



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

**MEMORANDUM**

Date: 20-MAR-2003

Subject: Imazapyr. Section 3 Registration Request for Use in/on Aquatic Sites and Grasslands. Summary of Analytical Chemistry and Residue Data.

DP Barcode: D275561

Case No.: 293089

PC Code: 128821

Submission: S598694

40 CFR 180. 500

Petition No.: 0F06166

From: William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

Through: G. Jeffrey Herndon, Branch Senior Scientist  
Registration Action Branch 1 (RAB1)  
Health Effects Division (HED) (7509C)

*G. Jeffrey Herndon*

To: Jim Tompkins, PM Team 25  
Registration Division (RD) (7505C)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

BASF has submitted a petition is proposing uses for imazapyr (2 lb ae/gal AS) on pasture and rangeland grasses for the control of undesirable vegetation and on aquatic freshwater sites for the control of floating or emergent vegetation. In conjunction with these uses, BASF is proposing the establishment of permanent tolerances for residues of imazapyr [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid] applied as the isopropylamine salt, in/on the following raw plant and animal commodities:

|                                                                           |           |
|---------------------------------------------------------------------------|-----------|
| Grass, forage .....                                                       | 125.0 ppm |
| Grass, hay .....                                                          | 35.0 ppm  |
| Fish, freshwater finfish .....                                            | 1.0 ppm   |
| Shellfish .....                                                           | 0.1 ppm   |
| Fat of cattle, sheep, goats, and horses .....                             | 0.05 ppm  |
| Kidney of cattle, sheep, goats, and horses .....                          | 0.5 ppm   |
| Meat byproducts (except kidney) of cattle, sheep, goats, and horses ..... | 0.05 ppm  |



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|                                                                               |           |
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| Grass, hay . . . . .                                                          | 35.0 ppm  |
| Fish, freshwater finfish . . . . .                                            | 1.0 ppm   |
| Shellfish . . . . .                                                           | 0.1 ppm   |
| Fat of cattle, sheep, goats, and horses . . . . .                             | 0.05 ppm  |
| Kidney of cattle, sheep, goats, and horses . . . . .                          | 0.5 ppm   |
| Meat byproducts (except kidney) of cattle, sheep, goats, and horses . . . . . | 0.05 ppm  |

|                                                    |          |
|----------------------------------------------------|----------|
| Meat of cattle, sheep, goats, and horses . . . . . | 0.05 ppm |
| Milk . . . . .                                     | 0.01 ppm |

## Executive Summary

Provided that 1) the petitioner submits an acceptable fish metabolism study, 2) the petitioner submits adequate corn or grass storage stability information, 3) the petitioner specifies the identity and quantity of spray additives used in all of the grass field trials, 4) the analytical enforcement method is determined to be adequate, 5) Section F is revised to include the recommended tolerance levels, and 6) Section B is revised to prohibit use of treated waters on irrigated crops or residue data is provided for irrigated crops, HED concludes there are no residue chemistry data requirements that would preclude the establishment of the recommended tolerance levels. A human-health risk assessment will be prepared as a separate document.

## Residue Chemistry Deficiencies

The following residue chemistry deficiencies should be resolved before any new permanent tolerances are established for imazapyr:

- Revised Section B or irrigated crop residue data
- Revised Section F
- Enforcement method validation

The following residue chemistry deficiencies should be resolved as a condition of registration:

- Fish metabolism study
- Corn or grass storage stability information/study
- Additional spray additive information supporting the grass field trials

## Background

Imazapyr is a broad spectrum, imidazolinone herbicide used for pre- and post-emergence control of annual and perennial grass and broadleaf weeds, brush, vines, and deciduous trees. Imazapyr is currently registered to BASF Corporation for use on non-cropland areas and on imidazolinone-resistant field corn. End-use products currently registered to BASF include aqueous solution (AS) and ready-to-use (RTU) formulations of imazapyr, formulated as a isopropylamine salt.

For the use on field corn, the HED Metabolism Committee concluded (N. Dodd, 2/26/97) that the residues of concern in field corn and animal commodities included only the parent compound. Tolerances for residues of imazapyr in or on plant and animal commodities are currently expressed as parent imazapyr. Permanent tolerances have been established for residues in/on field corn forage, grain and stover each at 0.05 ppm [40 CFR §180.500].

For the proposed uses of imazapyr on grasses and aquatic systems, the HED Metabolism Assessment Review Committee (MARC) determined that the residue of concern for risk assessment purposes and the tolerance expression is imazapyr *per se* in/on the following matrices: plants (primary and rotational crops), livestock commodities, fish and shellfish, and drinking water (TXR# 0051641, W. Donovan and E. Rinde, 13-MAR-2003).

### 860.1200 Directions for Use

| Table 1. Summary of Proposed Directions for Use of Imazapyr.                            |                                            |                                                |                            |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-----------------------------------------------------------------------------------------|--------------------------------------------|------------------------------------------------|----------------------------|---------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Applic. Timing, Type, and Equip.                                                        | Formulation <sup>a</sup><br>[EPA Reg. No.] | Maximum Applic. Rate<br>(lb ae/A) <sup>b</sup> | Max. No. Applic/<br>Season | PHI<br>(days) | Use Directions and Limitations <sup>c</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Grass Pasture and Rangeland</b>                                                      |                                            |                                                |                            |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <u>Spot treatment</u> ground application<br>Ground equipment                            | 2 lb ae/gal EC<br>[241-346]                | 0.75 lb ae/<br>acre                            | NS                         | 7             | Applications may not exceed more than 1/10 of a given acre, therefore the maximum rate per acre is 0.075 lb ae/A. Do not cut forage for hay for 7 days after application.<br>Rotational crops: 12 months after application, a successful field bioassay must be completed. If no crop injury is evident in the bioassay, then the intended rotational crop may be planted the following year.<br>Postemergence applications require the addition of a spray adjuvant (nonionic surfactant or methylated seed oils or vegetable oil concentrates). |
| <b>Aquatic (fresh water) <sup>d</sup></b>                                               |                                            |                                                |                            |               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Broadcast application to aquatic areas or draw down area<br>Surface or aerial equipment | 2 lb ae/gal EC<br>[241-346]                | 1.5                                            | NS                         | NA            | Do not apply to marine or estuarine areas. Do not apply within ½ mile (standing water) or within ½ mile upstream (flowing water) of an <u>active</u> irrigation or potable water intake.<br>For application, within ½ mile of a water intake, the water intake must be turned off for a minimum of 48 hours after the application.<br>Allow 1 hour after treatment before refilling draw down area.<br>Apply in a minimum of volume of 5 gal/A.                                                                                                   |

<sup>a</sup> The product is an isopropylamine salt of imazapyr, the active ingredient is listed as equivalent to 2 lb ae/gal.

<sup>b</sup> Applications require the use of a nonionic surfactant (minimum 0.25% v/v) or a methylated seed oil or vegetable oil concentrate (2 pints/A).

<sup>c</sup> The label specifies a restricted entry interval of 12 hours.

<sup>d</sup> Aquatic use sites included: lakes, rivers, streams, ponds, seeps, drainage ditches, canals, reservoirs, swamps, bogs, marshes, transitional areas between terrestrial and aquatic sites, seasonal wet areas, and similar non-crop areas.

Provided that the petitioner submits residue data for irrigated crops or adds a label restriction that prohibits the use of treated water for irrigation purposes for 120 days following application or demonstrates non-detectable residue levels of imazapyr in irrigation water by laboratory analysis prior to use, the proposed use directions adequately reflect the use pattern from the grass field trials and the data from the aquatic studies.

### **860.1300 Nature of the Residue - Plants**

For purposes of this petition, the qualitative nature of imazapyr residues in plants is understood based upon the adequate corn and grass metabolism studies. An acceptable corn metabolism study was submitted in conjunction with the earlier petition for use on corn (D222027, N. Dodd, 6/26/96), and an adequate bermuda grass metabolism study was submitted with the current petition (discussed below). In both the corn and grass metabolism studies, imazapyr was the principal residue identified in each commodity. Based on the minor metabolites identified, the metabolism of imazapyr in plants primarily involves esterification of the carboxylic acid of the parent molecule to form CL 240000 and/or CL 247087 (cyclization product), and hydrolysis of the imidazolinyl ring to form pyridine dicarboxylic acid (CL 9140).

For the use on field corn, the HED Metabolism Committee has previously concluded (N. Dodd, 2/26/97) that the residues of concern in field corn commodities include only the parent compound. In a meeting of the HED Metabolism Assessment Review Committee (MARC) held 26-FEB-2003, the residue of concern in plants (primary and rotational crops) was determined to be imazapyr *per se* (TXR# 0051641, W. Donovan and E. Rinde, 13-MAR-2003).

#### **45119715.der.wpd**

Field-grown bermuda grass was treated with a single broadcast foliar application of [<sup>14</sup>C-6-pyridine]imazapyr at 1.5 lb ae/A (2x proposed use rate). Samples of grass foliage were collected 0, 4, 10, 15, and 21 days after treatment (DAT); hay was not collected.

Total radioactive residues (TRR) were highest at 0 DAT (64.29 ppm) and declined to 17.5-24.9 ppm at 4-15 DAT. TRR levels increased substantially at 21 DAT (47.7 ppm); however, the petitioner attributed this increase to water loss from senescence and phytotoxicity in the sample.

Methanolic extraction released 85.8-97.3% of the TRR from all foliage samples and subsequent enzymatic extraction and/or acid and base hydrolyzation released an additional 1.0-8.9% of the TRR. Radioactivity remaining in the residual solids accounted for <1.5% of the TRR. Isolated metabolites or fractions were characterized and identified by TLC and HPLC analysis with reference standards and by GC/MS or LC/MS. Analyses of solvent extracts identified parent imazapyr and three metabolites, along with numerous minor unknown components. The major extractable <sup>14</sup>C-residue was identified as parent (78.1-96.7% TRR). All other metabolites

accounted for a substantially lower percentage of the TRR: CL 9140 (0.08-12.8% TRR), combined CL 240000 and CL 247087 (0.20-10.5% TRR), and minor discrete unknowns (each <3% TRR).

### 860.1300 Nature of the Residue - Livestock

Adequate studies are available depicting the metabolism of imazapyr in poultry and ruminants. These studies were previously submitted and reviewed in conjunction with the petition for use on field corn (PP#6F04641, D222027, N. Dodd, 6/26/96).

In the poultry metabolism study, two groups of hens were dosed orally for 7 days with [6-pyridine-<sup>14</sup>C]imazapyr at levels equivalent to 1.98 ppm or 9.72 ppm (~50x and 240x the maximum theoretical dietary burden (MTDB)). The TRR were <0.01 ppm (<LOD) in eggs, liver, kidneys, muscle, and skin with adhering fat from both dose groups. Imazapyr *per se* was the sole radioactive component identified in the excreta of treated hens.

In the goat metabolism study, two goats were dosed orally for 7 days with [6-pyridine-<sup>14</sup>C]imazapyr at levels equivalent to 17.7 ppm or 42.5 ppm in the diet (0.7x or 1.8x the MTDB). The TRR ranged from <0.01 ppm-0.02 ppm in milk and were 0.08 ppm and 0.11 ppm, respectively, in the kidneys of the low and high dose goats. The TRR in the remaining tissues (fat, liver, and leg and loin muscle) were nondetectable (<0.05 ppm) and were not further characterized. The study sufficiently characterized and identified detectable residues in extracts of milk and kidney by TLC and HPLC. Imazapyr *per se* was the sole radioactive component identified, comprising ~50% of TRR in milk and ~95% of TRR in kidney.

The petitioner conducted a second goat metabolism study (discussed below) to generate <sup>14</sup>C-labeled samples for radiovalidation of the proposed enforcement method. In this study, a single goat was dosed orally for 7 days with [imidazole-5-<sup>14</sup>C]imazapyr at a level equivalent to 46.9 ppm in the diet (2x) and only milk and kidney samples were collected for analysis. TRR were 0.014-0.016 ppm in milk and 0.074 ppm in kidneys, and parent compound accounted for 65.6% of the TRR in milk and 81.9% of the TRR in kidney. This study was also adequate and confirmed the finding of the earlier goat metabolism study.

The HED Metabolism Committee concluded (N. Dodd, 2/26/97) that the residues of concern in animal commodities included only the parent compound. In a meeting of the HED MARC held 26-FEB-2003, the residue of concern in livestock (ruminants) was confirmed to be imazapyr *per se* (TXR# 0051641, W. Donovan and E. Rinde, 13-MAR-2003).

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A single lactating goat was dosed orally once a day for 7 consecutive days via gelatin capsules with [imidazole-5-<sup>14</sup>C]imazapyr at a dose of 90.6 mg ae/day, equivalent to 46.9 ppm in the diet. Urine, feces, and milk were collected daily and kidney samples were collected at sacrifice, ~21 hours after administering the final dose.

A total of 93.3% of the dosed radioactivity was excreted in the urine (58.7% dose) and feces (34.4% dose), and another 0.04% of the dose was excreted in the milk. Radioactivity remaining in the kidneys at sacrifice accounted for <0.01% of the dose. TRR were 0.014-0.016 ppm in milk and 0.074 ppm in kidneys. Radioactive residues in milk plateaued by Day-1 at 0.015 ppm.

Analyses of solvent extracts (82.0-93.5% TRR) identified parent imazapyr (AC 243997) as the major extractable <sup>14</sup>C-residue, and 3-5 unknown HPLC regions and/or peaks. Parent compound accounted for 65.6% of the TRR in 7-Day milk and 81.9% of the TRR in kidney. Minor unknown regions and/or peaks accounted for <6.5% TRR.

