



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

**MEMORANDUM**

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

DATE: 12-JUN-2001

SUBJECT: PP# 9F05092. Imazapic use on Pasture and Rangeland Grasses.  
**Evaluation of Residue Data and Analytical Methods.** MRID#s 440151-01, 444561-02 thru -04, 444561-08, 444561-12, and 451709-01. Barcode D269038. Chemical#s 128943 & 129041. Case 291904. Submission S581930.

FROM: William H. Donovan, Ph.D., Chemist *William H. Donovan*  
Registration Action Branch 1 (RAB1)  
Health Effects Division (HED) (7509C)

THRU: G. Jeffrey Herndon, Branch Senior Scientist *G. Jeffrey Herndon*  
RAB1/HED (7509C)

TO: Jim Tompkins/James Stone, PM Team 25  
Registration Division (7505C)

American Cyanamid Company has submitted a registration application for use of imazapic on pasture and rangeland grasses, along with a petition to establish permanent tolerances for residues in/on grass forage and hay and livestock commodities as a result of the proposed uses. Section F of the current petition proposes to establish permanent tolerances for residues of imazapic ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid applied either as the free acid or its ammonium salt and its metabolite ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-hydroxymethyl-3-pyridinecarboxylic acid both free [CL 263284] and conjugated [CL 189215] in/on:

- Grass, forage :..... 35 ppm
- Grass, hay ..... 15 ppm

Tolerances are also being proposed for residues of imazapic and its metabolite CL 263284 in the following livestock commodities:

- Milk ..... 0.1 ppm
- Meat of cattle, sheep, goats, and horses ..... 0.1 ppm

Fat of cattle, sheep, goats, and horses .....	0.1 ppm
Meat byproducts (except kidney) of cattle, sheep, goats, and horses .....	0.1 ppm
Kidney of cattle, sheep, goats, and horses .....	2.0 ppm

Permanent tolerances are currently established under §180.490(a) for residues of imazapic and its metabolites CL 263284 and CL 189215 in/on peanut, nutmeat at 0.1 ppm. Time-limited tolerances set to expire 12/31/01 are established under §180.490(b) in connection with Section 18 emergency exemptions (99NE0009) for residues of imazapic and its metabolites CL 263284 and CL 189215 for grass forage at 30 ppm, grass hay at 15 ppm, milk at 0.010 ppm, fat, meat, and meat byproducts (except kidney) of cattle, goats, hogs, horses, and sheep at 0.10 ppm, and kidney of cattle, goats, hogs, horses, and sheep at 1.0 ppm.

The attached contractor's document (Attachment 1) has been reviewed and revised to reflect current HED policy.

**Executive Summary of Chemistry Deficiencies**

- Agency validation of the analytical method for grass and livestock.
- Additional grass field trials.
- Goat metabolism or bovine feeding study using <sup>14</sup>C-labeled CL 263284 or CL 263284, respectively.
- Revised Section B or additional rotational crop data.
- Revised Section F.

**RECOMMENDATIONS**

Provided that Agency validation of the analytical method is successful (Conclusions 5 & 6b) and that Sections B (Conclusions 2c & 13e) and F (Conclusions 10h & 11d) are modified as requested, HED concludes there are no residue chemistry data requirements that would preclude establishment of permanent tolerances for imazapic and its hydroxymethyl metabolites in/on the following commodities:

<b>Grass, forage</b>	<b>30 ppm</b>
<b>Grass, hay</b>	<b>15 ppm</b>
<b>Milk</b>	<b>0.10 ppm</b>
<b>Meat*</b>	<b>0.10 ppm</b>
<b>Fat*</b>	<b>0.10 ppm</b>
<b>Meat byproducts (except kidney)*</b>	<b>0.10 ppm</b>
<b>Kidney*</b>	<b>1.0 ppm</b>

\* Of cattle, sheep, goats, and horses

However, registration of Plateau™ Herbicide for use on pastures and rangeland should be made conditional upon the submission of additional data as specified in Conclusions 4c, and 10a - 10c. Registration of Plateau™ DG Herbicide for use on pastures and rangeland should be delayed until the petitioner has submitted the requested field trial data supporting its use. A human-health risk assessment will be prepared as a separate document.

List of Attachments

- |               |  |
|---------------|--|
| Attachment 1. | Contractor review.   |
| Attachment 2. | Chemical names and structures of imazapic and its metabolites in plants and livestock. |
| Attachment 3. | IRLS sheet for imazapic.   |

cc (with Attachments): W. Donovan, O. Odior  
RDI: RAB Chemists (31-MAY-2001), G. Herndon (12-JUN-2001)  
W.H. Donovan:806R:CM#2:(703)305-7330:7599C:RAB

**IMAZAPIC  
PC Code 128943  
(DP Barcode D269038)**

**PP#9F05092: Evaluation Of Residue Chemistry Data To Support  
Permanent Tolerances For Use Of Imazapic  
On Pasture and Rangeland Grasses**

**February 26, 2001**

**Contract No. 68-W-99-053**

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

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

# IMAZAPIC

## PP#9F05092: EVALUATION OF RESIDUE CHEMISTRY DATA TO SUPPORT

### PERMANENT TOLERANCES FOR USE OF IMAZAPIC

#### ON PASTURE AND RANGELAND GRASSES

(DP BARCODE D269038)

### INTRODUCTION

American Cyanamid Company has submitted an amended registration application for use of imazapic on pasture and rangeland grasses, along with a petition to establish permanent tolerances for residues in/on grass forage and hay and livestock commodities as a result of the proposed uses. Imazapic is also known by the petitioner's code AC or CL 263222, or as CADRE®. The chemical structure of imazapic is the same as that of the imidazolinone herbicides imazethapyr and imazamox except that position 5 of the pyridine ring is occupied by a methyl group rather than the ethyl and methoxymethyl groups of imazethapyr and imazamox, respectively. Section F of the current petition proposes to establish permanent tolerances for residues of imazapic ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid applied either as the free acid or its ammonium salt and its metabolite ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-hydroxymethyl-3-pyridinecarboxylic acid both free and conjugated in/on:

Grass, forage .....	35 ppm
Grass, hay .....	15 ppm

Tolerances are also being proposed for residues of imazapic ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid and its metabolite ( $\pm$ )-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-hydroxymethyl-3-pyridinecarboxylic acid in the following livestock commodities:

Milk .....	0.1 ppm
Meat of cattle, sheep, goats, and horses .....	0.1 ppm
Fat of cattle, sheep, goats, and horses .....	0.1 ppm
Meat byproducts (except kidney) of cattle, sheep, goats, and horses .....	0.1 ppm
Kidney of cattle, sheep, goats, and horses .....	2.0 ppm

Concurrently, the petitioner is requesting for amended Section 3 registration of two end-use products containing imazapic as the active ingredient: the 2 lb ae/gal ammonium salt soluble concentrate (SC) formulation (Product Name = Plateau™ Herbicide; EPA Reg. No. 241-365)

and the 0.0625 lb ai/packet water dispersible granular (WDG) formulation in water soluble packets (Product Name = Plateau™ DG Herbicide; EPA Reg. No. 241-393). These products are currently registered for weed control, native grass establishment and turf growth suppression on roadside and other noncrop areas.

Imazapic is an imidazolinone herbicide which is currently registered for use on peanuts. The herbicidal activity of imazapic, as an imidazolinone, is due to the inhibition of acetohydroxyacid synthase, which is a key plant enzyme in the biosynthesis of the amino acids leucine, isoleucine, and valine. Livestock lack this biosynthetic pathway and must obtain these amino acids from their diet. The fact that the herbicidal mode of action of imazapic is through inhibition of a biosynthetic pathway not present in livestock is one of the factors contributing to the low toxicity of imazapic to livestock.

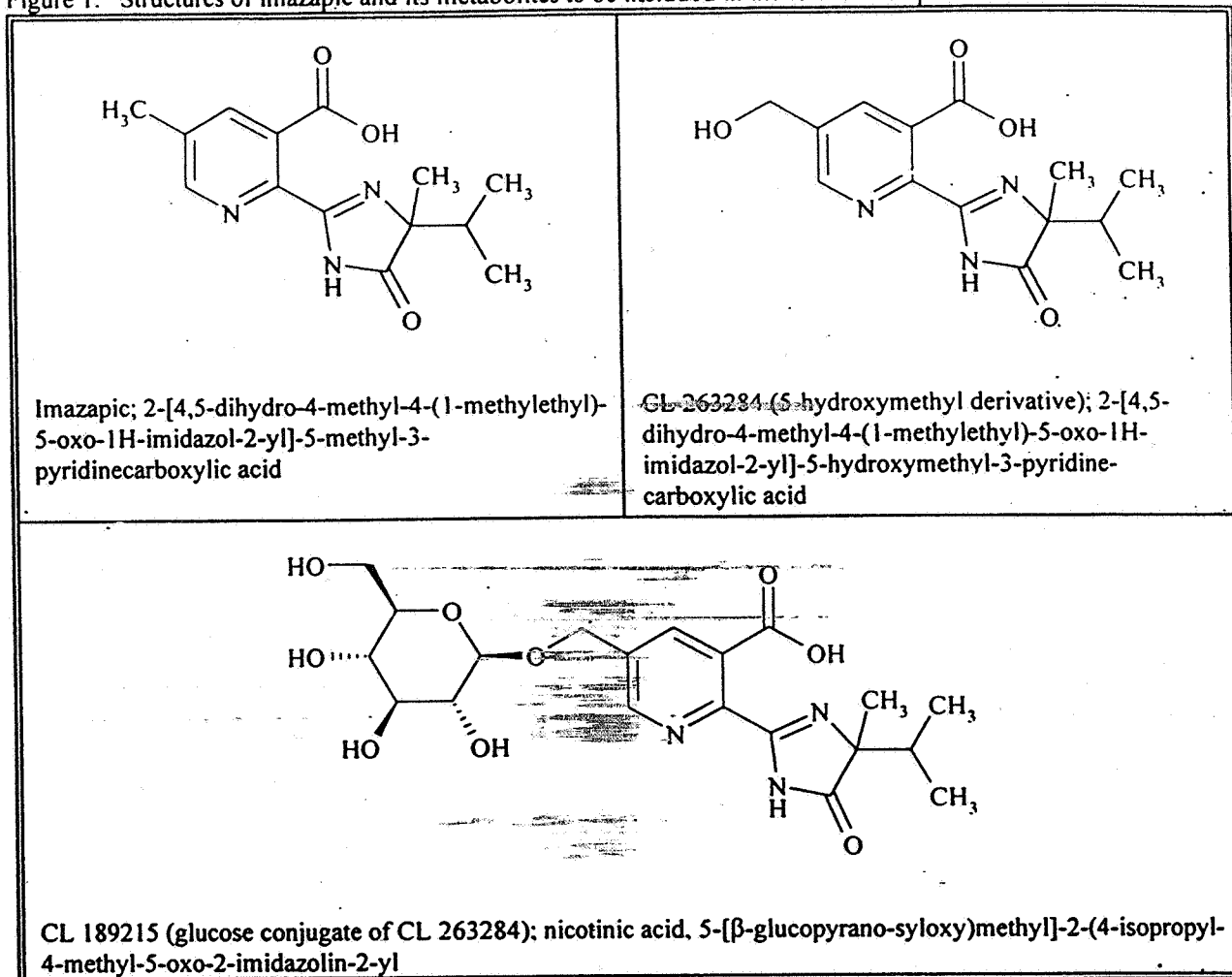
Data were previously submitted under PP#3G4203/3H5669 for an experimental use permit and establishment of temporary tolerances, and under PP#4F4390 for establishment of permanent tolerances. In conjunction with these petitions, HED concluded that the nature of the residue in peanuts was adequately understood. The Metabolism Committee determined (9/18/95) that the residues of concern in plants are imazapic and its hydroxymethyl metabolite, both free and conjugated (metabolites CL 263284 and CL 189215). The nature of the residue in livestock (ruminants) was considered to be understood for the use on peanuts. The residues of concern were determined to be imazapic and its hydroxymethyl metabolite (free only; metabolite CL 263284); however, HED required that additional ruminant metabolism studies be conducted at a higher dose level if future uses resulted in higher residues on feed items (DP Barcodes D207019, D207047, D207079, D209892, D209899, D211500, and D211980, 9/15/95, J. Garbus).

Tolerances are currently established for imazapic under §180.490(a) for the residues of the herbicide (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid applied as its ammonium salt and its metabolite (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-hydromethyl-3-pyridinecarboxylic acid both free and conjugated in/on peanut, nutmeat at 0.1 ppm. Time-limited tolerances set to expire 12/31/01 are established under §180.490(b) in connection with Section 18 emergency exemptions (99NE0009) for residues of imazapic [(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid], applied as its ammonium salt and its metabolite (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-hydromethyl-3-pyridinecarboxylic acid, both free and conjugated for grass forage at 30 ppm, grass hay at 15 ppm, milk at 0.010 ppm, fat, meat, and meat byproducts (except kidney) of cattle, goats, hogs, horses, and sheep at 0.10 ppm, and kidney of cattle, goats, hogs, horses, and sheep at 1.0 ppm.

**Note to PM: The metabolite name is incorrect in 40 CFR: 5-hydromethyl (underlined above) should be corrected to 5-hydroxymethyl.**

The chemical names and structures of imazapic and the metabolites proposed for inclusion in the tolerance expression are depicted below in Figure 1.

Figure 1. Structures of imazapic and its metabolites to be included in the tolerance expression.



Associated with this petition are 11 volumes of residue chemistry submissions which are evaluated in this document.

## CONCLUSIONS

### OPPTS 830 Series GLNs: Product Properties

1. The petitioner has previously submitted product chemistry data in conjunction with PP#3G4203/3H5669 (DP Barcodes D195919 and D198247, 2/2/94, F. Griffith) and

PP#4F4390 (DP Barcodes D207019, D207047, D207079, D209892, D209899, D211609, and D211980, 9/15/95, J. Garbus). Product chemistry data for the imazapic technical grade of the active ingredient (TGAI) are adequate; no additional data are required in support of this petition.

OPPTS GLN 860.1200: Proposed Uses

- 2a. The proposed Section B is not adequate. No field trial data have been submitted reflecting application of the 0.0625 lb ai/packet acid WDG formulation which was previously registered for noncrop uses only. The petitioner should provide field trial data using the WDG formulation or remove the proposed pasture and rangeland use from the Plateau™ DG Herbicide label.
- 2b. Both Plateau labels allow imazapic to be used with other pesticides in tank mixes. RD should ensure that all tank mix active ingredients have established tolerances or tolerance exemptions for use on grass commodities.
- 2c. With respect to the proposed plantback intervals (PBIs), the 4-month PBI for rye and wheat and the 9-month PBI for legume vegetables are not supported by rotational crop data reflecting the current maximum application rate for grasses. PBIs of 6 months for small grains, 11 months for root and tuber crops, and 12 months for leafy vegetables are supported by the available data (see OPPTS 860.1850: Confined Accumulation in Rotational Crops). **The petitioner should submit additional rotational crop data (confined or field) reflecting the proposed grass use rate and desired PBI or a revised Section B with updated rotational crop intervals for rye and wheat (6 months), and legume vegetables (12 months).**

OPPTS GLN 860.1300: Nature of the Residue in Plants

- 3a. The Bermuda grass metabolism study is acceptable. The total radioactive residues (TRR; expressed as parent equivalents) were 0.77-8.25 ppm and 0.92 ppm in/on grass forage and hay samples, respectively, following a single postemergence broadcast application of [<sup>14</sup>C]imazapic labeled at the 6-position of the pyridine ring at 0.181 lb ae/A (~1x the maximum proposed seasonal rate) to a plot of established Bermuda grass. In forage, TRR decreased with increasing harvest intervals, from 8.25 ppm in 0-DAT forage to 0.77 ppm in 49-DAT forage.
- 3b. Approximately 84% to >100% of the TRR were characterized/identified in grass forage and hay. The parent, imazapic, was the major residue component identified in 0-DAT grass forage (89.3% TRR, 7.37 ppm). Residues of imazapic declined significantly to 5.9% TRR (0.27 ppm) in 15-DAT forage, and continued to decline with longer harvest intervals. Imazapic was identified in 68-DAT hay (2.3% TRR, 0.02 ppm) as a minor residue. Metabolites CL 263284 and CL 189215 were identified, respectively, in 0-DAT



forage at 0.7% and 1.0% TRR (0.06 ppm and 0.08 ppm); in 15-DAT forage at 30.2% and 3.9% TRR (1.38 ppm and 0.17 ppm); in 32-DAT forage at 22.0% and 5.3% TRR (0.60 ppm and 0.14 ppm); in 49-DAT forage at 20.5% and 9.2% TRR (0.16 ppm and 0.08 ppm); and in 68-DAT hay at 8.4% and 9.2% TRR (0.08 ppm each). As residues of imazapic declined in forage, residues of the hydroxymethyl analog metabolite CL 263284 and its glucose conjugate CL 189215 increased.

- 3c. The majority of radioactivity in 15- to 49-DAT forage (20.6-26.8% TRR, 0.20-0.95 ppm) and 68-DAT hay (26.9% TRR, 0.25 ppm) was characterized as consisting of highly polar metabolites comprised of multi-substituted derivatives of the natural product nicotinic acid. Of these metabolites, several were tentatively identified in 15-DAT forage as 5-hydroxymethyl-2,3-pyridinedicarboxamide, hydroxy-pyridinedicarboxylic acid, 5-hydroxy-2,3-pyridine-dicarboxylic anhydride, and 2-formyl-5-hydroxy-nicotinic acid or its tautomeric form of 3,7-dihydroxy-furo[3,4-b]pyridine-5(7H)-one. Additional minor components present in forage and hay, each at  $\leq 0.02$  ppm, were tentatively identified in 15-DAT forage as hydroxy-imidazopyrrolopyridine and imidazopyrrolopyridine tricyclic derivatives and pyridodiazocine and hydroxy-pyridodiazocine derivatives. We note that, although imazapic is a double-ring compound, based on the results of the grass and peanut metabolism studies, cleavage between the pyridine and imidazole rings is not expected; therefore, a metabolism study reflecting labeling in the second ring is not required.
- 3d. Metabolism of imazapic in grass forage and hay is similar to that in peanuts, in which imazapic metabolizes to hydroxymethyl and glucoside derivatives; however, further oxidation of the hydroxymethyl metabolite to highly polar nicotinic acid derivatives is more extensive in Bermuda grass than in peanuts. The dicarboxylic acid derivative (CL 312622) identified in the peanut metabolism study was not observed in the Bermuda grass metabolism study.
- 3e. The HED MARC determined that the residues of concern in grass commodities are imazapic, CL 263284, and CL 189215 (D275136, W. Donovan and W. Dykstra, 07-JUN-2001).

#### OPPTS GLN 860.1300: Nature of the Residue in Livestock

- 4a. Following oral administration of [ $^{14}$ C]imazapic to a lactating goat for 5 consecutive days at a feeding level of 174.7 ppm, the total radioactive residues (TRR) were 0.012-0.078 ppm in whole milk, 0.275 ppm in kidney, 0.033 ppm in liver, 0.010 ppm in muscle, and 0.003 ppm in fat. Residues in milk were highest in the p.m. sample following each treatment and declined to the lowest levels in each of the following a.m. samples prior to the next treatment. Residues in composited daily milk gradually increased with each subsequent treatment day from 0.026 ppm to 0.037 ppm. Residues in tissues were highest in kidney and lowest in fat.

- 4b. Approximately 74.3-92.4% of the TRR were characterized/identified in goat milk and tissues (kidney and liver); only 37.3% of the TRR were characterized/identified in goat muscle because of low residue levels (TRR = 0.010 ppm). The parent, imazapic, was the only residue identified in milk and tissues. Imazapic residues accounted for 65.0-66.8% TRR (0.039-0.051 ppm) in whole milk, 85.0% TRR (0.234 ppm) in kidney, 49.1% TRR (0.016 ppm) in liver, and 33.8% TRR (0.003 ppm) in muscle. Most of the remaining radioactivity in milk and tissues consisted of low level, diffuse residues which did not form distinct peaks; no individual peak area accounted for more than 3.8% TRR (0.004 ppm).
- 4c. Because CL 263284 is a significant grass metabolite and may be consumed by ruminants, a goat metabolism study using <sup>14</sup>C-labeled CL 263284 is needed to adequately delineate the nature of the residue in ruminants. Alternatively, a bovine feeding study where cattle are fed CL 263284 at 1x, 3x, and 10x the maximum theoretical dietary burden (MTDB) may be conducted.
- 4d. The HED MARC determined that for the tolerance expression the residues of concern in/on livestock commodities are imazapic and its metabolite CL 263284 (D275136, W. Donovan and W. Dykstra, 07-JUN-2001).

OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

5. The petitioner has proposed CE method M 3114 for the enforcement of tolerances for grass forage and hay. This method was used for the determination of residues of imazapic and its metabolites CL 263284 and CL 189215 in/on grass forage and hay samples collected from the grass field trials and is similar to the peanut enforcement method CE M 2379. Concurrent method recoveries submitted in conjunction with the grass field trials indicate that this method adequately recovers residues of imazapic and its metabolites CL 263284, and CL189215 from grass forage and hay. Adequate independent method validation and radiovalidation data have been submitted for this method. This method was forwarded to ACB/BEAD for petition method validation (D271474, W. Donovan, 17-JAN-2001).

OPPTS GLN 860.1340: Residue Analytical Methods - Livestock Commodities

- 6a. The petitioner has proposed CE methods M 3188 and M 3222 for the enforcement of tolerances of imazapic and CL 263284 in milk (M 3188) and livestock tissues (M 3222), and HPLC/MS method M 3233 for the enforcement of tolerances in fat. These methods were used for the determination of residues of imazapic and CL 263284 in samples from the ruminant feeding study. Concurrent method recoveries submitted in conjunction with the ruminant feeding study indicate that these methods adequately recover residues of imazapic and CL 263284 from ruminant tissues and milk.

- 6b. Adequate independent method validation have been submitted in support of all livestock methods. Adequate radiovalidation data have been submitted for the CE enforcement methods. Although no radiovalidation data were submitted in support of HPLC/MS method M 3233 for determination of residues of imazapic in fat, in consideration of the concurrent validation data, and the fact that TRR in goat fat from the metabolism study (0.003 ppm) were below the method LOQ for fat of 0.050 ppm, no radiovalidation data are required for this method. The livestock methods have been forwarded to ACB/BEAD for a petition method validation (D271474, W. Donovan, 17-JAN-2001).

OPPTS GLN 860.1360: Multiresidue Method

7. The petitioner previously submitted data pertaining to the multiresidue methods testing of imazapic and its metabolites CL 263284 and CL 189215 in conjunction with PP#4F4390 (DP Barcode D211846, 2/9/95, F. Griffith). Methods testing indicated that residues of imazapic and its metabolites CL 263284 and CL 189215 are not likely to be recovered by the multiresidue methods. The results of the multiresidue testing for imazapic and its metabolites CL 263284 and CL189215 were forwarded for inclusion in PAM Volume I.

OPPTS GLN 860.1380: Storage Stability Data

8. Grass forage and hay samples from the submitted field studies were stored frozen from harvest to analysis for up to 24 months. The petitioner submitted storage stability data for wheat that indicated that residues of imazapic and its metabolites CL 263284 and CL 189215 are relatively stable under frozen storage conditions in/on fortified samples of wheat forage, hay, straw, and grain for up to 24 months. Because the commodities of wheat forage and hay are similar to pasture and rangeland grass forage and hay, the submitted storage stability data are adequate to support the storage conditions and intervals of the samples from the grass field trial studies.
9. Milk and cow tissue samples from the submitted ruminant feeding study were stored frozen from collection to analysis for up 6.1 months for whole milk, 8.3 months for milk fat, and 7.8 months for tissues. The submitted storage stability data indicate that residues of imazapic and its metabolite CL 263284 are relatively stable under frozen storage conditions in/on fortified samples of whole milk for up to 6 months and in/on fortified samples of cow kidney, liver, and muscle for up to 8 months. Although no data were submitted depicting storage stability of residues of imazapic and CL 263284 in fat, no data are required because TRR in fat in the goat metabolism study were quite low in comparison to TRR in other tissues, indicating that residues of imazapic and CL 263284 are not likely to be found in fat samples. The submitted storage stability data are adequate to support the storage conditions and intervals of the samples from the ruminant feeding study.

