UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Date: March 20, 2003

MEMORANDUM

SUBJECT: **Benfluralin Reregistration.** GLN#: 860.1850: Confined Rotational Crop Data on Lettuce, Mustard, Radishes, and Wheat.

Reregistration Case No.: 2030.
PC Code: 084301.
DP Barcode Nos.: D226790.
MRID Nos.: 44019801.

FROM: Sherrie L. Kinard, Chemist
Reregistration Branch II
Health Effects Division (7509C)

THROUGH: Alan Nielsen, Branch Senior Scientist
Reregistration Branch II
Health Effects Division (7509C)

TO: Rich Griffin, Risk Assessor
Reregistration Branch II
Health Effects Division (7509C)

Attached is a review of benfluralin confined rotational crop data on lettuce, mustard, radishes, and wheat prepared by Dynamac Corporation for the reregistration eligibility decision of benfluralin. This information has undergone secondary review in Reregistration Branch 2 and is consistent with current Agency policies.

CONCLUSIONS AND RECOMMENDATIONS

The submitted confined rotational crop study is adequate to satisfy data requirements for OPPTS 860.1850 pending submission of additional storage stability data and label amendments. These data demonstrate differences in the metabolism of benfluralin in rotational crops versus primary crops. RRB2 concludes that a 12-month plant-back interval would be appropriate for all crops and applying to all regions of the U.S.

DEFICIENCIES

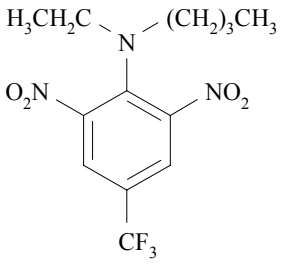
The registrant must submit the dates of the final analyses of samples as well as data demonstrating that the metabolic profile in each rotational crop commodity did not change over the intervals during which samples were stored.

The registrant must modify their product label to specify a 12-month plant-back interval for crops that may be rotated and to make it clear that rotational crop restrictions apply to all regions of the U.S. If the registrant wishes a rotational crop restriction of less than 12 months for leafy vegetables, then limited field rotational crop studies (OPPTS 860.1900) must be conducted.

cc: Sherrie L. Kinard (RRB2), Benfluralin Reg. Standard File, Benfluralin SF, RF, LAN.
RD/I: RRB2 Chemistry Team Review (3/17/03), Alan Nielsen (3/20/03).

7509C: RRB2: S. Kinard: CM2: Rm 712M: 703-305-0563

BENFLURALIN



Shaughnessy No. 084301; Case 2030

(CBRS No. 17297; DP Barcode D226790)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

Platte Chemical Company has submitted a confined rotational crop study (1996; MRID 44019801) which is evaluated in this document for adequacy in fulfilling the residue chemistry reregistration requirements for OPPTS Guideline No. 860.1850.

The qualitative nature of the residue in plants is adequately understood based on metabolism studies conducted on alfalfa, lettuce, and peanuts. The residue of concern in plants is benfluralin. The qualitative nature of the residue in animals is adequately understood based on metabolism studies conducted on dairy cattle and laying hens. There is no reasonable expectation of finite residues in meat, milk, poultry, and eggs from the registered uses of benfluralin.

Tolerances are established for negligible residues of benfluralin (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine) in/on the raw agricultural commodities (RACs) of alfalfa, birdsfoot trefoil, clover, lettuce, and peanuts at 0.05 ppm [40 CFR §180.208]. No Codex MRLs have been established for benfluralin; therefore, there are no issues of compatibility between U.S. tolerances and Codex MRLs. An adequate method is available for the purposes of tolerance enforcement. A GC method using electron capture detection is listed as Method A in PAM, Vol. II, for the determination of benfluralin in/on plant commodities.

CONCLUSIONS AND RECOMMENDATIONS

1. The submitted confined rotational crop study is adequate to satisfy data requirements for OPPTS 860.1850 pending submission of additional storage stability data. The registrant must submit the dates of the final analyses of samples as well as data demonstrating that

the metabolic profile in each rotational crop commodity did not change over the intervals during which samples were stored.

2. The total radioactive residues (TRR) accumulated at levels above 0.01 ppm in/on the rotational commodities of lettuce, mustard, radishes, and wheat that were planted 28, 91, or 364 days following application of [^{14}C]benfluralin to sandy loam soil at 1x the maximum registered seasonal rate. In general, accumulation of total radioactive residues was highest (0.03-0.34 ppm) in samples collected 91-days after treatment (DAT) and decreased (0.01-0.07 ppm) at the 364-DAT interval.