In the currently reviewed study, only milk and kidney were analyzed and the dose rate was slightly higher than the previous study (47 vs 43 ppm). The study was conducted to generate residues in the kidney and milk to be used to conduct an extraction efficiency/accountability study. The current goat metabolism study is adequate for this purpose.

### **860.1340 Residue Analytical Methods**

Two methods are currently listed in the Pesticide Analytical Manual (PAM) Vol. II for enforcing tolerances of imazapyr in/on corn commodities. Method M 2468 is a GC/MS method with a limit of quantitation (LOQ) of ~0.01 ppm for imazapyr in/on corn grain, forage and fodder, and Method M 2657 is a capillary electrophoresis (CE) method with UV detection that has a LOQ of 0.05 ppm for imazapyr in/on corn grain, forage and fodder.

The petitioner is proposing a series of CE/UV Methods as enforcement methods for determining imazapyr in/on grass forage and hay (Method M 3023), in livestock tissues (Method M 3184), in milk and milk fat (Methods M 3075 and M 3223), and in fish and shellfish tissues (Method M 3066). These methods are similar to the current enforcement method M 2657, and each of these methods also include directions for a confirmatory analysis using LC/MS.

Based on concurrent method recovery data submitted with the grass field trials, the cattle feeding study, and the bioaccumulation studies for fish and shellfish, the above CE/UV methods are adequate for collecting data on residues of imazapyr in grass forage and hay, cattle tissues and milk, and fish and shellfish (see discussion below). The validated LOQs for imazapyr in the respective CE/UV methods are 0.5 ppm in/on grass forage and hay, 0.05 ppm in cattle tissues, fish and shellfish, and 0.01 ppm in milk and milk fat.

Each of the above methods has undergone a successful independent laboratory validation (ILV) trial. Adequate radiovalidation data were also submitted for CE/UV methods M 3066, M 3075, and M 3185, demonstrating the efficiency of these methods in extracting residues from aged samples.

The CE/UV Methods M 3023, M 3184, M 3075, and M 3066 have been forwarded to the Analytical Chemistry (ACB) for petition method validation (PMV) trials (D288863, W. Donovan, 14-MAR-2003). Conclusions regarding the suitability of the proposed enforcement methods will be deferred until completion of the PMV trials.

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A series of ILV trials were conducted on the following potential enforcement methods: (i) a CE/UV detection method (Method M 3023) for determining imazapyr in grass forage and hay; (ii) a CE/UV method (Method M 3184) for determining imazapyr in cattle tissues; (iii) a CE/UV method (Method M 3075) for determining imazapyr in milk; (iv) a CE/UV method (Method M 3223) for determining imazapyr in milk fat; and (v) a CE/UV method (Method M 3066) for determining imazapyr in fish and crayfish. In addition, three of these CE/UV methods (M 3075, M 3184, and M 3066) were radiovalidated using samples from the goat and oyster metabolism studies.

Each of these CE/UV methods was successfully validated by an independent laboratory. The average recovery of imazapyr from grass forage and hay fortified at 0.5-50.0 ppm was  $84 \pm 2.4\%$  (n=16) using CE/UV Method M 3023. The average recoveries of imazapyr from cattle tissues fortified at 0.05-0.50 ppm were  $80 \pm 4.8\%$  from muscle (n=6),  $80 \pm 4.4\%$  from liver (n=6),  $79 \pm 5.4\%$  from kidneys (n=6), and  $82 \pm 6.2\%$  from fat (n=6) using CE/UV Method M 3184. The average recovery of imazapyr from milk fortified at 0.01-0.50 ppm was  $90 \pm 5.6\%$  (n=6) using CE/UV Method M 3075, and the average recovery of imazapyr from milk fat fortified at 0.01-0.10 ppm was  $78 \pm 9.5\%$  (n=6) using CE/UV Method M 3223. The average recoveries of imazapyr from fish and shellfish fortified at 0.05-0.50 ppm were  $84 \pm 4.1\%$  from fish fillets (n=6) and  $84 \pm 11\%$  from crayfish meat (n=6) using CE/UV Method M 3066, which is essentially identical to Method M 3184.

The validated LOQs for imazapyr are 0.5 ppm in/on grass forage and hay, 0.05 ppm in cattle tissues, fish and shellfish, and 0.01 ppm in milk and milk fat.

Adequate radiovalidation data were also submitted for the proposed CE/UV methods (M 3066, M 3075, and M 3185) and demonstrated the efficiency of the methods in extracting residues from aged samples.

**860.1360 Multiresidue Methods**

FDA multi-residue methods do not exhibit sufficient detectability and sensitivity to other imidazolinone herbicides, and thus there is no reasonable expectation that these methods would prove to be useful for determining residues of imazapyr.

**860.1380 Storage Stability**

In the grass field trials, total frozen ( $\leq -10$  C) storage intervals for forage and hay ranged from 11-24 months. These storage intervals could be supported by referenced storage stability data on

field corn indicating that imazapyr is stable in frozen forage and fodder for up to 27 months (ACCO Protocol AR94PT01, Report # RES 95-163). However, until these data are submitted, adequate storage stability has not been demonstrated to support the grass field trials.

In the ruminant feeding study, total frozen ( $\leq -10$  C) storage intervals were 1-3 months for milk and milk fat samples and 6-7 months for tissue samples. These storage intervals are supported by the available storage stability data (discussed below), which indicate that imazapyr is stable in frozen liver and muscle for up to 8 month and in frozen milk for up to 6 months.

In the clam bioaccumulation study and the aquatic field tests, the total frozen ( $\leq -10$  C) storage intervals were  $\leq 8$  months in clams and  $\leq 21$  months in fish and crayfish tissue samples. These storage intervals are supported by the available storage stability data (discussed below), which indicate that imazapyr is stable in fish muscle for up to 21 months.

Samples of pond water from the aquatic field tests were stored frozen ( $\leq -10$  C) for up to 27 months prior to analysis of imazapyr and the two degradates, CL 9140 and CL 119040, and samples of pond sediments were stored frozen ( $\leq -10$  C) for up to 21 months prior to the analysis for imazapyr. Data from an ongoing storage stability study (discussed below) indicate that imazapyr is stable in frozen ( $\leq -10$  C) sediments and pond water for up to 12 months and that the degradates are stable in frozen water for up to 15 months. Additional storage stability data are required to support the residue data on pond water and sediments from the current field trials.

#### **45119719.der.wpd**

Control samples of homogenized cattle muscle and liver tissue and milk were fortified with 0.1 ppm of imazapyr, then stored frozen for up to 6 or 8 months, with analysis at 0, 3, and 6-8 months. Control samples of fish muscle were homogenized and fortified with 0.5 ppm of imazapyr, then stored frozen for up to 21 months, with analysis at 0, 3, 6, 12, 18, and 21 months. Residues of imazapyr were stable for up to 6 months in frozen milk, 8 months in frozen ( $< -15$  C) cattle tissue, and up to 21 months in frozen fish muscle.

#### **860.1400 Water, Fish, and Irrigated Crops**

To support the proposed aquatic use of imazapyr for control of floating and emergent weed on freshwater sites, BASF submitted two studies examining the potential for bioaccumulation of [ $^{14}\text{C}$ ]imazapyr in oysters and shrimp, a bioaccumulation study on freshwater clams, and two field trials examining imazapyr residues in water, sediments, fish and shellfish following an application at the proposed use rate.

No studies depicting imazapyr residue levels in irrigated crops have been submitted and presently no label restriction prohibits use of treated waters for irrigated crops. Accordingly, the petitioner should submit residue data for irrigated crops or add a label restriction that prohibits the use of treated water for irrigation purposes for 120 days following application or demonstrates non-detectable residue levels of imazapyr in irrigation water by laboratory analysis prior to use.

In the two adequate [ $^{14}\text{C}$ ]bioaccumulation studies (discussed below), oysters and grass shrimp were exposed to [ $^{14}\text{C}$ ]imazapyr at an average concentration of  $\sim 0.25$  mg a.i./L for 28 days, followed by a 14-day depuration period. For both species, uptake of radioactivity was low and variable, with the majority of samples having  $^{14}\text{C}$ -residues below the LOQ. By day 28,  $^{14}\text{C}$ -residues were 0.073-0.105 ppm in oysters and  $<0.128$ -0.160 ppm in shrimp. Due to the low levels of radioactivity in the tissues, bioconcentration factor values and uptake and depuration rates were not calculated, and  $^{14}\text{C}$ -residues in tissues were not further characterized. However, considering that all of the oyster or shrimp tissue samples had concentrations lower than in the saltwater, [ $^{14}\text{C}$ ]imazapyr does not appear to accumulate in shellfish.

In the clam bioaccumulation study (discussed below), freshwater clams were raised for 28 days in recirculating tanks treated with imazapyr at a rate equivalent to 1.5 lb ae/A (1x proposed rate). Following application, residues of imazapyr in the water remained relatively consistent (0.074-0.085 ppm) over the 28-day period and residues in clams were  $<0.05$  ppm ( $<\text{LOQ}$ ) in all samples.

In aquatic field trials at two location (FL and MO) imazapyr was applied at a broadcast rate of 1.6 lb ae/A to either the banks and water's edge of a pond (Treatment I) or to the banks and the entire pond surface (Treatment II). Ponds were stocked with fish (bluegill, bass, tilapia, and catfish) and crayfish prior to treatment. Samples of each animal and water were collected at various intervals up to 42 DAT and samples of pond sediments were collected at up to 180 DAT.

Residues of imazapyr were  $<0.05$  ppm ( $<\text{LOQ}$ ) in all samples of each non-target species from Treatment I at both test sites and from Treatment II at the MO test site. For Treatment II at the FL site, imazapyr residues were detected only in bluegill (0.636 ppm), tilapia (0.233 ppm), catfish (0.068 ppm), crayfish (0.059 ppm) at 3 hours post-treatment.

For Treatment I, average imazapyr residues in water peaked at 0.028 ppm by 3 hours post-treatment in the FL test and declined to  $<0.001$  ppm by 36 DAT, and average imazapyr residues in water peaked at 0.029 ppm by 1 hour post-treatment in the MO test and declined to 0.002 ppm by 42 DAT. For Treatment II, the average imazapyr residues in water peaked at 0.164 ppm by 12 hours post-treatment in the FL test and declined to  $<0.001$  ppm by 27 DAT, and the average imazapyr residues in water peaked at 0.196 ppm by 1 hour post-treatment in the MO tests, and declined to 0.025 ppm by 42 DAT. The half-life of imazapyr in the pond water was 3.9 days at the FL test site and 15 days at the MO test site. The petitioner attributed the increased half-life to higher levels of suspended sediments at the MO site, which reduced light penetration, and decreased the potential for photolytic degradation. The degradates CL 9140 and CL 119060 were  $<0.002$  ppm ( $<\text{LOQ}$ ) in all water samples from Treatment I collected at both the FL and MO test sites and were  $\leq 0.003$  ppm in samples from Treatment II in MO. For Treatment II at the FL site, CL 9140 and CL 119060 and detected 0.002-0.006 ppm in water at 1-5 DAT and were  $<0.002$  ppm at all subsequent sampling intervals. Residues of imazapyr in pond sediments were low ( $<0.02$  ppm) in both tests at each site at each interval.

In a meeting of the HED MARC held 26-FEB-2003, the residue of concern in fish and shellfish was tentatively determined to be imazapyr *per se* (TXR# 0051641, W. Donovan and E. Rinde, 13-MAR-2003). This conclusion will need to be confirmed by submission of an acceptable fish

metabolism study as a condition of registration. The proposed tolerances of 1.0 and 0.1 ppm for freshwater finfish and shellfish are adequate based on the residues observed in bluegills (0.636 ppm) and crayfish (0.059 ppm) from the FL field trial.

#### 45119709.der.wpd

Two studies were conducted to examine the potential for bioaccumulation of [pyridine-5-<sup>14</sup>C]imazapyr in shellfish (oysters and grass shrimp). These studies were designed to determine (i) the steady-state bioconcentration factor (BCF) of [<sup>14</sup>C]imazapyr in each species; (ii) uptake and depuration rate constants; and (iii) the metabolism of [<sup>14</sup>C]imazapyr in these animals.

Two replicate populations each of oysters (52 animals/tank) and grass shrimp (410 animals/tank) were exposed to [<sup>14</sup>C]imazapyr at an average concentration of ~0.25 mg a.i./L for 28 days, followed by a 14-day depuration period. Actual average [<sup>14</sup>C]imazapyr concentrations in saltwater during the uptake phase were 0.244 mg a.i./L for oysters and 0.268 mg a.i./L for shrimp, and HPLC analyses of the water samples indicated that [<sup>14</sup>C]imazapyr was stable in the tank water during the tests.

In the oyster test, concentrations of radioactivity in tissues during the uptake phase were low and variable, with the majority of samples having <sup>14</sup>C-residues below the limit of quantitation (LOQ, 0.072 ppm). <sup>14</sup>C-Residues in oysters were <0.072-0.078 ppm on Day 7, 0.074-0.120 ppm on Day 14, <0.072-0.101 ppm on Day 21, and 0.073-0.105 ppm on Day 28. Concentrations of radioactivity were <LOQ in all samples collected during the depuration phase and in all control samples.

