized/Identified	**98.5**	**0.0171**
Non-extractable	0.8	0.0001

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Table 12. Summary of metabolites characterized/identified in liver of hens orally dosed once daily with NPR-labeled and CPR-labeled acifluorfen for five consecutive days, at 111x and 92x, respectively.

Metabolite	[^{14}C]Sodium Acifluorfen Equivalents	
	%TRR	ppm
CPR-label		
Identified		
Acifluorfen	5.2	0.0048
Acifluorfen acetamide	15.4	0.0142
Descarboxy acifluorfen	15.7	0.0144
Total Identified	**36.3**	**0.0334**
Characterized		
CF3LAW1	1.4	0.0013
CF3LAW2	5.3	0.0049
CF3LAW3	6.9	0.0064
CF3LAW5	0.8	0.0007
CF3LAW7	1.9	0.0017
CF3LAW8	1.5	0.0014
CF3LAW9	9.7	0.0089
CF3LAW10	3.0	0.0028
Other peaks	14.5	0.0131
MeOH soluble	8.7	0.0080
Hexane soluble	0.8	0.0007
Total Characterized/Identified	**90.8**	**0.0833**
Non-extractable	15.4	0.0141
NPR-label		
Identified		
Acifluorfen	11.4	0.0092
Acifluorfen acetamide	8.9	0.0073
Total Identified	**20.3**	**0.0165**
Characterized		
NO2LAW1	1.9	0.0015
NO2LAW2	1.8	0.0014
NO2LAW3	8.0	0.0065
NO2LAW5	3.6	0.0029
NO2LAW7	1.0	0.0009
NO2LAW8	2.6	0.0021
NO2LAW9	1.5	0.0013

(continued; footnotes follow)

Table 12 *(continued)*.

Metabolite	[^{14}C]Sodium Acifluorfen Equivalents	
	%TRR	ppm
NPR-label (continued)		
NO2LAW10	10.9	0.0088
NO2LAW11	1.0	0.0008
NO2LAW12	6.1	0.0049
Other peaks	15.8	0.0125
MeOH soluble	6.3	0.0050
Hexane soluble	3.5	0.0028
Total Characterized/Identified	**84.3**	**0.0679**
Non-extractable	11.6	0.0094

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Table 13. Summary of metabolites characterized/identified in thigh muscle of hens orally dosed once daily with NPR-labeled and CPR-label acifluorfen for five consecutive days, at 111x and 92x, respectively.

Metabolite	[^{14}C]Sodium Acifluorfen Equivalents	
	%TRR	ppm
CPR-label		
Identified		
Acifluorfen	1.9	0.0004
Acifluorfen acetamide	2.1	0.0004
Descarboxy acifluorfen	49.7	0.0096
Total Identified	**53.7**	**0.0104**
Characterized		
CF3TAW1	3.2	0.0006
CF3TAW2	2.3	0.0004
CF3TAW5	1.5	0.0003
CF3TAW6	7.7	0.0015
Other Peaks	19.9	0.0037
Total Characterized/Identified	**88.3**	**0.0169**
Non-extractable	7.3	0.0014
NPR-label		
Identified		
Acifluorfen	17.8	0.0013
Acifluorfen acetamide	20.6	0.0015
Descarboxy acifluorfen	5.5	0.0004
Total Identified	**43.9**	**0.0032**
Characterized		
NO2TAW1	5.0	0.0004
NO2TAW2	2.6	0.0002
NO2TAW3	4.7	0.0004
NO2TAW6	3.8	0.0003
NO2TAW7	21.4	0.0016
NO2TAW8	10.9	0.0008
NO2TAW10	1.3	0.0001
NO2TAW11	1.6	0.0001
Other Peaks	11.0	0.0005
Total Characterized/Identified	**106.2**	**0.0076**
Non-extractable	14.0	0.0010