3. Although the TRR were $0.01 ppm in/on all rotational crop commodities at all plant-back intervals tested, the registrant chose to conduct metabolite characterization/identification procedures only on the following selected commodities: the 28- and 364-DAT lettuce, the 91-DAT mustard greens, the 364-DAT radish roots and tops, the 364-DAT wheat forage, and the 28- and 91-DAT wheat straw. Approximately 86-97% of TRR in these commodities were characterized/identified by a combination of chromatographic techniques (HPLC, TLC, LC/MS). The parent, benfluralin, was detected at low levels in 28-DAT lettuce (13.20% TRR, 0.009 ppm), 91-DAT mustard greens (6.21% TRR, 0.005 ppm), 364-DAT radish roots (10.30% TRR, 0.002 ppm), and 91-DAT wheat straw (1.30% TRR, 0.004 ppm). The major metabolite, referred to as Metabolite A and identified as trifluoroacetic acid in mustard greens, was detected in all tested commodities at 27.88-76.92% of TRR (0.006-0.137 ppm). An additional 19 unidentified metabolites were resolved by HPLC analyses; these metabolites were each present at <0.05 ppm.

4. There appears to be differences in the metabolism of benfluralin in rotational crops versus primary crops. In plant metabolism studies conducted on alfalfa, lettuce, and peanuts, the majority of total radioactivity was found to be incorporated into lignin and cellulose with the remainder of the radioactivity due to numerous minor unidentified metabolites. Only small amounts of benfluralin were identified in lettuce and peanut hulls, and trifluoroacetic acid was not identified at all.

5. The results of the current confined rotational crop study indicate that a 12-month plant-back interval would be appropriate for all crops. Currently, the product label specifies a 10-month plant-back interval for wheat, barley, oats, rye, other grasses, onions, corn, milo (grain sorghum), spinach, red beets, sugar beets, or other root crops, and the label implies that this rotational crop restriction applies only to arid, irrigated areas of the Western United States. The registrant must modify their product label to specify a 12-month plant-back interval for crops that may be rotated and to make it clear that rotational crop restrictions apply to all areas of the U.S. If the registrant wishes a rotational crop restriction of less than 12 months for leafy vegetables, then limited field rotational crop studies (OPPTS 860.1900) must be conducted.

DETAILED CONSIDERATIONS

Registered Use Patterns

A REFS search, conducted on 10/21/97, identified one benfluralin end-use product registered to Platte Chemical Company for use on crops which may be rotated, the 60% DF formulation (EPA Reg. No. 34704-746). The maximum application rate listed on the product label for rotatable crops (lettuce, alfalfa, birdsfoot trefoil, and clover) is 1.5 lb ai/A for fine soils and 1.2 lb ai/A for medium and coarse soils. The product label specifies the following rotational crop restrictions:

> "In arid, irrigated areas of the Western United States-Arizona, California, Idaho, Montana, Nevada, Oregon, Utah, Washington, and Wyoming-certain crops are susceptible to injury when planted in soil previously treated with BALAN DF. To avoid such injury, do not plant wheat, barley, oats, rye, other grasses, onions, corn, milo (grain sorghum), spinach, red beets, sugar beets, or other root crops for 10 months following an application of BALAN DF."

Confined Rotational Crop Study

In-life phase

Platte submitted (1996; MRID 44019801) a confined rotational crop study with [^{14}C]benfluralin on lettuce, mustard, radishes, and wheat. The field phase of the study was conducted by American Agricultural Services, Inc. (Cary, NC) and the analytical phases of the study were conducted by Battelle (Columbus, OH; TRR determinations) and XenoBiotic Laboratories (XBL; Plainsboro, NJ; metabolite characterization and identification). Uniformly ring-labeled [^{14}C]benfluralin (radiochemical purity 98.5%, specific activity 8.97 µCi/mg) was mixed with nonlabeled benfluralin and dissolved in methanol. The test substance was applied using a sprayer at a field-equivalent rate of 1.26 lb ai/A to sandy loam soil (68-70% sand, 24-28% silt, 4-6% clay, 1.3-1.4% organic matter, pH 6.3-6.9, cation exchange capacity 5.4-5.7 meq/100 g) in 8 ft x 3 ft x 2 ft tanks maintained outdoors. The application was then tilled into the top 3 inches of soil. The application rate of 1.26 lb ai/A is 1x the maximum registered seasonal application rate for sandy loam (medium) soil. Lettuce, radish, and wheat (winter) were planted 28 and 364 days after treatment (DAT) and mustard, radish, and wheat were planted 91 DAT. Prior to rotational crop plantings, the soil in the tanks was tilled to a depth of 2-4 inches. Twelve tanks were used: six control tanks and six tanks for treatment. Lettuce/mustard and radishes were planted together and wheat was planted in a separate tank for each rotational interval. The crops were fertilized and watered as necessary; adequate information pertaining to the agronomic and climatic conditions during the study was provided.

Wheat forage was sampled 42-94 days after planting. Mature lettuce, mustard, radishes (separated into roots and tops), and wheat (separated into grain and straw) were harvested 42-45, 84, 32-88, and 175-258 days after planting, respectively. Lettuce, mustard, and wheat forage samples were harvested by cutting the plants above the soil. Radish roots were shaken after

harvest to remove adhering soil particles. Wheat grain heads were threshed to separate the grain, and the threshed heads and chaff were combined with the straw sample. Samples were stored frozen at the field facility, during shipment (overnight on dry ice or via ACDS freezer truck) to Battelle, and at Battelle (#-20E C) prior to analysis. Samples which were then sent to XBL for further analysis were shipped frozen (on dry ice overnight) and stored frozen (-10E C) prior to analysis.