OPPTS GLN 860.1500: Crop Field Trials

- 10a. The submitted grass field trial data are inadequate to support the proposed uses of imazapic on the crop group Grass Forage, Fodder, and Hay (Crop Group 17). The petitioner has not provided adequate residue data reflecting the maximum proposed use pattern of imazapic on grasses (a single postemergence application at 0.1875 lb ae/A with a 0-day PHI for forage and a 7-day PHI for hay). Only eight grass field trials were conducted according to the maximum proposed use pattern; the Agency (Table 5 of OPPTS 860.1500) requires a total of 12 trials (geographic distribution unspecified) for the establishment of tolerances for residues in/on grass commodities, with four trials each to be conducted on the representative cultivars of Bermuda grass, bluegrass, and bromegrass or fescue for establishment of crop group tolerances (Table 2 of OPPTS 860.1500). Also, no field trial data were submitted in support of the 0.0625 lb ai/packet WDG acid formulation, which represents a different formulation class as well as a different chemical form of imazapic; under current Agency policy, the results of trials reflecting a representative of each formulation type and/or major form of an active ingredient (e.g., the acid vs. salt) must be compared to determine if there is an effect of formulation type/chemical form on the relationship between application rate and residue level.
- 10b. In support of postemergence use of imazapic on grasses, the petitioner should conduct four additional field trials reflecting a single postemergence application of the 2 lb ae/gal ammonium salt SC formulation at 0.1875 lb ae/A. Because it appears that residues may be higher in trials conducted in late summer or fall, the petitioner is advised to conduct the required trials during these seasons, preferably in Regions 7 and 8.
- 10c. In support of the 0.0625 lb ai/packet WDG acid formulation, field trials reflecting a 25% reduction in the number of trials (i.e., 9 instead of 12) would be appropriate to support registration of the WDG acid formulation for use on grasses. **Should the petitioner choose to conduct these trials side-by-side with the maximum rate ammonium salt SC formulation trials, then only 4 side-by-side trials are necessary.**
- 10d. The petitioner conducted 12 trials reflecting postemergence application to a variety of grass cultivars, including Bermuda grass, big blue stem grass, bromegrass, and mixtures of big bluestem and bromegrass, little bluestem and blue grass, bluegrass and little bluestem grass, and bluegrass and bromegrass. In eight trials, established grass stands received a single postemergence broadcast application made in the summer of the 2 lb ae/gal ammonium salt SC formulation at 0.2 lb ae/A (~1.1x the maximum proposed seasonal application rate). In three additional trials grasses received two postemergence broadcast applications of the 2 lb ae/gal ammonium salt SC formulation, with the first application made in the late summer or fall at 0.14-0.15 lb ae/A (~0.8x the maximum proposed seasonal application rate), and a second application made in the spring (following late summer application) or summer (following fall application) at 0.07 lb ae/A (0.4x the maximum proposed seasonal application rate); retreatment intervals were 9.3-9.4 months. In one trial, grasses received a single postemergence broadcast

application made in the fall of the 2 lb ae/gal ammonium salt SC formulation at 0.14 lb ae/A (~0.7x the maximum proposed seasonal application rate).

- 10e. The submitted data indicate that the combined residues of imazapic and its metabolites, CL 263284 and CL 189215, were <8.2-<23 ppm in/on grass forage harvested 0 days and 2.31-8.21 ppm in/on grass hay harvested 7 days following a single postemergence broadcast application of the 2 lb ae/gal ammonium salt SC formulation, made in the summer, at 0.20-0.21 lb ae/A (~1.1x the maximum proposed seasonal application rate). We note that in the four trials in which a postemergence application was made at 0.14-0.15 lb ae/A (~0.8x) during the late summer or fall, combined residues were 9.10-<25.12 ppm in/on grass forage harvested on the day of that application and <1.82-9.9 ppm in/on grass hay harvested 7 days after application.
- 10f. With respect to residue decline requirements, based on data from additional sampling intervals (7, 14, 28, and 54-57 days) following postemergence application, residues of imazapic do not increase in/on grass forage and hay with increasing PHI. Residues of imazapic *per se* declined to levels below the method LOQ within 7-28 days of application, while residues of CL 263284 appeared to increase from Day 0 to Days 7-14, and then decrease to below the method LOQ by Days 14-28. Residues of CL 189215 were below the method LOQ at all sampling intervals.
- 10g. The submitted data indicate that the combined residues of imazapic and its metabolites CL 263284 and CL 189215 were less than the LOQ (<1.50 ppm) in/on both grass forage and hay harvested 69-71 days following a single preemergence (at seeding) application of the 2 lb ae/gal ammonium salt SC formulation at 0.22 lb ae/A (~1.1x the maximum proposed seasonal application rate).
- 10h. The available field trial data support tolerance levels for residues of imazapic and its metabolites CL 263284 and CL 189215 in/on grass forage at 30 ppm and grass hay at 15 ppm. However, these levels may be adjusted as necessary when the requested additional data have been submitted and evaluated. In the tolerance expression, HED recommends that the petitioner remove references to the form of imazapic applied. **A revised Section F should be submitted.**

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

Ruminants:

- 11a. The submitted dairy cattle feeding study is tentatively determined to be acceptable; however, the recommended tolerance levels in livestock commodities are subject to revision upon assessment of additional crop field trial data required under OPPTS 860.1500. When the field trial data have been submitted and evaluated, HED will reevaluate the feeding study and update recommended tolerances for livestock commodities as necessary based on any changes to the MTDB for beef and dairy cattle.

- 11b. In the submitted study, Holstein dairy cows were dosed orally once daily for 28 consecutive days with imazapic at dose levels equivalent to 67 ppm, 223 ppm, and 676 ppm. Detectable residues of imazapic were observed in samples of milk, milk fat, and kidney at all dose levels; residues of imazapic were less than the LOQ (<0.05 ppm) in samples of fat, liver, and muscle at the 67-ppm dosing level. Residues of the metabolite CL 263284 were less than the respective LOQs in all samples of milk and milk fat (<0.01 ppm), and tissues (<0.05 ppm).
- 11c. Overall, residues of imazapic increased in milk and tissues with increasing in dose level. Residues in whole milk appear to plateau at Day 1 and did not significantly increase with subsequent doses. Residues in milk fat were lower than those in whole milk, confirming that residues do not tend to partition into fats. In tissues, residues were lowest in fat and highest in kidney.
- 11d. Based on the information presently available, the appropriate tolerance level for meat, fat, milk and meat byproducts (except kidney) is 0.10 ppm. For kidney, the appropriate tolerance level is 1.0 ppm. However, these levels may be adjusted as necessary when the requested additional grass field trial data have been submitted and evaluated. A revised Section F should be submitted.

Poultry:

12. There are no poultry feed items associated with the proposed uses of imazapic on pasture and rangeland grasses; therefore, a poultry feeding study is not required, and tolerances on eggs and poultry tissues need not be proposed in conjunction with this petition request.

OPPTS GLN 860.1850: Confined/Accumulation in Rotational Crops

- 13a. The petitioner submitted a new confined rotational crop study in support of the grass petition. The TRRs, expressed as [<sup>14</sup>C]imazapic equivalents, did not accumulate at levels ≥0.01 ppm in/on the RACs of winter wheat, spring wheat, carrots, and lettuce planted in sandy loam soil 181, 318, 318, and 363 days, respectively, days after treatment (DAT) of the soil with [<sup>14</sup>C]imazapic at 0.195 lb ae/A (~1x the maximum proposed seasonal rate for grasses).
- 13b. Although the TRR in all rotational crops at the various PBIs were <0.01 ppm, the petitioner subjected crop commodities with TRR >0.003 ppm to characterization/identification procedures. Residues of imazapic, CL 263284, CL 189215, and CL 312622 were identified in all rotational crop matrices at ≤0.001 ppm. Imazapic accounted for 0.9-13.0% TRR in rotational crop commodities, and was the major metabolite identified in 318-DAT carrot root. CL 263284 accounted for 1.5-24.5% TRR, and was the major metabolite identified in 181-DAT winter wheat hay and straw, 318-DAT spring wheat hay and straw, and 363-DAT lettuce. CL 189215 accounted for 1.5-8.9% TRR, and was the major metabolite identified in 318-DAT spring wheat grain.

CL 312622 was identified at  $\leq 2.1\%$  TRR, and CL 397695 was identified ( $\leq 5.3\%$  TRR,  $< 0.001$  ppm) in all rotational crop matrices except for lettuce. Based on the components identified, the study results suggest that imazapic is metabolized through the same routes in rotational crops as in the primary crop.

- 13c. Currently, the label for the 2 lb ae/gal ammonium salt SC formulation specifies the following minimum PBIs for rotational crops: 4 months following application for Bahiagrass, rye, and wheat; 9 months following application for field corn, snap beans, southern peas, soybeans, and tobacco; 18 months following application for barley, cotton, sorghum grain, oats, and sweet corn; and 40 months following application for canola, potatoes, red table beets, and sugar beets. All other crops for which a minimum PBI is not specified may be planted 26 months following application.
- 13d. HED notes that the 4-month PBI for bahiagrass, rye, and wheat was based on confined rotational crop data submitted in support of the peanut petition (use rate = 0.0625 lb ae/A). As the present petition for pasture and rangeland use involves an application rate three times higher than the peanut use rate, the confined rotational crop data at 0.0625 lb ae/A can not be translated to the Plateau labels for grass use.
- 13e. The submitted rotational crop data reflecting the grass use rate support establishment of a 6-month PBI for small grains, an 11-month PBI for root and tuber crops, and a 12-month PBI for leafy vegetables. The proposed 4-month PBI for rye and wheat and 9-month PBI for legume vegetables are not currently supported by rotational crop data. If the petitioner wishes to establish PBIs less than those reflected in the current confined rotational crop study, limited field rotational crop studies or additional confined rotational crop studies making use of the grass application rate and desired PBI are recommended. Alternatively, the petitioner may increase the rye and wheat PBIs from 4 to 6 months, and increase the PBI for legume vegetables to 12 months. The petitioner should submit additional rotational crop data or a revised Section B with updated rotational crop intervals for rye, wheat, and legume vegetables.

#### Codex Issues

14. There are currently no established Codex, Canadian, or Mexican maximum residue limits (MRLs) for residues of imazapic in/on plant or livestock commodities. Therefore, no compatibility issues exist with regard to the proposed U.S. tolerances discussed in this petition review. An International Residue Limit Status sheet is attached (Attachment 3).

#### RECOMMENDATIONS

**Provided that Agency validation of the analytical method is successful (Conclusions 5 & 6b) and that Sections B (Conclusions 2c & 13e) and F (Conclusions 10h & 11d) are modified as**

requested, HED concludes there are no residue chemistry data requirements that would preclude establishment of permanent tolerances for imazapic and its hydroxymethyl metabolites in/on the following commodities:

Grass, forage	30 ppm
Grass, hay	15 ppm
Milk	0.10 ppm
Meat*	0.10 ppm
Fat*	0.10 ppm
Meat byproducts (except kidney)*	0.10 ppm
Kidney*	1.0 ppm

\* Of cattle, sheep, goats, and horses

However, registration of Plateau™ Herbicide for use on pastures and rangeland should be made conditional upon the submission of additional data as specified in Conclusions 4c, and 10a - 10c. Registration of Plateau™ DG Herbicide for use on pastures and rangeland should be delayed until the petitioner has submitted the requested field trial data supporting its use. A human-health risk assessment will be prepared as a separate document.

#### DETAILED CONSIDERATIONS

##### OPPTS 830 Series GLNs: Product Properties

The petitioner has previously submitted product chemistry data in conjunction with PP#3G4203/3H5669 (DP Barcodes D195919 and D198247, 2/2/94, F. Griffith) and PP#4F4390 (DP Barcodes D207019, D207047, D207079, D209892, D209899, D211609, and D211980, 9/15/95, J. Garbus). Product chemistry data for the imazapic technical grade of the active ingredient (TGAI) are adequate; no additional data are required in support of this petition.

##### OPPTS GLN 860.1200: Proposed Uses

The petitioner provided specimen labels for two imazapic end-use products: the 2 lb ae/gal ammonium salt soluble concentrate (SC) formulation (EPA Reg. No. 241-365; Product Name = Plateau™ Herbicide), and the 0.0625 lb ai/packet imazapic acid water dispersible granule (WDG) formulation in water soluble packets (EPA Reg. No. 241-393; Product Name = Plateau™ DG Herbicide). We note that the SC formulation was referred to in the crop field trial submission as the 2 AS formulation (22.2% ae). For both formulations, the petitioner is proposing to amend the previously registered uses for weed control, native grass establishment and turf growth suppression on roadsides and other noncrop areas, to include use on pasture and rangeland grasses.



The 2 lb ae/gal ammonium salt-SC formulation is proposed for one or more preemergence or postemergence spot or broadcast applications to pasture and rangeland grasses at 2-12 oz. product (0.03125-0.1875 lb ae/A/application) with a maximum seasonal rate of 0.1875 lb ae/A. The 0.0625 lb ai/packet acid WDG formulation is proposed for one or more preemergence or postemergence spot or broadcast applications to pasture and rangeland grasses at 0.0625-0.1875 lb ai/A/application with a maximum seasonal rate of 0.1875 lb ai/A. Both labels state that the treated area may not be cut for hay within 7 days after treatment.

Applications may be made using ground equipment (minimum of 2 gal of water/A (GPA)) or aerial equipment (minimum of 5 GPA). Postemergence applications are to be made using a spray adjuvant (methylated seed oils, vegetable oil concentrates, nonionic surfactants, silicone-based surfactants, or fertilizer/surfactant blends). In addition, the labels allow tank mixes with dicamba, diuron, glufosinate-ammonium, glyphosate, imazapyr, N-methyl pyrrolidone, metsulfuron-methyl, MSMA, pendimethalin, sulfometuron-methyl, and triclopyr. Tank mixing with organophosphate insecticides or use of organophosphate insecticides during the same year as imazapic applications is prohibited.

The following minimum PBIs for rotational crops are proposed for both products: 4 months following application for Bahiagrass, rye, and wheat; 9 months following application for field corn, snap beans, southern peas, soybeans, and tobacco; 18 months following application for barley, cotton, sorghum grain, oats, and sweet corn; and 40 months following application for canola, potatoes, red table beets, and sugar beets. All other crops for which a minimum PBI is not specified may be planted 26 months following application.

#### Comments

The proposed Section B is not adequate. No field trial data have been submitted reflecting application of the 0.0625 lb ai/packet acid WDG formulation which was previously registered for noncrop uses only. The petitioner should provide field trial data using the WDG formulation or remove the proposed pasture and rangeland use from the Plateau™ DG Herbicide label.

Both Plateau labels allow imazapic to be used with other pesticides in tank mixes. RD should ensure that all tank mix active ingredients have established tolerances or tolerance exemptions for use on grass commodities.

With respect to the proposed plantback intervals (PBIs), the 4-month PBI for rye and wheat and the 9-month PBI for legume vegetables are not supported by rotational crop data reflecting the current maximum application rate for grasses. PBIs of 6 months for small grains, 11 months for root and tuber crops, and 12 months for leafy vegetables are supported by the available data (see OPPTS 860.1850: Confined Accumulation in Rotational Crops). **The petitioner should submit additional rotational crop data (confined or field) reflecting the proposed grass use rate and desired PBI or a revised Section B with updated rotational crop intervals for rye and wheat (6 months), and legume vegetables (12 months).**

## OPPTS GLN 860.1300: Nature of the Residue in Plants

### Grass

American Cyanamid has submitted a Bermuda grass metabolism study (citation listed below) in support of the current petition. The field portion of the study was conducted at American Agricultural Services, Inc. (Lucama, NC), and the analytical phase of the study was performed by the Agricultural Products Research Division of American Cyanamid (Princeton, NJ).

44817707 Fung, C.H. (1999) CL 263222: Metabolism of CL 263222 in Bermudagrass under Field Conditions. Laboratory Project Identification MET 98-015. Unpublished study prepared by American Cyanamid Company. 301 p.

The test substance, [<sup>14</sup>C]imazapic labeled at the 6-position of the pyridine ring (radiochemical purity >99%) was mixed with [<sup>13</sup>C]imazapic and formulated as an ammonium salt formulation containing approximately 22.9% active ingredient (final specific activity 10.94  $\mu$ Ci/mg). The formulated test substance was diluted with water and applied as a single postemergence broadcast spray to a small field plot of established Bermuda grass at 0.181 lb ae/A (~1x the maximum proposed seasonal application rate). Application was made when the grass was approximately 20 inches tall (62 days after sprigging) using a hand-held N<sub>2</sub>-pressurized sprayer. A separate plot received a single application of formulation blank for controls. The grass plots were watered and weeded by hand as needed.

Bermuda grass forage was harvested from control and treatment plots on the treatment day within 3 hours of application and 15, 32, and 49 days following the application. Bermuda grass for hay was cut 68 days following treatment and dried in the field for 3 days. Due to poor drying conditions, hay samples were dried an additional 3 days in a greenhouse. Forage and hay samples were cut by hand near the soil surface. The collected samples were immediately frozen and shipped frozen via FedEx to the analytical laboratory where they remained frozen (-35 to -10 C) until processed (homogenized) for analysis. The study report provided adequate information pertaining to plot design, preparation and calculation of the test substance, and key study dates.

### Total radioactive residues (TRR)

The collected Bermuda grass forage and hay samples were ground in the presence of dry ice and subjected to combustion/liquid scintillation counting (LSC) to determine total radioactive residues. The reported limit of detection, based on [<sup>14</sup>C]imazapic fortified control samples, was 0.005 ppm. The TRR (expressed as parent equivalents) in/on treated forage and hay samples are presented in Table 1.

Table 1. TRR in/on Bermuda grass forage and hay harvested at various intervals following a single postemergence broadcast application of [<sup>14</sup>C]imazapic at 0.181 lb ae/A (~ 1x the maximum proposed seasonal rate).

Commodity	Sampling interval, DAT <sup>a</sup>	TRR, ppm [ <sup>14</sup> C]imazapic equivalents
Grass, forage	0	8.25
	15	4.58
	32	2.73
	49	0.77
Grass, hay	68 (+6 days drying)	0.92

<sup>a</sup> DAT = days after treatment that the sample was harvested.

### Extraction of residues

Bermuda grass forage and hay samples were subjected to extraction and/or hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below.

Samples of ground forage and hay were extracted overnight with methanol:acetone:water (1:1:1, v:v:v), homogenized in a Polytron, and centrifuged. Nonextractable residues were extracted again with methanol:acetone:water for 2 hours and centrifuged. Remaining nonextractable residues were then extracted overnight with acetone:water (1:1, v:v) and centrifuged. The methanol:acetone:water and acetone:water supernatants were combined and concentrated for HPLC analysis.

The remaining nonextractable residues were subjected to acid and base hydrolyses. Nonextractable residues were sequentially extracted with methanol:water:HCl (200:50:27, v:v:v), 1 N HCl, and 1 N NaOH (overnight at 37 C in a water bath) and centrifuged. The supernatants were each adjusted to pH 7 with 50% sodium hydroxide or 6 N HCl and combined. Nonextractable residues were washed with methanol:water (1:1, v:v). The combined supernatants and methanol:water wash were each concentrated for HPLC analysis.

Nonextractable residues remaining after acid/base hydrolysis were subjected to enzyme hydrolysis (cellulase and pepsin). Nonextractable residues were incubated with cellulase (in 0.05 M acetate buffer, pH 5, at 37 C for 48 hours) and centrifuged. The supernatant was collected, and the precipitate was resuspended in 0.1 N HCl with pepsin and incubated at 37 C for another 48 hours then centrifuged. The precipitate following enzyme hydrolysis was then subjected to sequential hydrolysis with strong base (10 N NaOH at reflux for 8 hours) and acid (6 N HCl at reflux for 8 hours).

The distribution of <sup>14</sup>C-activity in the extracts of Bermuda grass forage and hay is presented in Table 2.

### Characterization/identification of residues

Grass forage and hay extracts were analyzed by HPLC. Reverse phase HPLC analysis was performed on a system equipped with a Supelco C18 column, with a gradient mobile phase of methanol and water, both containing 1% acetic acid, and a UV (254 nm) and radiodetector. Samples were co-chromatographed with nonradiolabeled reference standards of imazapic, CL 263284, CL 312622 (5-acid imidazolinone metabolite), CL 312622, CL 290610, and CL 397695. Chemical names and structures for identified metabolites are presented in Attachment 2.

Eleven radioactive regions (M1-M10) were identified in forage and hay extracts by reverse phase HPLC; a twelfth region was detected in 0-day forage at <0.5% of the TRR. Fractions M1 and M2 (containing very polar components) from 15-, 32-, and 49-DAT forage samples were separately pooled and concentrated. Sodium chloride was precipitated from the M1 fraction with cold methanol in a freezer (-20 C). The aqueous phase was subjected to strong cation exchange (SCX) SPE cleanup. The SCX eluate was concentrated for HPLC analysis. HPLC analysis of M1 and M2 fractions was conducted on a system equipped with a Hypersil Hypercarb column, a gradient mobile phase of tetrahydrofuran (THF):water (50:49, v:v) and water, both containing 1% acetic acid, a diode array detector (254 nm), and a fraction collector. HPLC analysis indicated that the M1 fraction was comprised of 12 components; further analysis of these minor metabolites by HPLC demonstrated that each contained multiple components.

The major metabolites M1-B and M1-C were isolated from the 15-DAT forage M1 fraction and metabolites M2-A and M2-B were isolated from the 15-DAT forage M2 fraction. These metabolites were further purified by additional HPLC separations using the Hypercarb column and varying gradient mobile phases of acidic water and THF. The purified metabolites M1-B, M1-C, M2-A, and M2-B were analyzed by MS. Based on liquid chromatography electrospray positive ion mass spectrometry (LC-ESP-PIMS), the metabolites were tentatively identified as multi-substituted pyridine derivatives: 5-hydroxymethyl-2,3-pyridinedicarboxamide (M1-B), hydroxy-pyridinedicarboxylic acid (M1-C), 5-hydroxy-2,3-pyridine-dicarboxylic anhydride (M2-A), and 2-formyl-5-hydroxy-nicotinic acid or its tautomer 3,7-dihydroxy-furo[3,4-b]pyridine-5(7H)-one (M2-B).

Fractions M4, M5, and M7 from 15-DAT forage were isolated by C18 HPLC using acidic water and methanol gradient mobile phases. The M4 and M7 isolates were further purified through a monoclonal antibody affinity column; residues were eluted with methanol:water (30:70, v:v). Identifications of metabolites M4, M5, and M7 as CL 189215, CL 263284, and unchanged imazapic, respectively, were confirmed by LC-ES-PIMS of the isolated fractions.

Fraction M6 of 15-DAT forage was isolated by HPLC, further purified on a Supelco C18 column, and subjected to affinity column chromatography. Fraction M6 did not bind to anti-imidazolinone ring monoclonal antibody, suggesting it lacked the free imidazolinone ring. The M6 fraction was further separated into many discernible components by sequential HPLC

separation using a Phenomenex LUNA C18 column with a gradient mobile phase of ACN:water each with 1% acetic acid and a Hypercarb column with a gradient mobile phase of THF, ACN, and water, each containing 1% acetic acid. Metabolites M6-D, M6-H, M6-J2, and M6-J3, each present at  $\leq 0.02$  ppm, were tentatively identified by LC-ES-PIMS as hydroxy-imidazopyrrolopyridine (M6-D or M6-H) and imidazopyrrolopyridine (M6-J3) tricyclic derivatives and pyridodiazocine (M6-D or M6-H) and hydroxy-pyridodiazocine (M6-J2) derivatives.

Analysis of fractions M8, M9, and M10 by reversed phase HPLC indicated that these unknowns were less polar than the parent imazapic; each fraction was comprised of multiple minor components at low concentrations.

A summary of the characterized and identified  $^{14}\text{C}$ -residues in Bermuda grass is presented in Table 3. The chemical structures of identified metabolites in grass forage and hay are depicted in Attachment 2.

Table 2. Distribution and characterization radioactive residues in/on Bermuda grass forage harvested 0, 15, 32, and 49 days or hay harvested 68 days following a single broadcast postemergence application with  $^{14}\text{C}$ imazapic at 0.181 lb ae/A (~1x the maximum proposed seasonal rate).

Fraction	% TRR	ppm	Characterization/Identification *
<b>Bermuda grass, forage (0 DAT; TRR = 8.25 ppm)</b>			
Methanol:acetone:water	97.6	8.05	<u>HPLC analysis resolved:</u>
			Imazapic 88.1% TRR 7.27 ppm
			CL 263284 0.6% TRR 0.05 ppm
			CL 189215 0.9% TRR 0.08 ppm
			M1 2.7% TRR 0.22 ppm
			M2 1.0% TRR 0.08 ppm
			M3 0.5% TRR 0.04 ppm
			M3A 0.3% TRR 0.02 ppm
			M6 0.8% TRR 0.07 ppm
			M8 1.3% TRR 0.11 ppm
			M9 0.5% TRR 0.04 ppm
			M10 0.5% TRR 0.04 ppm
			M11 0.4% TRR 0.03 ppm

Fraction	% TRR	ppm	Characterization/Identification *
Combined methanol:water:HCl/ 1 N HCl/1 N NaOH extracts	2.7	0.22	<u>HPLC analysis resolved:</u>
			Imazapic 1.2% TRR 0.10 ppm
			CL 263284 0.1% TRR <0.01 ppm
			CL 189215 0.1% TRR <0.01 ppm
			M1 0.3% TRR 0.02 ppm
			M2 0.3% TRR 0.02 ppm
			M3 <0.1% TRR <0.01 ppm
			M3A 0.1% TRR <0.01 ppm
			M6 0.1% TRR 0.01 ppm
			M8 0.2% TRR 0.02 ppm
			M9 0.1% TRR <0.01 ppm
			M10 0.1% TRR 0.01 ppm
M11 0.1% TRR <0.01 ppm			
Methanol:water rinsate	0.3	0.02	Not further analyzed (N/A).
Nonextractable	<0.1	<0.01	Subjected to sequential enzyme (cellulase and pepsin) hydrolysis.
Enzyme hydrolysate	<0.1	<0.01	N/A.
Nonextractable	NR <sup>b</sup>	NR	N/A.