Volatile metabolite study

The registrant observed lower total recoveries of ^{14}C-residues from edible matrices and excreta of hens treated with CPR-label test substance than from tissues from hens treated with NPR-label test substance. Therefore, a study was designed to determine if the lower recoveries observed for CPR-labeled hens resulted from the loss of volatile metabolites. Two hens were orally dosed once daily with CPR-labeled acifluorfen at 10 ppm in a gelatin capsule. The hens were placed in enclosed cages designed to collect volatile ^{14}C-residues. Expired air was passed through three separate bottles, respectively containing trapping solutions of ethylene glycol, 0.01% sulfuric acid, and 2N sodium hydroxide. The trapping solutions were analyzed daily by LSS. The registrant indicated that no ^{14}C-residues were detected in the trapping solutions throughout the study. Hens were sacrificed ~22 hours following the last dose, but tissues were not analyzed for radioactivity.

Storage stability data

Hen matrices used in this study were stored for ~12 months at -20 C prior to analysis. To determine the stability of acifluorfen in frozen storage, liver samples from CPR-labeled hens were extracted after ~3 months and ~12 months frozen storage using methods previously described, and were analyzed by HPLC System II (previously described). The registrant noted a 2-3 minute retention time shift for the chromatographic profiles between the 3-month and 12-month analyses; however, all major metabolites were identified by comparison of the retention times with those of the appropriate reference standards. The data indicate that acifluorfen, acifluorfen acetamide, and descarboxy acifluorfen are stable in liver samples for up to 12 months at ~-20 C. Storage stability data are presented in Table 14.

Table 14. Stability of acifluorfen, acifluorfen acetamide, and descarboxy acifluorfen in liver samples from hens orally dosed with CPR-labeled acifluorfen at 111x for five consecutive days; samples were stored for 3 and 12 months at ~ -20 C prior to analysis.

Metabolite *	3-Month Analysis		12-Month Analysis	
	%TRR	ppm	%TRR	ppm
Acifluorfen	5.2	0.0048	5.1	0.0047
Acifluorfen acetamide	15.4	0.0142	16.6	0.0153
Descarboxy acifluorfen	15.7	0.0144	19.3	0.0177

* Metabolites were identified and quantified in ACN:water extracts using HPLC System II.

In summary, the qualitative nature of the residue in poultry is adequately understood. The study involved dosing hens separately with CPR- and NPR-labeled [^{14}C]sodium acifluorfen. No metabolites were identified which indicate cleavage of the diphenyl ether bond. Acifluorfen was a major residue in egg white (up to 40% of the total residue), egg yolk (up to 22%), liver (up to 11%), and thigh muscle (up to 18%). Acifluorfen acetamide accounted for up to 20% of the residue in egg white, 15% in liver, and 20% in thigh muscle. Descarboxy acifluorfen comprised up to 11% of the residue in egg white, 30% in egg yolk, 15% in liver, and 50% in thigh muscle. Descarboxy acifluorfen was the major residue in fat accounting for 50-70% of the residue.

The study successfully characterized/identified the following levels of radioactivity in matrices from hens orally dosed with CPR- and NPR-labeled acifluorfen: egg white (87.2-90.7% TRR), egg yolk (64.5-74.5% TRR), fat (89.9-98.5% TRR), liver (84.3-90.8% TRR), and thigh muscle (88.3-106.2% TRR).

Except for descarboxy acifluorfen (0.2246 ppm) in fat of CPR-labeled hens, residues of acifluorfen and its metabolites, acifluorfen acetamide and descarboxy acifluorfen, were present at levels below 0.04 ppm. Combined residues of acifluorfen, acifluorfen acetamide, and descarboxy acifluorfen were: egg white (0.0060-0.0064 ppm), egg yolk (0.0139-0.0506 ppm), fat (0.0104-0.2246 ppm), liver (0.0165-0.0334 ppm), and thigh (0.0032-0.0114 ppm). Unidentified extractable and non-extractable components in all matrices each were $\leq$0.0173 ppm.

