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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 7 1996

MEMORANDUM:

SUBJECT: ***PP5G4466 & PP5F4578: Glufosinate-ammonium in/on Transgenic Corn and Soybeans. Experimental Use Permit, Temporary Tolerance and Permanent Tolerance Petitions. Evaluation of Analytical Method and Residue Data.***
CBTS #s 16154 & 15081
DP Barcode #s D219069 & D211531
*** MRID #s for Permanent Petition:**
437784-01, 437669-01, 437669-02, 437669-03, 437669-04, 437669-05, 437669-06, 437669-07, 437669-08, 437669-09, 437669-10, 437669-11, 437669-12, 437669-22, 437669-27, 437669-25, 437669-24, 437669-20, 437669-19, 437669-18, 437669-17, 437669-21, 437669-26, 437669-23.
*** MRID #s for Temporary Petition:**
435156-01, 435156-06, 435156-08, 435156-09, 435156-07, 435156-06, 435156-06 (3 Volumes), 435156-02, 435156-03, 435156-04, 435246-01, 435156-05.

FROM: María Isabel Rodríguez, Chemist
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March 7, 1996.

THROUGH: Edward Zager, Acting Chief
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TO: Joanne I. Miller/Eugene Wilson
Product Management Team #23
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and

Karen Whitby, Section Head
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The petitioner, AgrEvo USA Company, in letter dated January 18, 1995 from Victor A. Dorr - Manager, Regulatory Affairs - to Joanne I. Miller, is applying for a crop destruct Experimental Use Permit (EUP) for the use of Liberty Herbicide on corn and soybeans for the 1996 season. The EUP request has permit #45639-EUP-LA and is proposed from March 1, 1996 to October 1, 1996. A total of 4327 pounds of the active ingredient are proposed to be used on 6695 acres of corn and soybeans.

Herbicide (0.73 lb ai) on corn or soybeans per growing season.

The proposed use of glufosinate-ammonium as Liberty Herbicide on Liberty Link corn and soybeans is not adequately described. The label should clearly specify the individual application rate as well as seasonal maxima. Pre-harvest intervals for each plant part (e.g., forage, hay, fodder, grain) in Table II (September 1995) need to be listed. The use pattern needs to be supported by the crop field trials. The feeding/grazing restrictions on treated soybean straw and vines should be deleted from the label as these commodities are not in Table II (September 1995) and tolerances are not required. A plantback interval is also needed for rotational crops (see the Rotational Crops Section for details). A revised label should be submitted for review by CBTS.

NATURE OF THE RESIDUE:

a. **Plants:**

1. **Corn:**

The following study was submitted for review as part of PP5G4466, the temporary tolerance petition.

> Burnett, T.J. December 16, 1994. *^{14}C-Glufosinate-ammonium: Nature of the Residue in Field Corn.* Study performed by Pan-Agricultural Labs., Inc., Madera, CA and submitted by AgrEvo USA Company, Wilmington, DE. Lab Project ID #93-0025, Study Report #93260. MRID #435156-02. Guidelines Reference #171-4(a).

Transgenic field corn was grown in a small fenced outdoor plot at Madera, California. 3,4-^{14}C-Glufosinate-ammonium was applied post-emergent to the corn using a N_2 backpack sprayer. The test substance was prepared as Ignite 150 SL formulation (now known as Liberty). Two foliar applications of 0.45 lb ai/acre each were performed for a total of 0.90 lb ai/acre. The first application was done at the sixteen-inch average corn height and the second application was done 10 days later at the twenty-four-inch corn height.

Single corn plants were collected within an hour of each application [Coded 0 days after treatment (DAT)-1 and 0 DAT-2] and five days after each application (5 DAT-1 and 5 DAT-2). Two plants were collected at the forage stage (28 DAT), six plants were collected at the pre-dent stage (55 DAT, corn silage sample) and the remaining plants were collected at the hard dent stage (103 DAT). Whole plants were collected at the first six samplings. At the final harvest (103 DAT), the corn was separated into fodder, husks, cobs, and corn grain.

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The time interval between sampling and frozen (-10 °C) storage was less than one hour. The samples were processed to a fine powder with dry ice in a food processor, the dry ice sublimed, samples weighed, and aliquots analyzed (in triplicate) by oxidation and extraction to determine the total radioactive residue (TRR). The TRR levels in corn tissues were as follows.