Table 2 (continued).

Fraction	% TRR	ppm	Characterization/Identification *
<b>Bermuda grass, forage (15 DAT; TRR = 4.58 ppm)</b>			
Methanol:acetone:water	68.6	3.14	<u>HPLC analysis resolved:</u> Imazapic 4.3% TRR 0.20 ppm CL 263284 28.1% TRR 1.29 ppm CL 189215 2.7% TRR 0.12 ppm M1 17.2% TRR 0.79 ppm M2 5.0% TRR 0.23 ppm M3 3.1% TRR 0.14 ppm M3A 1.6% TRR 0.08 ppm M6 2.9% TRR 0.13 ppm M8 1.5% TRR 0.07 ppm M9 1.1% TRR 0.05 ppm M10 0.8% TRR 0.03 ppm
Combined methanol:water:HCl/ 1 N HCl/1 N NaOH extracts	12.6	0.58	<u>HPLC analysis resolved:</u> Imazapic 1.4% TRR 0.06 ppm CL 263284 2.0% TRR 0.09 ppm CL 189215 1.0% TRR 0.04 ppm M1 2.4% TRR 0.11 ppm M2 1.3% TRR 0.06 ppm M3 0.8% TRR 0.04 ppm M3A 0.5% TRR 0.02 ppm M6 0.7% TRR 0.03 ppm M8 0.8% TRR 0.04 ppm M9 0.6% TRR 0.03 ppm M10 0.7% TRR 0.03 ppm
Methanol:water rinsate	3.0	0.14	<u>HPLC analysis resolved:</u> Imazapic 0.2% TRR <0.01 ppm CL 263284 0.1% TRR <0.01 ppm CL 189215 0.2% TRR 0.01 ppm M1 1.0% TRR 0.05 ppm M2 0.1% TRR <0.01 ppm M3 0.1% TRR <0.01 ppm M3A 0.1% TRR <0.01 ppm M6 0.4% TRR 0.02 ppm M8 0.4% TRR 0.02 ppm M9 0.1% TRR <0.01 ppm M10 0.1% TRR <0.01 ppm
Nonextractable	2.7	0.12	Subjected to sequential enzyme (cellulase and pepsin) hydrolysis; and strong base (10 N NaOH) and acid (6 N HCl) hydrolysis at reflux.
Enzyme hydrolysate	0.1	<0.01	N/A.
Reflux base/acid hydrolysate	0.8	0.04	N/A.
Nonextractable	0.3	0.02	N/A.

Table 2 (continued).

Fraction	% TRR	ppm	Characterization/Identification *
<b>Bermuda grass, forage (32 DAT; TRR = 2.73 ppm)</b>			
Methanol:acetone:water	62.1	1.70	<u>HPLC analysis resolved:</u> Imazapic 1.7% TRR 0.05 ppm CL 263284 18.7% TRR 0.51 ppm CL 189215 4.1% TRR 0.11 ppm M1 18.9% TRR 0.52 ppm M2 3.6% TRR 0.10 ppm M3 4.6% TRR 0.13 ppm M3A 2.8% TRR 0.08 ppm M6 2.6% TRR 0.07 ppm M8 2.3% TRR 0.06 ppm M9 1.6% TRR 0.04 ppm M10 1.1% TRR 0.03 ppm
Combined methanol:water:HCl/ 1 N HCl/1 N NaOH extracts	15.6	0.43	<u>HPLC analysis resolved:</u> Imazapic 1.2% TRR 0.03 ppm CL 263284 2.2% TRR 0.06 ppm CL 189215 0.7% TRR 0.02 ppm M1 3.6% TRR 0.10 ppm M2 1.0% TRR 0.03 ppm M3 1.8% TRR 0.05 ppm M3A 0.9% TRR 0.02 ppm M6 1.2% TRR 0.03 ppm M8 1.3% TRR 0.04 ppm M9 0.5% TRR 0.01 ppm M10 1.0% TRR 0.03 ppm
Methanol:water rinsate	7.2	0.20	<u>HPLC analysis resolved:</u> Imazapic 0.2% TRR <0.01 ppm CL 263284 1.1% TRR 0.03 ppm CL 189215 0.5% TRR 0.01 ppm M1 1.7% TRR 0.05 ppm M2 0.3% TRR <0.01 ppm M3 0.5% TRR 0.01 ppm M3A 0.3% TRR <0.01 ppm M6 0.6% TRR 0.02 ppm M8 0.8% TRR 0.02 ppm M9 0.6% TRR 0.02 ppm M10 0.5% TRR 0.01 ppm
Nonextractable	2.3	0.06	Subjected to sequential enzyme (cellulase and pepsin) hydrolysis: and strong base (10 N NaOH) and acid (6 N HCl) hydrolysis at reflux.
Enzyme hydrolysate	1.0	0.03	N/A.
Reflux base/acid hydrolysate	0.6	0.02	N/A.
Nonextractable	1.3	0.04	N/A



Table 2 (continued).

Fraction	% TRR	ppm	Characterization/Identification *
<b>Bermuda grass, forage (49 DAT; TRR = 0.77 ppm)</b>			
Methanol:acetone:water	51.2	0.39	<u>HPLC analysis resolved:</u> Imazapic 1.3% TRR <0.01 ppm CL 263284 16.9% TRR 0.13 ppm CL 189215 3.3% TRR 0.03 ppm M1 17.5% TRR 0.13 ppm M2 1.3% TRR 0.01 ppm M3 1.5% TRR 0.01 ppm M3A 3.2% TRR 0.02 ppm M6 1.9% TRR 0.01 ppm M8 1.1% TRR <0.01 ppm M9 1.4% TRR 0.01 ppm M10 1.4% TRR 0.01 ppm
Combined methanol:water:HCl/ 1 N HCl/1 N NaOH extracts	27.3	0.21	<u>HPLC analysis resolved:</u> Imazapic 1.3% TRR 0.01 ppm CL 263284 3.6% TRR 0.03 ppm CL 189215 5.9% TRR 0.05 ppm M1 9.3% TRR 0.07 ppm M2 3.6% TRR 0.03 ppm M3 1.5% TRR 0.01 ppm M3A 3.9% TRR 0.03 ppm M6 5.2% TRR 0.04 ppm M8 1.2% TRR <0.01 ppm M9 1.8% TRR 0.01 ppm M10 2.3% TRR 0.02 ppm
Methanol:water rinsate	13.7	0.11	M3 1.5% TRR 0.01 ppm M3A 3.9% TRR 0.03 ppm M6 5.2% TRR 0.04 ppm M8 1.2% TRR <0.01 ppm M9 1.8% TRR 0.01 ppm M10 2.3% TRR 0.02 ppm
Nonextractable	10.2	0.08	Subjected to sequential enzyme (cellulase and pepsin) hydrolysis; and strong base (10 N NaOH) and acid (6 N HCl) hydrolysis at reflux.
Enzyme hydrolysate	2.1	0.02	N/A.
Reflux base/acid hydrolysate	4.3	0.03	N/A.
Nonextractable	2.8	0.02	N/A.
<b>Bermuda grass, hay (68 DAT; TRR = 0.92 ppm)</b>			
Methanol:acetone:water	34.8	0.32	<u>HPLC analysis resolved:</u> Imazapic 0.7% TRR <0.01 ppm CL 263284 5.7% TRR 0.05 ppm CL 189215 7.7% TRR 0.07 ppm M1 11.5% TRR 0.11 ppm M2 0.6% TRR <0.01 ppm M3 1.1% TRR 0.01 ppm M3A 2.2% TRR 0.02 ppm M6 1.3% TRR 0.01 ppm M8 1.9% TRR 0.02 ppm M9 0.7% TRR <0.01 ppm M10 0.7% TRR <0.01 ppm

Table 2 (continued).

Fraction	% TRR	ppm	Characterization/Identification <sup>a</sup>
Combined methanol:water:HCl/ 1 N HCl/1 N NaOH extracts	27.0	0.25	<u>HPLC analysis resolved:</u> Imazapic 0.9% TRR <0.01 ppm CL 263284 2.0% TRR 0.02 ppm CL 189215 0.7% TRR <0.01 ppm M1 13.3% TRR 0.12 ppm M2 0.8% TRR <0.01 ppm M3 0.9% TRR <0.01 ppm M3A 1.2% TRR 0.01 ppm M6 1.7% TRR 0.02 ppm M8 2.0% TRR 0.02 ppm M9 1.3% TRR 0.01 ppm M10 1.4% TRR 0.01 ppm
Methanol:water rinsate	12.4	0.11	<u>HPLC analysis resolved:</u> Imazapic 0.7% TRR <0.01 ppm CL 263284 0.7% TRR <0.01 ppm CL 189215 0.8% TRR <0.01 ppm M1 2.1% TRR 0.02 ppm M2 1.2% TRR 0.01 ppm M3 0.5% TRR <0.01 ppm M3A 0.6% TRR <0.01 ppm M6 1.8% TRR 0.02 ppm M8 0.9% TRR <0.01 ppm M9 1.1% TRR <0.01 ppm M10 0.7% TRR <0.01 ppm
Nonextractable	31.2	0.29	Subjected to sequential enzyme (cellulase and pepsin) hydrolysis; and strong base (10 N NaOH) and acid (6 N HCl) hydrolysis at reflux.
Enzyme hydrolysate	4.7	0.04	N/A.
Reflux base/acid hydrolysate	8.1	0.07	N/A.
Nonextractable	2.9	0.03	N/A.

<sup>a</sup> Residues of imazapic, CL 263284, and CL 189215 were identified by HPLC and confirmed by MS; metabolites M1 (M1-B and M1-C), M2 (M2-A and M2-B), M6 (M6-D, M6-H, M6-J2, and M6-J3) were tentatively identified by MS. See Figure 2 (Attachment II) for structures and chemical names of identified metabolites.

<sup>b</sup> NR = not reported.

Table 3. Identification/characterization of radioactive residues in/on Bermuda grass, forage harvested 0, 15, 32, and 49 days and hay harvested 68 days following a single broadcast postemergence application with [<sup>14</sup>C]imazapic at 0.181 lb ae/A (0.9x the maximum proposed seasonal rate).

Metabolite/Fraction	Grass, forage; 0 DAT (TRR = 8.25 ppm)		Grass, forage; 15 DAT (TRR = 4.58 ppm)		Grass, forage; 32 DAT (TRR = 2.73 ppm)		Grass, forage; 49 DAT (TRR = 0.77 ppm)		Grass, hay; 68 DAT (TRR = 0.92 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
<b>Identified *</b>										
Imazapic	89.3	7.37	5.9	0.27	3.1	0.08	2.6	0.02	2.3	0.02
CL 263284	0.7	0.06	30.2	1.38	22.0	0.60	20.5	0.16	8.4	0.08
CL 189215	1.0	0.08	3.9	0.17	5.3	0.14	9.2	0.08	9.2	0.08
<b>Total Identified</b>	<b>91.0</b>	<b>7.51</b>	<b>40.0</b>	<b>1.82</b>	<b>30.4</b>	<b>0.82</b>	<b>32.3</b>	<b>0.26</b>	<b>19.9</b>	<b>0.18</b>
<b>Characterized</b>										
M1 (>12 components including M1-B and M1-C)	3.0	0.24	20.6	0.95	24.2	0.67	26.8	0.20	26.9	0.25
M2 (M2-A and M2-B)	1.3	0.10	6.4	0.29	4.9	0.13	4.9	0.04	2.6	0.02
M3 (>5 components)	0.5	0.04	4.0	0.18	6.9	0.19	3.0	0.02	2.5	0.02
M3A (>28 components)	0.4	0.03	2.2	0.10	4.0	0.11	7.1	0.05	4.0	0.04
M6 (>12 components)	0.9	0.08	4.0	0.18	4.4	0.12	7.1	0.05	4.8	0.04
M8 (>6 components)	1.5	0.13	2.7	0.13	4.4	0.12	2.3	0.02	4.6	0.04
M9 (>18 components)	0.6	0.05	1.8	0.08	2.7	0.07	3.2	0.02	3.0	0.03
M10 (>16 components)	0.6	0.05	1.6	0.07	2.6	0.07	3.7	0.03	2.8	0.03
M11	0.5	<0.04	--	--	--	--	--	--	--	--
Methanol:water rinsate	0.3	0.02	--	--	--	--	--	--	--	--
Enzyme hydrolysate	<0.1	<0.01	0.1	<0.01	1.0	0.03	2.1	0.02	4.7	0.04
Base/Acid reflux	--	--	0.8	0.04	0.6	0.02	4.3	0.03	8.1	0.07
<b>Total Characterized/Identified</b>	<b>&lt;100.7</b>	<b>&lt;8.3</b>	<b>84.2</b>	<b>&lt;3.85</b>	<b>86.1</b>	<b>2.35</b>	<b>96.8</b>	<b>0.74</b>	<b>83.9</b>	<b>0.76</b>
Nonextractable	NR	NR	0.3	0.02	1.3	0.04	2.8	0.02	2.9	0.03

\* Residues of imazapic, CL 263284, and CL 189215 were identified by HPLC and confirmed by MS; metabolites M1 (M1-B and M1-C), M2 (M2-A and M2-B), M6 (M6-D, M6-H, M6-J2, and M6-J3) were tentatively identified by MS. See Attachment 2 for structures and chemical names of identified metabolites.

### Storage stability

The Bermuda grass forage and hay samples were stored frozen prior to analysis. Analysis of grass forage and hay samples was completed within 115 days (3.8 months) of sample collection. The methanol:acetone:water extract of 15-DAT forage was analyzed 109, 369, and 1064 days following collection. No significant changes in the metabolic profiles were observed following -31 months of frozen storage between the 109- and 1064-day analyses. Because samples were stored for <4 months, RAB1 concludes that storage stability is not an issue with regard to determining the acceptability of the submitted grass metabolism study.

### Proposed metabolic pathway

Based on the Bermuda grass metabolism study, the petitioner proposes that imazapic is rapidly metabolized by oxidative hydroxylation of the 5-methyl substituent to form a 5-hydroxymethyl metabolite (CL 263284). CL 263284 is either rapidly conjugated with glucose to form CL 189215 or further metabolized to hydroxy-imidazopyrrolopyridine and imidazopyrrolopyridine tricyclic derivatives, and hydroxy-pyridodiazocine and pyridodiazocine derivatives. The imidazopyrrolopyridine and pyridodiazocine metabolites are quickly degraded to form highly polar multi-substituted derivatives of the natural product nicotinic acid. This pathway is similar to the pathway proposed for metabolism of imazapic in peanuts (DP Barcodes D191715, D191694, and D191710, 3/10/94, F. Griffith).

### Summary

The Bermuda grass metabolism study is acceptable. The total radioactive residues (TRR: expressed as parent equivalents) were 0.77-8.25 ppm and 0.92 ppm in/on grass forage and hay samples, respectively, following a single postemergence broadcast application of [<sup>14</sup>C]imazapic labeled at the 6-position of the pyridine ring at 0.181 lb ae/A (~1x the maximum proposed seasonal rate) to a plot of established Bermuda grass. In forage, TRR decreased with increasing harvest intervals, from 8.25 ppm in 0-DAT forage to 0.77 ppm in 49-DAT forage.

Approximately 84% to >100% of the TRR were characterized/identified in grass forage and hay. The parent, imazapic, was the major residue component identified in 0-DAT grass forage (89.3% TRR, 7.37 ppm). Residues of imazapic declined significantly to 5.9% TRR (0.27 ppm) in 15-DAT forage, and continued to decline with longer harvest intervals. Imazapic was identified in 68-DAT hay (2.3% TRR, 0.02 ppm) as a minor residue. Metabolites CL 263284 and CL 189215 were identified, respectively, in 0-DAT forage at 0.7% and 1.0% TRR (0.06 ppm and 0.08 ppm); in 15-DAT forage at 30.2% and 3.9% TRR (1.38 ppm and 0.17 ppm); in 32-DAT forage at 22.0% and 5.3% TRR (0.60 ppm and 0.14 ppm); in 49-DAT forage at 20.5% and 9.2% TRR (0.16 ppm and 0.08 ppm); and in 68-DAT hay at 8.4% and 9.2% TRR (0.08 ppm each). As residues of imazapic declined in forage, residues of the hydroxymethyl analog metabolite CL 263284 and its glucose conjugate CL 189215 increased.

The majority of radioactivity in 15- to 49-DAT forage (20.6-26.8% TRR, 0.20-0.95 ppm) and 68-DAT hay (26.9% TRR, 0.25 ppm) was characterized as consisting of highly polar metabolites comprised of multi-substituted derivatives of the natural product nicotinic acid. Of these metabolites, several were tentatively identified in 15-DAT forage as 5-hydroxymethyl-2,3-pyridinedicarboxamide, hydroxy-pyridinedicarboxylic acid, 5-hydroxy-2,3-pyridine-dicarboxylic anhydride, and 2-formyl-5-hydroxy-nicotinic acid or its tautomeric form of 3,7-dihydroxy-furo[3,4-b]pyridine-5(7H)-one. Additional minor components present in forage and hay, each at  $\leq 0.02$  ppm, were tentatively identified in 15-DAT forage as hydroxy-imidazopyrrolopyridine and imidazopyrrolopyridine tricyclic derivatives and pyridodiazocine and hydroxy-pyridodiazocine derivatives. We note that, although imazapic is a double-ring compound, based on the results of the grass and peanut metabolism studies, cleavage between the pyridine and imidazole rings is not expected; therefore, a metabolism study reflecting labeling in the second ring is not required.

Metabolism of imazapic in grass forage and hay is similar to that in peanuts, in which imazapic metabolizes to hydroxymethyl and glucoside derivatives; however, further oxidation of the hydroxymethyl metabolite to highly polar nicotinic acid derivatives is more extensive in Bermuda grass than in peanuts. The dicarboxylic acid derivative (CL 312622) identified in the peanut metabolism study was not observed in the Bermuda grass metabolism study.

In a meeting held 22-MAY-2001, the HED MARC determined that the residues of concern in grass include imazapic, CL 263284, and CL 189215 (D275136, W. Donovan and W. Dykstra, xx-JUN-2001).

### OPPTS GLN 860.1300: Nature of the Residue in Livestock

#### Ruminants

In support of the proposed use of imazapic on grasses, American Cyanamid has submitted a study (citation listed below) pertaining to the metabolism of [ $^{14}$ C]imazapic in lactating goats. The in-life and analytical phases of the study were conducted by Covance Laboratories, Inc. (Madison, WI). A ruminant metabolism study was previously submitted and reviewed in conjunction with the petition for tolerances of imazapic on peanuts. At that time, the nature of the residue in ruminants was considered to be understood. The residues of concern were determined to be imazapic and its hydroxymethyl metabolite (free only). The Agency commented that additional ruminant metabolism studies, conducted at a higher dose level, would be required if future uses were to result in higher residues of imazapic in/on feed items (DP Barcodes D207019, D207047, D207079, D209892, D209899, D211609, and D211980, 9/15/95, J. Garbus).

- 44817708 Sharp, D.; Thalacker, F.W. (1999) AC 263222 (Imazapic): Metabolic Fate of  $^{14}$ C AC 263222 in Lactating Goats. Laboratory Project Report Number MET 99-002: Covance Number 6123-241. Unpublished study prepared by American Cyanamid Company. 102 p.

The test substance, [<sup>14</sup>C]imazapic labeled at the 6-position of the pyridine ring (radiochemical purity >99%), was mixed with [<sup>13</sup>C]imazapic, imazapic, and isopropyl alcohol, and concentrated under nitrogen to a final specific activity of 12.48 μCi/mg. The concentrated solution of the test substance was dispensed into gelatin capsules containing dextrose. The capsules were administered orally to a lactating goat once per day following the morning milking for five consecutive days. Based on the actual feed consumption, the goat received 174.7 ppm [<sup>14</sup>C]imazapic per day in the diet. A second control goat was dosed with gelatin capsules containing only isopropyl alcohol and dextrose.

During the study the goat was fed both roughage (*ad libitum*) and a commercial grain-based milking ration twice per day at milking; water was provided *ad libitum*. The petitioner provided sufficient descriptions of preparation of dose capsules and livestock husbandry practices, as well as data concerning daily feed intake, body weights, and milk production.

Milk was collected twice daily (in the morning prior to treatment and in the afternoon); subsamples of the day's milk (p.m. and following a.m. samples) were composited. The goat was sacrificed ~23 hours after the final dose, and the following samples were collected: liver, kidneys, muscle (composite weight ratio of triceps, longissimus dorsi, and gastrocnemius), and fat (1:1 composite of omental and renal). All milk and tissue samples were stored frozen (~-20 C) until analysis.

#### Total radioactive residues (TRR)

Tissue samples were cut into small pieces and homogenized in the presence of dry ice. The TRR in replicate homogenized subsamples of liver, kidney, muscle, and fat were determined by combustion/LSC. Replicate aliquots of milk from separate and composited a.m. and p.m. samplings were analyzed directly by LSC. The TRR in goat milk and tissues are presented in Table 4. The LODs for TRR determinations were reported as <0.001 ppm for milk and 0.002-0.003 ppm for tissues.

Table 4. Total radioactive residues in samples of milk and edible tissues from a lactating goat following administration of [<sup>14</sup>C]imazapic at a feeding level of 174.7 ppm (1.7x) for 5 consecutive days.

Matrix	TRR, ppm [ <sup>14</sup> C]imazapic equivalents	
	Separate Samples	Composited Samples
Whole Milk		
Day 1 p.m.	0.045	0.026
Day 1 a.m.	0.012	
Day 2 p.m.	0.058	0.030
Day 2 a.m.	0.012	
Day 3 p.m.	0.058	0.030
Day 3 a.m.	0.013	
Day 4 p.m.	0.071	0.035
Day 4 a.m.	0.014	
Day 5 p.m.	0.078	0.037
Day 5 a.m.	0.013	
Liver		0.033
Kidney		0.275
Muscle		0.010
Fat		0.003

Samples of urine, feces, and cage washings were collected and analyzed for TRR. The data indicated that most of the radioactivity was excreted in the urine (91.7%) and feces (6.57%); 2.38% TRR were determined in the cage washings. TRR in milk and tissues accounted for only 0.03% and 0.01%, respectively, of the administered radioactivity.

#### Extraction and hydrolysis of residues

Samples of whole milk (Day 2 p.m. and Day 5 p.m.) and tissues (except fat) were subjected to extraction and hydrolysis procedures for residue characterization and identification. Fat samples were not extracted because the TRR were <0.010 ppm. The petitioner provided adequate descriptions of the fractionation procedures for each matrix. During the extraction and fractionation procedures, aliquots of extracts and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. Fractions containing significant residues were concentrated and reserved for chromatographic analysis. The general extraction and fractionation procedures are summarized below.

Aliquots of whole milk were extracted with acetone (2x) and centrifuged. The acetone extracts were combined, and the solvent was removed by evaporation under nitrogen. The remaining aqueous extract was then partitioned with hexane (2x). The aqueous phase was concentrated under nitrogen for HPLC and TLC analyses.

Aliquots of homogenized tissue (except fat) samples were sequentially extracted with acetonitrile (ACN; 2x) and methanol (2x), and centrifuged. The ACN and methanol supernatants were

combined and concentrated. To remove fat-like material in liver, the concentrated liver extract was partitioned with hexane and centrifuged. The resulting liver aqueous phase was re-extracted with ACN (2x) and methanol (2x), and all liver supernatants were combined and concentrated. The concentrated extracts from all tissues were diluted with water and/or ACN acidified with 1% formic acid. The acidified extracts were then applied to a C18 solid phase extraction (SPE) cartridge. Unretained (flow-through) radioactivity was collected, and retained residues were eluted with ACN followed by methanol. The ACN and methanol eluates were combined for HPLC and/or TLC analyses; the unretained SPE residues of muscle were also analyzed by HPLC.

The distribution of  $^{14}\text{C}$ -activity in the extracts of goat milk and tissues is presented in Table 5.