There were differences observed between residue levels in tissues from the CPR-labeled and NPR-labeled treated hens. In general, total radioactive residues (TRR) were greater from dosing with the CPR-label about 2x in muscle and liver and up to 10x in fat. The metabolite profiles differed as well in some cases. Egg yolks for example contained acifluorfen at 8% and descarboxy acifluorfen at almost 30% of the TRR from the CPR-label, whereas the NPR label resulted in about 20% acifluorfen and 3.6% descarboxy acifluorfen. As another example, CPR label residues in thigh muscle consisted of ~2% acifluorfen, 2% acifluorfen acetamide, and ~50% descarboxy acifluorfen; as NPR label residues, these compounds accounted for 18, 20, and 5.5% of the TRR, respectively. Addressing the differences in descarboxy acifluorfen in CPR-labeled fat (0.2246 ppm, 74.4% TRR) and NPR-labeled fat (0.0092 ppm, 52.8% TRR), the registrant pointed out (i) the higher administered dose of the CPR-labeled test substance than of the NPR-labeled test substance (11.1 ppm vs. 9.2 ppm); and (ii) a higher level of [^{14}C]descarboxy acifluorfen impurity in the CPR-labeled test substance than in the NPR-labeled test substance (2.2% vs. 0.3%).

The requirement for the radiovalidation of analytical methods is reserved until the HED Metabolism Committee determines the residues to be regulated.

Residue Analytical Methods

BASF Corporation (1993; MRID 42815702) submitted a method (Analytical Method No. D9205) for the determination of sodium acifluorfen and metabolites in/on soybean seed. Acifluorfen, salt and acid forms, and acifluorfen methyl ester are determined by GC following methylation and acifluorfen amine and acifluorfen amine methyl ester are quantified directly by HPLC. Analyses were conducted by BASF Corporation (Research Triangle Park, NC).

Using this method, soybean seeds are pulverized and soaked in 0.1 N sodium hydroxide (NaOH) for one hour and residues are extracted with 1% acetic acid in acetonitrile (ACN). The solids are rinsed with the same solvent and the combined acetic acid/ACN fractions are filtered. For analyzing acifluorfen and acifluorfen methyl ester, the extract is washed with heptane and ACN. The residues in ACN are concentrated and partitioned to dichloromethane (DCM). The DCM fraction is washed with 1 N HCl and the aqueous layer discarded. Residues are concentrated under a stream of nitrogen and methylated with trimethylsilyl diazomethane. The residues are cleaned up on a silica gel solid phase extraction (SPE) column and diluted with toluene. Acifluorfen methyl ester is determined using GC/ECD with a DB-5 column. The limit of quantitation was 0.02 ppm for each metabolite.

To determine acifluorfen amine and acifluorfen amine methyl ester, the residues in ACN are cleaned on a C18 column, if necessary, and analyzed directly by HPLC on a Nucleosil 5 C18 column equipped with a fluorometric detector (excitation wavelength, 350 nm; emission wavelength, 420

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nm). The isocratic mobile phase consisted of ACN:2.5% aqueous acetic acid (75:25, v:v). The limit of quantitation was 0.02 ppm for each metabolite.

The registrant stated that lactofen and its metabolites may be expected to interfere with GC and HPLC analyses because of chemical similarities with acifluorfen and its metabolites. However, no other interfering substances were observed.

Confirmatory methods: The registrant stated that residues of acifluorfen methyl ester obtained from the GC preparation steps and residues of amino acifluorfen and amino acifluorfen methyl ester obtained from the HPLC preparation steps, may be confirmed by GC/MS and HPLC/MS, respectively. For the purpose of distinguishing between residues of acifluorfen *per se* and acifluorfen methyl ester, GC/ECD analyses may be repeated without prior derivatization with diazomethane.

The registrant provided all sample calculations and data for procedural recoveries from reference standards and method validation data using fortified soybean grain samples. Representative chromatograms depicting GC analyses of acifluorfen and acifluorfen methyl ester in fortified grain samples, and HPLC analyses of amino acifluorfen and amino acifluorfen methyl ester in fortified grain samples were provided as well as standard curves for GC and HPLC analyses.