Sample	Total Radioactive Residue *(ppm)*	Distribution (%)[a]
0 DAT-1 Plant	23.0	N/A[b]
5 DAT-1 Plant	5.83	N/A
0 DAT-2 Plant	14.5	N/A
5 DAT-2 Plant	9.86	N/A
Forage (28 DAT-2)	2.64	N/A
Silage (55 DAT-2)	1.82	N/A
Mature (102 DAT-2)		
Fodder	2.01	93.7
Husk	0.872	2.3
Cob	0.251	1.5
Corn Grain	0.130	2.4

a Distribution of radioactivity in mature plant parts based on total dpm per plant part.
b Not applicable because these samples were not divided into separate tissues.

The TRR in each treated sample was significantly greater than 0.01 ppm in each case. The petitioner concluded that the decreasing ppm values observed with time are consistent with dilution of the test substance with plant growth. The bulk of the radioactivity (93.7%) is observed in the fodder. Only 2.4% of the radioactivity is in the corn grain.

All tissues containing radioactive residue levels greater than 0.01 ppm were extracted with water containing sodium azide (as a preservative to inhibit bacterial growth). The tissues were analyzed by oxidation and the liquid by liquid scintillation counting (LSC). The mean extractability of each plant sample was as follows. The % TRR in water ranged from 76.7 to 110.9%. The % TRR in the bound residues ranged from 1.5 to 22.1%. Total % TRR ranged from 94.6 to 112.5%.

The extraction efficiency was determined by separately fortifying control samples of forage, silage, fodder, and corn

grain with glufosinate-ammonium (Hoe-039866), N-acetyl-glufosinate (Hoe-061517), and MP-propionic acid (Hoe-099729), and analysis by HPLC. The efficiency ranged from 94.1 to 102.7% for glufosinate and 87.5% to 110.9% for the N-acetyl-L-glufosinate standards. Recoveries ranged from 97.8 to 102.7% for glufosinate and from 92.0 to 111.0% for N-acetyl-L-glufosinate.

After extraction, those residues that were greater than 9% of the TRR were further analyzed. Corn grain, fodder, and silage were subjected to base and acid hydrolyses. In the corn grain, the remaining residue was subjected to enzymatic digestion with α-amylase. The extracted and hydrolysed tissues were analyzed by oxidation analysis, and liquid samples by LSC. Results were as follows.

Sample Description	Extract	ppm	% TRR
55-DAT Corn Silage	NH_4OH extract	0.063	3.5
	0.1 N HCl extract	0.032	1.7
	0.1 N HCl hydrolysate	0.041	2.2
	Dry Residue	0.022	1.2
	Total	0.158	8.6
102-DAT Corn Grain	NH_4OH extract	0.004	2.8
	0.1 N HCl extract	0.001	1.0
	0.1 N HCl hydrolysate	0.012	9.3
	α-amylase hydrolysate	0.001	0.7
	Dry residue	0.008	6.1
	Total	0.026	19.9
102 DAT Corn Fodder	NH_4OH extract	0.047	2.3
	0.1 N HCl extract	0.026	1.3
	0.1 N HCl hydrolysate	0.057	2.8
	Dry residue	0.059	2.9
	Total	0.189	9.3

The major metabolite in the foliage (post-application) samples was N-acetyl-glufosinate and in the husks, cobs, and

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grain was MP-propionic acid.

The samples were analyzed using the analytical enforcement method described in the Analytical Methodology Section of this review, AgrEvo Analytical Method AE-24.

Confirmation of structural identities was achieved using fodder tissue since it contained relatively high residue levels. The HPLC profiles of this tissue indicated the presence of all major and minor metabolites found in other tissues. Mass spectra confirmed the identification of the test substance N-acetyl-glufosinate, MP-propionic, and MP-acetic acid, and identification of MP-butyric acid was inconclusive. Uncharacterized radioactivity was relatively low in all samples. The methylated metabolites were analyzed by GC-MS to confirm identification of peaks.

All rac samples were extracted and analyzed by HPLC within one month of collection. The petitioner indicated that storage stability of glufosinate-ammonium, N-acetyl-glufosinate and MP-propionic acid indicated that no significant degradation occurred during the duration of the test. Oxidation recoveries were between 95 and 105%.