#### Characterization and identification of residues

Whole milk and tissue extracts were analyzed by HPLC. Reversed phase HPLC analysis was performed using an Alltech Hypersil ODS C18 column with a gradient mobile phase of 0.01 M  $\text{KH}_2\text{PO}_4$  (pH 2.5) and ACN, and a UV (254 nm) and radiodetector. Samples were co-chromatographed with nonradiolabeled reference standards of imazapic, CL 263284 (imazameth alcohol metabolite), and CL 312622 (5-acid imidazolinone metabolite).

Imazapic was the only residue identified in milk and tissues. Identification of imazapic in milk, liver, and kidney extracts was confirmed by two-dimensional (2D) TLC. TLC plates were developed with ethyl acetate:n-propanol:water:acetic acid (60:100:30:10, v:v:v:v) and methanol:n-propanol:water:acetic acid (51:75:12:12, v:v:v:v). Residues were visualized by a radioanalytical imaging system. Imazapic residues in muscle were not confirmed by 2D TLC analysis due to low levels of radioactivity.

The initial HPLC analysis of milk indicated imazapic as the major metabolite with another peak present. Coinjection with radiolabeled imazapic increased both peaks, suggesting that both peaks were imazapic. The petitioner hypothesized that imazapic exists in equilibrium between two different ionized forms on the HPLC column. Identification of both peaks as imazapic was confirmed by 2D TLC.

A summary of the characterized and identified  $^{14}\text{C}$ -residues in goat matrices is presented in Table 6.



Table 5. Distribution of total radioactive residues in milk and tissues from a lactating goat dosed with [<sup>14</sup>C]imazapic at a feeding level of 174.7 ppm in the diet for 5 consecutive days.

Fraction	% TRR	ppm	Characterization-Identification <sup>a</sup>
<b>Milk, whole (Day 2 p.m.; TRR = 0.058 ppm)</b>			
Acetone	95.8	0.056	Evaporated to aqueous and partitioned with hexane.
Aqueous	90.4	0.052	HPLC analysis resolved: Imazapic 66.8% TRR 0.039 ppm Plus five peak areas accounting for 9.1% TRR (<0.006 ppm); each at ≤2.6% TRR (≤0.002 ppm).
Hexane	0.4	<0.001	Not further analyzed (N/A).
Nonextractable	4.5	0.003	N/A.
<b>Milk, whole (Day 5 p.m.; TRR = 0.078 ppm)</b>			
Acetone	91.5	0.071	Evaporated to aqueous and partitioned with hexane.
Aqueous	103.9	0.081	HPLC analysis resolved: Imazapic 65.0% TRR 0.051 ppm Plus eight peak areas accounting for 11.7% TRR (<0.011 ppm); each at ≤3.8% TRR (≤0.003 ppm).
Hexane	0.6	<0.001	N/A.
Nonextractable	5.5	0.004	N/A.
<b>Kidney (TRR = 0.275 ppm)</b>			
ACN Methanol	102.9	0.283	Concentrated, redissolved in ACN, diluted with water, acidified with 1% formic acid and subjected to C18 SPE; retained residues were eluted with ACN and methanol.
ACN methanol	93.1	0.256	HPLC analysis resolved: Imazapic 85.0% TRR 0.234 ppm Plus six peak areas accounting for 5.5% TRR (<0.016 ppm); each at ≤1.5% TRR (≤0.004 ppm).
Unretained (flow-through)	1.9	0.005	N/A.
Nonextractable	5.3	0.015	N/A.
<b>Liver (TRR = 0.033 ppm)</b>			
ACN Methanol	85.7	0.028	Concentrated and partitioned with hexane.
Hexane	12.9	0.004	N/A.
Aqueous	NR <sup>b</sup>	NR	Re-extracted with ACN and methanol, and extracts combined; concentrated, diluted with water, acidified with 1% formic acid and subjected to C18 SPE; retained residues were eluted with ACN and methanol.
ACN methanol	69.0	0.023	HPLC analysis resolved: Imazapic 49.1% TRR 0.016 ppm Plus four peak areas accounting for 3.0% TRR (<0.004 ppm); each at ≤0.9% TRR (≤0.001 ppm).
Unretained (flow-through)	13.3	0.004	N/A.
Nonextractable	50.7	0.017	N/A.

Table 5 (continued).

Fraction	% TRR	ppm	Characterization/Identification <sup>a</sup>
<b>Muscle (TRR = 0.010 ppm)</b>			
ACN/Methanol	105.6	0.011	Concentrated, redissolved in ACN, diluted with water, acidified with 1% formic acid and subjected to C18 SPE: retained residues were eluted with ACN and methanol.
ACN/methanol	51.6	0.005	<u>HPLC analysis resolved:</u> Imazapic 19.7% TRR 0.002 ppm Plus five peak areas accounting for 2.7% TRR (<0.005 ppm); each at $\leq 0.7\%$ TRR ( $\leq 0.001$ ppm).
Unretained (flow-through)	47.5	0.005	<u>HPLC analysis resolved:</u> Imazapic 14.1% TRR 0.001 ppm Plus one peak area accounting for 0.8% TRR (<0.001 ppm).
Nonextractable	31.5	0.003	N/A.

<sup>a</sup> Imazapic residues were identified by HPLC and confirmed by 2D TLC.

<sup>b</sup> NR = Not reported.

Table 6. Summary of radioactive residues characterized/identified in milk and tissues of a lactating goat orally dosed with [<sup>14</sup>C]imazapic at a feeding level of 174.7 ppm for 5 consecutive days.

Fraction	Milk Day 2 p.m. (TRR = 0.058 ppm)		Milk Day 5 p.m. (TRR = 0.078 ppm)		Kidney (TRR = 0.275 ppm)		Liver (TRR = 0.033 ppm)		Muscle (TRR = 0.010 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
<b>Identified<sup>a</sup></b>										
Imazapic	66.8	0.039	65.0	0.051	85.0	0.234	49.1	0.016	33.8	0.003
<b>Total identified</b>	66.8	0.039	65.0	0.051	85.0	0.234	49.1	0.016	33.8	0.003
<b>Characterized</b>										
Indiscrete peak areas <sup>b</sup>	9.1	<0.006	11.7	<0.011	5.5	<0.016	3.0	<0.004	3.5	<0.006
Hexane	0.4	<0.001	0.6	<0.001	--	--	12.9	0.004	--	--
Unretained (SPE flow-through)	--	--	--	--	1.9	0.005	13.3	0.004	--	--
<b>Total characterized/identified</b>	76.3	<0.046	77.3	<0.063	92.4	0.255	78.3	<0.028	37.3	<0.009
<b>Nonextractable</b>	4.5	0.003	5.5	0.004	5.3	0.015	50.7	0.017	31.5	0.003

<sup>a</sup> Imazapic was identified by HPLC and confirmed by 2D-TLC.

<sup>b</sup> No individual peak area exceeded 3.8% TRR (0.004 ppm).

### Storage stability

Based on the experimental start and completion dates, analysis of milk and tissue samples from the goat study was completed within 4 months of sample collection. No additional storage stability data are required to support the ruminant metabolism study because all analyses were performed within 4 months of sacrifice.

### Proposed metabolic pathway in ruminants

Based on the results of the goat metabolism study, the petitioner proposes that imazapic is eliminated primarily via the urine and feces. The parent, imazapic, was the only residue identified in goat milk and tissues.

### Study summary

The submitted goat metabolism study adequately delineates the nature of the residue in ruminants. Following oral administration of [<sup>14</sup>C]imazapic to a lactating goat for 5 consecutive days at a feeding level of 174.7 ppm, the total radioactive residues (TRR) were 0.012-0.078 ppm in whole milk, 0.275 ppm in kidney, 0.033 ppm in liver, 0.010 ppm in muscle, and 0.003 ppm in fat. Residues in milk were highest in the p.m. sample following each treatment and declined to the lowest levels in each of the following a.m. samples prior to the next treatment. Residues in composited daily milk gradually increased with each subsequent treatment day from 0.026 ppm to 0.037 ppm. Residues in tissues were highest in kidney and lowest in fat.

Approximately 74.3-92.4% of the TRR were characterized/identified in goat milk and tissues (kidney and liver); only 37.3% of the TRR were characterized/identified in goat muscle because of low residue levels (TRR = 0.010 ppm). The parent, imazapic, was the only residue identified in milk and tissues. Imazapic residues accounted for 65.0-66.8% TRR (0.039-0.051 ppm) in whole milk, 85.0% TRR (0.234 ppm) in kidney, 49.1% TRR (0.016 ppm) in liver, and 33.8% TRR (0.003 ppm) in muscle. Most of the remaining radioactivity in milk and tissues consisted of low level, diffuse residues which did not form distinct peaks; no individual peak area accounted for more than 3.8% TRR (0.004 ppm).

In a meeting held 22-MAY-2001, the HED MARC determined that the residues of concern in livestock commodities include imazapic and CL 263284 (D275136, W. Donovan and W. Dykstra, xx-JUN-2001).

### Poultry

There are no poultry feed items associated with the proposed uses of imazapic on grasses; therefore, the requirements for a poultry metabolism study do not apply to this petition.

A poultry metabolism study was previously submitted in conjunction with PP#3G4203/3H5669 (DP Barcodes D191715, D191694, and D191710, 3/10/94, F. Griffith). On the basis of that

study, the Agency concluded that there was no reasonable expectation of finite residues of imazapic in poultry commodities based on the use on peanuts [Category 3 of 40 CFR §180.6(a)], and therefore no need for secondary tolerances in poultry tissues and eggs.

### OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

#### Enforcement method - plant commodities

The petitioner is proposing capillary electrophoresis (CE) Method M 3114 (dated 11/12/97) for the enforcement of tolerances for residues of imazapic and its metabolites CL 263284 and CL 189215 in/on grass forage and hay. A detailed description of the method, updated with changes recommended as a result of independent laboratory validation, was included in MRID 44817709 (citation listed below). Under PP#4F4390 (DP Barcode D222184, 2/6/96, J. Garbus) the petitioner proposed a similar CE method, M 2379 as the enforcement method for determination of residues of imazapic in/on peanuts. Adequate method validation recoveries, radiovalidation, and independent method validation for residues of imazapic and its metabolites CL 263284 and CL 189215 in/on peanuts were submitted for this method, and the method was successfully validated at the Analytical Chemistry Laboratory (with suggested minor revisions) for enforcement of the 0.1 ppm peanut nutmeat tolerance. Minor revisions suggested by ACL were subsequently incorporated by the petitioner into CE method M 2379.02.

44817709 Nejad, H.; Miller, P.; Duan, B. (1999) Independent Laboratory Validation of Capillary Electrophoresis (CE) Determinative Method for CL 263222, CL 263284, and CL 189215 Residues in Grass, Radiovalidation, and Multiresidue Method. Laboratory Project Identification CY241: RES 98-144; RES 99-038. Unpublished study prepared by American Cyanamid Company. 151 p.

In CE method M 3114, grass samples are pulverized in dry ice and homogenized. Residues are extracted with hydrochloric acid:water:methanol (1:39:60, v:v:v) and vacuum filtered through Celite. The filtrate is concentrated by rotary evaporation. Lead acetate (10%) and 0.5 M sodium hydroxide are added to the concentrated filtrate, and the pH of the mixture is adjusted to pH 6.0-6.2 using 0.5 M sodium hydroxide before centrifugation at 4 C. The resulting supernatant is purified on a series of solid phase extraction (SPE) cartridges/columns using a vacuum manifold as follows. Residues are eluted from a Poly-Prep AG1-X8 cartridge to a C18 SPE cartridge with 1% formic acid in water, from the C18 cartridge to a strong cation exchange (SCX) cartridge with methanol, and from the SCX cartridge with saturated potassium chloride in methanol. The purified eluate is concentrated, redissolved in 1% formic acid in water, and applied again to a C18 SPE cartridge; residues are eluted with methanol, and the eluate is evaporated to dryness, redissolved in water, and sonicated for analysis by CE using a bare fused silica capillary, pH 7.3 separation buffer (20 mM dodecyltrimethyl-ammonium bromide:10 mM tris-(hydroxymethyl)-aminomethane:10 mM NaH<sub>2</sub>PO<sub>4</sub>), and a high sensitivity optical flow cell with a UV detector (240 nm). Residues are quantitated by direct comparison with peak heights of external standards. The validated method limit of quantitation (LOQ) is 0.50 ppm for each analyte in grass matrices.

Identification of residues of imazapic and its metabolites CL 263284 and CL 189215 is confirmed by LC/MS. A brief description of the confirmatory LC/MS method was included.

The petitioner conducted method validation concurrently with the field trial studies using fortified samples of grass forage and hay. Method recoveries are presented in Table 9. Sample calculations and representative electropherograms were included in the submission.

#### Independent Laboratory Validation

American Cyanamid submitted data (MRID 44817709) pertaining to independent laboratory validation of the proposed CE tolerance enforcement method for grass commodities, Method M 3114 (draft dated 11/12/97). The ILV was performed by Centre Analytical Laboratories, Inc. (State College, PA).

Replicate untreated samples (received from American Cyanamid) of Bermuda grass, big bluestem grass, and a grass mixture (little bluestem, bluegrass, and bromegrass) forage and hay were fortified with imazapic, CL 263284, and CL 189215, each at 0.50 ppm (LOQ), 1.0 ppm, and 50 ppm. Unfortified grass forage and hay samples were also assayed.

Successful validation recoveries were obtained in the first trial for all samples except the grass mixture hay. Communication occurred between Centre Analytical Laboratories and American Cyanamid to discuss the implementation of minor modifications to the method. With these minor modifications (initial extract adjusted to pH 6-6.2 and adjustment of the amount of methanol used to wash the SCX cartridge) successful validation recoveries were obtained in the second trial for the grass mixture hay. Apparent residues of imazapic, CL 263284, and CL 189215 were each less than the method LOQ in/on two unfortified samples each of Bermuda grass forage and hay, bluestem grass forage and hay, and the grass mixture forage and hay. The results of the ILV study are presented in Table 7. Following revisions to the method for analysis of mixture grasses, adequate recoveries were obtained at all fortification levels.

The laboratory reported that the extraction of eight samples required approximately 8 person-hours, and CE analysis for eight samples with five standards required approximately 6 hours. Representative sample calculations and electropherograms were provided.

Table 7. Independent laboratory validation recoveries of imazapic and its metabolites, CL 263284 and CL 189215, from analysis of fortified samples of untreated grass forage and hay analyzed using CE method M 3114.

Matrix	Fortification Level, ppm	Percent Recoveries *		
		Imazapic	CL 263284	CL 189215
Bermuda grass, forage	0.50	85.85	87.87	88.89
	1.0	77.83	78.89	80.87
	50	82.92	86.94	86.93
	Mean ± s.d.	84 ± 4.9 (n=6)	87 ± 5.2 (n=6)	87 ± 4.3 (n=6)
Bermuda grass, hay	0.50	76.78	77.78	76.80
	1.0	78.84	80.88	79.91
	50	61.69	63.72	61.68
	Mean ± s.d.	74 ± 8.1 (n=6)	76 ± 8.4 (n=6)	76 ± 10.4 (n=6)
Bluestem grass, forage	0.50	79.81	74.79	79.83
	1.0	80.87	79.86	82.88
	50	71.74	69.72	72.75
	Mean ± s.d.	79 ± 5.6 (n=6)	77 ± 6.1 (n=6)	80 ± 5.8 (n=6)
Bluestem grass, hay	0.50	71.75	68.70	70.72
	1.0	72.73	83.84	84.84
	50	69.73	70.73	70.73
	Mean ± s.d.	72 ± 2.0 (n=6)	75 ± 7.0 (n=6)	76 ± 6.7 (n=6)
Mixture grass, forage	0.50	74.80	79.85	76.84
	1.0	86.96	89.96	87.97
	50	81.86	89.93	82.85
	Mean ± s.d.	84 ± 7.4 (n=6)	89 ± 6.0 (n=6)	85 ± 6.9 (n=6)
Mixture grass, hay (first attempt)	0.50	59.63	63.65	66.68
	1.0	66.71	69.76	73.81
	50	62.68	72.76	64.72
	Mean ± s.d.	65 ± 4.4 (n=6)	70 ± 5.5 (n=6)	71 ± 6.1 (n=6)
Mixture grass, hay (second attempt)	0.50	80.87	81.87	88.92
	1.0	74.82	72.82	78.88
	50	74.80	72.80	79.86
	Mean ± s.d.	80 ± 5.0 (n=6)	79 ± 5.9 (n=6)	85 ± 5.5 (n=6)

\* Means and standard deviations were calculated by the petitioner.

#### Radiovalidation of the enforcement method

Radiovalidation data for CE method M 3114 were included in the independent laboratory validation submission (MRID 44817709). A sample of 15-DAT Bermuda grass forage from the plant metabolism study was subjected to extraction and analysis using the CE method.

Prior to analysis by the enforcement method, a subsample of the treated forage was combusted and analyzed by LSC to determine the total radioactive residues (TRR). In the metabolism study, the sample TRR were 4.58 ppm; on reanalysis, TRR were 11.1 ppm. The petitioner attributed the increase in TRR to the extended period of freezer storage (~3.5 years) and resulting desiccation of the sample. The results of the radiovalidation study are presented in Table 8.

The petitioner reported that 59% TRR were extracted from the treated forage with 1 N hydrochloric acid:water:methanol (1:39:60, v:v:v) as specified in the residue method, versus 84% extractability in the metabolism study. Concurrent recoveries of 86%, 86%, and 74%, respectively, were reported for imazapic, CL 263284, and CL 189215 based on analysis of fortified untreated Bermuda grass samples, and residues were less than the method LOQ (<0.50 ppm) for each analyte in an untreated Bermuda grass sample. The submitted radiovalidation data confirm that CE method M 3114 adequately extracts aged (weathered) residues from grass forage.

Table 8. Results of radiovalidation of the proposed CE enforcement method (Method M 3114) using samples from the Bermuda grass metabolism study.

Matrix	Analyte	Residues from Metabolism Study, ppm		Residues from Method M 3114, ppm
		TRR = 4.58 ppm	TRR = 11.1 ppm *	
15-DAT Bermuda grass, forage	Imazapic	0.27	0.65	<0.5
	CL 263284	1.38	3.3	2.55
	CL 189215	0.17	0.41	<0.5

\* Residues values from the metabolism study were corrected for the increase in TRR on re-assay to 11.1 ppm for the radiovalidation study.

#### Residue data collection methods - plant commodities

Samples from the grass field trials were analyzed using the proposed enforcement method, CE method M 3114 (draft dated 11/12/97) by Centre Analytical Laboratories (CAL: State College, PA). Samples from selected field trials were initially analyzed by CE method M 2702, which is similar to CE method M 3114 except that it does not include analysis for metabolite CL189215. The petitioner submitted concurrent method recovery for both methods with the field trial data (MRID 44817713). Samples of untreated commodities from the field trials were fortified with imazapic and its metabolites and analyzed with the field trial samples. The concurrent method recoveries are presented in Table 9. Sample calculations and representative electropherograms were provided. The reported method LOQs for both methods were 0.50 ppm each for residues of imazapic, CL 263284, and CL 189215 in/on grass forage and hay; method LODs of 0.050-0.060 ppm were reported.

Wheat samples from the storage stability study were analyzed by CAL using CE method M 2463, which is essentially the same as CE method M 3114. Residues were extracted from



homogenized samples with acidic water and methanol, and purified using clean-up procedures which included precipitation, centrifugation, and solid-phase extraction techniques. Residues were quantitated by CE using a high sensitivity flow cell and a UV detector at 240 nm. A method LOQ of 0.1 ppm was reported. Concurrent method recoveries (fresh fortification recoveries) are reported under the Storage Stability section.

Table 9. Concurrent method recoveries of residues of imazapic, CL 263284, and CL 189215 from fortified samples of grass commodities analyzed using CE methods M 3114 and M 2702 (MRID 44817713).

Grass variety	Fortification Level, ppm	Statistic	Imazapic	CL 263284	CL 189215
CE Method M 3114					
Bermuda grass, forage	0.50-50	Average (%)	89	89	85
		Recovery Range (%)	78-109	77-105	63, 67; 70-105
		SD (%)	7	6	9
		Number	31	31	31
Bermuda grass, hay	0.50-50	Average (%)	90	88	84
		Recovery Range (%)	80-108	66; 81-104	70-96
		SD (%)	7	8	7
		Number	30	30	30
Big bluestem grass, forage	0.50-50	Average (%)	86	85	84
		Recovery Range (%)	81-91	79-89	75-92
		SD (%)	4	3	5
		Number	9	9	13
Big bluestem grass, hay	0.50-50	Average (%)	85	85	82
		Recovery Range (%)	67; 73-95	68; 73-95	69; 72-94
		SD (%)	9	8	8
		Number	11	11	15
Bromegrass, forage	0.50-50	Average (%)	86	87	82
		Recovery Range (%)	68; 85-93	84-91	73-89
		SD (%)	8	3	7
		Number	8	5	5

Table 9 (continued).

Grass variety	Fortification Level, ppm	Statistic	Imazapic	CL 263284	CL 189215
Bromegrass, hay	0.50-50	Average (%)	91	90	78
		Recovery Range (%)	79-100	78-100	65, 65; 81-88
		SD (%)	9	8	10
		Number	6	6	6
Bluegrass and bromegrass, forage	0.50-50	Average (%)	93	91	86
		Recovery Range (%)	81-109	81-106	74-101
		SD (%)	8	8	8
		Number	17	17	17
Bluegrass and bromegrass, hay	0.50-50	Average (%)	98	92	83
		Recovery Range (%)	76-116	75-103	69; 79-100
		SD (%)	10	7	6
		Number	17	17	17
Little bluestem and bluegrass, forage	0.50-50	Average (%)	95	92	83
		Recovery Range (%)	82-114	79-109	74-95
		SD (%)	10	9	7
		Number	18	18	18
Little bluestem and bluegrass, hay	0.50-50	Average (%)	88	86	75
		Recovery Range (%)	74-101	71-99	64, 65, 66, 66; 70-90
		SD (%)	8	8	8
		Number	18	18	18
Big bluestem and bromegrass, forage	0.50-50	Average (%)	85	84	85
		Recovery Range (%)	80-93	80-94	82-93
		SD (%)	5	5	4
		Number	6	6	6
Big bluestem and bromegrass, hay	0.50-50	Average (%)	82	80	80
		Recovery Range (%)	77-92	73-89	73-87
		SD (%)	5	6	5
		Number	6	6	6

Table 9 (continued).

Grass variety	Fortification Level, ppm	Statistic	Imazapic	CL 263284	CL 189215
CE Method M 2702					
Big bluestem grass, forage	0.50, 50	Average (%)	88	76	n/a <sup>a</sup>
		Recovery Range (%)	84-95	72-79	n/a
		SD (%)	5	3	n/a
		Number	4	4	n/a
Big bluestem grass, hay	0.50, 50	Average (%)	83	75	n/a
		Recovery Range (%)	79-87	71-78	n/a
		SD (%)	3	3	n/a
		Number	4	4	n/a

<sup>a</sup> n/a = Not analyzed; samples were not analyzed for CL 189215 using method M 2702.

### Comments

The petitioner has proposed CE method M 3114 for the enforcement of tolerances for grass forage and hay. This method was used for the determination of residues of imazapic and its metabolites CL 263284 and CL 189215 in/on grass forage and hay samples collected from the grass field trials and is similar to the peanut enforcement method CE M 2379. Concurrent method recoveries submitted in conjunction with the grass field trials indicate that this method adequately recovers residues of imazapic and its metabolites CL 263284 and CL 189215 from grass forage and hay. Adequate independent method validation and radiovalidation data have been submitted for this method. This method was forwarded to ACB/BEAD for petition method validation (D271474, W. Donovan, 17-JAN-2001).

### OPPTS GLN 860.1340: Residue Analytical Methods - Livestock Commodities

#### Enforcement method - livestock commodities

American Cyanamid is proposing capillary electrophoresis (CE) methods M 3188 (dated 5/20/98) and M 3222 for the enforcement of tolerances for residues of imazapic and its metabolite CL 263284 in milk and livestock tissues, and HPLC/MS method M 3233 for the enforcement of tolerances for fat. Descriptions of the methods were included in MRID 44817710 (citation listed below).

44817710 Nejad, H.; Miller, P.; Sweeney, R.A.; Boner, P.L. (1999) CL 263222 (Imazapic): Independent Laboratory Validation of Methods to Measure CL 263222 and CL 263284 Residues in Cattle Muscle, Kidney, Liver Tissue, Milk, Bovine Milk Fat, and Tissue Fat and Multiresidue Method. Laboratory Project Identification CY239: RES 98-201, RES 98-235, RES 99-004, and RES 99-007. Unpublished study prepared by American Cyanamid Company. 251 p.