Total radioactive residues (TRR)

Subsamples of the rotational crop commodities were homogenized in liquid nitrogen. Triplicate aliquots were analyzed for TRR by liquid scintillation counting (LSC) following combustion. The LSC limit of detection was 0.01 ppm. The apparent TRR in/on all control samples were each below the detection limit. The TRR in/on treated rotational crop commodities are presented in Table 1.

Selected rotational crop commodities were sent to XBL for metabolite characterization and identification. The TRR in these commodities were redetermined at XBL; these values are also presented in Table 1. For XBL determinations, the LSC limit of detection was 0.001 ppm.

Table 1. Total radioactive residues (TRR) in/on rotational crops grown in sandy loam soil treated with [^{14}C]benfluralin at 1.26 lb ai/A (1x the maximum registered seasonal rate).

	TRR (ppm [^{14}C]benfluralin equivalents) [a]		
Substrate	28 DAT [b]	91 DAT	364 DAT
Lettuce	0.06 - Battelle 0.071 - XBL	--	<0.01 - Battelle 0.011 - XBL
Mustard greens	--	0.07 - Battelle 0.073 - XBL	--
Radish roots	0.10	0.06	0.02 - Battelle 0.021 - XBL
Radish tops	0.05	0.09	0.03 - Battelle 0.031 - XBL
Wheat forage (immature)	0.05	0.05	0.02 - Battelle 0.019 - XBL
Wheat grain	0.02	0.03	0.01
Wheat straw	0.23 - Battelle 0.239 - XBL	0.31 - Battelle 0.344 - XBL	0.07

[a] Reported residues are the mean of triplicate analyses. When TRR determinations were conducted at Battelle and at XBL, the results of both analyses are presented.

[b] DAT = Days after treatment.

The TRR data indicate that ^{14}C-residues accumulated at levels above 0.01 ppm in/on the commodities of lettuce, mustard, radishes, and wheat planted 28, 91, or 364 days following

application of [^{14}C]benfluralin to soil at 1x the maximum registered seasonal rate. In general, accumulation of residues was highest in 91-DAT samples and decreased at the 364-DAT interval.

Extraction and hydrolysis of ^{14}C-residues

The following crop samples were subjected to extraction procedures: 28- and 364-DAT lettuce; 91-DAT mustard greens; 364-DAT radish roots and tops; 364-DAT wheat forage; and 28- and 91-DAT wheat straw. The distribution of the TRR in the extracts and nonextractable fractions was determined by LSC and combustion/LSC, respectively. The registrant stated that because there is already a rotational crop restriction on the product label for root crops and small grains, it was decided to focus on leafy vegetables at all plant-back intervals.

Crop samples were sequentially extracted with methanol:water (11:5, v:v) and chloroform (2x). The extracts were combined and allowed to phase separate into a chloroform phase and a methanol:water phase. For 28-DAT lettuce and wheat forage, 91-DAT mustard greens and wheat straw, and 364-DAT radish tops, the chloroform fractions were evaporated to near dryness by rotary evaporation and then partitioned with hexane:acetonitrile (1:1, v:v) to yield hexane and acetonitrile phases.

The nonextractable residues were subjected to enzyme hydrolysis with cellulase (in pH 5 acetate buffer at 38EC for 24-73 hours). The hydrolysate was isolated by filtration or centrifugation and reserved for HPLC analysis (28-DAT lettuce, 28- and 91-DAT wheat straw, and 91-DAT mustard greens only). The remaining solids were acid hydrolyzed (in 1 N HCl at room temperature for 5 days under nitrogen for 28-DAT lettuce and 91-DAT mustard greens, or in 1 N HCl at reflux for 3.5-4.5 hours under nitrogen for 28- and 91-DAT wheat straw, 364-DAT radish root, and 364-DAT wheat forage) and the hydrolysate was isolated by filtration or centrifugation and reserved for HPLC analysis.

For 28-DAT lettuce and 91-DAT mustard greens, the acid hydrolyzed solids were subjected to mild base hydrolysis (in 0.25 N NaOH at room temperature for 24 hours under nitrogen) and the hydrolysate was isolated by centrifugation and reserved for HPLC analysis.

The acid hydrolyzed solids (28- and 91-DAT wheat straw, and 364-DAT radish root) and base hydrolyzed solids (28-DAT lettuce and 91-DAT mustard greens) were subjected to strong base hydrolysis (in 1 N NaOH at reflux for 4 to 30 hours under nitrogen). The hydrolysate was isolated by filtration or centrifugation. For lettuce and mustard greens, the hydrolysate was partitioned with ethyl acetate (EtOAc), then adjusted to pH 5 and partitioned with EtOAc, and then adjusted to pH 1 and partitioned with EtOAc. The EtOAc phases were combined for HPLC analysis. For wheat straw samples, the hydrolysate was adjusted to pH <2 and partitioned with EtOAc.