The petitioner concluded that glufosinate-ammonium is significantly degraded in transgenic corn. The proposed metabolic pathway for the degradation of glufosinate in transgenic corn is presented as Attachment #2 to this review. The initial, rapid acetylation of L-glufosinate occurs within 5 days after the application of herbicide to transgenic plants as indicated by the identification of approximately one-half of the radioactivity in the 5-DAT-1 and 5-DAT-2 samples as N-acetyl-glufosinate. The proportion of metabolites and parent found in forage, silage, and fodder was very similar, indicating that the metabolism did not occur extensively in the later days of the study. Further metabolism results in MP-propionic acid, MP-acetic acid, and MP-butyric acid. The petitioner indicated that these products have been observed in cell cultures of wheat, soybeans, and maize and *in vivo* plant metabolism studies with transgenic tobacco and carrots. As reported by the petitioner, the proposed intermediates, 4-methylphosphinico-2-oxo-butyric acid and 4-methylphosphinico-2-hydroxy-butyric acid were not observed during this study but have been identified in other studies.

The nature of the residues of glufosinate-ammonium in/on transgenic field corn is considered to be understood.

2. **Soybeans:**

The following study was submitted for review as part of PP5G4466, the temporary tolerance petition.

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Rupprecht, J.K. and Smith, S.S. December 29, 1994. *Metabolism of [^{14}C]-Glufosinate-ammonium in Soybeans, Treated Under Normal Field Conditions.* Study performed by AgrEvo USA Company, Pikeville, NC and submitted by AgrEvo USA Company, Wilmington, DE. Study #500BK. MRID #435156-03. Guidelines Reference #171-4(a).

Transgenic soybeans grown under field conditions in three plots situated within a field cage were treated with 3,4-^{14}C-glufosinate-ammonium formulated as Liberty Herbicide (Ignite 200 SI). The test site was an AgrEvo farm in Pikeville, North Carolina. The treatment was made in two spray applications of 0.45 lb ai/acre each for a total of 0.90 lb ai/acre. One application was at the third trifoliate leaf stage and the second one at full bloom. This rate represents a slight exaggeration over the proposed 0.80 lb ai/acre for the temporary petition.

Plant samples were harvested for Day Zero analysis immediately after each treatment. The forage was harvested just prior to the second application. Mature plants were harvested 85 days after the second treatment and separated into straw, pods, and beans.

The harvested parts were rinsed with water and then by an appropriate organic/aqueous and/or organic solvents of decreasing polarity. The radioactivity in each extract was quantitated by liquid scintillation counting (LSC). The unextractable residues were determined by combustion. Extractable residues were identified by comparative HPLC using authentic standards. Bound residues were released by acid or base hydrolysis methods and the hydrolysates were analyzed by HPLC. Additional confirmation of the identity of the extractable residue was obtained by application of the standard residue method.

A summary of total glufosinate-ammonium equivalent residue levels in treated soybeans is as follows. It should be noted that three replicates of each Day Zero Treatment and two forage samples were extracted on the day of harvest but that only one replicate was extracted further and is included in this table.

Matrix	Replicate	Extractable Residue		Fiber Bound Residue[1]		Total Radioactive Residue	
		% Total	ppm	% Total	ppm	% Total	ppm
Whole Plant Treatment 1	1	99.8	70.4	0.2	0.2	100	70.6
Whole Plant Treatment 2	1	99.4	28.0	0.6	0.2	100	28.2

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Matrix	Replicate	Extractable Residue		Fiber Bound Residue[1]		Total Radioactive Residue	
		% Total	ppm	% Total	ppm	% Total	ppm
Forage	1	92.5	1.8	7.5	0.1	100	1.9
Straw	1	94.0	3.0	6.0	0.2	100	3.2
	2	94.1	2.9	5.9	0.2	100	3.1
	Mean	94.1	3.0	6.0	0.2	100	3.2
Pods	1	96.3	4.3	3.7	0.2	100	4.5
	2	96.2	4.8	3.8	0.2	100	5.0
	Mean	96.3	4.6	3.8	0.2	100	4.8
Beans	1	82.5	1.1	17.5	0.2	100	1.3
	2	77.4	1.1	22.6	0.3	100	1.4
	Mean	80.0	1.1	20.1	0.3	100	1.4

1 Bound prior to acid/base hydrolysis.

Total mean residues in each tissue, expressed as glufosinate-ammonium equivalents, were 1.9 ppm in forage, 3.2 in straw, 4.8 ppm in pods, and 1.4 ppm in beans.

The distribution of the radioactivity residue levels in the analyzed soybean samples following hydrolysis is as follows.