In the shrimp test, concentrations of radioactivity in tissues were also low and variable, with the majority of samples having residues <LOQ (<0.128 ppm). During the uptake phase, <sup>14</sup>C-residues in shrimp were <0.128 ppm on Days 0, 7, and 14, and were <0.128-0.147 ppm on Day 21 and <0.128-0.160 ppm on Day 28. During the depuration phase, <sup>14</sup>C-residues in shrimp were <0.128-0.137 ppm up to Day 3, ≤0.132 ppm by Day 7, and <0.128 ppm at the later sampling intervals. Concentrations of radioactivity were <LOQ in all control samples.

Due to the low levels of radioactivity in the oyster and shrimp tissues, a BCF value and uptake and depuration rates were not calculated, and <sup>14</sup>C-residues in tissues were not further characterized. However, considering that all of the oyster or shrimp tissue samples had concentrations lower than in the saltwater, [<sup>14</sup>C]imazapyr does not appear to accumulate in shellfish.

#### 45119722.der.wpd

In a clam bioaccumulation study, a recirculating tank system containing a layer of sediment and clams (*Corbicula fluminea*) was treated with a single application of imazapyr (2 lb ae/gal AS) at a rate equivalent to 1.5 lb ae/A. Clam and water samples were collected at 0 (4 hours), 1, 3, 7, 14, 21, and 28 days after treatment (DAT), and sediment samples were collected at 0, 14, and 28 DAT. Total frozen (temperature not specified) storage intervals for water, clam, and sediment

samples were  $\leq 8$  months, an interval which is supported by the available storage stability data. Samples were analyzed for residues of imazapyr using the adequate capillary electrophoresis/ultraviolet detection (CE/UV) methods M 3001 for water, M 3066 for clam tissues, and M 3014 for sediments. The method limits of quantitation (LOQ) for imazapyr are 0.001 ppm in water and sediments and 0.05 ppm in clams.

Following application of imazapyr to the recirculating tank system, residues of imazapyr in the water remained relatively consistent, ranging from 0.074-0.085 ppm over the 28-day treatment period. Residues in sediments were  $<0.001$  ppm immediately after application (4 hours), and were 0.029 ppm at 14 and 28 DAT. Imazapyr did not appear to accumulate in clam tissues, as residues were  $<0.05$  ppm ( $<\text{LOQ}$ ) in composited samples collected at 0, 1, 7, and 28 DAT.

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At two locations in FL and MO during 1996, a single application of imazapyr (2 lb ae/gal AS) was made to the banks (4-6 feet) and water's edge (4-6 feet) of a fresh water pond at a broadcast rate of 1.6 lb ae/A (Treatment I). A second pond at each site received a single application of imazapyr (2 lb ae/gal AS) to the banks (4-6 feet) and the entire pond surface at a broadcast rate of 1.6 lb ae/A (Treatment II). Prior to treatment, each pond was stocked with non-target organisms (bluegill, bass, tilapia, catfish, and crayfish), and five sampling stations were established in various areas of each pond. Following treatment, water samples were collected from each station per pond at various intervals from 0 to 42 days after treatment (DAT), and single composite samples of each non-target organism were collected from each pond at intervals from 0 to 42 DAT. Sediment samples were also collected from each sampling station in each pond at various intervals from 0 to  $\sim 180$  DAT.

Fish and shellfish samples were stored frozen for up to 21 months, an interval which is supported by available storage stability data. Sediment and water samples were stored frozen for up to 27 months. Additional storage stability data are needed to support this storage interval, although sediment and water issues are under the purview of EFED. Imazapyr residues were determined using the adequate CE/UV methods BASF M 3001 (water), M 3014 (sediment), and M 3066 (fish and shellfish). The method limits of quantitation (LOQ) for imazapyr are 0.001 ppm in water and sediments and 0.05 ppm in fish and shellfish tissues. Residues of the degradate CL 9140 in water were determined using an adequate CE/UV method BASF M 3097, and residues of the degradate CL 119060 were determined using an adequate LC/MS/MS Method BASF M 2672. The LOQ for both degradates in water is 0.002 ppm.

For Treatment I, average imazapyr residues in water peaked at 0.028 ppm by 3 hours post-treatment in the FL test and declined to  $<0.001$  ppm by 36 DAT, and average imazapyr residues in water peaked at 0.029 ppm by 1 hour post-treatment in the MO test and declined to 0.002 ppm by 42 DAT. The highest concentration observed for any one water sample was 0.067 ppm (1 hour) from the FL test and 0.081 ppm (1 hour) from the MO test.

For Treatment II, the average imazapyr residues in water peaked at 0.164 ppm by 12 hours post-treatment in the FL test and declined to  $<0.001$  ppm by 27 DAT, and the average imazapyr

residues in water peaked at 0.196 ppm by 1 hour post-treatment in the MO tests, and declined to 0.025 ppm by 42 DAT. The highest residues observed in water samples from Treatment II were 0.186 ppm (12 hours) from the FL test and 0.309 ppm (1 hour) from the MO test. For Treatment II, the registrant calculated the half-life of imazapyr in the pond water to be 3.9 days at FL test site and 15 days at the MO test site. The registrant attributed the increased half-life to higher levels of suspended sediments at the MO site, which reduced light penetration, and decreased the potential for photolytic degradation.

The degradates CL 9140 and CL 119060 were <0.002 ppm (<LOQ) in all water samples from Treatment I collected at both the FL and MO test sites. For Treatment II at the FL site, CL 9140 was detected at 0.002-0.005 ppm at 1-5 DAT and CL 119060 was detected at 0.002-0.006 ppm at 1-5 DAT. For Treatment II at the MO site, CL 9140 was detected in only one sample at 0.003 ppm at 14 DAT, and CL 119060 was not detected (<0.002 ppm) in any samples.

Residues of imazapyr in pond sediments were generally low in both FL tests. For the Treatment I in FL, average imazapyr residues in sediments were 0.003-0.005 ppm from 6 hours to 1 day post-treatment and were  $\leq 0.001$  ppm at all subsequent intervals. For Treatment II in FL, average imazapyr residues in sediments declined from 0.011 ppm at 6 hours post-treatment to  $\leq 0.001$  ppm by 22 DAT. Imazapyr residues in sediments from the MO test were more variable and showed little or no decline over time. For the Treatment I in MO, average imazapyr residues in sediments were initially the highest (0.034 ppm) of any treatment; however, average residues at all subsequent intervals (1-180 DAT) were 0.001-0.005 ppm. For Treatment II in MO, average imazapyr residues in sediments averaged 0.009-0.018 ppm at 0.25-180 DAT.

Residues of imazapyr were <0.05 ppm (<LOQ) in all samples of each non-target species from Treatment I at both test sites and from Treatment II at the MO test site. For Treatment II at the FL site, imazapyr residues were detected in bluegill (0.636 ppm), tilapia (0.233 ppm), catfish (0.068 ppm), crayfish (0.059 ppm) from the 3 hour post-treatment interval, for an average concentration of 0.249 ppm (FL test) in edible fish and shellfish tissues. Residues were higher in the mid-level species (bluegill and tilapia), than in the bottom feeding species (catfish and crayfish). At all subsequent sampling intervals (1-42 DAT), imazapyr residues were <0.005 ppm in all species from this test.

The water, fish, and sediment residue data are adequate, provided supporting storage stability data are provided for residues of imazapyr in water and sediments stored for up to 27 months. A storage stability study for residues of imazapyr in frozen water and sediments is ongoing. The currently available data indicate that imazapyr is stable in frozen ( $\leq -10$  C) sediments and pond water for up to 12 months and that the degradates CL 9140 and CL 119040 are stable in frozen water for up to 15 months.

### **860.1460 Food Handling**

As there are no food handling uses for imazapyr, this guideline requirement is not relevant to the current petition.

### **860.1480 Meat, Milk, Poultry, and Eggs**

Registration requirements for magnitude of the residue in meat, milk, poultry, and eggs are fulfilled. An adequate ruminant feeding study is available for imazapyr, and a poultry feeding study is not required for this petition as detectable <sup>14</sup>C-residues were not found in eggs and tissues from the poultry metabolism study conducted at a dose level equivalent to 9.72 ppm (~240x) in the diet.

The maximum theoretical dietary burdens (MTDBs) for livestock are calculated below in Table 2. Based on a diet including corn grain at 80% of the diet, the MTBD is 0.04 ppm for both for poultry and swine. For cattle the recommended tolerances on grass forage (100 ppm) and hay (30 ppm) have the major impact on the dietary burden for cattle. However, these tolerances are based on a broadcast application rate of 0.75 lb ae/A, and the label directions specify spot applications at 0.75 lb ae/treated acre, with no more than 10% of an acre being treated. Accordingly, the calculation of the MTDB for cattle includes multiplication by a 10% correction factor to account for the label restriction limiting the spot treatment of grasses to a maximum of 10% of any given acre. This approach is consistent with a previous HED Chemistry Science Advisory Council (ChemSAC) decision to set the grass forage and hay tolerance levels on residue levels in the treated area alone, while calculating dietary burdens for livestock based on the average residue across the whole crop (i.e., the % area treated is multiplied by the residue in the treated spot) [Minutes of 1/20/99 ChemSAC meeting].

Based on a MTDB of 24.0 ppm and comparison with the maximum residues levels observed in the 58 ppm dose group in the cattle feeding study, the maximum expected imazapyr residues in cattle commodities are <0.010 ppm in milk, <0.050 ppm in muscle, fat, and liver; and 0.15 ppm in kidney. Accordingly, the appropriate tolerance levels for imazapyr residues in cattle tissues are as follows: milk 0.01 ppm; meat, fat, and meat byproduct (except kidney) 0.05 ppm; kidney 0.20 ppm.

Based upon a MTDB of 0.04 ppm for swine, the 58 ppm dose level in the ruminant feeding study is equivalent to 1450x the MTDB for swine. Based on the maximum residues detected in the 58 ppm dose group, there is no reasonable expectation of finding quantifiable residues of imazapyr in hog tissues [40 CFR 180.6(a)(3)]. Thus, tolerances for imazapyr residues in hog commodities are not necessary.

As indicated above, total <sup>14</sup>C-residues were <0.01 ppm in eggs and poultry tissues from hens dosed in the poultry metabolism study at a level equivalent to 9.72 ppm (~240x) in the diet. Therefore, there is no reasonable expectation of finding quantifiable residues of imazapyr in poultry commodities [40 CFR 180.6(a)(3)] and tolerances are not required.

| Table 2. Calculation of maximum dietary burdens of livestock animals for Imazapyrs |                           |                     |                                             |                                  |                                                   |
|------------------------------------------------------------------------------------|---------------------------|---------------------|---------------------------------------------|----------------------------------|---------------------------------------------------|
| Feed Commodity                                                                     | % Dry Matter <sup>a</sup> | % Diet <sup>a</sup> | Recommended or established Tolerances (ppm) | % Area treated/acre <sup>b</sup> | Potential Dietary Contribution (ppm) <sup>c</sup> |
| <b>Beef and Dairy Cattle</b>                                                       |                           |                     |                                             |                                  |                                                   |
| Grass forage                                                                       | 25                        | 60                  | 100                                         | 10                               | 24.0                                              |
| Grass hay                                                                          | 88                        | 60                  | 30                                          | 10                               | 2.05                                              |
| Corn grain                                                                         | 88                        | 80                  | 0.05                                        | NA                               | 0.05                                              |
| Corn forage                                                                        | 40                        | 50                  | 0.05                                        | NA                               | 0.06                                              |
| Corn stover                                                                        | 83                        | 25                  | 0.05                                        | NA                               | 0.02                                              |
| TOTAL BURDEN                                                                       |                           |                     |                                             |                                  | <b>24.0</b> <sup>d</sup>                          |
| <b>Poultry</b>                                                                     |                           |                     |                                             |                                  |                                                   |
| Corn grain                                                                         | NA                        | 80                  | 0.05                                        | NA                               | 0.04                                              |
| TOTAL BURDEN                                                                       |                           |                     |                                             |                                  | <b>0.04</b>                                       |
| <b>Swine</b>                                                                       |                           |                     |                                             |                                  |                                                   |
| Corn grain                                                                         | NA                        | 80                  | 0.05                                        | NA                               | 0.04                                              |
| TOTAL BURDEN                                                                       |                           |                     |                                             |                                  | <b>0.04</b>                                       |

<sup>a</sup> Table 1 (August 1996).

<sup>b</sup> For the spot application to grasses, the label specifies that no more than 10% of any given acre is to be treated.

<sup>c</sup> Contribution = [tolerance / % DM (if cattle)] X % diet X % area treated (for grass).

<sup>d</sup> Based on a diet consisting of 60% grass forage and 40% corn grain.

NA = not applicable.

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In the cattle feeding study, four groups of dairy cows (3 cows/group) were dosed orally with imazapyr in capsules at 2, 6, 20, or 60 mg ai/kg body weight/day for 28 consecutive days. Based on their feed consumption, these dose levels are equivalent to 57, 157, 607, and 1680 ppm of imazapyr in the diet. Cows were milked twice daily and samples from Day -1 (pretreatment) and Days 2, 3, 6, 8, 10, 13, 17, 24, and 27 used for analysis. Subsamples of milk from Days 8, 15, and 22 were centrifuged to obtain milk fat samples. After 28 days of treatment, cows were sacrificed and muscle, omental fat, kidney, and liver samples were collected. Samples were stored frozen for a maximum of 7 months, an interval which is supported by available storage stability data. Samples were analyzed for residues of imazapyr using adequate CE/UV methods (Methods M 3075, M 3223, or M 3184). The LOQ for imazapyr is 0.01 ppm in milk and milk fat and 0.05 ppm in tissues.