**CE Method M 3188:** CE method M 3188 is intended for the determination of residues of imazapic and its metabolite CL 263284 in milk. Residues are extracted from whole milk with 6 N hydrochloric acid:water (0.1:20, v:v). Following the addition of lead acetate (10%) to the extract, the mixture is vortexed and centrifuged, and formic acid is added to the supernatant. The supernatant is purified on a series of SPE cartridges/columns using a vacuum manifold. Residues are eluted from a C18 SPE cartridge with methanol to an SCX cartridge, and from the SCX cartridge with saturated potassium chloride in methanol. The eluate is concentrated, redissolved in 1% formic acid in water, and applied again to a C18 SPE cartridge; residues are eluted with methanol. The eluate is evaporated to dryness, redissolved in water, and sonicated for analysis by CE using a bare fused silica capillary, pH 11.2 separation buffer (20 mM dodecyltrimethyl-ammonium bromide:10 mM sodium citrate dihydrate:10 mM sodium phosphate monobasic, monohydrate), and a high sensitivity optical flow cell with a UV detector (240 nm). If interfering peaks occur, the following alternative separation buffers, also at pH 11.2, may be used: 20 mM dodecyltrimethyl-ammonium bromide:10 mM sodium citrate dihydrate:10 mM 3-cyclohexylaminol-propanesulfonic acid, or 20 mM dodecyltrimethyl-ammonium bromide:10 mM sodium L-tartrate dihydrate:10 mM sodium phosphate monobasic. Residues are quantitated by direct comparison with peak heights of external standards. The validated method LOQ is 0.010 ppm for each analyte in milk.

**CE Method M 3222:** CE method M 3222 is used for determination of residues of imazapic and its metabolite CL 263284 in cattle muscle, kidney, and liver. Tissue samples are pulverized in dry ice and homogenized. Residues are extracted from the homogenized samples with 1 N hydrochloric acid:water:methanol (1:39:60, v:v:v) and centrifuged. The supernatant is applied to an ENV C18 SPE cartridge, and residues are eluted with methanol through a strong anion exchange (SAX) cartridge to an SCX cartridge. Residues are eluted from the SCX cartridge with saturated potassium chloride in methanol, and the eluate is concentrated, redissolved in 1% formic acid in water, and applied again to a C18 SPE cartridge. Residues are eluted from the C18 cartridge with methanol, and the eluate is evaporated to dryness, redissolved in water, and sonicated for analysis by CE using a bare fused silica capillary, pH 11.1 separation buffer (20 mM dodecyltrimethyl-ammonium bromide:10 mM sodium citrate dihydrate:10 mM sodium phosphate monobasic, monohydrate), and a high sensitivity optical flow cell with a UV detector (240 nm). This method proposes use of the alternative separation buffers mentioned above for method M 3188 if interfering peaks occur. Residues are quantitated by direct comparison with peak heights of external standards. The validated method LOQ is 0.050 ppm for each analyte in cattle muscle, kidney, and liver.

For both CE methods, identification of imazapic and CL 263284 is confirmed by LC/MS. A brief description of the confirmatory LC/MS method and representative mass spectra for fortified milk, muscle, kidney, and liver samples were included.

HPLC/MS Method M 3233: HPLC/MS method M 3233 is used for determination of residues of imazapic and its metabolite CL 263284 in tissue fat and milk fat. Tissue fat samples are pulverized in dry ice and homogenized, and milk fat is separated from whole milk by centrifugation. Residues are extracted from the fat samples with 1% formic acid in acetonitrile (ACN):hexane (2:1, v:v) and filtered. The filtrate is partitioned with 1% formic acid in ACN followed by ACN. The ACN phases are combined and evaporated at 35-40 C under low vacuum. Methanol is added to the concentrated residues, and dichloromethane is added after vortexing. Residues are then applied to an SCX cartridge and eluted with saturated potassium chloride in methanol. The eluate is concentrated, redissolved in 1% formic acid in water, and applied to a C18 SPE cartridge. Residues are eluted from the C18 cartridge with methanol. The eluate is evaporated to dryness, redissolved in water, and sonicated for analysis by HPLC/positive ion electrospray ionization tandem mass spectrophotometry. The LC system utilizes a C18 column and a gradient mobile phase of water and methanol, both acidified with 1% acetic acid. The validated method LOQ for each analyte is 0.050 ppm for tissue fat and 0.010 ppm for milk fat.

Identification of residues of imazapic and CL 263284 is confirmed by LC/MS/MS. A brief description of the confirmatory LC/MS/MS method and representative mass spectra for fortified milk fat and tissue fat samples were included.

The petitioner conducted method validation for all three methods concurrently with the ruminant feeding study, using fortified samples of milk, milk fat, tissue fat, kidney, liver, and muscle. Method recoveries are presented in Table 12.

#### Independent Laboratory Validation

American Cyanamid submitted data (MRID 44817710) pertaining to independent laboratory validation of the proposed tolerance enforcement methods for milk (CE method M 3188), cattle tissues (CE method M 3222), and tissue fat (HPLC/MS method M 3233). The ILV of the CE methods was performed by ABC Laboratories, Columbia, MO, and the ILV of the HPLC/MS method was performed by XenoBiotic Laboratories (XBL), Inc., Plainsboro, NJ.

CE Method M 3188: Replicate untreated samples of bovine milk (unspecified source) were fortified with imazapic and CL 263284, each at 0.010 ppm (LOQ), 0.020 ppm, and 1.000 ppm. Successful validation recoveries were obtained with the first attempt for milk samples. Apparent residues of imazapic and CL 263284 were each less than the method LOQ in two unfortified samples of milk. The laboratory reported that the extraction of eight milk samples required approximately 8 person-hours, and that CE analysis of the eight samples with nine standards required approximately 12 hours; 1.5 calendar days are required to analyze eight milk samples.

CE Method M 3222 (dated 8/10/98): Replicate untreated samples of cow muscle, kidney, and liver (supplied by American Cyanamid) were fortified with imazapic and CL 263284, each at 0.050 ppm (LOQ), 0.100 ppm, and 1.000 ppm. Successful validation recoveries were obtained with the first attempt for all tissue matrices; however, the petitioner requested that additional kidney samples be analyzed at the 0.050 ppm fortification level due to a low recovery value (47%) obtained in the first trial. The low recovery value was later determined to be a statistical outlier, and was not included in the data summaries. Apparent residues of imazapic and CL 263284 were each less than the method LOQ in two unfortified samples each of cattle muscle, kidney, and liver. The laboratory reported that the extraction of eight tissue samples required approximately 11 person-hours, and CE analysis of the eight samples with nine standards required approximately 11 hours; 3 calendar days were required to analyze eight tissue sample.

HPLC/MS Method M 3233: Replicate untreated samples of milk fat (supplied by American Cyanamid) were fortified with imazapic and CL 263284, each at 0.010 ppm (LOQ), 0.020 ppm, and 0.200 ppm, and replicate untreated samples of tissue fat (supplied by American Cyanamid) were fortified with imazapic and CL 263284, each at 0.050 ppm (LOQ), 0.100 ppm, and 1.000 ppm. Unfortified milk and tissue fat samples were also assayed. Successful validation recoveries were obtained with the first attempt for tissue fat. The first two attempts were unsuccessful for milk fat. Upon consultation with the petitioner, the following modifications were made to the method: the vacuum conditions for concentration of the milk fat extract were adjusted to reduce loss, and the sample load applied to the SCX cartridge was reduced. Successful validation recoveries were obtained for milk fat with the third attempt; recoveries from the first two attempts were not included in the submission. The method modifications for milk fat have been incorporated into the final method procedures. Apparent residues of imazapic and CL 263284 were each less than the method LOQ in two unfortified samples each of milk fat and tissue fat. The laboratory reported that the extraction of eight samples required approximately 20 person-hours, and HPLC/MS analysis of the eight samples with five standards required approximately 3.5 hours.

The results of the ILV studies are presented in Table 10. Following revisions to the method for milk and tissue fat, adequate recoveries were obtained at all fortification levels. We note that the fortification range for kidney did not include the proposed tolerance level of 2 ppm. Typically, the Agency requires that ILV show adequate results for samples fortified at the LOQ and at the proposed tolerance for each matrix. Provided that a successful PMV is conducted by ACB/BEAD reflecting analysis of kidney fortified at 2 ppm, no additional ILV data will be required for kidney.

Representative sample calculations and electropherograms or chromatograms were provided for all methods.

Table 10. Independent laboratory validation recoveries of imazapic and its metabolite CL 263284 from analysis of fortified samples of untreated milk and cattle tissue analyzed using the proposed tolerance enforcement methods.

Matrix	Fortification Level. ppm	Percent Recoveries <sup>a</sup>	
		Imazapic	CL 263284
<b>CE Method M 3188</b>			
Milk	0.010	87. 90	85. 89
	0.020	92, 95. 97	91. 91. 95
	1.000	89. 92	89. 92
	Mean ± s.d.	92 ± 3.5 (n=7)	90 ± 3.1 (n=7)
<b>CE Method M 3222</b>			
Muscle	0.050	74, 74	88. 89
	0.100	73, 75	75. 78
	1.000	79, 82	76. 77
	Mean ± s.d.	76 ± 3.5 (n=6)	81 ± 6.3 (n=6)
Liver	0.050	77. 80	76. 80
	0.100	83, 83	80, 86
	1.000	80, 83	77, 79
	Mean ± s.d.	81 ± 2.4 (n=6)	80 ± 3.5 (n=6)
Kidney	0.050	78, 80, 80	80, 90, 94
	0.100	74, 83	80, 82
	1.000	81, 89	80, 87
	Mean ± s.d.	80.7 ± 4.6 (n=7)	85 ± 5.7 (n=7)
All tissues (muscle, liver, and kidney)	Mean ± s.d.	79 ± 4.2 (n=19)	82 ± 5.5 (n=19)
<b>HPLC/MS Method M 3233</b>			
Tissue fat	0.050	87, 101	93, 106
	0.100	87, 101	85, 91
	1.000	94, 114	92, 101
	Mean ± s.d.	96 ± 11.7 (n=6)	95 ± 7.6 (n=6)
Milk fat	0.010	95, 117	86, 113
	0.020	100, 103	103, 105
	0.200	98, 109	85, 99
	Mean ± s.d.	104 ± 8.1 (n=6)	99 ± 11.1 (n=6)

<sup>a</sup> Means and standard deviations were calculated by the petitioner, except for those for individual tissues, which were calculated by the study reviewer.

## Radiovalidation

Radiovalidation data for the proposed CE enforcement methods (M 3188 and M 3222) were included in the independent laboratory validation submission (MRID 44817710). Samples of milk and kidney from the goat metabolism study were subjected to extraction and analysis using the enforcement methods. The results of the radiovalidation study are presented in Table 11. The results of the analyses for residues of imazapic in milk and kidney by the CE residue methods are in good agreement with the HPLC radioassay from the goat metabolism study: residues of CL 263284 were nondetectable in the metabolism study.

The petitioner reported that 94% TRR were extracted from radiolabeled milk samples with 6 N hydrochloric acid:water:10% lead acetate (0.1:20:2, v:v:v), and 88% of TRR were extracted from radiolabeled kidney samples with 1 N hydrochloric acid:water:methanol (1.5:58.5:90, v:v:v) as specified in the residue methods. Using the CE methods, respective concurrent recoveries of imazapic and CL 263284 were 88% and 98% for fortified milk, and 81% and 100% for fortified kidney. Residues of imazapic and CL 263284 were each less than the respective method LOQ for each analyte in one untreated milk and kidney sample.

Table 11. Results of radiovalidation of the proposed CE enforcement methods M 3188 (milk) and M 3222 (kidney) using samples from the goat metabolism study.

Matrix	Analyte	HPLC Radioassay Method <sup>14</sup> C, ppm	Residues from Methods M 3188/M 3222, <sup>a</sup> ppm
Goat, milk	Imazapic	0.051	0.060
	CL 263284	n.d. <sup>b</sup>	n.d.
Goat, kidney	Imazapic	0.234	0.271
	CL 263284	n.d.	n.d.

<sup>a</sup> The average of duplicate analyses is reported. Milk was analyzed using CE method M 3188 and kidney was analyzed using CE method M 3222.

<sup>b</sup> n.d. = Not detected

We note that no radiovalidation data were submitted in support of HPLC/MS method M 3233 for determination of residues of imazapic in fat. In consideration of the adequate concurrent validation data presented below, and the fact that TRR in goat fat from the metabolism study (0.003 ppm) were below the method LOQ for fat of 0.050 ppm, no radiovalidation data are required.

## Residue data collection methods - livestock commodities

Samples of cattle milk and tissues from the submitted feeding study were analyzed by ABC Laboratories (Columbia, MO) and XenoBiotic Laboratories (Plainsboro, NJ) using the CE methods proposed for enforcement of tolerances in livestock commodities. Whole milk and tissue (except fat) samples were analyzed for residues of imazapic and CL 263284 by ABC



Laboratories using CE methods M 3188 (milk) and M 3222 (tissues). The reported method LOQs for each analyte were 0.010 ppm for whole milk and 0.050 ppm for tissues. Samples of milk fat and tissue fat were analyzed for residues of imazapic and CL 263284 by XenoBiotic Laboratories using HPLC/MS method M 3233. The reported method LOQs for each analyte were 0.010 ppm for milk fat and 0.050 ppm for tissue fat. The petitioner submitted concurrent method recoveries in conjunction with the feeding study. Concurrent method recoveries were generated from untreated cow milk and tissue samples fortified with imazapic and CL 263284. Concurrent method recoveries are presented in Table 12. Sample calculations and representative electropherograms and chromatograms were provided.

Samples of milk, kidney, liver, and muscle from the storage stability study were also analyzed using CE methods M 3188 and M 3222 by ABC Laboratories. The method LOQs were reported as 0.010 ppm for milk and 0.050 ppm for tissues. Concurrent method recoveries (fresh fortification recoveries) are reported under the Storage Stability section.

Table 12. Concurrent method recoveries of residues of imazapic and CL 263284 from fortified samples of cattle commodities analyzed using the CE or HPLC/MS methods (MRID 44817714).

Commodity (Analytical Laboratory)	Fortification Level, ppm	Statistic	Imazapic	CL 263284
<b>CE Method M 3188</b>				
Milk (ABC Labs)	0.010-1.00	Average (%)	91	91
		Recovery range (%)	80-111	81-111
		SD (%)	6.5	7.5
		Number	27	27
<b>CE Method M 3222</b>				
Kidney (ABC Labs)	0.100-5.00	Average (%)	82	83
		Recovery range (%)	80-84	78-88
		SD (%)	1.8	4.2
		Number	4	4
Liver (ABC Labs)	0.050-0.100	Average (%)	79	90
		Recovery range (%)	74-84	84-103
		SD (%)	4.3	8.8
		Number	4	4
Muscle (ABC Labs)	0.050-0.100	Average (%)	87	81
		Recovery range (%)	65-99	67-87
		SD (%)	14.7	8.3
		Number	5	5

Table 12 (continued).

Commodity (Analytical Laboratory)	Fortification Level, ppm	Statistic	Imazapic	CL 263284
<b>HPLC/MS Method M 3233</b>				
Milk fat (XenoBiotic Labs)	0.010, 0.200	Average (%)	95	89
		Recovery range (%)	89-102	81-94
		SD (%)	6.6	7.2
		Number	3	3
Tissue fat (XenoBiotic Labs)	0.050-1.00	Average (%)	100	94
		Recovery range (%)	97-105	86-107
		SD (%)	4.4	11.6
		Number	3	3

Comments

The petitioner has proposed CE methods M 3188 and M 3222 for the enforcement of tolerances of imazapic and CL 263284 in milk (M 3188) and livestock tissues (M 3222), and HPLC/MS method M 3233 for the enforcement of tolerances in fat. These methods were used for the determination of residues of imazapic and CL 263284 in samples from the ruminant feeding study. Concurrent method recoveries submitted in conjunction with the ruminant feeding study indicate that these methods adequately recover residues of imazapic and CL 263284 from ruminant tissues and milk.

Adequate independent method validation have been submitted in support of all methods. Adequate radiovalidation data have been submitted for the CE enforcement methods. Although no radiovalidation data were submitted in support of HPLC/MS method M 3233 for determination of residues of imazapic in fat, in consideration of the concurrent validation data, and the fact that TRR in goat fat from the metabolism study (0.003 ppm) were below the method LOQ for fat of 0.050 ppm, no radiovalidation data are required for this method. The methods were forwarded to ACB/BEAD for petition method validation (D271474, W. Donovan, 17-JAN-2001).

**OPPTS GLN 860.1360: Multiresidue Method**

The petitioner previously submitted data pertaining to the multiresidue methods testing of imazapic and its metabolites CL 263284 and CL 189215 in conjunction with PP#4F4390 (DP Barcode D211846, 2/9/95, F. Griffith). Methods testing indicated that residues of imazapic and its metabolites CL 263284 and CL 189215 do not fluoresce; therefore, no additional work was done for Protocol A. Because all three compounds are nicotinic acid derivatives, they were methylated by Protocol B and analyzed by GC as described by Protocol C; however, no response

was observed using GC/ECD analysis, and multiple peaks were observed only at the 1- $\mu$ g level, indicating thermal decomposition. Methylated imazapic was detected by nitrogen-phosphorus detection; however, no response was observed with a Florisil column, thus no further work was done with Protocols D and E. The results of the multiresidue testing for imazapic and its metabolites CL 263284 and CL 189215 were forwarded for inclusion in PAM Volume I.

### OPPTS GLN 860.1380: Storage Stability Data

#### Plant sample storage conditions and intervals

Samples of grass forage and hay collected from the field trials were frozen at the field; hay samples were dried for 1-8 days prior to collection. The collected samples were frozen (-37 to -0.6 C) at the field within 2-4 hours of harvest and were shipped frozen within 44 days of harvest to the petitioner. Samples were stored at American Cyanamid frozen ( $\leq$ -10 C) prior to sample preparation. RAC samples were homogenized with dry ice and frozen. The homogenized samples were shipped to Centre Analytical Laboratories (State College, PA), where they were stored frozen until analysis. Maximum total storage intervals from harvest to analysis were 727 days (~24 months) for grass forage and hay samples.

#### Plant storage stability data

In support of the storage intervals and conditions of samples from the grass field trial studies, American Cyanamid submitted storage stability data (citation listed below) for wheat commodities. Storage stability data, previously submitted in conjunction with PP#4F4390 (DP Barcode D222184, 2/6/96, J. Garbus), indicated that residues of imazapic, CL 263284, and CL 189215 are relatively stable under frozen storage conditions in/on fortified samples of peanut commodities for up to 12 months.

44817711 Nejad, H. (1999) CL 263222 (Imazapic): Freezer Storage Stability of Residues of CL 263222, CL 263284, and CL 189215 in Wheat Green Forage, Wheat Hay, Wheat Straw, and Wheat Grain. Laboratory Project Number RES 98-161. Unpublished study prepared by American Cyanamid Company. 71 p.

At American Cyanamid, untreated samples of wheat forage, hay, straw, and grain were fortified with imazapic, CL 263284, and CL 189215, each at 0.5 ppm, and stored frozen ( $\leq$ -10 C). Fortified samples were shipped frozen to Centre Analytical Laboratories (CAL; State College, PA) after ~14 months of frozen storage at American Cyanamid. Samples were stored frozen ( $\leq$ -10 C) at CAL and were analyzed at the 14-, 18-, and 24-month storage intervals.

Fortified samples of wheat matrices were analyzed for residues of imazapic, CL 263284, and CL 189215 using CE method M 2463, a method similar to the proposed enforcement method. Control samples were fortified with imazapic, CL 263284 and CL 189215 at 10 ppm at the time of analysis for concurrent recoveries. Apparent residues of imazapic, CL 263284, and CL 189215 were each less than the method LOQ (<0.10 ppm) in/on three untreated samples each.

of wheat forage, hay, straw, and grain. The results of the storage stability study are presented in Table 13.

Table 13. Frozen storage stability and concurrent method recoveries (fresh fortification recovery) of residues of imazapic and its metabolites CL 263284 and CL 189215 from samples of fortified wheat commodities.

Matrix	Fortification Level, ppm	Storage Period, months	Fresh Fortification Recovery, %	Apparent Recovery in Stored Samples, %	Corrected Recovery in Stored Samples, % <sup>a</sup>
<b>Imazapic</b>					
Wheat, forage	0.5	14	88	80, 90	91, 102
		18	90	82, 89	91, 99
		24	85	69, 72	82, 85
Wheat, hay	0.5	14	90	83, 85	92, 94
		18	92	69, 79	75, 86
		24	103	84, 100	82, 97
Wheat, straw	0.5	14	88	87, 87	99, 99
		18	92	82, 89	88, 96
		24	87	87, 97	100, 111
Wheat, grain	0.5	14	90	87, 94	97, 104
		18	108	82, 95	76, 88
		24	100	98, 105	98, 105
<b>CL 263284</b>					
Wheat, forage	0.5	14	87	75, 83	84, 93
		18	87	91, 102	105, 117
		24	87	67, 73	77, 84
Wheat, hay	0.5	14	92	82, 82	89, 89
		18	89	71, 79	80, 89
		24	108	75, 92	69, 85
Wheat, straw	0.5	14	91	90, 93	99, 102
		18	98	89, 96	91, 98
		24	92	87, 97	95, 105
Wheat, grain	0.5	14	91	91, 91	100, 100
		18	104	96, 107	92, 103
		24	106	110, 113	104, 107

Table 13 (continued).

Matrix	Fortification Level, ppm	Storage Period, months	Fresh Fortification Recovery, %	Apparent Recovery in Stored Samples, %	Corrected Recovery in Stored Samples, % <sup>a</sup>
<b>CL 189215</b>					
Wheat, forage	0.5	14	85	74.84	87.99
		18	95	80.87	84.92
		24	90	69.72	77.80
Wheat, hay	0.5	14	92	80.85	87.92
		18	95	71.82	75.86
		24	108	83.93	77.86
Wheat, straw	0.5	14	78	79.79	101.101
		18	89	76.84	85.94
		24	77	83.90	108.117
Wheat, grain	0.5	14	90	82.84	91.93
		18	98	76.87	78.89
		24	703	83.87	81.84

<sup>a</sup> Apparent storage stability recovery divided by fresh fortification recovery.

Study summary

Grass forage and hay samples from the submitted field studies were stored frozen from harvest to analysis for up to 24 months. The petitioner submitted storage stability data for wheat that indicated that residues of imazapic and its metabolites CL 263284 and CL 189215 are relatively stable under frozen storage conditions in/on fortified samples of wheat forage, hay, straw, and grain for up to 24 months. Because the commodities of wheat forage and hay are similar to pasture and rangeland grass forage and hay, the submitted storage stability data are adequate to support the storage conditions and intervals of the samples from the grass field trial studies.

Livestock sample storage conditions and intervals

Samples of milk from the ruminant feeding study were refrigerated (2.2-5.8 C) following collection until the next day, when subsamples were frozen ( $\leq -10$  C) for shipment to ABC Laboratories. Subsamples of milk collected on Days 8, 15, and 22 and composited from each treatment group were separated into milk fat and skim milk by centrifugation. Milk fat samples were frozen and shipped to XenoBiotic Laboratories for analysis. Samples of liver, kidney, omental fat, and loin muscle from the ruminant feeding study were sliced and frozen (-15.1 to -19.7 C) until the next day, when tissue samples were ground in the presence of dry ice and stored frozen ( $\leq -10$  C). Tissue samples (except fat) were shipped to ABC Laboratories, and fat samples were shipped to XenoBiotic Laboratories for analysis. The storage conditions at the analytical laboratories were not specified; however, the maximum storage intervals from

collection until analysis were 185 days (6.1 months) for whole milk, 253 days (8.3 months) for milk fat, and 237 days (7.8 months) for tissues.

#### Livestock storage stability data

In support of the storage intervals and conditions of samples from the ruminant feeding study, American Cyanamid has submitted storage stability data (citations listed below) for livestock commodities.

44817712 Naumann, L.; Nejad, H. (1999) CL 263222 (Imazapic): Freezer Storage Stability of CL 263222 and Metabolite CL 263284 Residues in Milk and Cattle Muscle, Kidney, and Liver Tissue. Report Number CY240: RES 99-036 and RES 99-039. Unpublished study prepared by American Cyanamid Company. 84 p.

45075901 Nejad, H.; Sweeney, R.A. (1999) CL 263222 (Imazapic): Freezer Storage Stability of CL 263222 and Metabolite CL 263284 Residues in Cattle Muscle, Kidney, and Liver Tissue - Final Report. Report Number RES 99-036.01. Unpublished study prepared by American Cyanamid Company. 59 p.

Control samples of milk (from the University of Missouri dairy) and cow tissues (kidney, liver, and muscle; supplied by American Cyanamid) were fortified with imazapic and its metabolite CL 263284, each at 1.00 ppm, and stored frozen ( $\leq -10$  C) at ABC Laboratories. Milk samples were analyzed at 1-, 3-, and 6-month storage intervals and tissue samples were analyzed at 1-, 3-, 5-, and 8-month storage intervals:

Milk samples were analyzed for residues of imazapic and CL 263284 in milk using the proposed CE enforcement method M 3188; samples of kidney, liver, and muscle were analyzed using the proposed CE enforcement method M 3222. Control milk and tissue samples were fortified with imazapic and CL 263284, each at 1.00 ppm at the time of analysis for concurrent recoveries. Apparent residues of imazapic and CL 263284 were each less than the respective method LOQ ( $<0.010$  ppm for milk and  $<0.050$  ppm for tissues) in/on three untreated samples of whole milk and in/on four untreated samples each of cow kidney, liver, and muscle. The results of the storage stability studies are presented in Table 14.