Procedural recoveries are presented in Table 15. The registrant also presented method validation data, presented in Table 16.

Table 15. Procedural recoveries of acifluorfen and its metabolites using BASF Method D9205.

Metabolite	Fortification Level (ppm)	Percent Recovery
Acifluorfen (GC/ECD)	0.02	62, 76
	0.20	73, 76
Acifluorfen methyl ester (GC/ECD)	0.02	72, 85
	0.20	82, 91
Acifluorfen amine (HPLC)	0.02	66, 71
	0.20	76, 77
Acifluorfen amine methyl ester (HPLC)	0.02	66, 73
	0.20	69, 72

Table 16. Method recoveries of acifluorfen and its metabolites from fortified soybean grain samples using BASF Method D9205.

Metabolite	Fortification Level (ppm)	Percent Recovery
Acifluorfen (GC/ECD)	0.02	68, 71
	0.20	78, 89
Acifluorfen methyl ester (GC/ECD)	0.02	84, 91
	0.20	89, 93
Acifluorfen amine (HPLC)	0.02	67, 79
	0.20	87, 88
Acifluorfen amine methyl ester (HPLC)	0.02	93, 94
	0.20	88, 93

In summary, the submitted method is adequate for data collection on the combined residues of acifluorfen, its amine metabolite, and their methyl esters in soybean grain. To be considered for tolerance enforcement, the method must be validated by an independent laboratory and submitted to EPA for Agency validation.

Magnitude of the Residue in Soybeans

A tolerance of 0.1 ppm has been established for 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 analogues) in/on soybeans [40 CFR §180.383].

A REFs search indicated that two 2 lb/gal SC/L formulations (EPA Reg. Nos. 7969-79 and 7969-80) are registered for 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. *[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). 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 (EPA Reg. No. 7969-76) formulation is registered for postemergence use on soybeans at the 2-3 leaf stage at 0.25 lb ai/A/application and a maximum seasonal rate of 0.25 lb ai/A. A 0.67 lb/gal MAI SC/L (EPA Reg. No. 7969-77) formulation is registered for postemergence use on soybeans at 0.17 lb ai/A/application at the 1-2 leaf stage and a seasonal rate of 0.25 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. In case of crop failure, soybeans may be replanted immediately. 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].

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BASF Corporation (1993; MRID 42815701) submitted data from six tests conducted in IA(2), IL(2), and MN(2) depicting residues of the sodium salt of acifluorfen and its metabolites (the corresponding acid, methyl ester, and amino analogues) in/on soybean grain harvested 48-53 days following the second of two postemergence applications of the 2 lb/gal SC/L formulation using ground equipment. The first application was made at a field-equivalent rate of 0.126-0.127 lb ai/A and the second application was made at 0.38-0.391 lb ai/A, for a seasonal rate of ~0.5 lb ai/A (1x the maximum revised seasonal use rate). In one MN test, the crop was still green at harvest and samples from this trial were not analyzed.

Samples from the field trial were stored for a maximum of 6 months at <-5 C prior to analysis. The registrant initiated a frozen storage stability study for acifluorfen, its amino metabolite, and their methyl esters in or on soybean grain in support of the field residue data. The results are shown in Table 17.

Table 17. Percent recoveries of acifluorfen and its metabolites from soybean grain samples fortified with each metabolite at 0.02 ppm and stored at <-5 C.

Metabolite	Storage Interval (months) [a,b,c]		
	0	1.5	2.5
Acifluorfen	100	106	106
Acifluorfen methyl ester	100	121	92
Acifluorfen amine	100	27	24
Acifluorfen amine methyl ester	100	104	89

[a] Values are corrected for procedural recoveries: acifluorfen, 71-83%; acifluorfen methyl ester, 76-88%; acifluorfen amine, 75-86%; and acifluorfen amine methyl ester, 61-92%.
[b] 0-month samples were extracted without a pre-soak in 0.1 N sodium hydroxide prior to solvent extraction; values are from a single analysis.
[c] 1.5- and 2.5-month samples were from duplicate analyses.