In each dose group, imazapyr residues in milk remained steady over the entire 28 day dosing period. In the 58 ppm dose group, imazapyr residues in milk were  $\leq 0.013$  ppm, with only 4 out of 27 samples having quantifiable residues. Average residues in milk were 0.028, 0.092, and

0.258 ppm for the 157, 607, and 1680 ppm dose groups, respectively. Residues in milk increased proportionally with the increases in the dose level, and residues in milk fat were approximately 0.4-0.5x lower than in whole milk.

In each dose group, residues in tissue were lowest in muscle and fat, and highest in kidney, and residues levels in generally increased proportionally with the dose level. In muscle, average residues were <0.05 ppm (<LOQ) in the 58 and 157 ppm dose groups, and were 0.097 and 0.234 ppm in the 607 and 1680 ppm dose groups. In fat, average residues were <0.05 ppm in the 58 and 157 ppm dose groups, and were 0.067 and 0.092 ppm in the 607 and 1680 ppm dose groups, respectively. In liver, average residues were <0.05 ppm in the 58 ppm dose groups, and were 0.057, 0.097 and 0.234 ppm in the 157, 607 and 1680 ppm dose groups. Quantifiable residues were detected in kidneys of each dose group. Average imazapyr residues in kidneys were 0.246, 0.519, 4.36, and 7.51 ppm for the 58, 157, 607, and 1680 ppm dose groups, respectively.

### 860.1500 Crop Field Trials

The grass field trial data are adequate, pending the submission of acceptable supporting storage stability data on corn forage and fodder, which may be used to support the grass field trials; and clarification of the identity and quantity of spray additives utilized in the grass field trials. The available data indicate that the proposed 125 and 35 ppm tolerances for grass forage and hay are too high. As forage and hay are only used as livestock feed items, tolerances of 100 and 30 ppm for grass forage and hay would be adequate.

#### Grass forage and hay

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| Table 3. Summary of Residue Data in/on Grass from Crop Field Trials with Imazapyr. |                                |                  |                               |         |      |                   |              |
|------------------------------------------------------------------------------------|--------------------------------|------------------|-------------------------------|---------|------|-------------------|--------------|
| Total Applic.<br>Rate (lb ae/A)                                                    | Applic.<br>Method <sup>a</sup> | PHI<br>(days)    | Imazapyr Residue Levels (ppm) |         |      |                   |              |
|                                                                                    |                                |                  | Minimum                       | Maximum | HAFT | Mean <sup>b</sup> | Std.<br>Dev. |
| Forage                                                                             |                                |                  |                               |         |      |                   |              |
| 0.73-0.78                                                                          | Broadcast<br>Foliar            | 0.1 <sup>c</sup> | 26.9                          | 98.0    | 97.0 | 53.6              | 19.5         |
|                                                                                    |                                | 7                | 0.59                          | 12.2    | 11.2 | 5.83              | 2.51         |
|                                                                                    |                                | 14               | <0.50                         | 10.6    | 10.5 | 3.63              | 2.34         |
|                                                                                    |                                | 28               | <0.50                         | 6.41    | 6.25 | 1.80              | 1.44         |
| Hay                                                                                |                                |                  |                               |         |      |                   |              |
| 0.73-0.78                                                                          | Broadcast<br>Foliar            | 0.1              | 64.5                          | 277     | 269  | 142               | 53.5         |
|                                                                                    |                                | 7                | 0.88                          | 27.1    | 25.4 | 15.1              | 6.41         |
|                                                                                    |                                | 14               | 0.51                          | 19.6    | 17.6 | 7.90              | 4.22         |
|                                                                                    |                                | 28               | 0.50                          | 8.56    | 8.22 | 3.19              | 1.86         |

- <sup>a</sup> A single broadcast application of imazapyr (2 lb ae/gal AS) was made for all tests.
- <sup>b</sup> N = 28, except at the 28-day sampling interval (N=26).
- <sup>c</sup> Data for the proposed PHIs for forage (0-day) and hay (7-day) are **bolded**.

In fourteen tests conducted in AR, CO, GA, ID, IN, MI, NE (2), OR (2), PA, TX (2), and WI during 1996 and 1997, imazapyr (2 lb/gal AS) was applied as a single broadcast foliar application to established grass (bermuda, tall fescue, bluegrass, and brome grass) at 0.73-0.78 lb ae/A in 20-60 gallons of water per acre with a 0.25% (v/v) nonionic surfactant. Grass forage and hay samples were harvested 0.1, 7, 14, and 28 days after application, and the hay samples were allowed to field dry for 1-17 days after cutting. Samples were stored frozen for a maximum of 24 months and then analyzed for residues of imazapyr using the adequate CE/UV Method M 3023.

Residues of imazapyr were 27-98 ppm in/on grass forage harvested immediately following application (0.1 day), which is the proposed PHI. Residues in/on forage declined steadily at subsequent sampling intervals to 0.59-12.2 ppm by 7 DAT, <0.5-10.6 ppm by 14 DAT, and <0.5-6.25 ppm by 28 DAT. Residues of imazapyr were 65-277 ppm in/on hay harvested immediately following application, and declined to 0.88-27.1 ppm in/on hay harvested at 7 DAT, which is the proposed PHI. Residues in/on hay continued to decline at later sampling intervals; 0.51-19.6 ppm by 14 DAT and <0.5-8.56 ppm by 28 DAT.

#### **860.1520 Processed Food and Feed**

As there are no processed food or feed items associated with the proposed aquatic or grassland uses of imazapyr, this guideline requirement is not relevant to the current petition.

#### **860.1650 Submittal of Analytical Reference Standards**

The analytical reference standard for imazapyr has been submitted to the EPA National Pesticide Standards Repository.

#### **860.1850/1900 Confined and Field Accumulation in Rotational Crops**

An adequate confined rotational crop field trial was previously submitted to support the use on field corn at 0.014 lb ae/A (D222027, N. Dodd, 6/26/96). This earlier study was conducted at an application rate of 0.025 lb ae/A. To support the currently purposed use on grass pastures and rangeland, the petitioner has submitted a new confined rotational crop field trial (discussed below) reflecting the higher proposed use rate on grass (0.75 lb ae/treated acre).

Although supporting storage stability data were not provided, and metabolite identities were not confirmed using a second analytical method, the submitted confined rotational crop study is adequate. Based on the low TRR levels observed (<0.02 ppm) in each crop at the ~12 and 18 month plant-back intervals, and the HPLC analyses of solvent extracted <sup>14</sup>C-residues, any residues

of concern in rotational crops at the proposed 12-month PBI would be  $<0.005$  ppm. In addition, while the use rate in the current study (0.79 lb ae/A) is equivalent to the maximum proposed rate for spot treatments, the total amount applied per full acre is limited to 0.075 lb ae/A, since the spot treatment is only permitted on a maximum of one tenth (1/10) of a given acre. Therefore, the maximum average exposure of rotational crops to imazapyr should reflect a rate of 0.075 lb ae/A, and the present study may be considered an exaggerated rate study conducted at 10x.

Accordingly, limited field rotational crop trials and rotational crop tolerances will not be required to support the proposed use on grasses.

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In a field study, [ $^{14}\text{C}$ -6-pyridinyl] imazapyr was applied to a sandy loam soil at a rate of 0.79 lb ae/A ( $\sim 10\text{x}$ ). Rotational crops of carrots, lettuce, and wheat were planted at approximately 12 and 18 months post-treatment, and the appropriate commodities were collected from each crop at each plant-back interval (PBI). TRR were generally low in all commodities from both PBIs. TRRs were  $<0.005$  ppm ( $<\text{LOD}$ ) in carrots (tops and roots) and lettuce from the  $\sim 12$ -month PBI, carrot roots from the 18-month PBI, and wheat forage from the 12- and  $\sim 18$ -month PBIs, and were 0.005-0.009 ppm in lettuce and carrot tops from the 18-month PBI, and wheat hay, grain, and straw from the 12-month PBI. The only commodities with TRRs  $>0.01$  ppm were wheat hay (0.018 ppm), grain (0.016 ppm), and straw (0.015 ppm) from the  $\sim 18$ -month PBI.

Based on HPLC retention times, 5.2-25.1% of the TRR was tentatively identified in lettuce, carrot tops, and wheat hay, grain, and straw. The parent, imazapyr, was the major residue identified in rotational crop commodities, accounting for 1.1-19.5% TRR ( $\leq 0.003$  ppm). The Metabolites CL 247087, CL 252974, CL17226, and CL 119060 were all present as minor components, each at 0.3-3.6% TRR ( $<0.001$  ppm). HPLC analyses of the solvent extracts also detected numerous (10-17) unknowns, but these are all present at  $\leq 0.002$  ppm.

Based on the limited identification of  $^{14}\text{C}$ -residues in this study, the petitioner proposes that imazapyr was extensively metabolized in rotational crops by hydrolysis of the imidazolinoyl ring, and cyclization of the carboxyl group with the imidazolinoyl nitrogen.

## 860.1550 Proposed Tolerances

The HED Metabolism Committee previously concluded that tolerances for imazapyr in plants and animals should include only the parent compound (N. Dodd, 2/26/97). This conclusion was confirmed by the HED MARC and extended to include fish and shellfish (TXR# 0051641, W. Donovan and E. Rinde, 13-MAR-2003). Permanent tolerances have been established at 0.05 ppm for residues in/on corn forage, fodder, and grain [40 CFR §180.500]. Recommended tolerances for imazapyr residues are presented in Table 4. Based on the available residue data, the proposed tolerances for grass forage and hay; and kidneys of cattle, goats, horses, and sheep can be lowered to the recommended levels indicated in Table 4.

| Table 4. Tolerance Summary for Imazapyr                                |                          |                             |                                                                                                                                                                                                            |
|------------------------------------------------------------------------|--------------------------|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Commodity                                                              | Proposed Tolerance (ppm) | Recommended Tolerance (ppm) | Comments<br>(Correct commodity definition)                                                                                                                                                                 |
| Grass, forage                                                          | 125                      | 100                         | The available residue data indicate that the proposed tolerances are too high. Particularly as the field trial data reflect a spot treatment rate which is 10x the maximum per full acre application rate. |
| Grass, hay                                                             | 35                       | 30                          |                                                                                                                                                                                                            |
| Fish, freshwater finfish                                               | 1.0                      | 1.0                         | The available residue data support the proposed tolerance; however the commodity definition should be revised to "Fish".                                                                                   |
| Shellfish                                                              | 0.1                      | 0.10                        | The available residue data support the proposed tolerance.                                                                                                                                                 |
| Fat of cattle, sheep, goats, and horses                                | 0.05                     | 0.05                        | The available residue data support the proposed tolerance.                                                                                                                                                 |
| Kidney of cattle, sheep, goats, and horses                             | 0.5                      | 0.20                        | The available residue data indicate that the proposed tolerance is too high.                                                                                                                               |
| Meat byproducts, excluding kidney, of cattle, sheep, goats, and horses | 0.05                     | 0.05                        | The available residue data support the proposed tolerances.                                                                                                                                                |
| Meat, of cattle, sheep, goats, and horses                              | 0.05                     | 0.05                        |                                                                                                                                                                                                            |
| Milk                                                                   | 0.01                     | 0.01                        |                                                                                                                                                                                                            |

## International Tolerances

There are no Codex, Canadian or Mexican tolerances for imazapyr residues in/on corn, grass, fish, shellfish, or livestock commodities (see Attachment 1). Thus, international harmonization of tolerances is not an issue at this time.

**References**

DP Barcode: D222027  
Subject: PP#6F04641 - Imazapyr on Field Corn. Review of Analytical Methods and Residue Data. First Food Use Review.

From: N. Dodd  
To: D. McCall  
Dated: 6/26/96  
MRID(s): 43861502-43861506, and 43861516-43861518

DP Barcode: None  
Subject: Imazapyr Metabolism in Field Corn. The HED Metabolism Committee Meeting Held on 2/25/97.  
From: N. Dodd  
To: HED Metabolism Committee  
Dated: 2/26/97  
MRID(s): None

DP Barcode: D288678  
TXR#: 0051641  
Subject: Imazapyr: Health Effects Division (HED) Metabolism Assessment Review Committee (MARC) Decision Document.  
From: W.H. Donovan  
To: Y. Donovan  
Dated: 3/13/2003  
MRID(s) None

DP Barcode: D288863  
Subject: PP# 0F06166. Imazapyr in/on Grass and Fish. Request for Petition Method Validation (PMV).  
From: W.H. Donovan  
To: F.D. Griffith, Jr.  
Dated: 3/14/2003  
MRID(s) None

List of Attachments

- Attachment 1. IRLS sheet for imazapyr.
- Attachment 2. 45119707.der.wpd.
- Attachment 3. 45119709.der.wpd.
- Attachment 4. 45119715.der.wpd.
- Attachment 5. 45119716.der.wpd.
- Attachment 6. 45119717.der.wpd.
- Attachment 7. 45119718.der.wpd.
- Attachment 8. 45119719.der.wpd.
- Attachment 9. 45119720.der.wpd.
- Attachment 10. 45119721.der.wpd.
- Attachment 11. 45119722.der.wpd.

cc (with Attachments): W. Donovan  
cc (without Attachments): D. Vogel  
RDI: G. Herndon (14-MAR-2003)  
W.H. Donovan:821B:CM#2:(703)305-7330:7509C:RRB3