The distribution of radioactivity in the extracts of rotational crop commodities is presented in Tables 2 (lettuce/mustard), 3 (radishes), and 4 (wheat).

Metabolite characterization and identification

The extracts of rotational crop commodities were analyzed by HPLC and/or TLC to identify/characterize residues. The extracts were cleaned up or fractionated on a C-18 solid-phase extraction column as necessary prior to analysis. The identities of individual metabolites isolated by HPLC were confirmed by co-chromatography and/or comparison of retention times with those of standards of benfluralin, 2,2,2-trifluoroacetamide, trifluoroacetic acid, oxalic acid, [1-^{14}C]pyruvic acid, and [1-^{14}C]acetic acid.

HPLC analyses were conducted using an Rx C-8 column and a gradient mobile phase of ACN and 0.01 M ammonium acetate (pH 7), or an Rx C-8 column and a gradient mobile phase of ACN and 0.01 M ammonium acetate (pH 4). Nonlabeled reference compounds were detected by UV (210 or 254 nm) and radioactivity was detected and quantified by fraction collection/LSC. Unknowns were isolated and purified using one or more of the following HPLC systems: an NH_2 column and a gradient mobile phase of water, ACN, and 0.005 M ammonium acetate (pH 3.5 or 4.0); an Rx C-8 column and a gradient mobile phase of ACN and 0.005 M ammonium acetate (pH 3.5); a Nucleosil C18 column and an isocratic mobile phase of water and ACN; or an Aminex ion exclusion column with an isocratic mobile phase of 0.01 N sulfuric acid.

TLC analyses were used for confirmation of metabolite identifications and for purification of metabolites. One- or two-dimensional TLC analyses were performed on normal-phase silica gel plates using one or two of the following solvent systems: EtOAc:ethanol:acetic acid:water (60:20:5:5, v:v:v:v); isopropanol:acetic acid:water (70:20:10, v:v:v); hexane:methanol:acetone (70:30:10 (v:v:v); chloroform:EtOAc (80:20, v:v); or EtOAc:ethanol:acetic acid:water (70:10:2:2, v:v:v:v). Radioactive residues were visualized by use of a radiographic scanner system. Nonlabeled standards were visualized using a UV lamp. Extracts were first purified/fractionated by HPLC. When TLC was used for purification purposes, metabolites were isolated by scraping bands from the plates and eluting the scraped material with methanol.

TLC analyses were used to confirm the identification of benfluralin in 28-DAT lettuce and to confirm that Metabolite A found in 91-DAT mustard greens, 364-DAT lettuce, 364-DAT radish roots and tops, 364-DAT wheat forage, and 28- and 91-DAT wheat straw was the same compound as found in 28-DAT lettuce.

HPLC/TLC analyses indicated that Metabolite A was the major metabolite in rotational crop commodities. The registrant isolated Metabolite A from 91-DAT mustard greens for further characterization and identification (the registrant noted that although wheat straw samples contained larger amounts of Metabolite A, these samples were not available when characterization and identification of Metabolite A was initiated). A subsample of mustard greens was extracted as described previously and the methanol:water phase was concentrated to remove methanol. The extract was acidified to pH - 1 using 2 N or 6 N HCl and then partitioned three times with diethyl ether. The diethyl ether fraction was cleaned up by preparative HPLC and TLC for LC/MS analyses, which were conducted using an Rx C8 column, an isocratic

mobile phase of 0.4% formic acid and methanol, and negative electrospray ionization. Based on these analyses, the registrant concluded that Metabolite A was trifluoroacetic acid.

Summaries of the characterized and identified ^{14}C-residues in lettuce/mustard, radish, and wheat matrices are presented in Tables 5, 6, and 7, respectively. The chemical names and structures of the identified metabolites are presented in Figure 1.

Table 2. Distribution of total radioactive residues (TRR) in **lettuce/mustard** grown in sandy loam soil treated with [^{14}C]benfluralin at 1.26 lb ai/A (1x the maximum registered seasonal application rate).