Although no data were submitted depicting storage stability of residues of imazapic and CL 263284 in fat, no data are required because TRR in fat in the goat metabolism study were quite low in comparison to TRR in other tissues, indicating that residues of imazapic and CL 263284 are not likely to be found in fat samples.

Table 14. Frozen storage stability and concurrent method recoveries (fresh fortification recovery) of residues of imazapic and its metabolite CL 263284 from samples of fortified ruminant commodities.

Matrix	Fortification Level, ppm	Storage Period, months	Fresh Fortification Recovery, %	Apparent Recovery in Stored Samples, %	Corrected Recovery in Stored Samples, % <sup>a</sup>
<b>Imazapic</b>					
Whole milk	1.00	1	87	89.89	102.102
		3	97	92.97	95.100
		6	90	86.89	96.99
Kidney	1.00	1	87	83.87	95.100
		3	90	86.87	96.97
		5	90	84.87	93.97
		8	82	77.77	94.94
Liver	1.00	1	80	79.83	99.104
		3	88	78.85	89.97
		5	82	77.80	94.98
		8	77	75.81	97.105
Muscle	1.00	1	84	82.85	98.101
		3	83	75.81	90.98
		5	80	75.85	94.106
		8	79	79.79	100.100
<b>CL 263284</b>					
Milk	1.00	1	88	87.87	99.99
		3	97	92.98	95.101
		6	90	91.92	101.102
Kidney	1.00	1	82	72.77	88.94
		3	86	83.83	97.97
		5	93	83.86	89.92
		8	78	75.77	96.99
Liver	1.00	1	72	76.79	106.110
		3	86	77.87	90.101
		5	81	87.90	107.111
		8	76	80.82	105.108
Muscle	1.00	1	72	59.72	82.100
		3	76	79.81	104.107
		5	78	81.83	104.106
		8	77	74.76	96.99

<sup>a</sup> Apparent storage stability recovery divided by fresh fortification recovery.

## Study summary

Milk and cow tissue samples from the submitted ruminant feeding study were stored frozen from collection to analysis for up to 6.1 months for whole milk, 8.3 months for milk fat, and 7.8 months for tissues. The submitted storage stability data indicate that residues of imazapic and its metabolite CL 263284 are relatively stable under frozen storage conditions in/on fortified samples of whole milk for up to 6 months and in/on fortified samples of cow kidney, liver, and muscle for up to 8 months. Although no data were submitted depicting storage stability of residues of imazapic and CL 263284 in fat, no data are required because TRR in fat in the goat metabolism study were quite low in comparison to TRR in other tissues, indicating that residues of imazapic and CL 263284 are not likely to be found in fat samples. The submitted storage stability data are adequate to support the storage conditions and intervals of the samples from the ruminant feeding study.

## OPPTS GLN 860.1500: Crop Field Trials

### Grass Forage, Fodder, and Hay Group

American Cyanamid submitted grass field trial data (citation listed below) reflecting application of imazapic to Bermuda grass, bromegrass, Big Bluestem grass, and grass mixtures to support the establishment of proposed group tolerances for residues of imazapic and its metabolites CL 263284 and CL 189215 in/on grass forage at 35 ppm and grass hay at 15 ppm.

44817713 Hallman, D.C.; Leonard, R.C. (1999) CL 263222 (Imazapic): Residues of CL 263222, CL 263284, and CL 189215 in Grass Forage and Hay After Postemergence or Preemergent Treatment with PEA FEAU® Herbicide. Report No. CY232: RES 98-077, RES 98-078, RES 98-070, RES 96-109, RES 99-034, RES 98-071, RES 98-094, RES 98-147, RES 98-101, RES 98-102, RES 98-115, RES 98-148, RES 98-116. Unpublished study prepared by American Cyanamid Company. 1754 p.

A total of 13 grass field trials were conducted during the 1996 and 1997 growing season in CO(1 trial), GA(2), IA(3), FL(1), MS(1), NE(1), SD(1), TX(1), and WA(2). Four different treatment and sampling schemes were represented in the field trials.

In eight trials conducted in 1996-1997 in CO, FL, GA (2 trials), IA, MS, TX, and WA, grass forage and hay were harvested 0, 7, 14, 28, and 54-56 days following a single postemergence broadcast application, made in the summer, of the 2 lb ae/gal ammonium salt SC formulation at 0.2 lb ae/A (~1.1x the maximum proposed seasonal application rate).

In three trials conducted in IA, NE, and WA, grasses received two postemergence broadcast applications of the 2 lb ae/gal ammonium salt SC formulation. In these trials, the first application was made in the late summer or fall of 1996 at 0.14 lb ae/A, and a second application was made in the spring (following late summer application) or summer (following fall application) of 1997 at 0.07 lb ae/A; retreatment intervals were 9.3-9.4 months. Grass forage and



hay samples were collected 0, 7, 14, 28-33, and 55-57 days after the first application, and 0, 7, 14, 28, and 56 days following the second application. The total application rate was 0.21 lb ae/A (~1.1x the maximum proposed seasonal application rate). We note that although the total application rate in these trials corresponds to the maximum proposed seasonal application rate, because of the length of the retreatment interval between first and second applications, the trials are not representative of the maximum proposed use pattern (a single application at 0.1875 lb ae/A).

In one trial conducted in 1996 in SD, grass forage and hay were harvested 0, 7, 14, and 28 days following a single postemergence broadcast application, made in the fall, of the 2 lb ae/gal ammonium salt SC formulation at 0.14 lb ae/A (~0.7x the maximum proposed seasonal application rate), and in one trial conducted in 1996, the 2 lb ae/gal ammonium salt SC formulation was applied preemergence in the summer to newly seeded grass at 0.2 lb ae/A (~1.1x the maximum proposed seasonal application rate). Grass forage was collected 69, 83, and 106 DAT, and hay was collected 71, 83, and 106 DAT.

Applications were made in 19.2-25.1 gal/A of water using ground equipment (backpack or tractor-mounted sprayers); a nonionic surfactant (0.25%) was added to the application sprays for all postemergence applications. A separate plot at each trial site was left untreated to provide control samples.

One untreated and duplicate treated samples were collected (manually or using commercial harvesting equipment) from each test site. Following harvest, grass samples for hay were dried in the field, in grain bins, on drying racks, or in mesh bags in a sheltered area for 1-8 days prior to collection. The moisture content of dried hay samples typically ranged 10.5%-39.5%; however, the moisture content of dried hay from the SD trial was 31.5%-71.5%. The collected samples were frozen (-37 to -0.6 °C) at the field within 2-4 hours of harvest and were shipped frozen within 44 days of harvest to the petitioner. Samples were stored frozen ( $\leq -10$  °C) at American Cyanamid prior to sample preparation. RAC samples were homogenized in the presence of dry ice and frozen. The homogenized samples were shipped to Centre Analytical Laboratories (CAL; State College, PA) for analysis, and were stored frozen at the analytical laboratory until analysis. Maximum total storage intervals from harvest to analysis were 727 days (~24 months) for grass forage and hay samples.

At CAL, the samples from selected field trials were initially analyzed for residues of imazapic and CL 263284 only using CE method M 2702 previously described under the Residue Analytical Methods section. These samples were subsequently re-analyzed, together with samples from the remaining field trials, to include analysis for CL 189215 using the proposed enforcement method, CE method M 3114. For methods M 3114 and M 2702, the reported method LOQ and LOD were, respectively, 0.50 ppm and 0.05-0.06 ppm, for each analyte in all grass matrices. Apparent residues of imazapic, CL 263284, and CL 189215 were each less than the LOQ in/on all untreated samples of grass forage (n=76) and hay (n=76). The results of the imazapic field trials are reported in Table 15.

The petitioner reported that for the NE field trial, in which samples from the first application were initially analyzed by method M 2702, there was such significant loss of moisture in stored forage samples that residue values for these samples on re-analysis with method M 3114 were felt to be inflated. Moisture content was 43.0-62.5% on first analysis and 12.6-32.7% on second analysis. For forage and hay samples from the first application for this trial only, the reported residue values in Table 15 for imazapic and CL 263284 reflect analysis by method M 2702, and the reported residue value for CL 189215 reflects analysis by method M 3114. The petitioner maintains that the residue levels for CL 189215 for these samples are artificially inflated.

Table 15. Residues of imazapic in/on grass forage and hay harvested following a single preemergence application or 1-2 postemergence applications of the 2 lb ae/gal ammonium salt SC formulation.

Test Site (Year; EPA Region; ID No.)	Grass Type	No./Timing of Apps. <sup>a</sup>	Application Rate	PHI, days	Residues, ppm <sup>b</sup>			Total
					Imazapic	CL 263284	CL 189215	
<b>Grass Forage</b>								
<b>Preemergence Application</b>								
Hamilton County, IA (1996; Region 5; RES 98-078)	Big bluestem grass	1 (summer)	0.22 lb ae/A	69	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
					<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
					<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
<b>Postemergence Application</b>								
Macon County, GA (1996; Region 2; RES 98-077)	Bermuda grass	1 (summer)	0.21 lb ae/A	0	14, 15	0.64, 0.56	<0.50, <0.50	<15.14, <16.06
				1	<0.50, <0.50	2.5, 3.0	<0.50, <0.50	<3.5, <4.0
				14	<0.50, <0.50	1.8, 1.9	<0.50, <0.50	<2.8, <2.9
				28	<0.50, <0.50	1.2, 1.3	<0.50, <0.50	<2.2, <2.3
				54	<0.50, <0.50	<0.50, 0.64	<0.50, <0.50	<1.50, <1.64
Pulaski County, GA (1997; Region 2; RES 98-102)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	7.5, 10	<0.50, <0.50	<0.50, <0.50	<8.5, <11
				7	<0.50, <0.50	0.91, 0.93	<0.50, <0.50	<1.91, <1.93
				14	<0.50, <0.50	0.81, 0.95	<0.50, <0.50	<1.81, <1.95
				28	<0.50, <0.50	0.64, 0.77	<0.50, <0.50	<1.64, <1.77
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Marion County, FL (1997; Region 3; RES 98-101)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	7.2, 7.4	<0.50, <0.50	<0.50, <0.50	<8.2, <8.4
				7	<0.50, <0.50	1.4, 1.7	<0.50, <0.50	<2.4, <2.7
				14	<0.50, <0.50	1.1, 1.3	<0.50, <0.50	<2.1, <2.3
				28	<0.50, <0.50	0.65, 0.73	<0.50, <0.50	<1.65, <1.73
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50

(continued; footnotes follow)

Table 15 (continued).

Test Site (Year; EPA Region; ID No.)	Grass Type	No./Timing of Apps. <sup>a</sup>	Application Rate	PHI, days	Residues, ppm <sup>b</sup>			
					Imazapic	CL 263284	CL 189215	Total
Washington County, MS (1996; Region 4; RES 96-109)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	15, 15	<0.50, <0.50	<0.50, <0.50	<16.0, <16.0
				7	<0.50, <0.50	0.58, 0.61	<0.50, <0.50	<1.58, <1.61
				14	<0.50, <0.50	0.81, 1.1	<0.50, <0.50	<1.81, <2.1
				28	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	11, 12	<0.50, <0.50	<0.50, <0.50	<12, <13
Hamilton County, IA (1997; Region 5; RES 98-115)	Big bluestem and bromegrass	1 (summer)	0.20 lb ae/A	7	0.52, 0.54	<0.50, <0.50	<0.50, <0.50	<1.52, <1.54
				14	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				28	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	8, 1	<0.50, <0.50	<0.50, <0.50	<9, 10
				1	17	<0.50	<0.50	<18, 0
Hamilton County, IA (1996; Region 5; RES 98-070)	Bluegrass and bromegrass	1 (late summer)	0.15 lb ae/A	7	1.7, 1.9	1.5, 1.7	<0.50, <0.50	<3.70, <4.10
				14	<0.50, <0.50	1.8, 1.9	<0.50, <0.50	<2.80, <2.90
				33	<0.50, <0.50	1.2, 1.3	<0.50, <0.50	<2.2, <2.3
				57	<0.50, <0.50	<0.50, 0.56	<0.50, <0.50	<1.50, <1.56
				0	3.7, 4.2	<0.50, <0.50	<0.50, <0.50	<4.7, <5.2
		7	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
		14	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
		28	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
		56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
		2	(late summer + spring) RTT - 9.4 mos	0.22 lb ae/A (0.15 + 0.07)				

Table 15 (continued).

Test Site (Year, EPA Region; ID No.)	Grass Type	No./Timing of Apps. *	Application Rate	PHI, days	Residues, ppm <sup>b</sup>			Total
					Imazapic	CL 263284	CL 189215	
York County, NE <sup>c</sup> (1996; Region 5; RES 99-034)	Big bluestem grass	1 (fall)	0.14 lb ae/A	0	0.50, 0.62	<0.50, <0.50	<25.0, <25.12	
				7	2.7, 3.0	4.3, 4.0	7.72, 7.90	
		2 (fall + summer)	0.21 lb ae/A (0.14 + 0.07)	14	<0.50, 0.58	<0.50, <0.50	<0.50, <0.50	<2.70, <2.88
				0	7.4, 7.8	<0.50, <0.50	<8.40, <8.80	
		RTI = 9.3 mos		7	<0.50, <0.50	0.52, <0.61	<0.50, <0.50	<1.52, 1.61
				14	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				28	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				0	9.6, 9.9	<0.50, <0.50	<10.6, 10.9	
				7	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
Marshall County, SD (1996; Region 5; RES 98-071)	Bluegrass and brome grass	1 (fall)	0.14 lb ae/A	14	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				28	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				0	21, 22	<0.50, <0.50	<22, <23	
				7	0.64, 0.67	2.6, 2.4	<0.50, <0.50	
				14	<0.50, <0.50	1.8, 2.5	<0.50, <0.50	
				28	<0.50, <0.50	1.3, 1.6	<0.50, <0.50	
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				0	12, 14	<0.50, <0.50	<13.0, <15.0	
Waller County, TX (1997; Region 6; RES 98-148)	Bermuda grass	1 (summer)	0.20 lb ae/A	7	1.8, 1.8	0.91, 1.0	<3.21, <3.30	
				14	0.96, 1.1	1.1, 1.1	<2.56, <2.70	
				28	<0.50, <0.50	0.56, 0.66	<1.56, <1.66	
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				0	12, 14	<0.50, <0.50	<13.0, <15.0	
				7	1.8, 1.8	0.91, 1.0	<3.21, <3.30	
Delta County, CO (1997; Region 9; RES 98-147)	brome grass	1 (summer)	0.20 lb ae/A	14	0.96, 1.1	1.1, 1.1	<2.56, <2.70	
				28	<0.50, <0.50	0.56, 0.66	<1.56, <1.66	
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				0	12, 14	<0.50, <0.50	<13.0, <15.0	

(continued; footnotes follow)

Table 15 (continued).

Test Site (Year; EPA Region; ID No.)	Grass Type	No./Timing of Apps.*	Application Rate	PHI, days	Residues, ppm <sup>b</sup>			Total
					Imazapic	CL 263284	CL 189215	
Grant County, WA (1997; Region 11; RES 98-116)	Little bluestem and bluegrass	1 (summer)	0.20 lb ae/A	0	6.5, 8.5	<0.50, <0.50	<0.50, <0.50	<7.5, <9.5
				7	<0.50, <0.50	<0.50, 0.54	<0.50, <0.50	<1.50, <1.54
				14	<0.50, <0.50	0.50, 0.60	<0.50, <0.50	<1.50, <1.60
				28	<0.50, <0.50	0.55, 0.63	<0.50, <0.50	<1.55, <1.63
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	9.5, 11	<0.50, <0.50	<0.50, <0.50	<10.5, <12.0
Grant County, WA (1996; Region 11; RES 98-094)	Bluegrass and little bluestem grass	1 (late summer)	0.14 lb ae/A	7	<0.50, <0.50	1.0, 1.1	<0.50, <0.50	<2.0, <2.1
				14	<0.50, <0.50	0.87, 1.1	<0.50, <0.50	<1.87, <2.1
				28	<0.50, <0.50	<0.50, 0.62	<0.50, <0.50	<1.50, <1.62
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	2.5, 2.6	<0.50, <0.50	<0.50, <0.50	<3.50, <3.60
				7	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Grant County, WA (1996; Region 11; RES 98-094)	Bluegrass and little bluestem grass	2 (late summer + spring) RTI = 9.4 mos	0.21 lb ae/A (0.14 + 0.07)	14	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				28	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
<b>Grass Hay</b>								
<b>One Preemergence Application</b>								
Hamilton County, IA (1996; Region 5; RES 98-078)	Big bluestem	1 (summer)	0.22 lb ae/A	71	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				83	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				106	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50

(continued; footnotes follow)

Table 15 (continued)

Test Site (Year, EPA Region, ID No.)	Grass Type	No./Timing of Apps. *	Application Rate	PHI, days	Residues, ppm <sup>b</sup>			
					Imazapic	Cl. 263284	Cl. 189215	Total
<b>Postemergence Application</b>								
Macon County, GA (1996; Region 2; RES 98-077)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	21, 28	11, 8.9	0.67, <0.50	32.67, <37.4
				7	<0.50, <0.50	2.7, 3.2	<0.50, <0.50	<3.7, <4.2
				14	0.50, 0.50	3.1, 5.0	0.63, 0.87	4.23, 6.37
				28	<0.50, <0.50	1.5, 2.2	<0.50, <0.50	<2.50, <3.20
				56	<0.50, <0.50	<0.50, 0.50	<0.50, <0.50	<1.50, <1.50
Pulaski County, GA (1997; Region 2; RES 98-102)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	22, 23	2.8, 3.6	<0.50, <0.50	<25.3, <27.1
				7	<0.50, <0.50	1.6, 2.0	<0.50, <0.50	<2.6, <3.0
				14	<0.50, <0.50	1.4, 1.7	<0.50, <0.50	<2.4, <2.7
				28	<0.50, <0.50	1.1, 1.3	<0.50, <0.50	<2.1, <2.3
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Marion County, FL (1997; Region 3; RES 98-01)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	12, 13	2.3, 2.9	<0.50, <0.50	<14.8, <16.4
				7	<0.50, <0.50	1.8, 2.4	<0.50, <0.50	<2.8, <3.4
				14	<0.50, <0.50	1.9, 2.3	<0.50, <0.50	<2.9, <3.3
				28	<0.50, <0.50	1.2, 1.5	<0.50, <0.50	<2.2, <2.5
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Washington County, MS (1996; Region 4; RES 96-109)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	17, 17	2.4, 2.5	<0.50, <0.50	<19.9, <20.0
				7	0.64, 0.72	1.8, 1.6	<0.50, <0.50	<2.94, <2.82
				14	<0.50, <0.50	1.1, 1.2	<0.50, <0.50	<2.10, <2.20
				28	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50

(continued, footnotes follow)

Table 15 (continued).

Test Site (Year; EPA Region; ID No.)	Grass Type	No./Timing of Apps. *	Application Rate	PHI, days	Residues, ppm <sup>b</sup>				Total
					Imazapic	CL 263284	CL 189215		
Hamilton County, IA (1997; Region 5; RES 98-115)	Big bluestem and bromegrass	1 (summer)	0.20 lb ae/A	0	2.9, 3.5	<0.50, <0.50	<24.4, <25.0		
				7	0.95, 1.0	<0.50, <0.50	<2.31, <2.50		
				14	<0.50, <0.50	<0.50, <0.50	<1.78, <1.85		
				28	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
				0	13	1.6	39.6		
Hamilton County, IA (1996; Region 5; RES 98-070)	Bluegrass and bromegrass	1 (late summer)	0.15 lb ae/A	1	8.4	1.3	22.7		
				7	4.4, 5.1	0.84, 1.0	8.74, 9.9		
				14	2.8, 3.0	<0.50, 0.53	<3.80, <4.03		
				33	1.7, 1.7	0.53, 0.55	<2.73, <2.75		
				57	0.59, 0.66	<0.50, <0.50	<1.59, <1.66		
				0	8.7, 9.2	<0.50, <0.50	<9.7, <10.2		
		2 (late summer + spring) RTI = 9.4 mos	0.22 lb ae/A (0.15 + 0.07)	7	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
				14	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
				28	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50		

(continued; footnotes follow)



Table 15 (continued).

Test Site (Year; EPA Region; ID No.)	Grass Type	No./Timing of Apps.	Application Rate	PHL, days	Residues, ppm <sup>b</sup>			
					Imazapic	CL 263284	CL 189215	Total
York County, NE <sup>c</sup> (1996; Region 5; RES 99-034)	Big bluestem grass	1 (fall)	0.14 lb ae/A	0	46, 49	3.1, 3.2	<0.50, <0.50	<49.6, <52.7
				7	1.1, 1.6	4.0, 5.2	<0.50, <0.50	<5.60, <7.30
				14	0.89, 0.92	2.9, 3.9	<0.50, <0.50	<4.29, <5.32
				21	14, 15	4.5, 4.8	<0.50, <0.50	<19.0, <20.3
				28	<0.50, <0.50	1.5, 1.7	<0.50, <0.50	<2.50, <2.70
				35	<0.50, <0.50	0.71, 0.78	<0.50, <0.50	<1.71, <1.78
				55	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Marshall County, SD (1996; Region 5; RES 98-071)	Bluegrass and brome grass	1 (fall)	0.14 lb ae/A	0	16, 26	3.8, 6.5	<0.50, 0.67	<20.3, 33.17
				7	0.82, 0.84	<0.50, <0.50	<0.50, <0.50	<1.82, <1.84
				14	<0.50, <0.50	<0.50, 0.51	<0.50, <0.50	<1.50, <1.51
				28	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Waller County, TX (1997; Region 6; RES 98-148)	Bermuda grass	1 (summer)	0.20 lb ae/A	0	35, 36	12, 12	0.93, 0.64	47.93, 48.64
				7	<0.50, 0.57	2.4, 3.1	<0.50, <0.50	<3.4, <4.17
				14	<0.50, <0.50	3.0, 3.9	<0.50, <0.50	<4.0, <4.9
				28	<0.50, <0.50	2.0, 2.6	<0.50, <0.50	<2.0, <3.6
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
Delta County, CO (1997; Region 9; RES 98-147)	brome grass	1 (summer)	0.20 lb ae/A	0	30, 39	4.9, 5.5	<0.50, <0.50	<35.4, <45.0
				7	4.0, 4.2	3.6, 2.8	0.61, 0.51	8.21, 7.51
				14	2.2, 2.3	2.3, 2.7	0.50, 0.69	5.00, 5.69
				28	<0.50, <0.50	1.2, 1.2	<0.50, <0.50	<2.20, <2.20
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50

(continued; footnotes follow)

Table 15 (continued).

Test Site (Year; EPA Region; ID No.)	Grass Type	No./Timing of Apps. <sup>a</sup>	Application Rate	PHI, days	Residues, ppm <sup>b</sup>			Total
					Imazapic	CL 263284	CL 189215	
Grant County, WA (1997; Region 11; RES 98-116)	Little bluestem and bluegrass	1 (summer)	0.20 lb ae/A	0	0.77, 0.96	<0.50, <0.50	<18.27, <18.46	
				7	<0.50, <0.50	1.4, 1.4	<0.50, <0.50	<2.4, <2.4
				14	<0.50, <0.50	1.1, 1.2	<0.50, <0.50	<2.1, <2.2
				28	<0.50, <0.50	0.71, 0.78	<0.50, <0.50	<1.71, <1.78
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	19, 24	1.7, 1.6	<0.50, <0.50	<21.2, <26.1
Grant County, WA (1996; Region 11; RES 98-094)	Bluegrass and little bluestem grass	1 (late summer)	0.14 lb ae/A	7	<0.50, <0.50	1.7, 2.1	<0.50, <0.50	<2.70, <3.10
				14	<0.50, <0.50	1.3, 1.6	<0.50, <0.50	<2.30, <2.60
				28	<0.50, <0.50	0.79, 0.81	<0.50, <0.50	<1.79, <1.81
				56	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
				0	3.8, 6.7	<0.50, <0.50	<0.50, <0.50	4.8, 7.7
				7	<0.50, <0.50	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50
		2 (late summer + spring) RTI = 9.4 mos	0.21 lb ae/A (0.14 + 0.07)	14	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				28	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	
				56	<0.50, <0.50	<0.50, <0.50	<1.50, <1.50	

<sup>a</sup> Where two applications were made, retreatment intervals (RTIs) are provided.

<sup>b</sup> Residue values for imazapic, CL 263284, and CL 189215 are listed respectively for each sample. Residue values reflecting the maximum proposed use pattern are **bolded**.