## Attachment 1

| INTERNATIONAL RESIDUE LIMIT STATUS                                                                                        |                          |                                                                                                                                   |                 |
|---------------------------------------------------------------------------------------------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Chemical Name:<br>2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid             | Common Name:<br>Imazapyr | X <input type="checkbox"/> Proposed tolerance<br><input type="checkbox"/> Reevaluated tolerance<br><input type="checkbox"/> Other | Date: 12/9/02   |
| Codex Status (Maximum Residue Limits)                                                                                     |                          | U. S. Tolerances                                                                                                                  |                 |
| X No Codex proposal step 6 or above<br><input type="checkbox"/> No Codex proposal step 6 or above for the crops requested |                          | Petition Number: 0F06166<br>DP Barcode: D275561<br>Other Identifier:                                                              |                 |
| Residue definition (step 8/CXL):<br>N/A                                                                                   |                          | Reviewer/Branch: W. Donovan/RRB3                                                                                                  |                 |
|                                                                                                                           |                          | Residue definition: Imazapyr <i>per se</i>                                                                                        |                 |
| Crop (s)                                                                                                                  | MRL (mg/kg)              | Crop(s)                                                                                                                           | Tolerance (ppm) |
|                                                                                                                           |                          | grass forage                                                                                                                      | 125             |
|                                                                                                                           |                          | grass hay                                                                                                                         | 35              |
|                                                                                                                           |                          | fish                                                                                                                              | 1.0             |
|                                                                                                                           |                          | shellfish                                                                                                                         | 0.1             |
|                                                                                                                           |                          | meat, fat, and meat byproducts (except kidney)                                                                                    | 0.05            |
|                                                                                                                           |                          | kidney                                                                                                                            | 0.5             |
|                                                                                                                           |                          | milk                                                                                                                              | 0.01            |
| Limits for Canada                                                                                                         |                          | Limits for Mexico                                                                                                                 |                 |
| X No Limits<br><input type="checkbox"/> No Limits for the crops requested                                                 |                          | <input type="checkbox"/> No Limits<br>X No Limits for the crops requested                                                         |                 |
| Residue definition: N/A<br>Registered for use on noncrop land.                                                            |                          | Residue definition:<br>imazethapyr                                                                                                |                 |
| Crop(s)                                                                                                                   | MRL (mg/kg)              | Crop(s)                                                                                                                           | MRL (mg/kg)     |
|                                                                                                                           |                          |                                                                                                                                   |                 |
| Notes/Special Instructions:<br>S. Funk, 12/09/02.                                                                         |                          |                                                                                                                                   |                 |

Rev. 1998

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** Danette Drew, Chemist  
RRB3/HED (7509C)

*Danette Drew*

**Petition Number:** PP#0F06166

**DP Barcode:** D275561

**Citation:** 45119722 Christensen, G.; Madsen, T.; Skorczynski, S. et al. (1999) Field Accumulation Study of Arsenal Herbicide in Freshwater Clam: Lab Project Number: ECO 98-197: 44881: 954-98-1973. Unpublished study prepared by American Cyanamid Company and ABC Laboratories, Inc. 62 p.

**Sponsor:** BASF (formerly American Cyanamid Company)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

In a clam bioaccumulation study, a recirculating tank system containing a layer of sediment and clams (*Corbicula fluminea*) was treated with a single application of imazapyr (2 lb acid equivalents (ae)/gal AS [aqueous solution]) at a rate equivalent to 1.5 lb ae/A. Clam and water samples were collected at 0 (4 hours), 1, 3, 7, 14, 21, and 28 days after treatment (DAT), and sediment samples were collected at 0, 14, and 28 DAT. Total frozen (temperature not specified) storage intervals for water, clam, and sediment samples were  $\leq 8$  months, an interval which is supported by the available storage stability data. Samples were analyzed for residues of imazapyr using the adequate capillary electrophoresis/ultraviolet detection (CE/UV) methods M 3001 for water, M 3066 for clam tissues, and M 3014 for sediments. The method limits of quantitation (LOQ) for imazapyr are 0.001 ppm in water and sediments and 0.05 ppm in clams.

Following application of imazapyr to the recirculating tank system, residues of imazapyr in the water remained relatively consistent, ranging from 0.074-0.085 ppm over the 28-day treatment period. Residues in sediments were  $<0.001$  ppm immediately after application (4 hours), and were 0.029 ppm at 14 and 28 DAT. Imazapyr did not appear to accumulate in clam tissues, as residues were  $<0.05$  ppm ( $<LOQ$ ) in composited samples collected at 0, 1, 7, and 28 DAT. The clam bioaccumulation study is adequate.

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** Danette Drew, Chemist  
RRB3/HED (7509C)

**Petition Number:** PP#0F06166

**DP Barcode:** D275561

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## Executive Summary

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## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Test Substance

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid

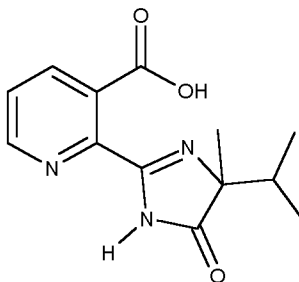
CAS Number: 81334-34-1

Company Name: AC 243997 or CL 243997

Other Synonyms: Arsenal Herbicide

Formulation: 2 lb ae/gal AS, 22.6% free acid

EPA Registration No.: 241-346



### 1.2. Test Animal

Animal/Species: Freshwater Clam/ *Corbicula fluminea*

Size:  $28 \pm 1.5$  mm; shucked wet weight of  $1.104 \pm 0.289$  g

Number/Group: 400 clams per tank (2 treated reps for a total of 800 clams)

Diet: Standard feed and various vitamins were added to each tank system once a day

Acclimation period: not reported

Mortality: <1% during the acclimation and study periods.

### 1.3. Test Site/System

Location: ABC Laboratories, Columbia, MO, in an open-sided greenhouse.

Tank system: Static tank system with recirculation of water. Two tank systems were used; one control and one treated. Each tank system consisted of two 6 feet diameter cylindrical tanks, used for treatment, interconnected by pipes with a oblong tank (4 x2 feet) used as a recirculation basin. Each tank was filled with water to a depth of ~14 inches. The two cylindrical treatment tanks were layered with sediment (2.5-3 cm), and 400 clams were added to each tank (800/tank system). Water was recirculated through the tank system throughout the study period.

Water: Well water; pH 8.1-8.5; hardness 130-160 mg/L as CaCO<sub>3</sub>; dissolved oxygen 7.3 ppm at 23 C and 10.8 ppm at 17 C.

Environmental Conditions: Natural sunlight and ambient temperatures; water temp. 13-25 C

Tank Sediment: 2.5-3 cm depth; loamy sand (86% sand, 12% silt, 2% clay ) with 0.7% organic matter

Study dates: 9/21/98-10/19/98

### 1.4 Application

Rate: Imazapyr (2 lb ae/gal AS) was diluted with water and added to the treated tanks at a rate of 1.5 lb ae/A, which is equivalent a target concentration of 0.091 ppm assuming a 6-foot water depth.

### 1.5. Post-harvest Procedures

Duplicate water samples were collected from each pool system at 0 (4 hours post-application), 1, 3, 7, 14, 21, and 28 days after application and the samples were composited by pool system and interval. Clams from both tanks were also sampled at 0 (4 hours post-application), 1, 3, 7, 14, 21, and 28 days after application. Clams were shucked, drained, composited by interval, and homogenized. Sediment samples were collected from each tank at 0, 14, and 28 days post-application and composited by interval. Samples were frozen (temperature not specified) until analysis by ABC Laboratories (Columbia, MO). Frozen storage intervals were not reported; however, based on the start and completion dates provided, samples were stored a maximum of 8 months.

### 1.6. Analytical Methods

Residues of imazapyr in clam samples from 0, 1, 7, and 28 DAT were analyzed using the CE/UV method, Method M 3066. Residues were extracted with acidic acetone:water (1:3, v/v), centrifuged, filtered, and partitioned into methylene chloride. Residues were then purified using solid-phase extraction (SPE) cartridges, and the final fraction is analyzed by CE/UV (240 nm). The LOQ for imazapyr in shellfish meat is 0.05 ppm, and the LOD is approximately 0.005 ppm.

Water samples from the replicate tanks were analyzed for residues of imazapyr using the CE/UV method, Method M 3001. For this method, residues of imazapyr are extracted from acidified water and purified using SPE cartridges. Residues are then determined by CE/UV (240 nm). The reported LOQ is 0.001 ppm; the LOD is 0.0002 ppm.

Sediment samples were analyzed for residues of imazapyr using the CE/UV method, Method M 3014. Residues were extracted with 0.5 N NaOH, acidified, and partitioned into methylene chloride. Residues are then cleaned

up using SPE cartridges, and quantified by CE/UV (240 nm). The method LOQ is 0.001 ppm for imazapyr in soil, and the LOD is approximately 0.0001 ppm.

## 2. Results

| Table 2.1. Summary of Concurrent Analytical Method Validation after Fortification with Imazapyr. |                           |                   |                         |                        |
|--------------------------------------------------------------------------------------------------|---------------------------|-------------------|-------------------------|------------------------|
| Matrix                                                                                           | Fortification Level (ppm) | Number of samples | Range of Recoveries (%) | Mean Recovery $\pm$ SD |
| Clam                                                                                             | 0.05-0.50                 | 6                 | 73-93                   | 85 $\pm$ 8             |
| Water                                                                                            | 0.001-0.10                | 7                 | 71-97                   | 85 $\pm$ 8             |
| Sediment                                                                                         | 0.001-0.10                | 5                 | 78-121 (1) <sup>a</sup> | 91 $\pm$ 17            |

<sup>a</sup> Number of recoveries outside the 70-120% range are in parentheses.

| Table 2.2. Residues of Imazapyr in Water, Clams, and Sediment following a Single Application of Imazapyr (2 lb ae/gal AS) at a rate equivalent to 1.5 lb ae/A. |                  |                                       |                                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|---------------------------------------|-----------------------------------|
| Matrix                                                                                                                                                         | Rate (lb ae/A)   | Sampling Interval (days) <sup>a</sup> | Imazapyr Residues (ppm)           |
| Water                                                                                                                                                          | 1.5 <sup>b</sup> | 0 (4 hours)                           | 0.081, 0.081 (0.081) <sup>c</sup> |
|                                                                                                                                                                |                  | 1                                     | 0.083, 0.083 (0.083)              |
|                                                                                                                                                                |                  | 3                                     | 0.084, 0.085 (0.085)              |
|                                                                                                                                                                |                  | 7                                     | 0.077, 0.078 (0.078)              |
|                                                                                                                                                                |                  | 14                                    | 0.074, 0.081 (0.078)              |
|                                                                                                                                                                |                  | 21                                    | 0.074, 0.079 (0.077)              |
|                                                                                                                                                                |                  | 28                                    | 0.077, 0.074 (0.076)              |
| Clams                                                                                                                                                          | 1.5              | 0 (4 hours)                           | <0.05 <sup>d</sup>                |
|                                                                                                                                                                |                  | 1                                     | <0.05                             |
|                                                                                                                                                                |                  | 7                                     | <0.05                             |
|                                                                                                                                                                |                  | 28                                    | <0.05                             |
| Sediment                                                                                                                                                       | 1.5              | 0 (4 hours)                           | <0.001 <sup>d</sup>               |
|                                                                                                                                                                |                  | 14                                    | 0.029                             |
|                                                                                                                                                                |                  | 28                                    | 0.029                             |

<sup>a</sup> Number of days after treatment.

<sup>b</sup> A single dose was applied at 1.5 lb ae/A, which was reported to be equivalent to a 0.091 ppm concentration based on a 6-foot water depth.

<sup>c</sup> Average water residues are presented in parentheses.

<sup>d</sup> The LOQs are 0.001 ppm in water and sediment and 0.05 ppm in clams.

### **3. Discussion**

#### **3.1. Methods**

The methods used to conduct the study and to analyze for residues were adequate. Total frozen (temperature not specified) storage intervals for clam tissue samples were not reported, however, based on the start and completion dates provided, samples were stored a maximum of 8 months. This interval is supported by available storage stability data, which indicate that imazapyr is stable in frozen fish tissues for up to 21 months (45119719.der, under review). Based on the concurrent method recoveries, the CE/UV methods (BASF Methods M 3066, M 3001, and M 3014) are adequate for collecting data on imazapyr residues in water, sediment, and clam tissue samples. Concurrent method recoveries were all within the acceptable 70-120% range, except one sediment sample (121%). Residues were also <0.001 ppm in 2 water samples and 3 sediment samples, and <0.05 ppm in 2 control clam tissue samples. The method LOQs are 0.001 ppm in water and sediment, and 0.05 ppm in clam tissues. Sample calculations and chromatograms were provided.

#### **3.2. Results**

Following a single application of imazapyr (2 lb ae/gal AS) at a rate of 1.5 lb ae/A to a recirculating tank system containing freshwater clams, residues of imazapyr in the water remained relatively consistent, starting at 0.081 ppm at 4 hours post-treatment, increasing to 0.085 ppm by 3 days, and then slowly declining to 0.075 ppm by 28 days post-treatment. Residues in sediments were <0.001 ppm immediately after application, and were 0.029 ppm at 14 and 28 days post-treatment. Imazapyr did not appear to accumulate in clam tissues, as residues were <0.05 ppm (<LOQ) at 0, 1, 7, and 28 DAT.

The clam bioaccumulation study is adequate.

### **4. Deficiencies**

None

### **5. References**

None

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

*David Soderberg*

**Petition Number:** PP#0F06166

**DP Barcode:** D275561

**Citation:** 45119721 Khunachak, A. (1999) Arsenal (Imazapyr-CL 243997): Magnitude of CL 243997 in Milk, Milk Fat and Edible Tissues from Dairy Cattle After Oral Administration for at Least 28 Days: Lab Project Number: RES 99-100: AR97PT07: 44390. Unpublished study prepared by American Cyanamid Company, ABC Labs Inc., Fort Dodge Animal Health, and Maxim Technologies. 232 p.