Fraction	% TRR	ppm	Characterization/Identification
28-DAT Lettuce (TRR = 0.071 ppm)			
Methanol:water	44.54	0.032	HPLC analysis resolved: Metabolite A [a] 32.70% TRR 0.023 ppm Metabolite B 11.84% TRR 0.008 ppm
Chloroform	29.64	0.021	Concentrated and partitioned with hexane and ACN.
Hexane	7.73	0.005	Not further analyzed (N/A).
ACN	21.91	0.016	HPLC analysis resolved: Benfluralin 12.36% TRR 0.009 ppm Metabolite E 2.79% TRR 0.002 ppm Metabolite F 4.32% TRR 0.003 ppm Metabolite G 1.75% TRR 0.001 ppm Metabolite H 0.69% TRR <0.001 ppm
Nonextractable	25.81	0.018	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	4.72	0.003	HPLC analysis resolved: Metabolite D 4.72% TRR 0.003 ppm
Solids	21.09	0.015	Subjected to acid hydrolysis.
Acid hydrolysate	2.14	0.002	N/A.
Solids	18.95	0.013	Subjected to mild base hydrolysis.
Base hydrolysate	5.61	0.004	N/A.
Solids	13.34	0.009	Subjected to strong base hydrolysis.
Base hydrolysate	9.86	0.007	Partitioned with EtOAc; adjusted to pH 5 and partitioned with EtOAc; and adjusted to pH 1 and partitioned with EtOAc. The EtOAc fractions were combined.
EtOAc	7.55	0.005	HPLC analysis resolved: Metabolite A 2.82% TRR 0.002 ppm Metabolite B 4.73% TRR 0.003 ppm
Aqueous	2.31	0.002	HPLC analysis resolved: Benfluralin 0.84% TRR <0.001 ppm Metabolite A 0.97% TRR <0.001 ppm
Solids	3.48	0.002	N/A.
91-DAT Mustard Greens (TRR = 0.073 ppm)			
Methanol:water	58.41	0.043	HPLC analysis resolved: Metabolite A 48.30% TRR 0.035 ppm Metabolite B 4.77% TRR 0.003 ppm Metabolite C 5.33% TRR 0.004 ppm
Chloroform	11.78	0.009	Concentrated and partitioned with hexane and ACN.
Hexane	1.29	0.001	N/A.
ACN	10.49	0.008	HPLC analysis resolved: Benfluralin 6.21% TRR 0.005 ppm Metabolite E 4.28% TRR 0.003 ppm

(continued; footnotes follow)

Table 2 (*lettuce/mustard, continued*).

Fraction	% TRR	ppm	Characterization/Identification
Nonextractable	29.81	0.022	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	5.78	0.004	HPLC analysis resolved: Metabolite A 5.78% TRR 0.004 ppm
Solids	24.03	0.018	Subjected to acid hydrolysis.
Acid hydrolysate	3.43	0.003	N/A.
Solids	20.60	0.015	Subjected to mild base hydrolysis.
Base hydrolysate	5.15	0.004	N/A.
Solids	15.45	0.011	Subjected to strong base hydrolysis.
Base hydrolysate	7.96	0.006	Partitioned with EtOAc; adjusted to pH 5 and partitioned with EtOAc; and adjusted to pH 1 and partitioned with EtOAc. The EtOAc fractions were combined.
EtOAc	4.99	0.004	HPLC analysis resolved: Metabolite A 0.92% TRR 0.001 ppm Metabolite B 3.05% TRR 0.002 ppm Metabolite I 1.02% TRR 0.001 ppm
Aqueous	2.97	0.002	HPLC analysis resolved: Metabolite A 1.95% TRR 0.001 ppm Metabolite J 1.02% TRR 0.001 ppm
Solids	7.49	0.005	N/A.
364-DAT Lettuce (TRR = 0.011 ppm)			
Methanol:water	56.24	0.006	HPLC analysis resolved: Metabolite A 56.24% TRR 0.006 ppm
Chloroform	24.65	0.003	HPLC analysis resolved: Metabolite E 24.65% TRR 0.003 ppm
Nonextractable	19.11	0.002	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	5.37	0.001	N/A.
Solids	13.74	0.001	N/A.

[a] Metabolite A was identified as trifluoroacetic acid in 91-DAT mustard greens.

Table 3. Distribution of total radioactive residues (TRR) in the commodities of **radishes** grown in sandy loam soil treated with [^{14}C]benfluralin at 1.26 lb ai/A (1x the maximum registered seasonal rate).

Fraction	% TRR	ppm	Characterization/Identification
364-DAT Roots (TRR = 0.021 ppm)			
Methanol:water	33.98	0.007	HPLC analysis resolved: Metabolite A [a] 33.98% TRR 0.007 ppm
Chloroform	17.06	0.004	HPLC analysis resolved: Benfluralin 10.30% TRR 0.002 ppm Metabolite F 2.93% TRR 0.001 ppm
Nonextractable	48.96	0.010	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	5.84	0.001	Not further analyzed (N/A).
Solids	43.12	0.009	Subjected to acid hydrolysis.
Acid hydrolysate	22.09	0.005	N/A.
Solids	21.03	0.004	Subjected to strong base hydrolysis.
Base hydrolysate	14.77	0.003	N/A.
Solids	6.26	0.001	N/A.
364-DAT Tops (TRR = 0.031 ppm)			
Methanol:water	76.92	0.024	HPLC analysis resolved: Metabolite A 76.92% TRR 0.024 ppm
Chloroform	10.33	0.003	Concentrated and partitioned with hexane and ACN.
Hexane	2.49	0.001	N/A.
ACN	7.84	0.002	HPLC analysis resolved: Metabolite E 7.84% TRR 0.002 ppm
Nonextractable	12.76	0.004	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	3.72	0.001	N/A.
Solids	9.04	0.003	N/A.

[a] Metabolite A was identified as trifluoroacetic acid in 91-DAT mustard greens.