For this application in this trial, residue values for imazapic and CL 263284 reflect analysis by CE method M 2702; residue values for CL 189215 reflect analysis by CE method M 3114.

Geographic representation of the data is inadequate for the purposes of this petition. The Agency (Table 5 of OPPTS 860.1500) requires a total of 12 trials (geographic distribution unspecified) for the establishment of tolerances for residues in/on grass commodities, with four trials each to be conducted on the representative cultivars of Bermuda grass, bluegrass, and bromegrass or fescue for establishment of crop group tolerances (Table 2 of OPPTS 860.1500). In support of postemergence application of imazapic, although the petitioner conducted 12 trials reflecting postemergence application to a variety of grass cultivars, including Bermuda grass, big blue stem grass, bromegrass, and mixtures of big bluestem and bromegrass, little bluestem and blue grass, bluegrass and little bluestem grass, and bluegrass and bromegrass, only eight of the trials reflected application according to the maximum proposed use pattern (a single application at 0.1875 lb ae/A and a 0-day PHI for forage or a 7-day PHI for hay). In support of preemergence application, only a single trial was conducted. According to current Agency policy, if the label specifies separate postemergence and preemergence application of a pesticide, the full number of trials must be conducted for each application type, with the exception that if side-by-side trials show a consistent pattern between residues from preemergence and postemergence use, fewer total trials may be acceptable. No field trial data were submitted in support of use of the 0.0625 lb ai/packet WDG acid formulation.

#### Study summary

The submitted grass field trial data are inadequate to support the proposed use of imazapic on the crop group Grass Forage, Fodder, and Hay (Crop Group 17). The petitioner has not provided adequate residue data reflecting the maximum proposed use pattern of imazapic on grasses (a single postemergence application at 0.1875 lb ae/A and a 0-day PHI for forage or a 7-day PHI for hay). Only eight grass field trials were conducted according to the maximum proposed use pattern; the Agency (Table 5 of OPPTS 860.1500) recommends a total of 12 trials (geographic distribution unspecified) for the establishment of tolerances for residues in/on grass commodities, with four trials each to be conducted on the representative cultivars of Bermuda grass, bluegrass, and bromegrass or fescue for establishment of crop group tolerances (Table 2 of OPPTS 860.1500). In addition, only a single trial was conducted in support of preemergence application of imazapic; according to current Agency policy, if the label specifies separate postemergence and preemergence application of a pesticide, the full number of trials must be conducted for each application type; except when side-by-side trials show a consistent pattern between residues from preemergence and postemergence use, fewer total trials may be acceptable. Finally, no field trial data were submitted in support of the 0.0625 lb ai/packet WDG acid formulation, which represents a different formulation class as well as a different chemical form of imazapic; under current Agency policy, the results of trials reflecting a representative of each formulation type and/or major form of an active ingredient (e.g., the acid vs. salt) must be compared to determine if there is an effect of formulation type/chemical form on the relationship between application rate and residue level.

In support of postemergence use of imazapic on grasses, the petitioner should conduct four additional field trials reflecting a single postemergence application of the 2 lb ae/gal ammonium salt SC formulation at 0.1875 lb ae/A. Because it appears that residues may be higher in trials conducted in late summer or fall, the petitioner is advised to conduct the required trials during

these seasons. Additional field trial data are also required to support preemergence use of imazapic; however, because data from the single preemergence trial indicate that residues of imazapic and metabolites were below the method LOQ, and because residue decline data indicated that residues of imazapic in grass commodities declined fairly quickly following application, a reduced number of trials will be acceptable. Four additional trials are required reflecting preemergence application of the 2 lb ae/gal ammonium salt SC formulation at 0.1875 lb ae/A. These trials may be conducted side-by-side with the trials required to support postemergence use. In support of the 0.0625 lb ai/packet WDG acid formulation, field trials reflecting a 25% reduction in the number of trials required for the SC salt formulation would be appropriate to support registration of the acid formulation for use on grasses. Both postemergence and preemergence application should be represented, and side-by-side trials conducted with those required for the SC formulation are recommended.

The petitioner conducted 12 trials reflecting postemergence application to a variety of grass cultivars, including Bermuda grass, big blue stem grass, bromegrass, and mixtures of big bluestem and bromegrass, little bluestem and blue grass, bluegrass and little bluestem grass, and bluegrass and bromegrass. In eight trials, established grass stands received a single postemergence broadcast application made in the summer of the 2 lb ae/gal ammonium salt SC formulation at 0.2 lb ae/A (~1.1x the maximum proposed seasonal application rate). In three additional trials grasses received two postemergence broadcast applications of the 2 lb ae/gal ammonium salt SC formulation, with the first application made in the late summer or fall at 0.14-0.15 lb ae/A (~0.8x the maximum proposed seasonal application rate), and a second application made in the spring (following late summer application) or summer (following fall application) at 0.07 lb ae/A (0.4x the maximum proposed seasonal application rate); retreatment intervals were 9.3-9.4 months. In one trial, grasses received a single postemergence broadcast application made in the fall of the 2 lb ae/gal ammonium salt SC formulation at 0.14 lb ae/A (~0.7x the maximum proposed seasonal application rate).

The submitted data indicate that the combined residues of imazapic and its metabolites, CL 263284 and CL 189215, were <8.2-<23 ppm in/on grass forage harvested 0 days and 2.31-8.21 ppm in/on grass hay harvested 7 days following a single postemergence broadcast application of the 2 lb ae/gal ammonium salt SC formulation, made in the summer, at 0.20-0.21 lb ae/A (~1.1x the maximum proposed seasonal application rate). We note that in the four trials in which a postemergence application was made at 0.14-0.15 lb ae/A (~0.8x) during the late summer or fall, combined residues were 9.10-<25.12 ppm in/on grass forage harvested on the day of that application and <1.82-9.9 ppm in/on grass hay harvested 7 days after application.

With respect to residue decline requirements, based on data from additional sampling intervals (7, 14, 28, and 54-57 days) following postemergence application, residues of imazapic do not increase in/on grass forage and hay with increasing PHI. Residues of imazapic *per se* declined to levels below the method LOQ within 7-28 days of application, while residues of CL 263284 appeared to increase from Day 0 to Days 7-14, and then decrease to below the method LOQ by Days 14-28. Residues of CL 189215 were below the method LOQ at all sampling intervals.

The submitted data indicate that the combined residues of imazapic and its metabolites CL 263284 and CL 189215 were less than the LOQ (<1.50 ppm) in/on both grass forage and hay harvested 69-71 days following a single preemergence (at seeding) application of the 2 lb ae/gal ammonium salt SC formulation at 0.22 lb ae/A (~1.1x the maximum proposed seasonal application rate).

The available field trial data support tolerance levels for residues of imazapic and its metabolites CL 263284 and CL 189215 in/on grass forage at 30 ppm and grass hay at 15 ppm. However, these levels may be adjusted as necessary when the requested additional data have been submitted and evaluated. In the tolerance expression, HED recommends that the petitioner remove references to the form of imazapic applied. A revised Section F should be submitted.

### OPPTS GLN 860.1480: Meat/Milk/Poultry/Eggs

#### Dairy Cattle Feeding Study.

American Cyanamid has submitted a study (citation shown below) depicting the magnitude of imazapic residues in the milk and tissues of dairy cattle. The in-life phase of the study was conducted by American Cyanamid (Princeton, NJ), and the analytical phase was performed by Xenobiotic Laboratories (Plainsboro, NJ) for analysis of fat and milk fat and by ABC Laboratories (Columbia, MO) for milk and tissue analysis.

44817714 Leonard, R.C. (1999) CL 263222 (imazapic): Determination of CL 263222 and CL 263284 Residues in Milk, Milk Fat and Edible Tissues from Dairy Cattle After Oral Administration of CL 263222 for 28 Consecutive Days. Laboratory Project Identification CL 229; Cyanamid Number RES 98-129; ABC Labs Number 44768 and 44864; Xenobiotic Lab Number RPT00445. Unpublished study prepared by American Cyanamid Company. 212 p.

Three groups of Holstein dairy cows (3 cows/group) were dosed orally for 28 consecutive days with imazapic at target rates of 1.73 g/day, 5.2 g/day, and 17.3 g/day, equivalent to 84 ppm, 252 ppm, and 840 ppm, respectively, in the diet assuming a daily feed intake of 20 kg dry matter/day/livestock. An additional group of three cows served as the control. Actual mean doses were calculated to be 67 ppm, 232 ppm, and 676 ppm in the diet.

Following an acclimation period of ~3 weeks, lactating cows (individually stalled indoors) were dosed once daily with a gelatin capsule containing imazapic (96.9% a.i.). The capsules were administered via a balling gun following the afternoon milking. During the treatment period, cows were fed a commercial mixed dairy ration consisting of corn silage, alfalfa hay, and crude protein. The average daily feed consumption was recorded, and the dry matter was determined. Water was provided *ad libitum* throughout the study. The petitioner submitted adequate information pertaining to daily food consumption, milk production, and general health of the test livestock.

Cows were milked twice daily; p.m. and subsequent a.m. samples were composited for each livestock for each sampling day. Milk samples were stored refrigerated (2.2-5.8 C) until the next day, when subsamples were frozen ( $\leq -10$  C) for shipment to ABC Laboratories. Subsamples of milk collected on Days 8, 15, and 22 composited from each treatment group were separated into milk fat and skim milk by centrifugation. Milk fat samples were frozen and shipped to XenoBiotic Laboratories for analysis. Livestock were sacrificed within 24 hours of the final dose. Samples of liver, kidney, omental fat, and loin muscle were collected after sacrifice. Tissue samples were sliced and frozen (-15.1 to -19.7 C) until the next day, when tissue samples were ground in the presence of dry ice and stored frozen ( $\leq -10$  C). Tissue samples (except fat) were shipped to ABC Laboratories, and fat samples were shipped to XenoBiotic Laboratories for analysis. The storage conditions at the analytical laboratories were not specified; however the maximum storage intervals from collection until analysis were 185 days (6.1 months) for whole milk, 253 days (8.3 months) for milk fat, and 237 days (7.8 months) for tissues.

Based on the metabolites identified in the plant and livestock metabolism studies, milk and tissue samples were analyzed for residues of imazapic and its metabolite CL 263284. The collected whole milk and tissue (except fat) samples were analyzed for residues of imazapic and CL 263284 by ABC Laboratories using the previously described CE methods M 3188 (milk) and M 3222 (tissues except fat). The reported method LOQs for each analyte were 0.010 ppm for whole milk and 0.050 ppm for tissues (except fat). The collected milk fat and tissue fat samples were analyzed for residues of imazapic and CL 263284 by XenoBiotic Laboratories using the previously described HPLC/MS method M 3233. The reported method LOQs for each analyte were 0.010 ppm for milk fat and 0.050 ppm for tissue fat. Apparent residues of imazapic and its metabolite CL 263284 were each below the respective LOQ in all untreated whole milk (n=14), milk fat (n=3), liver (n=3), kidney (n=3), muscle (n=3), and fat (n=3) samples. Residues of imazapic and CL 263284 were detected in several untreated milk and tissue samples at levels below the method LOQ but above the method LOD; therefore, untreated kidney samples were analyzed by HPLC/MS at American Cyanamid to confirm that the apparent residues detected were not imazapic or CL 263284. Residues of imazapic and its metabolite CL 263284 in milk and tissue samples from all dosing levels are presented in Table 16; residue values are not corrected or adjusted for method recoveries.

Table 16. Residues of imazapic and its metabolite CL 263284 in milk and tissues from cows dosed daily with imazapic at levels equivalent to 67 (0.655x), 232 (2.19x), and 676 ppm (6.63x) in the diet for 28 consecutive days.

Dose Level, ppm	Dosing or Sampling Day	Uncorrected Residues, ppm		
		Imazapic	CL 263284	Total <sup>b</sup>
<b>Whole Milk</b>				
67	1	0.022, 0.0248 <sup>a</sup> , 0.030	<0.010, <0.010, <0.010	<0.032, <0.0348, <0.040
	2	0.020, 0.0302 <sup>a</sup> , 0.031	<0.010, <0.010, <0.010	<0.030, <0.0402, <0.041
	3	0.024, 0.0306 <sup>a</sup> , 0.026	<0.010, <0.010, <0.010	<0.034, <0.0406, <0.036
	6	0.021, 0.0135 <sup>a</sup> , 0.022	<0.010, <0.010, <0.010	<0.031, <0.0235, <0.032
	10	0.021, 0.029, 0.0258 <sup>a</sup>	<0.010, <0.010, <0.010	<0.031, <0.039, <0.0358
	15	0.0301 <sup>a</sup> , 0.035, 0.027	<0.010, <0.010, <0.010	<0.0401, <0.045, <0.037
	20	0.024, 0.023, 0.027	<0.010, <0.010, <0.010	<0.034, <0.033, <0.037
	24	0.030, 0.0337 <sup>a</sup> , 0.026	<0.010, <0.010, <0.010	<0.040, <0.0437, <0.036
223	27	0.021, 0.023, 0.0267 <sup>a</sup>	<0.010, <0.010, <0.010	<0.031, <0.033, <0.0367
	1	0.042, 0.070, 0.090	<0.010, <0.010, <0.010	<0.052, <0.080, <0.100
	2	0.055, 0.068, 0.120	<0.010, <0.010, <0.010	<0.065, <0.078, <0.130
	3	0.052, 0.073, 0.103	<0.010, <0.010, <0.010	<0.062, <0.083, <0.113
	6	0.065, 0.066, 0.096	<0.010, <0.010, <0.010	<0.075, <0.076, <0.106
	10	0.058, 0.078, 0.086	<0.010, <0.010, <0.010	<0.068, <0.088, <0.096
	15	0.066, 0.087, 0.093	<0.010, <0.010, <0.010	<0.076, <0.097, <0.103
	20	0.061, 0.072, 0.080	<0.010, <0.010, <0.010	<0.071, <0.082, <0.098
676	24	0.063, 0.084, 0.121	<0.010, <0.010, <0.010	<0.073, <0.094, <0.131
	27	0.075, 0.078, 0.085	<0.010, <0.010, <0.010	<0.085, <0.088, <0.095
	1	0.272, 0.277, 0.374	<0.010, <0.010, <0.010	<0.282, <0.287, <0.384
	2	0.220, 0.264, 0.316	<0.010, <0.010, <0.010	<0.230, <0.274, <0.326
	3	0.211, 0.339, 0.303	<0.010, <0.010, <0.010	<0.221, <0.349, <0.313
	6	0.192, 0.261, 0.289	<0.010, <0.010, <0.010	<0.202, <0.271, <0.299
	10	0.186, 0.260, 0.286	<0.010, <0.010, <0.010	<0.196, <0.270, <0.296
	15	0.171, 0.288, 0.354	<0.010, <0.010, <0.010	<0.181, <0.298, <0.364
20	0.195, 0.346, 0.316	<0.010, <0.010, <0.010	<0.205, <0.356, <0.316	
24	0.215, 0.307, 0.317	<0.010, <0.010, <0.010	<0.225, <0.317, <0.327	
27	0.226, 0.313, 0.304	<0.010, <0.010, <0.010	<0.236, <0.323, <0.314	

Dose Level, ppm	Dosing or Sampling Day	Uncorrected Residues, ppm		
		Imazapic	CL 263284	Total <sup>b</sup>
<b>Milk Fat (composited by group)</b>				
67	8	0.0147	<0.010	<0.0247
	15	0.0102	<0.010	<0.0202
	22	0.0142	<0.010	<0.0242
223	8	0.0391	<0.010	<0.0491
	15	0.0425	<0.010	<0.0525
	22	0.0302	<0.010	<0.0402
676	8	0.135	<0.010	<0.145
	15	0.121	<0.010	<0.131
	22	0.125	<0.010	<0.135
<b>Fat</b>				
67	29	<0.050, <0.050, <0.050	<0.050, <0.050, <0.050	<0.100, <0.100, <0.100
223		<0.050, <0.050, 0.054	<0.050, <0.050, <0.050	<0.100, <0.100, <0.104
676		<0.050, <0.050, 0.0532	<0.050, <0.050, <0.050	<0.100, <0.100, <0.1032
<b>Kidney</b>				
67	29	0.391 <sup>a</sup> , 0.436, 0.465	<0.050, <0.050, <0.050	<0.441, <0.486, <0.515
223		1.070, 1.430, 2.200	<0.050, <0.050, <0.050	<0.120, <1.480, <2.250
676		1.930, 2.560 <sup>a</sup> , 3.750	<0.050, <0.050, <0.050	<1.980, <2.610, <3.800
<b>Liver</b>				
67	29	<0.050, <0.050, <0.050	<0.050, <0.050, <0.050	<0.100, <0.100, <0.100
223		0.058, 0.0634, 0.126	<0.050, <0.050, <0.050	<0.108, <0.1134, <0.176
676		0.170, 0.176, 0.231	<0.050, <0.050, <0.050	<0.220, <0.226, <0.281
<b>Muscle</b>				
67	29	<0.050, <0.050, <0.050	<0.050, <0.050, <0.050	<0.100, <0.100, <0.100
223		<0.050, <0.050, 0.0674 <sup>a</sup>	<0.050, <0.050, <0.050	<0.100, <0.100, <0.1174
676		0.0831 <sup>a</sup> , 0.0780 <sup>a</sup> , 0.0857	<0.050, <0.050, <0.050	<0.1131, <0.1280, <0.1357

<sup>a</sup> The highest value of duplicate analyses is reported.

<sup>b</sup> Totals calculated by study reviewer.



### Maximum Theoretical Dietary Burden (MTDB)

Grass forage and hay constitute ruminant feed items (but are not fed to poultry or swine). Calculation of the maximum theoretical dietary burden (MTDB) based on the appropriate grass tolerance levels is given in Table 17.

Feed Item	Tolerance <sup>1</sup>	%DM <sup>2</sup>	% in Diet <sup>2</sup>	MTDB <sup>3</sup> (ppm)
			Beef and Dairy Cattle	Beef and Dairy Cattle
Grass Forage	30	25	60	72
Grass Silage <sup>4</sup>	30	40	40	30
TOTAL			100	102

<sup>1</sup> Tolerance level residue in ppm.

<sup>2</sup> The % dry matter (%DM) and % in diet values for each feed item were based on information contained in Table 1 of OPPTS Test Guidelines Series 860.1000.

<sup>3</sup> The maximum theoretical dietary burden for each feed item is calculated by multiplying (Tolerance/%DM) by the % of the feed item in the diet. The total MTDB is the sum of the individual feed item dietary burdens.

<sup>4</sup> Because no grass silage data were provided, the tolerance level from grass forage was translated as recommended by 860.1000.

Comparison of the MTDB to the maximum residue levels measured in the 28-day bovine feeding study shows that livestock commodity tolerances are needed since measured residues extrapolated to a 10x MTDB feeding rate exceed 0.010 ppm (Table 18). When normalizing the data in Table 18 to a 1x rate, the appropriate tolerance level for meat, fat, milk and meat byproducts is 0.10 ppm. For kidney, the expected residue at the 1x dose rate can be calculated using data from all three experimental dose rates:  $[(0.515 \text{ ppm}/0.655) + (2.25 \text{ ppm}/2.19) + (3.80 \text{ ppm}/6.63)] \div 3 = 0.80 \text{ ppm}$ . Thus, a tolerance level of 1.0 ppm should be adequate to cover the expected residue level in kidney based on the MTDB of 102 ppm.

Table 18. Maximum Residue Levels of Imazapic (CL 263222) and CL 263284 Found in 28-day Bovine Feeding Study Using the Indicated Imazapic Doses.

Matrix	66.8 ppm (0.655x) <sup>1</sup>			223 ppm (2.19x) <sup>1</sup>			676 ppm (6.63x) <sup>1</sup>		
	CL263222	CL263284	Total <sup>2</sup>	CL263222	CL263284	Total <sup>2</sup>	CL263222	CL263284	Total <sup>2</sup>
Muscle	<0.050	<0.050	<0.10	<0.0674	<0.050	<0.117	0.086	<0.050	<0.136
Liver	<0.050	<0.050	<0.10	0.126	<0.050	<0.176	0.231	<0.050	<0.281
Kidney	0.465	<0.050	<0.515	2.20	<0.050	<2.25	3.75	<0.050	<3.80
Fat	<0.05	<0.050	<0.10	<0.054	<0.050	<0.104	0.053	<0.050	<0.103
Milk	0.035	<0.010	<0.045	0.121	<0.010	<0.131	0.374	<0.010	<0.384

<sup>1</sup> Average dosing level of three dairy cows; exaggeration rate calculated by dividing dose level by the MTDB.

<sup>2</sup> Sum of CL263222 and CL263284 residues.

### Study summary

The submitted dairy cattle feeding study is tentatively determined to be acceptable; however, the tolerance levels in livestock commodities may need revision upon receipt of additional crop field trial data. When the field trial data have been submitted and evaluated, HED will reevaluate the feeding study and recommend tolerances for livestock commodities based on the MTDB for beef and dairy cattle.

In the submitted study, Holstein dairy cows were dosed orally once daily for 28 consecutive days with imazapic at dose levels equivalent to 67 ppm, 223 ppm, and 676 ppm. Detectable residues of imazapic were observed in samples of milk, milk fat, and kidney at all dose levels; residues of imazapic were less than the LOQ (<0.05 ppm) in samples of fat, liver, and muscle at the 67-ppm dosing level. Residues of the metabolite CL 263284 were less than the respective LOQs in all samples of milk and milk fat (<0.01 ppm), and tissues (<0.05 ppm) at all three dosing levels.

At the 67-ppm feeding level, the maximum combined residues of imazapic and CL 263284 were <0.045 ppm in milk, <0.0247 ppm in milk fat, <0.100 ppm in fat, liver, and muscle, and <0.515 ppm in kidney. At the 223-ppm feeding level, maximum combined residues were <0.131 ppm in milk, <0.0525 ppm in milk fat, <0.104 ppm in fat, <0.176 ppm in liver, <0.117 ppm in muscle, and <2.25 ppm in kidney. At the 676-ppm feeding level, maximum combined residues were <0.384 ppm in milk, <0.145 ppm in milk fat, <0.103 ppm in fat, <0.281 ppm in liver, <0.136 ppm in muscle, and <3.80 ppm in kidney.

Overall, residues of imazapic increased in milk and tissues with increasing in dose level. Residues in whole milk appear to plateau at Day 1 and did not significantly increase with subsequent doses. Residues in milk fat were lower than those in whole milk, confirming that residues do not tend to partition into fats. In tissues, residues were lowest in fat and highest in kidney.

### Conclusions

Based on the information presently available, the appropriate tolerance level for meat, fat, milk and meat byproducts (except kidney) is 0.10 ppm. For kidney, the appropriate tolerance level is 1.0 ppm. However, these levels may be adjusted as necessary when the requested additional grass field trial data have been submitted and evaluated. The HED MARC determined that for the tolerance expression the residues of concern in/on livestock commodities are imazapic and its metabolite CL 263284 (D275136, W. Donovan and W. Dykstra, 07-JUN-2001). **A revised Section F should be submitted.**

### Poultry Feeding Study

There are no poultry feed items associated with the proposed uses of imazapic on pasture and rangeland grasses; therefore, a poultry feeding study is not required, and tolerances on eggs and poultry tissues need not be proposed in conjunction with this petition request.

### OPPTS GLNs 860.1850: Confined Accumulation in Rotational Crops

American Cyanamid has submitted data from a study (citation listed below) investigating the metabolism of [<sup>14</sup>C]imazapic in rotational crops. The in-life phase of the study was conducted by American Agricultural Services, Inc. (Lucama, NC), and the analytical phase of the study was conducted by American Cyanamid Company (Princeton, NJ). A rotational crop study was previously submitted in conjunction with PP#3G4203/3H5669 (DP Barcodes D191715, D191694, and D191710, 3/10/94, F. Griffith). In that study, barley, carrots, cotton, corn, and lettuce were planted at 90- to 120-day plantback intervals (PBI) following soil treatment with [<sup>14</sup>C]imazapic in an aqueous soluble formulation at 0.064 lb ae/A.

44817706 Mallipudi, N. Moorthy (1999) CL 263222: Confined Rotational Crop Study with Carbon-14 CL 263222. Laboratory Project Identification MET 99-001: M97222NC1. Unpublished study prepared by American Cyanamid company. 288 p.