Acifluorfen, acifluorfen methyl ester, and acifluorfen amine methyl ester were stable over the 2.5 month preliminary reporting interval. Acifluorfen amine declined to 27% of the fortified level after 1.5 months and to 24% after 2.5 months. The registrant claims that the instability of acifluorfen amine should not effect the integrity of the field study because this metabolite was shown to be an insignificant component of the residue in a previous metabolism study. The significance of the instability of the amine metabolite will be determined when plant metabolism data are complete and the final storage stability report has been reviewed.

Samples from the field study were analyzed using BASF Method No. D9205 (previously described) which is based upon Rhone-Poulenc Method No. 160, and modified to include an initial soak in 0.1 N sodium hydroxide prior to extraction. The limit of quantitation is 0.02 ppm each for acifluorfen, its amine metabolite, and their methyl esters. Method recoveries from two soybean grain samples each, fortified with each metabolite at 0.02 ppm were: acifluorfen (78-93%), acifluorfen-methyl ester (87-104%), acifluorfen amine (60-70%), and acifluorfen amine methyl ester (83-105%). Analyses were conducted by BASF Corporation (Research Triangle Park, NC).

Combined residues of acifluorfen and its metabolites were <0.1 ppm (<0.02 ppm for each of the five regulated compounds) in/on 15 samples of soybean grain. Apparent combined residues of

acifluorfen also were <0.1 ppm in/on 15 untreated controls. All samples were stored frozen within 6.5 hours of harvest and shipped frozen to the analytical laboratory (BASF Agricultural Research Center, RTP, NC). Upon receipt at the analytical laboratory, all samples were stored frozen at <-5 C for up to 6 months prior to analysis.

Geographic representation is adequate. The test states of IA(17), IL(18), and MN(9) [representing IN(9), MO(6), ND(1), NE(4), SD(3), and WI(1)] together accounted for ~70% of the 1990 U.S. soybean production.

In summary, the combined residues of the sodium salt of acifluorfen and its metabolites (the corresponding acid, methyl ester, and amino analogues) were <0.1 ppm (<0.02 ppm for each metabolite) in/on soybeans treated at 1x the revised maximum seasonal rate. These data are adequate pending receipt of the completed storage stability study and a determination that amine metabolite instability does not appreciably affect the total combined residue level.

EPA MEMORANDA CITED IN THIS REVIEW

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

MASTER RECORD IDENTIFICATION NUMBERS

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

42828201 Nelsen, J., Steginsky, C.A., Campbell, L.H., Veley, K., and Powell, J. (1993) Nature of the Residue Study of ^{14}C-Radiolabeled Sodium Acifluorfen Using Egg-Laying White Leghorn Hens. Battelle Study Reference No. SC910216, BASF Study No. 91115, BASF Report No. M9309. Unpublished study conducted by Battelle Columbus, Columbus, OH, and sponsored by BASF Corporation, Research Triangle Park, NC. 237 p.

42815702 Klose, S.F. and Burkey, J.D. (1993) Method for Determination of Residues of Acifluorfen and Metabolites in Soybean grain by Gas Chromatography and Liquid Chromatography. BASF Registration Document No. BASF 93/5055, BASF Study No. 92161. Unpublished study conducted by BASF Corporation, Research Triangle Park, NC. 70 p.

42815701 Burkey, J.D. (1993) Magnitude of the Residue of Sodium Acifluorfen and its Metabolites in Soybean Grain Raw Agricultural Commodity Samples. BASF Registration Document No. BASF 93/5053, BASF Study Number 92098, BASF Report Number A9314. Unpublished study conducted by BASF Corporation, Research Triangle Park, NC. 175 p.