Table 4. Distribution of total radioactive residues (TRR) in the commodities of **wheat** grown in sandy loam soil treated with [^{14}C]benfluralin at 1.26 lb ai/A (1x the maximum registered seasonal rate).

Fraction	% TRR	ppm	Characterization/Identification
364-DAT Forage (TRR = 0.019 ppm)			
Methanol:water	68.34	0.013	HPLC analysis resolved: Metabolite A [a] 68.34% TRR 0.013 ppm
Chloroform	11.81	0.002	HPLC analysis resolved: Metabolite E 8.76%TRR 0.002 ppm
Nonextractable	19.85	0.004	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	9.62	0.002	Not further analyzed (N/A).
Solids	10.23	0.002	Subjected to acid hydrolysis.
Acid hydrolysate	6.86	0.001	N/A.
Solids	3.37	0.001	N/A.
28-DAT Straw (TRR = 0.239 ppm)			
Methanol:water	38.41	0.092	HPLC analysis resolved: Metabolite A 38.41% TRR 0.092 ppm
Chloroform	10.44	0.025	Concentrated and partitioned with hexane and ACN.
Hexane	1.55	0.004	N/A.
ACN	8.89	0.021	HPLC analysis resolved: Metabolite M 3.61% TRR 0.009 ppm Metabolite N 5.28% TRR 0.013 ppm
Nonextractable	51.15	0.122	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	11.00	0.026	HPLC analysis resolved: Metabolite A 11.00% TRR 0.026 ppm
Solids	40.15	0.096	Subjected to acid hydrolysis.
Acid hydrolysate	20.07	0.048	HPLC analysis resolved: Metabolite A 7.84% TRR 0.019 ppm Metabolite P 12.23% TRR 0.029 ppm
Solids	20.08	0.048	Subjected to strong base hydrolysis.
Base hydrolysate	16.63	0.040	Adjusted to pH <2 and partitioned with EtOAc.
EtOAc	13.45	0.032	HPLC analysis resolved: Metabolite B 4.17% TRR 0.010 ppm Metabolite P 6.49% TRR 0.016 ppm Metabolite Q 2.78% TRR 0.007 ppm
Aqueous	3.19	0.008	N/A.
Solids	3.45	0.008	N/A.
91-DAT Straw (TRR = 0.344 ppm)			
Methanol:water	32.37	0.111	HPLC analysis resolved: Metabolite A 24.17% TRR 0.083 ppm Metabolite O 8.20% TRR 0.028 ppm
Chloroform	10.50	0.036	Concentrated and partitioned with hexane and ACN.
Hexane	1.00	0.003	N/A.

(continued; footnotes follow)

Table 4 *(wheat, continued)*.

Fraction	% TRR	ppm	Characterization/Identification
ACN	9.50	0.033	HPLC analysis resolved: Benfluralin 1.30% TRR 0.004 ppm Metabolite L 4.91% TRR 0.017 ppm Metabolite M 3.30% TRR 0.011 ppm
Nonextractable	57.13	0.197	Subjected to cellulase hydrolysis.
Cellulase hydrolysate	10.80	0.037	HPLC analysis resolved: Metabolite A 3.71% TRR 0.013 ppm Metabolite T 7.09% TRR 0.024 ppm
Solids	46.33	0.160	Subjected to acid hydrolysis.
Acid hydrolysate	21.26	0.073	HPLC analysis resolved: Metabolite M 3.00% TRR 0.010 ppm Metabolite O 3.00% TRR 0.010 ppm Metabolite P 11.45% TRR 0.039 ppm Metabolite Q 3.82% TRR 0.013 ppm
Solids	25.07	0.087	Subjected to strong base hydrolysis.
Base hydrolysate	19.92	0.069	Adjusted to pH <2 and partitioned with EtOAc.
EtOAc	13.58	0.047	HPLC analysis resolved: Metabolite C 3.12% TRR 0.011 ppm Metabolite O 1.71% TRR 0.006 ppm Metabolite Q 4.43% TRR 0.015 ppm Metabolite R 2.01% TRR 0.007 ppm Metabolite S [b] 2.31% TRR 0.008 ppm
Aqueous	6.34	0.022	N/A.
Solids	5.15	0.018	N/A.

[a] Metabolite A was identified as trifluoroacetic acid in 91-DAT mustard greens.
[b] The registrant believes this compound to be the same as Metabolite F; however, insufficient radioactivity was available for confirmation.

Table 5. Summary of radioactive residues identified in/on lettuce/mustard planted in soil treated with [^{14}C]benfluralin at 1x the maximum application rate.