The radioactive test substance, [<sup>14</sup>C]imazapic labeled in the 6-position of the pyridine ring (radiochemical purity 98.8%), was mixed with [<sup>13</sup>C]imazapic and formulated with water as a liquid end-use product to obtain an active ingredient with a specific activity of 11.5  $\mu$ Ci/mg. Prairie grass (a mixture of Cheyenne Indian grass and big bluestem grass) seed was planted in outdoor plots of sandy loam soil (61% sand, 22% silt, 17% clay, 1.0% organic matter, and cation exchange capacity 5.6 meq/100 g). The next day the test substance was applied as a broadcast spray to the soil surface at 0.195 lb ae/A (~1x the maximum proposed seasonal application rate) in a spray volume of 40 gal/A. A separate plot was treated with formulation blank for controls. The treated and untreated plots were divided into four subplots for rotational crops: the plots remained fallow with prairie grass until the specified plantback interval (PBI). Prior to planting the rotational crops, the prairie grass was killed with a glyphosate herbicide, the dried grass was cut and removed, and the subplot rottilled to create a seedbed. Winter wheat was planted 181 DAT, spring wheat and carrots were planted 318 DAT, and lettuce was planted 363 DAT. The crops were fertilized, irrigated, and received maintenance pesticides as necessary. Adequate information concerning preparation of the test substance, field conditions, and plant maintenance was provided.

Samples of immature winter and spring wheat forage (elongation to early boot growth stage) were collected 175 and 70 days after planting (DAP), respectively. Samples of immature winter and spring wheat for hay were collected 220 and 83 DAP, respectively, and were dried for 8 days in a greenhouse. The remaining RACs were collected at maturity: 51 DAP for lettuce; 102 DAP for carrots; and 239 and 102 DAP for winter and spring wheat grain/straw, respectively. Lettuce and wheat samples were cut close to the soil; mature wheat was separated into straw (stems, leaves, and chaff) and grain. Carrots were pulled from the soil, loose soil was brushed off, and the roots and tops were separated. All samples were stored frozen (-33 to -16 C) at the field until shipment to the analytical laboratory. At the analytical laboratory samples were stored frozen (-35 to -10 C) until processing.

## Total radioactive residues (TRR)

All crops were frozen in liquid nitrogen and processed to a fine powder. Replicate aliquots of each rotational crop commodity were then subjected to combustion/LSC for TRR determinations. The TRR in rotational crop commodities are presented in Table 19. TRR in all control samples determined by combustion/LSC were <0.003 ppm, the limit of detection (LOD) for combustion/LSC analyses.

Table 19. Total radioactive residues in samples of rotational crop commodities grown in soil treated with [<sup>14</sup>C]imazapic at an application rate of 0.195 lb ae/A (- 1x the proposed maximum seasonal rate).

Crop	Plantback Interval, days	Crop Commodity	TRR, ppm [ <sup>14</sup> C]imazapic equivalents
Winter wheat	181	forage	<0.003
		hay	0.003
		straw	0.004
		grain	<0.003
Spring wheat	318	forage	<0.003
		hay	0.006
		straw	0.004
		grain	0.004
Carrot	318	tops	0.004
		roots	0.003
Lettuce	363	leaf	0.003

## Extraction of residues

Although no rotational crop samples from any of the plantback intervals contained >0.01 ppm TRR, samples of 181-DAT winter wheat hay and straw, 318-DAT spring wheat hay, straw, and grain, 318-DAT carrot root, and 363-DAT lettuce were subjected to extraction procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts and nonextractable residues were analyzed for radioactivity by LSC or combustion/LSC. The general extraction procedures are summarized below.

Subsamples of rotational crop commodities were sequentially extracted with acetone:water (1:1, v:v) and acetone. The acetone:water and acetone extracts were combined and concentrated for HPLC analysis. Nonextractable residues were extracted further with acetone:water:HCl (50:50:2, v:v:v) and acetone. The acidic acetone:water and acetone extracts were combined and concentrated.

The distribution of <sup>14</sup>C-activity in the extracts and hydrolysates of rotational crop commodities is presented in Table 20.

## Characterization/identification of residues

The acetone:water extracts of 181-DAT winter wheat hay and straw, 318-DAT spring wheat hay, straw, and grain, 318-DAT carrot root, and 363-DAT lettuce were analyzed by HPLC to determine the metabolic profile. Reversed-phase HPLC analyses were conducted on a Supelcosil LC-18 column using a gradient mobile phase of methanol and water, each containing 1% acetic acid. The HPLC system was equipped with a diode array detector (254 nm), and fraction collector for determination of radioactivity by LSC. Residues were identified by comparison of peak retention times to nonlabeled reference standards of imazapic, CL 263284, CL 189215, CL 312622, and CL 397695. See Attachment 2 for chemical structures and names of identified metabolites.

Table 20. Distribution and characterization of radioactive residues in rotational crop commodities grown in soil treated with [<sup>14</sup>C]imazapic at 0.195 lb ae/A (- 1x the maximum proposed seasonal rate).

Fraction	% TRR	ppm	Characterization/Identification *		
<b>181-DAT Winter wheat, hay (TRR = 0.003 ppm)</b>					
Acetone:water	36.6	0.001	<u>HPLC analysis resolved:</u>		
			Imazapic	2.2% TRR	<0.001 ppm
			CL 263284	6.4% TRR	<0.001 ppm
			CL 189215	1.5% TRR	<0.001 ppm
			CL 312622	0.7% TRR	<0.001 ppm
			CL 397695	0.5% TRR	<0.001 ppm
			Unknowns (11)	18.6% TRR	<0.001 ppm
Acetone:water:HCl	25.6	0.001	Not further analyzed (N/A).		
Nonextractable	66.8	0.002	N/A.		
<b>181-DAT Winter wheat, straw (TRR = 0.004 ppm)</b>					
Acetone:water	20.3	0.001	<u>HPLC analysis resolved:</u>		
			Imazapic	0.9% TRR	<0.001 ppm
			CL 263284	4.4% TRR	<0.001 ppm
			CL 189215	1.5% TRR	<0.001 ppm
			CL 312622	0.9% TRR	<0.001 ppm
			CL 397695	1.2% TRR	<0.001 ppm
			Unknowns (13)	10.2% TRR	<0.001 ppm
Acetone:water:HCl	11.2	<0.001	N/A.		
Nonextractable	54.8	0.002	N/A.		
<b>318-DAT Spring wheat, hay (TRR = 0.006 ppm)</b>					
Acetone:water	33.7	0.002	<u>HPLC analysis resolved:</u>		
			Imazapic	1.7% TRR	<0.001 ppm
			CL 263284	13.7% TRR	0.001 ppm
			CL 189215	5.6% TRR	<0.001 ppm
			CL 312622	0.8% TRR	<0.001 ppm
			CL 397695	0.8% TRR	<0.001 ppm
			Unknowns (7)	13.0% TRR	0.001 ppm
Acetone:water:HCl	21.9	0.001	N/A.		
Nonextractable	38.8	0.002	N/A.		
<b>318-DAT Spring wheat, straw (TRR = 0.004 ppm)</b>					
Acetone:water	50.6	0.002	<u>HPLC analysis resolved:</u>		
			Imazapic	1.1% TRR	<0.001 ppm
			CL 263284	4.2% TRR	<0.001 ppm
			CL 189215	2.7% TRR	<0.001 ppm
			CL 312622	2.1% TRR	<0.001 ppm
			CL 397695	5.3% TRR	<0.001 ppm
			Unknowns (9)	33.9% TRR	0.001 ppm
Acetone:water:HCl	12.9	0.001	N/A.		
Nonextractable	62.2	0.002	N/A.		

Table 20 (continued).

Fraction	% TRR	ppm	Characterization/Identification <sup>a</sup>
<b>318-DAT Spring wheat, grain (TRR = 0.004 ppm)</b>			
Acetone:water	22.3	0.001	<u>HPLC analysis resolved:</u>
			Imazapic 3.2% TRR <0.001 ppm
			CL 263284 3.0% TRR <0.001 ppm
			CL 189215 4.5% TRR <0.001 ppm
			CL 312622 1.0% TRR <0.001 ppm
			CL 397695 0.4% TRR <0.001 ppm
Unknowns (11) 8.8% TRR <0.001 ppm			
Acetone:water:HCl	6.8	<0.001	N/A.
Nonextractable	70.3	0.003	N/A.
<b>318-DAT Carrot, root (TRR = 0.003 ppm)</b>			
Acetone:water	52.3	0.002	<u>HPLC analysis resolved:</u>
			Imazapic 13.0% TRR <0.001 ppm
			CL 263284 1.5% TRR <0.001 ppm
			CL 189215 0.9% TRR <0.001 ppm
			CL 312622 0.6% TRR <0.001 ppm
			CL 397695 1.2% TRR <0.001 ppm
Unknowns (11) 30.9% TRR 0.001 ppm			
Acetone:water:HCl	11.0	<0.001	N/A.
Nonextractable	33.7	0.001	N/A.
<b>363-DAT Lettuce (TRR = 0.003 ppm)</b>			
Acetone:water	78.4	0.002	<u>HPLC analysis resolved:</u>
			Imazapic 3.0% TRR <0.001 ppm
			CL 263284 24.5% TRR 0.001 ppm
			CL 189215 8.9% TRR <0.001 ppm
			CL 312622 2.0% TRR <0.001 ppm
			Unknowns (12) 33.3% TRR 0.001 ppm
Acetone:water:HCl	13.3	<0.001	N/A.
Nonextractable	45.6	0.001	N/A.

<sup>a</sup> Metabolites were identified by HPLC; refer to Attachment 2 for chemical names and structures of identified residues.

### Storage stability

Rotational crop samples were analyzed for TRR within 13-36 days of harvest, and extracts were analyzed by HPLC within 41-51 days of harvest. Storage stability data are not required to support the rotational crop study because samples were analyzed within 2 months of harvest.

### Proposed metabolic pathway

Based on the rotational crop study, the petitioner proposes that metabolism of imazapic in rotated crops is similar to that in the primary crop. Imazapic undergoes oxidative hydroxylation of the 5-methyl substituent of the pyridine ring to form the 5-hydroxymethyl metabolite CL 263284. CL 263284 is then either rapidly conjugated with glucose to form CL 189215 or further oxidized to form a 3,5-dicarboxylic acid derivative (CL 312622).

### Study summary

The submitted confined rotational crop study is adequate. The TRR, expressed as [<sup>14</sup>C]imazapic equivalents, did not accumulate at levels  $\geq 0.01$  ppm in/on the RACs of winter wheat, spring wheat, carrots, and lettuce planted in sandy loam soil 181, 318, and 363 days after treatment (DAT), respectively, of the soil with [<sup>14</sup>C]imazapic at 0.195 lb ae/A (~1x the maximum proposed seasonal rate for grasses).

Although the TRR in all rotational crops at the various PBIs were  $< 0.01$  ppm, the petitioner subjected crop commodities with TRR  $> 0.003$  ppm to characterization/identification procedures. Residues of imazapic, CL 263284, CL 189215, and CL 312622 were identified in all rotational crop matrices at  $\leq 0.001$  ppm. Imazapic accounted for 0.9-13.0% TRR in rotational crop commodities, and was the major metabolite identified in 318-DAT carrot root. CL 263284 accounted for 1.5-24.5% TRR, and was the major metabolite identified in 181-DAT winter wheat hay and straw, 318-DAT spring wheat hay and straw, and 363-DAT lettuce. CL 189215 accounted for 1.5-8.9% TRR, and was the major metabolite identified in 318-DAT spring wheat grain. CL 312622 was identified at  $\leq 2.1\%$  TRR, and CL 397695 was identified ( $\leq 5.3\%$  TRR,  $< 0.001$  ppm) in all rotational crop matrices except for lettuce. Based on the components identified, the study results suggest that imazapic is metabolized through the same routes in rotational crops as in the primary crop.

Currently, the label for the 2 lb ae/gal ammonium salt SC formulation specifies the following minimum PBIs for rotational crops: 4 months following application for Bahiagrass, rye, and wheat; 9 months following application for field corn, snap beans, southern peas, soybeans, and tobacco; 18 months following application for barley, cotton, sorghum grain, oats, and sweet corn; and 40 months following application for canola, potatoes, red table beets, and sugar beets. All other crops for which a minimum PBI is not specified may be planted 26 months following application.



HED notes that the 4-month PBI for bahiagrass, rye, and wheat was based on confined rotational crop data submitted in support of the peanut petition (use rate = 0.0625 lb ae/A). As the present petition for pasture and rangeland use involves an application rate three times higher than the peanut use rate, the confined rotational crop data at 0.0625 lb ae/A can not be translated to the Plateau labels for grass use.

The submitted rotational crop data reflecting the grass use rate support establishment of a 6-month PBI for small grains, an 11-month PBI for root and tuber crops, and a 12-month PBI for leafy vegetables. The proposed 4-month PBI for rye and wheat and 9-month PBI for legume vegetables are not currently supported by rotational crop data. If the petitioner wishes to establish PBIs less than those reflected in the current confined rotational crop study, limited field rotational crop studies or additional confined rotational crop studies making use of the grass application rate *and* desired PBI are recommended. Alternatively, the petitioner may increase the rye and wheat PBIs from 4 to 6 months, and increase the PBI for legume vegetables to 12 months. **The petitioner should submit additional rotational crop data or a revised Section B with updated rotational crop intervals for rye, wheat, and legume vegetables.**

#### Codex Issues

There are currently no established Codex, Canadian, or Mexican maximum residue limits (MRLs) for residues of imazapic in/on plant or livestock commodities (see Attachment 3). Therefore, no compatibility issues exist with regard to the proposed U.S. tolerances discussed in this petition review.

AGENCY MEMORANDA CITED IN THIS REVIEW

DP Barcodes: D195919 and D198247  
Subject: PP# 3G4203/3H5669 - CL 263.222, Ammonium Salt (Cadre®) In Peanut Nutmeats And Peanut Hulls. Review of Product Chemistry Data.  
From: F. Griffith  
To: R. Taylor  
Dated: 2/2/94  
MRID(s): 42711401-42711406.

DP Barcodes: D191715, D191694, and D191710  
Subject: PP# 3G4203/3H5669 - CL 263.222, Ammonium Salt (Cadre®) In Peanut Nutmeats And Peanut Hulls. Review of EUP Application, Residue Data, and Analytical Method.  
From: F. Griffith  
To: R. Taylor and A. Kocialski  
Dated: 3/10/94  
MRID(s): 42711439-42711443 and 42711447.

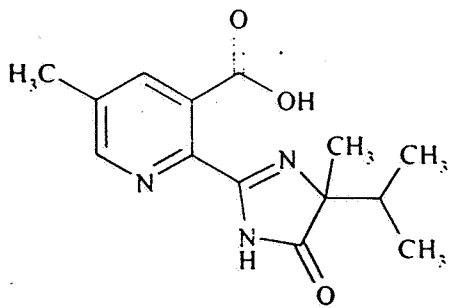
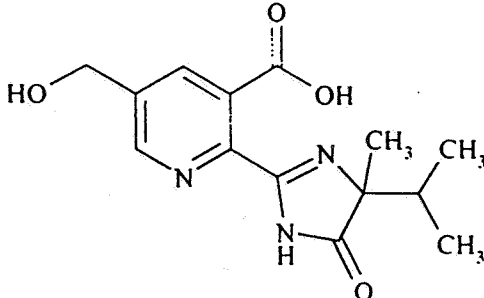
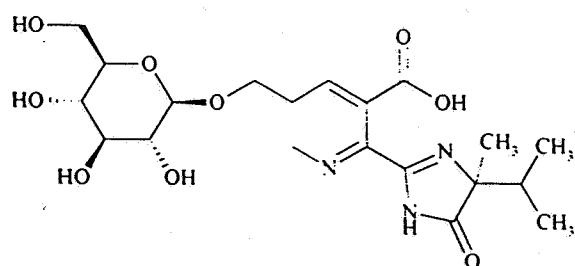
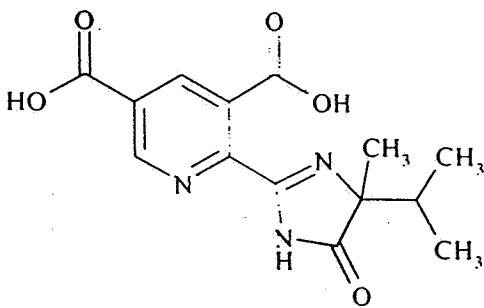
DP Barcodes: D211846  
Subject: Multiresidue Method (MRM) Validation Data for CL 263.222 (Cadre®).  
From: F. Griffith  
To: B. McMahon  
Dated: 2/9/95  
MRID(s): 43320322

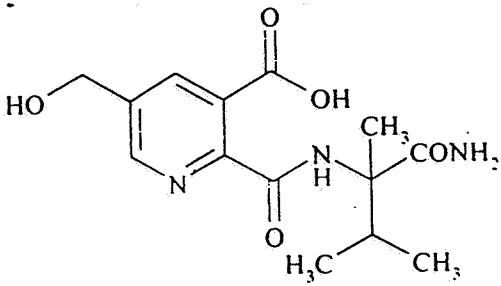
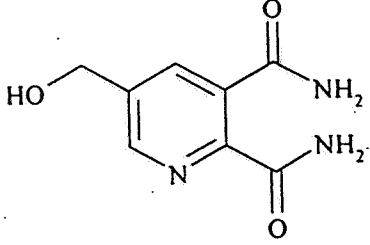
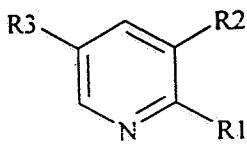
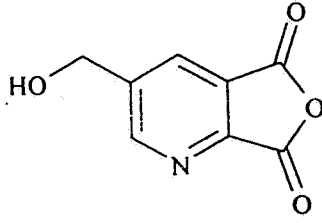
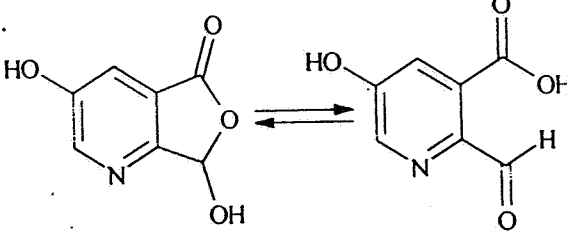
DP Barcodes: D207019, D207047, D207079, D209892, D209899, D211609, and D211980  
Subject: PP No. 4F4390: Ammonium Salt of CL 263,222 (CADRE) in/on Peanut RAC's. Evaluation of Analytical Methods and Residue Data.  
From: J. Garbus  
To: K. Hicks/R. Taylor and W. Hazel  
Dated: 9/15/95  
MRID(s): 43320300-43320303, 43320316-43320320, 43320324, and 43320325.

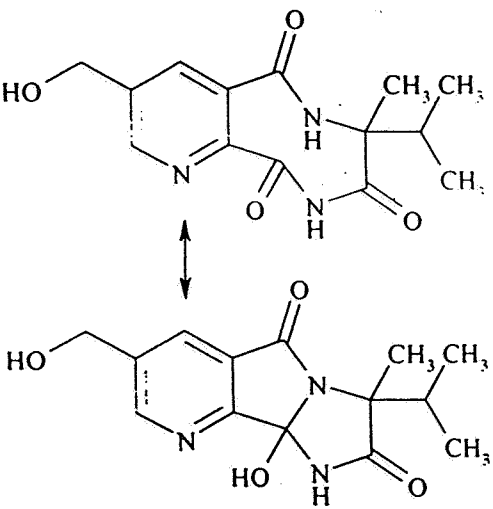
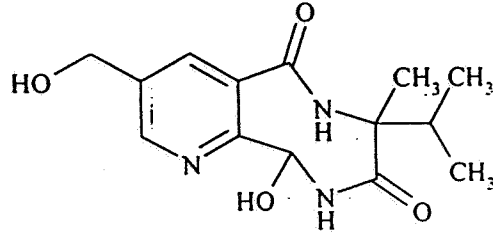
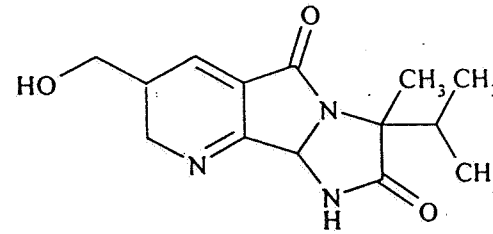
DP Barcodes: D222184  
Subject: PP No. 4F4390: Ammonium Salt of CL 263,222 (CADRE) in/on Peanut RAC's. Amendments Proposing Revised Sections B and F and Responses to Deficiencies Noted in CBTS's Review of 9/18/95.  
From: J. Garbus  
To: K. Hicks/R. Taylor and B. Madden  
Dated: 2/6/96  
MRID(s): None

DP Barcodes: D256433  
Subject: ID# 99NE0009. Section 18 Exemption For The Use Of Imazapic-Ammonium On  
**Pasture/Rangeland In Nebraska.**  
From: W. Dykstra, W. Donovan, and M. Christian  
To: L. Pemberton/R. Forrest  
Dated: 7/8/99  
MRID(s): None

Attachment 2. Chemical names and structures of imazapic and its metabolites in plants (grass and rotational crops) and livestock (goats).

Common Name/Number	Chemical Structure	Matrices
<p><b>Imazapic (CL 263222)</b></p> <p>2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid</p>		<p>Grass forage and hay</p> <p>Goat milk, kidney, liver, and muscle</p> <p>Rotated winter wheat hay and straw, spring wheat hay, straw, and grain, carrot root, and lettuce</p>
<p><b>CL 263284</b></p> <p>2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-hydroxymethyl-3-pyridinecarboxylic acid</p>		<p>Grass forage and hay</p> <p>Rotated winter wheat hay and straw, spring wheat hay, straw, and grain, carrot root, and lettuce</p>
<p><b>CL 189215</b></p> <p>nicotinic acid, 5-[β-glucopyranosyloxy)methyl]-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)</p>		<p>Grass forage and hay</p> <p>Rotated winter wheat hay and straw, spring wheat hay, straw, and grain, carrot root, and lettuce</p>
<p><b>CL 312622</b></p> <p>2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3,5-pyridine dicarboxylic acid</p>		<p>Rotated winter wheat hay and straw, spring wheat hay, straw, and grain, carrot root, and lettuce</p>

Common Name/Number	Chemical Structure	Matrices
<p><b>CL 397695</b></p> <p>nicotinic acid, 2-[(1-carbamoyl-1,2-dimethylpropyl)-carbamoyl]-5-hydroxymethyl-</p>		<p>Rotated winter wheat hay and straw, spring wheat hay, straw, and grain, and lettuce</p>
<p><b>M1-B<sup>a</sup></b></p> <p>5-hydroxymethyl-2,3-pyridine-dicarboxamide</p>		<p>Grass forage and hay</p>
<p><b>M1-C<sup>a</sup></b></p> <p>hydroxy-pyridinedicarboxylic acid</p>	 <p>R1 = COOH R2 = COOH R3 = OH  or R1 = COOH R2 = OH R3 = COOH  or R1 = OH R2 = COOH R3 = COOH</p>	<p>Grass forage and hay</p>
<p><b>M2-A<sup>a</sup></b></p> <p>5-hydroxy-2,3-pyridine-dicarboxylic anhydride</p>		<p>Grass forage and hay</p>
<p><b>M2-B<sup>a</sup></b></p> <p>2-formyl-5-hydroxy-nicotinic acid or its tautomer 3,7-dihydroxy-furo[3,4-b]pyridine-5(7H)-one</p>		<p>Grass forage and hay</p>

Common Name/Number	Chemical Structure	Matrices
<p><b>M6-D/M6-H <sup>‡</sup></b></p> <p>hydroxy-imidazopyrrolopyridine and pyridodiazocine derivatives.</p>		<p>Grass forage and hay</p>
<p><b>M6-J2 <sup>‡</sup></b></p> <p>hydroxy-pyridodiazocine derivative</p>		<p>Grass forage and hay</p>
<p><b>M6-J3 <sup>‡</sup></b></p> <p>imidazopyrrolopyridine derivative</p>		<p>Grass forage and hay</p>

<sup>‡</sup> Tentatively identified as a minor metabolite.

INTERNATIONAL RESIDUE LIMIT STATUS			
Chemical Name: (±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid	Common Name: Imazapic (ISO 1750 (provisional))	<input checked="" type="checkbox"/> Proposed tolerance <input type="checkbox"/> Reevaluated tolerance <input type="checkbox"/> Other	Date: 5/2/01
Codex Status (Maximum Residue Limits)		U. S. Tolerances	
<input checked="" type="checkbox"/> No Codex proposal step 6 or above <input type="checkbox"/> No Codex proposal step 6 or above for the crops requested		Petition Number: 9F05092 DP Barcode: D269038 Other Identifier:	
Residue definition (step 8/CXL): N/A		Reviewer/Branch: W. Donovan/RABI	
		Residue definition: Imazapic and its hydroxymethyl metabolites CL 263284 and CL 189215	
Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		grass forage	30
		grass hay	15
		meat, fat, meat byproducts (except kidney), and milk	0.10
		kidney	1.0
		peanuts	0.1
Limits for Canada		Limits for Mexico	
<input checked="" type="checkbox"/> No Limits <input type="checkbox"/> No Limits for the crops requested		<input checked="" type="checkbox"/> No Limits <input type="checkbox"/> No Limits for the crops requested	
Residue definition: N/A		Residue definition: N/A	
Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)
Notes/Special Instructions: S. Funk. 05/07/2001			