Matrix	Water Soluble (%)	Aqueous/organic Soluble (%)	Released by Hydrolysis (%)	Bound (%)
Forage	76.4	16.1	6.3	1.2
Straw	89.5	4.6	4.5	1.4
Pods	88.4	7.8	2.9	0.9
Beans	74.3	3.2	22.6	0.0

For all matrices, the majority (>77%) of the initial residue was solubilized by sequential washes and extractions. Then, following hydrolysis, over 99% of the residues were released. For all matrices, only less than 1.4% of the total residues

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remained bound.

A summary of the metabolic profile of the extractable radioactive residues as depicted by the chromatographic analysis by HPLC is as follows.

Matrix	Hoe 039866		Hoe 099729		Hoe 061517		Hoe 064619		Total Identified[2]	
	%	ppm	%	ppm	%	ppm	%	ppm	%	ppm
Forage	23.2	0.4	60.2	1.2	6.5	0.1	0.7	0.01	90.6	1.7
Straw	18.5	0.6	53.2	1.7	13.6	0.4	5.7	0.18	91.0	2.9
Pods	5.8	0.3	62.6	3.1	22.3	1.1	2.9	0.14	93.6	4.6
Beans[1]	6.2	0.1	60.8	0.9	16.0	0.2	7.1	0.10	90.1	1.3

1 Includes residue released.
2 No other single metabolite comprised >2.0% of total.

Analysis of the extractable residues showed a similar metabolic profile in all soybean tissues. The principal metabolites, in all matrices, were identified as N-acetyl-glufosinate (Hoe-099729), glufosinate (Hoe-039866), and MP-propionic acid (Hoe-061517). MP-Acetic acid (Hoe-064619) was obtained as a minor metabolite. Analysis of the residues released also showed a metabolic profile similar to that from the extractable residues. The petitioner indicated that due to extremely low levels of the residue released, accurate quantitation of the identified metabolites was only possible with the released residues from the beans.

Confirmation of the nature of the extractable residue was obtained by analyzing aliquots of the aqueous extracts from forage, straw, pods, and beans by the standard crop residue method, determination by GC, and then comparison to HPLC. The total residue levels from both methods as well as the accountability for the individual metabolites were in agreement.

Samples were extracted and initially analyzed by HPLC on the harvest day. The only exception was a bean sample that was extracted two days after harvest, which was stored at -15 °C prior to extraction. All extracts were stored at -15 °C or less prior to analysis. The study was completed within 3 months of harvest.

The petitioner concluded that the metabolic profile of glufosinate-ammonium in transgenic soybeans is similar to that reported in other transgenic plant systems. As is the case for

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transgenic corn (discussed above), glufosinate-ammonium is significantly degraded in transgenic soybeans. The proposed metabolic pathway for the degradation of glufosinate in transgenic soybeans is presented as Attachment #3 to this review.

The nature of the residues of glufosinate-ammonium in/on transgenic soybeans is considered to be understood.

3. **Rotational Crops:**

The following study was submitted for review as part of PP5F4578, the permanent tolerance petition.

> Meyer, B.N., Tull, P.J., and Rupprecht, J.K. July 14, 1995. *Uptake of ^{14}C-Glufosinate-ammonium Residues in Soil by Rotational Crops Under Confined Conditions.* Study performed by AgrEvo USA Company, Pikeville, NC and submitted by AgrEvo USA Company, Wilmington, DE. Lab Project ID #506BK, Study Report #A54272. MRID #437669-17. Guidelines Reference #165-1.

A confined rotational crop study was conducted with 3,4-^{14}C-glufosinate-ammonium formulated as Liberty Herbicide (formerly known as Ignite).

Lettuce, radish, and spring wheat as representatives of a leafy vegetable, a root crop, and a small grain, respectively, were the three major crop groups studied. The plants were grown in five rectangular stainless steel tanks; three for treatment and two for control. Additionally, untreated plants were grown in separate pots in close proximity to the treated plots in order to monitor for foliar uptake of volatiles. A single application at 0.9 lb ai/acre was performed. The soil (sandy loam) was aged 28 and 119 days before planting of the rotational crops. These time periods represent crop failure replanting and immediate re-cropping. The protocol also provides for a 300 day aging period and the petitioner indicated that an amended report will be submitted at a later date.

Samples of soil, fresh plants, and plant fiber were ground and then combusted and counted by LSC. HPLC was used for the confirmation and quantitation of metabolites.

Radish samples were divided into roots and tops. Wheat forage as well as straw (including hulls) and grain samples were taken for analysis. Lettuce was analyzed undivided. Soil samples were combusted and extracted but not further analyzed.

A summary of total glufosinate-ammonium equivalent residue levels in the treated radishes, lettuce, and wheat is as follows.

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