Fraction/Metabolite	28-DAT Lettuce (TRR = 0.071 ppm)		91-DAT Mustard Greens (TRR = 0.073 ppm)		364-DAT Lettuce (TRR = 0.011 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified						
Benfluralin	13.20	0.009	6.21	0.005	--	--
Metabolite A [a]	36.49	0.026	56.95	0.041	56.24	0.006
Total Identified	**49.69**	**0.035**	**63.16**	**0.046**	**56.24**	**0.006**
Characterized						
Metabolite B	16.57	0.012	7.82	0.005	--	--
Metabolite C	--	--	5.33	0.004	--	--
Metabolite D	4.72	0.003	--	--	--	--
Metabolite E	2.79	0.002	4.28	0.003	24.65	0.003
Metabolite F	4.32	0.003	--	--	--	--
Metabolite G	1.75	0.001	--	--	--	--
Metabolite H	0.69	<0.001	--	--	--	--
Metabolite I	--	--	1.02	0.001	--	--
Metabolite J	--	--	1.02	0.001	--	--
Metabolite K	0.49	<0.001	--	--	--	--
Hexane	7.73	0.005	1.29	0.001	--	--
Acid hydrolysate	2.14	0.002	3.43	0.003	--	--
Mild base hydrolysate	5.61	0.004	5.15	0.004	--	--
Cellulase hydrolysate	--	--	--	--	5.37	0.001
Total Identified/Characterized	**96.50**	**0.069**	**92.50**	**0.068**	**86.26**	**0.009**
Nonextractable	3.48	0.002	7.49	0.005	13.74	0.001

[a] Metabolite A was identified as trifluoroacetic acid in 91-DAT mustard greens.

Table 6. Summary of radioactive residues identified in/on radish matrices planted in soil treated with [^{14}C]benfluralin at 1x maximum application rate.

Fraction/Metabolite	364-DAT Roots (TRR = 0.021 ppm)		364-DAT Tops (TRR = 0.031 ppm)	
	% TRR	ppm	% TRR	ppm
Identified				
Benfluralin	10.30	0.002	--	--
Metabolite A [a]	33.98	0.007	76.92	0.024
Total Identified	**44.28**	**0.009**	**76.92**	**0.024**
Characterized				
Metabolite E	--	--	7.84	0.002
Metabolite F	2.93	0.001	--	--
Hexane	--	--	2.49	0.001
Cellulase hydrolysate	5.84	0.001	3.72	0.001
Acid hydrolysate	22.09	0.005	--	--
Base hydrolysate	14.77	0.003	--	--
Total Identified/Characterized	**89.91**	**0.019**	**90.97**	**0.028**
Nonextractable	6.26	0.001	9.04	0.003

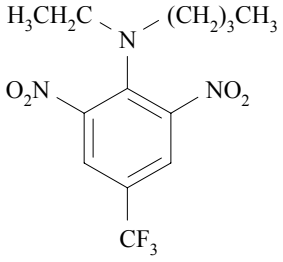
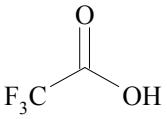
[a] Metabolite A was identified as trifluoroacetic acid in 91-DAT mustard greens.

Table 7. Summary of radioactive residues identified in/on wheat matrices planted in soil treated with [^{14}C]benfluralin at the 1x the maximum application rate.

Fraction/Metabolite	28-DAT Straw (TRR = 0.239 ppm)		91-DAT Straw (TRR = 0.344 ppm)		364-DAT Forage (TRR = 0.019 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified						
Benfluralin	--	--	1.30	0.004	--	--
Metabolite A [a]	57.25	0.137	27.88	0.096	68.34	0.013
Total Identified	**57.25**	**0.137**	**29.18**	**0.100**	**68.34**	**0.013**
Characterized						
Metabolite B	4.17	0.010	--	--	--	--
Metabolite C	--	--	3.12	0.011	--	--
Metabolite E	--	--	--	--	8.76	0.002
Metabolite L	--	--	4.91	0.017	--	--
Metabolite M	3.61	0.009	6.30	0.022	--	--
Metabolite N	5.28	0.013	--	--	--	--
Metabolite O	--	--	12.91	0.044	--	--
Metabolite P	18.72	0.045	11.45	0.039	--	--
Metabolite Q	2.78	0.007	8.25	0.028	--	--
Metabolite R	--	--	2.01	0.007	--	--
Metabolite S [b]	--	--	2.31	0.008	--	--
Metabolite T	--	--	7.09	0.024	--	--
Hexane	1.55	0.004	1.00	0.003	--	--
Cellulase hydrolysate	--	--	--	--	9.62	0.002
Acid hydrolysate	--	--	--	--	6.86	0.001
Aqueous	3.19	0.008	6.34	0.022	--	--
Total Identified/Characterized	**96.55**	**0.231**	**94.87**	**0.325**	**93.58**	**0.018**
Nonextractable	3.45	0.008	5.15	0.018	3.37	0.001

[a] Metabolite A was identified as trifluoroacetic acid in 91-DAT mustard greens.
[b] The registrant believes this compound to be the same as Metabolite F; however, insufficient radioactivity was available for confirmation.

Figure 1. Benfluralin and its metabolites in rotational crops.