**Sponsor:** BASF (formerly American Cyanamid Company)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

Four groups of dairy cows (3 cows/group) were dosed orally with imazapyr in capsules at 2, 6, 20, or 60 mg ai/kg body weight/day for 28 consecutive days. Based on their feed consumption, these dose levels are equivalent to 57, 157, 607, and 1680 ppm of imazapyr in the diet. Cows were milked twice daily and the morning and evening samples were combined into one sample for the day. Samples from Day -1 (pretreatment) and Days 2, 3, 6, 8, 10, 13, 17, 24, and 27 were analyzed. Subsamples of milk from Days 8, 15, and 22 were centrifuged to obtain milk fat samples. After 28 days of treatment, cows were sacrificed and muscle, omental fat, kidney, and liver samples were collected. Samples were stored frozen for a maximum of 7 months, an interval which is supported by available storage stability data. Samples were analyzed for residues of imazapyr using adequate capillary electrophoresis/ ultraviolet detection (CE/UV) methods (BASF Methods M 3075, M 3223, or M 3184). The limit of quantitation (LOQ) for imazapyr is 0.01 ppm in milk and milk fat and 0.05 ppm in tissues.

In each dose group, imazapyr residues in milk reached a plateau within two days and remained steady over the remainder of the 28 day dosing period. In the 58 ppm dose group, imazapyr residues in milk were  $\leq 0.013$  ppm, with only 4 out of 27 samples having quantifiable residues. Average residues in milk were 0.028, 0.092, and

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RRB3/HED (7509C)

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Four groups of dairy cows (3 cows/group) were dosed orally with imazapyr in capsules at 2, 6, 20, or 60 mg ai/kg body weight/day for 28 consecutive days. Based on their feed consumption, these dose levels are equivalent to 57, 157, 607, and 1680 ppm of imazapyr in the diet. Cows were milked twice daily and the morning and evening samples were combined into one sample for the day. Samples from Day -1 (pretreatment) and Days 2, 3, 6, 8, 10, 13, 17, 24, and 27 were analyzed. Subsamples of milk from Days 8, 15, and 22 were centrifuged to obtain milk fat samples. After 28 days of treatment, cows were sacrificed and muscle, omental fat, kidney, and liver samples were collected. Samples were stored frozen for a maximum of 7 months, an interval which is supported by available storage stability data. Samples were analyzed for residues of imazapyr using adequate capillary electrophoresis/ ultraviolet detection (CE/UV) methods (BASF Methods M 3075, M 3223, or M 3184). The limit of quantitation (LOQ) for imazapyr is 0.01 ppm in milk and milk fat and 0.05 ppm in tissues.

In each dose group, imazapyr residues in milk reached a plateau within two days and remained steady over the remainder of the 28 day dosing period. In the 58 ppm dose group, imazapyr residues in milk were  $\leq 0.013$  ppm, with only 4 out of 27 samples having quantifiable residues. Average residues in milk were 0.028, 0.092, and 0.258 ppm for the 157, 607, and 1680 ppm dose groups, respectively. Average residues in milk increased with increasing dose level, and residues in milk fat were approximately 0.4-0.5x lower than in whole milk.

In each dose group, residues in tissue were lowest in muscle and fat, and highest in kidney, and residues levels generally increased with the dose level. In muscle, average residues were <0.05 ppm (<LOQ) in the 58 and 157 ppm dose groups, and were 0.097 and 0.234 ppm in the 607 and 1680 ppm dose groups. In fat, average residues were <0.05 ppm in the 58 and 157 ppm dose groups, and were 0.067 and 0.092 ppm in the 607 and 1680 ppm dose groups, respectively. In liver, average residues were <0.05 ppm in the 58 ppm dose groups, and were 0.057, 0.097 and 0.234 ppm in the 157, 607 and 1680 ppm dose groups. Quantifiable residues were detected in kidneys of each dose group. Average imazapyr residues in kidneys were 0.246, 0.519, 4.36, and 7.51 ppm for the 58, 157, 607, and 1680 ppm dose groups, respectively.

The ruminant feeding study data are adequate. Based on a MTDB of 24.0 ppm and comparison with the maximum residues levels observed in the 58 ppm dose group in the cattle feeding study, the maximum expected imazapyr residues in cattle commodities are <0.010 ppm in milk, <0.050 ppm in muscle, fat, and liver; and 0.15 ppm in kidney. Accordingly, the appropriate tolerance levels for imazapyr residues in cattle tissues are as follows: milk 0.01 ppm; meat, fat, and meat byproduct (except kidney) 0.05 ppm; kidney 0.20 ppm.

## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Test Substance

#### Active Ingredient

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid

CAS Number: 81334-34-1

Company Name: AC 243997 or CL 243997

Chemical purity: 100%

## 1.2. Trial Animals

Breed: Holstein dairy cow

Groups/Dosage Rate: 1 group dose rate

Number/Group: 3 cows/group

Dosage Rates: 4 different dose levels were used: 2, 6, 20, and 60 mg ai/kg body weight. Based on the average daily dietary intake, these dose levels are equivalent to dietary levels of imazapyr of 58, 157, 607, and 1680 ppm.

Dose vehicle: The test substance was administered orally, once a day in gel capsules using a balling gun.

Diet/water: A total mixed ration (53% dry weight) was provide twice a day, and water was provided *ad libitum*

In-life Facility: American Cyanamid, Agricultural Research Center, Princeton, NJ.

## 1.3. Post-harvest Procedures

Cows were milked twice daily and the morning and evening samples were combined into one sample for the day. Samples from Day -1 (pretreatment) and Days 2, 3, 6, 8, 10, 13, 17, 24, and 27 after treatment were collected and refrigerated at 3-5.8 C until shipment to ABC Laboratories (Columbia, MO), where samples were stored frozen ( $\leq -10$  C) until analysis. Subsamples of milk (milk from all cows/group mixed together) from Days 8, 15, and 22 were centrifuged to obtain milkfat, then placed in frozen storage until analysis.

After 28 consecutive days of treatment, cows were sacrificed and muscle, omental fat, kidney, and liver samples were collected and stored frozen (-16 to -20 C) until shipment to Maxim Technologies, Inc. (Middleport, NY) where samples were stored frozen ( $\leq -10$  C) until analysis. The longest interval from extraction to analysis was 4 days. Table 1.3.1 summarizes the storage conditions for the cattle commodities involved in this study. Total frozen ( $\leq -10$  C) storage intervals were 1-3 months for milk and milk fat and 6-7 months for tissues. These storage intervals are supported by the available storage stability data (45119719.der.wpd), which indicate that imazapyr is stable in frozen liver and muscle for up to 8 months and in frozen milk for up to 6 months.

| Table 1.3.1. Summary of Storage Conditions |                                     |                   |
|--------------------------------------------|-------------------------------------|-------------------|
| Cattle Commodity or Matrix                 | Storage Temperature ( $^{\circ}$ C) | Duration (months) |
| Milk                                       | $\leq -10$                          | 1-3               |
| Milkfat                                    | $\leq -10$                          | 1-3               |
| Muscle                                     | $\leq -10$                          | 6-7               |
| Omental Fat                                | $\leq -10$                          | 6-7               |
| Kidney                                     | $\leq -10$                          | 6-7               |
| Liver                                      | $\leq -10$                          | 6-7               |

## 1.4. Analytical Methods

Milk samples were analyzed by ABC Laboratories (Columbia, MO) for residues of imazapyr using a CE/UV method (BASF Method M 3075). For this method, milk is diluted with 1M HCl (2:1) and centrifuged. The resulting supernatant is filtered and diluted (1:1) with 1M  $K_3PO_4 \cdot H_2O$ . Residues are then partitioned into methylene chloride, and cleaned up using strong cation exchange (SCX) and  $C_{18}$  solid phase extraction (SPE) cartridges. Residues in the final fraction are analyzed by CE/UV at 240 nm. The validated LOQ for imazapyr in milk is 0.01 ppm, and the LOD is approximately 0.001 ppm.

Milk fat samples were analyzed by American Cyanamid (Princeton, NJ) for residues of imazapyr a CE/UV method (BASF Method M 3223). For this method, milk fat is extracted with water:acetonitrile (ACN):hexane (2:5:10, v/v/v), filtered, and allowed to separate. The ACN/water fraction is then partitioned with hexane:methyl isobutyl ketone (1:1) with a phosphate buffer, draining off the aqueous phase. The organic fraction is then repartitioned with 0.2% phosphate buffer, and the resulting aqueous fraction is added to the initial aqueous fraction. The aqueous phase is acidified and residues are partitioned into methylene chloride. Residues are then cleaned up using SCX and  $C_{18}$  SPE cartridges. Residues in the final fraction are analyzed by CE/UV at 265 nm. The validated LOQ for imazapyr in milk fat is 0.01 ppm, and the LOD is approximately 0.001 ppm.

Tissue samples were analyzed by Maxim Technologies (Middleport, NY) for residues of imazapyr using a CE/UV method (BASF Method M 3184). For this method, residues were extracted with acidic acetone:water (1:3, v/v), partitioned into methylene chloride, and cleaned up using SCX and  $C_{18}$  SPE cartridges. Maxim indicated the following minor changes to the BASF Method: Celite 521 (from Aldrich) was used instead of Celite 545 AW (from Supelco). Both products are acid washed, but the particle size of Celite 521 is smaller (2  $\mu m$ ) than that of Celite 545 AW (17  $\mu m$ ). Also, the nitrogen evaporator was set at 35-40 C instead of ambient temperature, and some CE parameters were slightly modified. Residues in the final fraction were analyzed by CE/UV at 265 nm. The validated LOQ for imazapyr in livestock tissues is 0.05 ppm, and the LOD is approximately 0.005 ppm. Recoveries by all methods were acceptable and are summarized in Table 2.1.

## 2. Results

| Table 2.1. Summary of Concurrent Analytical Method Validation after Fortification with Imazapyr. |                           |              |                                      |                        |
|--------------------------------------------------------------------------------------------------|---------------------------|--------------|--------------------------------------|------------------------|
| Cattle Matrix                                                                                    | Fortification Level (ppm) | # of samples | Range of Recoveries (%) <sup>a</sup> | Mean Recovery $\pm$ SD |
| Milk                                                                                             | 0.01-0.50                 | 39           | 75-93                                | 85 $\pm$ 5             |
| Milk Fat                                                                                         | 0.01-0.20                 | 8            | 60-85 (1)                            | 76 $\pm$ 8             |
| Muscle                                                                                           | 0.05-0.50                 | 5            | 74-82                                | 78 $\pm$ 3             |
| Omental Fat                                                                                      | 0.05-0.50                 | 5            | 79-88                                | 84 $\pm$ 4             |
| Kidney                                                                                           | 0.05-10.0                 | 5            | 78-89                                | 84 $\pm$ 4             |
| Liver                                                                                            | 0.05-2.50                 | 5            | 80-85                                | 82 $\pm$ 2             |

<sup>a</sup> Number of recoveries outside the 70-120% range are in parentheses.

| Table 2.2.1. Imazapyr Residues in <b>Milk</b> and <b>Milk Fat</b> Following Daily Oral Dosing with Imazapyr. |                               |                                       |                                      |
|--------------------------------------------------------------------------------------------------------------|-------------------------------|---------------------------------------|--------------------------------------|
| Cattle Matrix                                                                                                | Dose level (ppm) <sup>a</sup> | Sampling Interval (days) <sup>b</sup> | Imazapyr Residues (ppm) <sup>c</sup> |
| Milk                                                                                                         | 58                            | 2                                     | <0.010, <0.010, 0.013                |
|                                                                                                              |                               | 3                                     | <0.010, <0.010, 0.012                |
|                                                                                                              |                               | 6                                     | <0.010, <0.010, <0.010               |
|                                                                                                              |                               | 8                                     | <0.010, <0.010, <0.010               |
|                                                                                                              |                               | 10                                    | <0.010, <0.010, <0.010               |
|                                                                                                              |                               | 13                                    | <0.010, <0.010, <0.010               |
|                                                                                                              |                               | 17                                    | <0.010, <0.010, <0.010               |
|                                                                                                              |                               | 24                                    | <0.010, <0.010, 0.013                |
|                                                                                                              |                               | 27                                    | <0.010, <0.010, 0.012                |
| Milk                                                                                                         | 157                           | 2                                     | 0.030, 0.039, 0.036                  |
|                                                                                                              |                               | 3                                     | 0.029, 0.021, 0.024                  |
|                                                                                                              |                               | 6                                     | 0.022, 0.029, 0.027                  |
|                                                                                                              |                               | 8                                     | 0.028, 0.028, 0.024                  |
|                                                                                                              |                               | 10                                    | 0.023, 0.036, 0.028                  |
|                                                                                                              |                               | 13                                    | 0.023, 0.025, 0.025                  |
|                                                                                                              |                               | 17                                    | 0.031, 0.033, 0.023                  |
|                                                                                                              |                               | 24                                    | 0.024, 0.025, 0.034                  |
|                                                                                                              |                               | 27                                    | 0.021, 0.031, 0.027                  |
| Milk                                                                                                         | 607                           | 2                                     | 0.073, 0.072, 0.117                  |
|                                                                                                              |                               | 3                                     | 0.108, 0.094, 0.121                  |
|                                                                                                              |                               | 6                                     | 0.090, 0.075, 0.112                  |
|                                                                                                              |                               | 8                                     | 0.083, 0.080, 0.125                  |
|                                                                                                              |                               | 10                                    | 0.067, 0.057, 0.121                  |
|                                                                                                              |                               | 13                                    | 0.077, 0.081, 0.126                  |
|                                                                                                              |                               | 17                                    | 0.110, 0.076, 0.101                  |
|                                                                                                              |                               | 24                                    | 0.111, 0.071, 0.099                  |
|                                                                                                              |                               | 27                                    | 0.083, 0.057, 0.086                  |
| Milk                                                                                                         | 1680                          | 2                                     | 0.239, 0.350, 0.350                  |
|                                                                                                              |                               | 3                                     | 0.238, 0.270, 0.297                  |
|                                                                                                              |                               | 6                                     | 0.213, 0.247, 0.270                  |
|                                                                                                              |                               | 8                                     | 0.191, 0.248, 0.229                  |
|                                                                                                              |                               | 10                                    | 0.209, 0.288, 0.317                  |
|                                                                                                              |                               | 13                                    | 0.180, 0.334, 0.297                  |
|                                                                                                              |                               | 17                                    | 0.169, 0.267, 0.231                  |
|                                                                                                              |                               | 24                                    | 0.252, 0.303, 0.252                  |
|                                                                                                              |                               | 27                                    | 0.180, 0.264, 0.288                  |

| Table 2.2.1. Imazapyr Residues in <b>Milk</b> and <b>Milk Fat</b> Following Daily Oral Dosing with Imazapyr. |                               |                                       |                                      |
|--------------------------------------------------------------------------------------------------------------|-------------------------------|---------------------------------------|--------------------------------------|
| Cattle Matrix                                                                                                | Dose level (ppm) <sup>a</sup> | Sampling Interval (days) <sup>b</sup> | Imazapyr Residues (ppm) <sup>c</sup> |
| Milk Fat                                                                                                     | 58                            | 8                                     | <0.010                               |
|                                                                                                              |                               | 15                                    | <0.010                               |
|                                                                                                              |                               | 22                                    | <0.010                               |
|                                                                                                              | 157                           | 8                                     | 0.012                                |
|                                                                                                              |                               | 15                                    | 0.011                                |
|                                                                                                              |                               | 22                                    | 0.015                                |
|                                                                                                              | 607                           | 8                                     | 0.039                                |
|                                                                                                              |                               | 15                                    | 0.041                                |
|                                                                                                              |                               | 22                                    | 0.032                                |
|                                                                                                              | 1680                          | 8                                     | 0.102                                |
|                                                                                                              |                               | 15                                    | 0.093                                |
|                                                                                                              |                               | 22                                    | 0.111                                |

<sup>a</sup> Cows were dosed once a day with imazapyr in capsules at doses of approximately 1.2, 3.6, 12, and 36 g ai/day, which were equivalent to 58, 157, 607, and 1680 ppm in the diet based on average daily feed consumption (on a dry matter basis determined as follows: dry matter consumed (kg) = average daily feed consumption (kg) x 53%) for the three cows in each dosing group.

<sup>b</sup> Number of dose days before collection of milk samples.

<sup>c</sup> The LOQ is 0.010 ppm for milk and milk fat. No corrections for concurrent recovery values were made.

| Table 2.2.2. Imazapyr Residues in Cattle <b>Tissues</b> Following Daily Oral Doses of Imazapyr for 28 Consecutive Days. |            |                                      |
|-------------------------------------------------------------------------------------------------------------------------|------------|--------------------------------------|
| Cattle Matrix                                                                                                           | Dose (ppm) | Imazapyr Residues (ppm) <sup>a</sup> |
| Muscle                                                                                                                  | 58         | <0.050, <0.050, <0.050               |
|                                                                                                                         | 157        | <0.050, <0.050, <0.050               |
|                                                                                                                         | 607        | 0.145, 0.083, 0.064                  |
|                                                                                                                         | 1680       | 0.186, 0.246, 0.269                  |
| Fat                                                                                                                     | 58         | <0.050, <0.050, <0.050               |
|                                                                                                                         | 157        | <0.050, <0.050, <0.050               |
|                                                                                                                         | 607        | 0.150, <0.050, <0.050                |
|                                                                                                                         | 1680       | 0.086, 0.111, 0.080                  |
| Kidney                                                                                                                  | 58         | 0.105, 0.356, 0.277                  |
|                                                                                                                         | 157        | 0.320, 0.899, 0.338                  |
|                                                                                                                         | 607        | 7.02, 3.91, 2.14                     |
|                                                                                                                         | 1680       | 7.34, 7.23, 7.97                     |
| Liver                                                                                                                   | 58         | <0.050, <0.050, <0.050               |
|                                                                                                                         | 157        | <0.050, 0.070, <0.050                |
|                                                                                                                         | 607        | 0.317, 0.386, 0.198                  |
|                                                                                                                         | 1680       | 0.552, 0.704, 1.17                   |

<sup>a</sup> Cows were dosed once a day with imazapyr in capsules at doses of approximately 1.2, 3.6, 12, and 36 g ai/day, which were equivalent to 58, 157, 607, and 1680 ppm in the diet based on average daily feed consumption (on a dry matter basis determined as follows: dry matter consumed (kg) = average daily feed consumption (kg) x 53%) for the three cows in each dosing group. The LOQ is 0.05 ppm for ruminant tissues. No corrections for concurrent recovery values were made.

| Table 2.3. Summary of Residue Data in/on Ruminant Milk and Tissues. |            |                                       |                         |                     |                   |                 |
|---------------------------------------------------------------------|------------|---------------------------------------|-------------------------|---------------------|-------------------|-----------------|
| Cattle Matrix                                                       | Dose (ppm) | Sampling Interval (days) <sup>a</sup> | Imazapyr Residues (ppm) |                     |                   |                 |
|                                                                     |            |                                       | Minimum                 | Maximum             | Mean <sup>b</sup> | Std. Dev.       |
| Milk                                                                | 58         | 2-27                                  | <0.010 <sup>c</sup>     | 0.013               | 0.010             | <0.001          |
|                                                                     | 157        | 2-27                                  | 0.021                   | 0.039               | 0.028             | 0.003           |
|                                                                     | 607        | 2-27                                  | 0.057                   | 0.126               | 0.092             | 0.009           |
|                                                                     | 1680       | 2-27                                  | 0.169                   | 0.350               | 0.258             | 0.029           |
| Milk Fat                                                            | 58         | 8-22                                  | <0.010                  | <0.010              | <0.010            | NA <sup>d</sup> |
|                                                                     | 157        | 8-22                                  | 0.011                   | 0.015               | 0.013             | NA              |
|                                                                     | 607        | 8-22                                  | 0.032                   | 0.041               | 0.037             | NA              |
|                                                                     | 1680       | 8-22                                  | 0.093                   | 0.111               | 0.102             | NA              |
| Muscle                                                              | 58         | 28                                    | <0.050                  | <0.050 <sup>c</sup> | <0.050            | NA              |
|                                                                     | 157        | 28                                    | <0.050                  | <0.050              | <0.050            | NA              |
|                                                                     | 607        | 28                                    | 0.064                   | 0.145               | 0.097             | NA              |
|                                                                     | 1680       | 28                                    | 0.186                   | 0.269               | 0.234             | NA              |
| Fat                                                                 | 58         | 28                                    | <0.050                  | <0.050              | <0.050            | NA              |
|                                                                     | 157        | 28                                    | <0.050                  | <0.050              | <0.050            | NA              |
|                                                                     | 607        | 28                                    | <0.050                  | 0.015               | 0.067             | NA              |
|                                                                     | 1680       | 28                                    | 0.080                   | 0.111               | 0.092             | NA              |
| Kidney                                                              | 58         | 28                                    | 0.105                   | 0.356               | 0.246             | NA              |
|                                                                     | 157        | 28                                    | 0.320                   | 0.899               | 0.519             | NA              |
|                                                                     | 607        | 28                                    | 2.14                    | 7.02                | 4.36              | NA              |
|                                                                     | 1680       | 28                                    | 7.23                    | 7.97                | 7.51              | NA              |
| Liver                                                               | 58         | 28                                    | <0.050                  | <0.050              | <0.050            | NA              |
|                                                                     | 157        | 28                                    | <0.050                  | 0.070               | <0.057            | NA              |
|                                                                     | 607        | 28                                    | 0.198                   | 0.386               | 0.300             | NA              |
|                                                                     | 1680       | 28                                    | 0.552                   | 1.17                | 0.809             | NA              |

<sup>a</sup> Cows received daily oral doses of imazapyr for 28 consecutive days before sacrifice. Milk was collected on treatment days 2, 3, 6, 8, 10, 13, 17, 24, and 27 and milk fat was sampled on treatment days 8, 15, and 22.

<sup>b</sup> N = 27 for milk and N = 3 for milk fat and tissues.

<sup>c</sup> The LOQs are 0.01 ppm for milk and milk fat and 0.05 ppm for ruminant tissues.

<sup>d</sup> NA = not applicable. Standard deviations were not calculated for milk fat and ruminant tissues because N = 3.

### 3. Discussion

#### 3.1. Methods

The number and concentration of feeding levels used in the current study are adequate. Based on the grass field trials (45119720.der.wpd) and the petitioners proposed use directions, the recommended tolerances for grass forage and hay are 100 and 30 ppm, respectively. However, these tolerances are based on a broadcast application rate of 0.75 lb ae/A, and the label directions specify spot applications at 0.75 lb ae/treated acre, with no more than 10% of an acre being treated. Based on a MTDB of 24.0 ppm and comparison with the maximum residues levels observed in the 58 ppm dose group in the cattle feeding study, the maximum expected imazapyr residues in cattle commodities are <0.010 ppm in milk, <0.050 ppm in muscle, fat, and liver; and 0.15 ppm in kidney. Accordingly, the appropriate tolerance levels for imazapyr residues in cattle tissues are as follows: milk 0.01 ppm; meat, fat, and meat byproduct (except kidney) 0.05 ppm; kidney 0.20 ppm.

| Feed Commodity               | % Dry Matter <sup>a</sup> | % Diet <sup>a</sup> | Recommended Tolerances (ppm) | % Area treated/acre <sup>b</sup> | Dietary Contribution (ppm) <sup>c</sup> |
|------------------------------|---------------------------|---------------------|------------------------------|----------------------------------|-----------------------------------------|
| <b>Beef and Dairy Cattle</b> |                           |                     |                              |                                  |                                         |
| Grass forage                 | 25                        | 60                  | 100                          | 10                               | 24.0                                    |
| Grass hay                    | 88                        | 60                  | 30                           | 10                               | 2.05                                    |
| Corn grain                   | 88                        | 80                  | 0.05                         | NA                               | 0.05                                    |
| Corn forage                  | 40                        | 50                  | 0.05                         | NA                               | 0.06                                    |
| Corn stover                  | 83                        | 25                  | 0.05                         | NA                               | 0.02                                    |
| TOTAL BURDEN                 |                           |                     |                              |                                  | <b>24.0<sup>d</sup></b>                 |

<sup>a</sup> OPPTS 860.1000 [Table 1 (August 1996)].

<sup>b</sup> For the spot application to grasses, the label specifies that no more than 10% of any given acre is to be treated.

<sup>c</sup> Contribution = [tolerance / % DM] X % diet X % area treated/A).

<sup>d</sup> Based on a diet consisting of 60% grass forage and 40% corn grain.

NA = not applicable

Based on the concurrent method recoveries, the CE/UV methods (BASF Methods M 3075, M 3223, and M 3184) are adequate for collecting data on imazapyr residues in milk, milk fat, and tissues. Concurrent method recoveries were all within the acceptable 70-120% range, except one milk fat sample (60%). Apparent residues of imazapyr were <0.001 ppm in all control samples of milk (n=39) and milk fat (n=3), except for one control sample of milk with apparent residues at 0.002 ppm. Apparent residues of imazapyr were <0.005 ppm in three control samples of each tissue, with the exception of one fat control sample with apparent residues at 0.014 ppm (<LOQ). The method LOQs for imazapyr are 0.01 ppm in milk and milk fat and 0.05 ppm in ruminant tissues. The method LODs for imazapyr are 0.001 ppm in milk and milk fat and 0.005 ppm in livestock tissues. Adequate sample calculations and chromatograms were provided.

### 3.2. Results

In each dose group, imazapyr residues in milk reached a plateau within two days and remained steady over the remainder of the 28 day dosing period. In the 58 ppm dose group, imazapyr residues in milk were <0.01 ppm in 23 of the 27 milk samples. Only four milk samples from one cow had quantifiable residues (0.012-0.013 ppm). Average residues in milk increased with increasing dose level. Average residues in milk were 0.028, 0.092, and 0.258 ppm for the 157, 607, and 1680 ppm dose groups, respectively. Imazapyr residues in milk fat were approximately 0.4-0.5x lower than in whole milk.

In each dose group, residues in tissue were lowest in muscle and fat, and highest in kidney, and residues levels generally increased with the dose level. In muscle, average residues were <0.05 ppm (<LOQ) in the 58 and 157 ppm dose groups, and were 0.097 and 0.234 ppm in the 607 and 1680 ppm dose groups. In fat, average residues were <0.05 ppm in the 58 and 157 ppm dose groups, and were 0.067 and 0.092 ppm in the 607 and 1680 ppm dose groups, respectively. In liver, average residues were <0.05 ppm in the 58 ppm dose group, and were 0.057, 0.097 and 0.234 ppm in the 157, 607 and 1680 ppm dose groups, respectively. Quantifiable residues were detected in kidneys of each dose group. Average imazapyr residues in kidneys were 0.246, 0.519, 4.36, and 7.51 ppm for the 58, 157, 607, and 1680 ppm dose groups, respectively.