Common Name Chemical Name	Structure	Substrate
Benfluralin (N-butyl-N-ethyl-α,α,α- trifluoro-2,6-dinitro-p-toluidine		Lettuce, mustard greens, radish roots and tops, and wheat straw
Metabolite A Trifluoroacetic acid		Lettuce, mustard greens, radish roots and tops, and wheat forage and straw

Storage stability

Samples of rotational crop commodities were stored frozen prior to analysis. TRR determinations at Battelle were conducted within 7-39 days of sample collection, and the re-determinations of TRR at XBL were conducted within 14-411 days of sample collection. The dates of initial HPLC analyses were provided; initial analyses were conducted within 25-157 days (~1-5 months) of sample collection for all samples except wheat straw, and within 423 days (~14 months) of collection for wheat straw samples. The dates of final analyses were not provided. The registrant stated that a previously submitted lettuce field trial study (MRID 43831902) included data demonstrating that benfluralin is stable in lettuce for up to 6 months.

The registrant must provide additional storage stability data to support this study. The dates of the final analyses of samples must be provided as well as data demonstrating that the metabolic profile in each rotational crop commodity did not change over the intervals during which samples were stored.

Proposed metabolic pathway

Based on the results of the study, the registrant proposed that benfluralin is metabolized by soil microbial enzymes to trifluoroacetic acid which is taken up by plants. The registrant also proposed that benfluralin could be converted by plant enzymes to a number of Phase I (unconjugated) metabolites (resulting from dealkylation, hydroxylation, amination, and oxidation) which undergo conjugation to form Phase II metabolites which are then further incorporated into biomolecules to form bound residues.

The registrant concluded that no rotational crop restrictions are required for rotational crops that are not susceptible to phytotoxic residues, such as leafy vegetables.

Study summary

The submitted confined rotational crop study is adequate to satisfy data requirements for OPPTS 860.1850 pending submission of additional storage stability data. The registrant must submit the dates of the final analyses of samples as well as data demonstrating that the metabolic profile in each rotational crop commodity did not change over the intervals during which samples were stored.

The total radioactive residues (TRR) accumulated at levels above 0.01 ppm in/on the rotational commodities of lettuce, mustard, radishes, and wheat that were planted 28, 91, or 364 days following application of [^{14}C]benfluralin to sandy loam soil at 1x the maximum registered seasonal rate. In general, accumulation of total radioactive residues was highest (0.03-0.34 ppm) in samples collected 91-days after treatment (DAT) and decreased (0.01-0.07 ppm) at the 364-DAT interval.

Although the TRR were $0.01 ppm in/on all rotational crop commodities at all plant-back intervals tested, the registrant chose to conduct metabolite characterization/identification procedures only on the following selected commodities: the 28- and 364-DAT lettuce, the 91-DAT mustard greens, the 364-DAT radish roots and tops, the 364-DAT wheat forage, and the 28- and 91-DAT wheat straw. Approximately 86-97% of TRR in these commodities were characterized/identified by a combination of chromatographic techniques (HPLC, TLC, LC/MS). The parent, benfluralin, was detected at low levels in 28-DAT lettuce (13.20% TRR, 0.009 ppm), 91-DAT mustard greens (6.21% TRR, 0.005 ppm), 364-DAT radish roots (10.30% TRR, 0.002 ppm), and 91-DAT wheat straw (1.30% TRR, 0.004 ppm). The major metabolite, referred to as Metabolite A and identified as trifluoroacetic acid in mustard greens, was detected in all tested commodities at 27.88-76.92% of TRR (0.006-0.137 ppm). An additional 19 unidentified metabolites were resolved by HPLC analyses; these metabolites were each present at <0.05 ppm.

There appears to be differences in the metabolism of benfluralin in rotational crops versus primary crops. In plant metabolism studies conducted on alfalfa, lettuce, and peanuts, the majority of total radioactivity was found to be incorporated into lignin and cellulose with the remainder of the radioactivity due to numerous minor unidentified metabolites. Only small amounts of benfluralin were identified in lettuce and peanut hulls, and trifluoroacetic acid was not identified at all.

The results of the current confined rotational crop study indicate that a 12-month plant-back interval would be appropriate for all crops. Currently, the product label specifies a 10-month plant-back interval for wheat, barley, oats, rye, other grasses, onions, corn, milo (grain sorghum), spinach, red beets, sugar beets, or other root crops, and the label implies that this rotational crop restriction applies only to arid, irrigated areas of the Western United States. The registrant must modify their product label to specify a 12-month plant-back interval for crops that may be rotated and to make it clear that rotational crop restrictions apply to all areas of the U.S. If the registrant wishes a rotational crop restriction of less than 12 months for leafy vegetables, then limited field rotational crop studies (OPPTS 860.1900) must be conducted.

MASTER RECORD IDENTIFICATION NUMBER

The citation for the MRID documents referred to in this review is presented below.

44019801 Singer, G. (1996) Confined Accumulation Study on Rotational Crops with (carbon 14)-Benefin: Final Report: Lab Project Number: AA930005: SC980240: XBL 94121. Unpublished study prepared by American Agricultural Services, Inc.; Battelle Labs; and XenoBiotic Labs, Inc. 443 p.