The ruminant feeding study data are adequate. Based on a MTDB of 240 ppm for cattle arising from the proposed use of imazapyr on grasses, and linear interpolation between the maximum residues levels observed in the 157 and 607 ppm dose groups, the maximum expected imazapyr residues in cattle commodities are 0.055 ppm in milk, 0.068 ppm in muscle and fat, 0.13 ppm in liver, and 2.0 ppm in kidney. These levels represent worst-case residue levels where cattle graze exclusively in treated areas, which the label restricts to 10% of any given acre of grasslands.

### 4. Deficiencies

None

### 5. References

None

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** Danette Drew, Chemist  
RRB3/HED (7509C)

*Drew*

**Petition Number:** PP#0F06166

**DP Barcode:** D275561

**Citation:** 45119720 Khunachak, A., Garrett, A., Baragary, N. (2000) Magnitude of the Residues of Imazapyr in Grass after Treatment with ARSENAL Herbicide: Lab Project Nos: RES 98-038 through RES 98-046 and RES 99-015 through RES 99-019. Unpublished study prepared by ABC Laboratories, Inc. and American Cyanamid Co. 1147 p.

**Sponsor:** BASF (formerly American Cyanamid Company)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

In fourteen tests conducted in AR, CO, GA, ID, IN, MI, NE (2), OR (2), PA, TX (2), and WI during 1996 and 1997, imazapyr (2 lb/gal AS [aqueous solution]) was applied as a single broadcast foliar application to established grass (bermuda, tall fescue, bluegrass, and brome grass) at 0.73-0.78 lb acid equivalents (ae)/A in 20-60 gallons of water per acre with a 0.25% (v/v) nonionic surfactant. Grass forage and hay samples were harvested 0.1, 7, 14, and 28 days after application, and the hay samples were allowed to field dry for 1-17 days after cutting. Samples were stored frozen for a maximum of 24 months and then analyzed for residues of imazapyr using an adequate capillary electrophoresis/ ultraviolet detection (CE/UV) method (BASF Method M 3023).

Residues of imazapyr were 27-98 ppm in/on 28 grass forage samples harvested immediately following application (0.1 day), which is the proposed PHI. Residues in/on forage declined steadily at subsequent sampling intervals to 0.59-12.2 ppm by 7 DAT (n=28), <0.5-10.6 ppm by 14 DAT (n=28), and <0.5-6.25 ppm by 28 DAT (n=26). Residues of imazapyr were 65-277 ppm in/on 28 hay samples harvested immediately following application, and declined to 0.88-27.1 ppm in/on 28 hay samples harvested at 7 DAT, which is the proposed PHI. Residues in/on hay continued to decline at later sampling intervals: 0.51-19.6 ppm by 14 DAT (n=28) and <0.5-8.56 ppm by 28 DAT (n=26).

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** Danette Drew, Chemist  
RRB3/HED (7509C)

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The grass field trial data are adequate, pending the submission of acceptable supporting storage stability data on corn forage and fodder, which will be used to support the grass field trials; and clarification of the identity and quantity of spray additives utilized in the grass field trials.

## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Imazapyr

#### Active Ingredient

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid

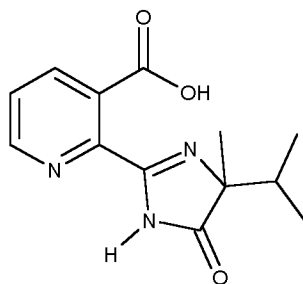
CAS Number: 81334-34-1

Company Name: AC 243997 or CL 243997

Other Synonyms: Arsenal Herbicide

Formulation: 2 lb ae/gal AS, 22.6% free acid

EPA Registration No.: 241-346



## 1.2. Trial Locations

| Table 1.2.1. Summary of Field Trial Numbers and Geographic Location. |                              |   |   |   |   |   |   |   |   |    |    |    |    |                        |
|----------------------------------------------------------------------|------------------------------|---|---|---|---|---|---|---|---|----|----|----|----|------------------------|
| Grass                                                                | Growing Region               |   |   |   |   |   |   |   |   |    |    |    |    | Total Number of Trials |
|                                                                      | 1                            | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |                        |
| Single broadcast foliar application at ~0.75 lb ae/A                 |                              |   |   |   |   |   |   |   |   |    |    |    |    |                        |
| Submitted                                                            | 1                            | 1 | - | 1 | 4 | 1 | 1 | 1 | 1 | -  | 1  | 2  | -  | 14                     |
| Requested                                                            | all areas across the country |   |   |   |   |   |   |   |   |    |    |    |    | 12                     |

Specific field trial information, including location, crop varieties, application method and application rate and timing, is listed in Table 2.2.

## 1.3. Post-harvest Procedures

Grass forage and hay was harvested 0.1, 7, 14, and 28 days after a single application at 13 of 14 locations (no 28 day harvest at one OR location). Additional grass forage and hay was harvested 56 or 58 days after application at two locations (ID and IN). Forage samples were placed in frozen storage shortly after collection. Hay samples were dried in the field or indoors for 1-17 days, then placed in frozen storage shortly after collection. Duplicate treated samples and a single control sample of grass forage and hay from each plot were kept frozen for 14-34 days until shipment via ACDS freezer truck to American Cyanamid (Princeton, NJ), where samples were stored frozen ( $\leq -10$  C) until shipment in dry ice via FedEx to the analytical laboratory (ABC Laboratories, Inc., Columbia, MO), where samples were stored at  $\leq -10$  C until analysis.

| Table 1.3.1. Summary of Storage Conditions |                |                          |                   |
|--------------------------------------------|----------------|--------------------------|-------------------|
| Matrix                                     | RAC            | Storage Temperature (°C) | Duration (months) |
| Grass                                      | Forage and Hay | $\leq -10$               | 11-24             |

## 1.4. Analytical Method

Samples of grass forage and hay were analyzed for residues of imazapyr using the CE/UV BASF Method M 3023. Briefly, residues are extracted with acidic acetone/water (1:4, v/v), concentrated, redissolved in methylene chloride, and cleaned up using solid phase extraction (SPE). The final extract is analyzed by CE/UV (240 nm) and quantitated by direct comparison of peak heights of the sample to those of external standards. The validated method LOQ is 0.5 ppm in/on grass forage and hay.

## 2. Results

| Table 2.1. Summary of Concurrent Analytical Method Validation after Fortification with Imazapyr. |                           |              |                                      |                        |
|--------------------------------------------------------------------------------------------------|---------------------------|--------------|--------------------------------------|------------------------|
| Grass Matrix                                                                                     | Fortification Level (ppm) | # of Samples | Range of Recoveries (%) <sup>a</sup> | Mean Recovery $\pm$ SD |
| Forage                                                                                           | 0.5-400                   | 79           | 72-127 (1)                           | 85 $\pm$ 10            |
| Hay                                                                                              | 0.5-250                   | 71           | 61-132 (2)                           | 87 $\pm$ 12            |

<sup>a</sup> Number of recoveries outside the 70-120% range are in parentheses.

| Table 2.2. Grass Crop and Field Trial Information. <sup>a</sup> |                              |               |                             |                             |                               |                |                |                |                  |
|-----------------------------------------------------------------|------------------------------|---------------|-----------------------------|-----------------------------|-------------------------------|----------------|----------------|----------------|------------------|
| EPA Region                                                      | Location (City, State, Year) | Grass Type    | Application Information     |                             |                               |                | Harvest Method | Plant Part     | PHI (days)       |
|                                                                 |                              |               | Adjuvant Added <sup>b</sup> | Rate (lb ae/A) <sup>c</sup> | Timing /Method                | Volume (gal/A) |                |                |                  |
| 4                                                               | Newport, AR, 1996            | Bermuda grass | NS <sup>d</sup>             | 0.74                        | Postemergence/BF <sup>e</sup> | 20             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 2                                                               | Hawkinsville, GA, 1996       | Bermuda grass | NS <sup>d</sup>             | 0.75                        | Postemergence/BF <sup>e</sup> | 26             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 6                                                               | Brookshire, TX, 1997         | Bermuda grass | Latron CS7 surfactant       | 0.77                        | Postemergence/BF <sup>e</sup> | 26             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 11                                                              | Payette, ID, 1996            | Tall Fescue   | NS <sup>d</sup>             | 0.76                        | Postemergence/BF <sup>e</sup> | 29             | Hand           | Forage and Hay | 0, 7, 14, 28, 58 |
| 1                                                               | Germansville, PA, 1996       | Tall Fescue   | NS <sup>d</sup>             | 0.78                        | Postemergence/BF <sup>e</sup> | 25             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 12                                                              | Hillsboro, OR, 1997          | Tall Fescue   | Nonionic surfactant         | 0.73                        | Postemergence/BF <sup>e</sup> | 22             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 8                                                               | Spearman, TX, 1996           | Tall Fescue   | LI 700, 0.25% v/v           | 0.74                        | Postemergence/BF <sup>e</sup> | 30             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 12                                                              | Halsey, OR, 1996             | Bluegrass     | NS <sup>d</sup>             | 0.75                        | Postemergence/BF <sup>e</sup> | 40             | Hand           | Forage and Hay | 0, 7, 14         |
| 5                                                               | Noblesville, IN, 1997        | Bluegrass     | X-77, 0.25%                 | 0.76                        | Postemergence/BF <sup>e</sup> | 23             | Hand           | Forage and Hay | 0, 7, 14, 28, 56 |
| 7                                                               | Grand Island, NE, 1997       | Bluegrass     | X-77                        | 0.76                        | Postemergence/BF <sup>e</sup> | 20             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 5                                                               | York, NE, 1996               | Bluegrass     | NS <sup>d</sup>             | 0.74                        | Postemergence/BF <sup>e</sup> | 20             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 5                                                               | Verona, WI, 1996             | Bromegrass    | NS <sup>d</sup>             | 0.75                        | Postemergence/BF <sup>e</sup> | 21             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 5                                                               | Sears, MI, 1996              | Bromegrass    | None <sup>f</sup>           | 0.74                        | Postemergence/BF <sup>e</sup> | 22             | Hand           | Forage and Hay | 0, 7, 14, 28     |
| 9                                                               | Read, Co, 1997               | Bromegrass    | Kinetic surfactant          | 0.75                        | Postemergence/BF <sup>e</sup> | 22             | Hand           | Forage and Hay | 0, 7, 14, 28     |

<sup>a</sup> The formulation used for all tests was a 2 lb/gal AS (aqueous solution).

<sup>b</sup> The study protocol specified the use of 0.25% (v/v) nonionic surfactant.

<sup>c</sup> The product is an isopropylamine salt of imazapyr, the active ingredient is listed as equivalent to 2 lb ae/gal AS.

<sup>d</sup> NS = Not specified.

<sup>e</sup> BF = Broadcast foliar.

<sup>f</sup> Listed as a protocol deviation due to field investigator oversight

| Table 2.3.1. Field Trial Results. <b>Grass Forage</b> |                              |                         |        |                                   |                         |                      |                                      |
|-------------------------------------------------------|------------------------------|-------------------------|--------|-----------------------------------|-------------------------|----------------------|--------------------------------------|
| EPA Region                                            | Location (City, State), Year | Application Information |        |                                   | PHI <sup>b</sup> (days) | Moisture Content (%) | Imazapyr Residues (ppm) <sup>c</sup> |
|                                                       |                              | Method                  | Number | Total Rate (lb ae/A) <sup>a</sup> |                         |                      |                                      |
| 4                                                     | Newport, AR, 1996            | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 67, 66               | <b>32.0, 41.5</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 58, 59               | 7.38 <sup>d</sup> , 7.88             |
|                                                       |                              |                         |        |                                   | 14                      | 53, 55               | 3.94, 4.25                           |
|                                                       |                              |                         |        |                                   | 28                      | 48, 49               | 1.50, 1.49                           |
| 2                                                     | Hawkinsville, GA, 1996       | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 72, 72               | <b>49.9, 57.3</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 66, 65               | 9.87, 6.82                           |
|                                                       |                              |                         |        |                                   | 14                      | 57, 56               | 4.32, 5.37                           |
|                                                       |                              |                         |        |                                   | 28                      | 64, 58               | 0.722, 0.951                         |
| 6                                                     | Brookshire, TX, 1997         | Broadcast Foliar        | 1      | 0.77                              | 0.1                     | 70, 68               | <b>59.6, 63.0</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 57, 57               | 10.2, 12.2                           |
|                                                       |                              |                         |        |                                   | 14                      | 52, 52               | 10.4, 10.6                           |
|                                                       |                              |                         |        |                                   | 28                      | 48, 48               | 6.41, 6.08                           |
| 11                                                    | Payette, ID, 1996            | Broadcast Foliar        | 1      | 0.76                              | 0.1                     | 71, 71               | <b>38.4, 37.8</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 71, 70               | 3.61, 3.86                           |
|                                                       |                              |                         |        |                                   | 14                      | 66, 66               | 1.58, 1.55                           |
|                                                       |                              |                         |        |                                   | 28                      | 61, 61               | 1.14, 1.04                           |
|                                                       |                              |                         |        |                                   | 58                      | 50                   | <0.50 <sup>e</sup>                   |
| 1                                                     | Germansville, PA, 1996       | Broadcast Foliar        | 1      | 0.78                              | 0.1                     | 82, 81               | <b>61.5, 38.1</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 78, 77               | 6.88, 6.82                           |
|                                                       |                              |                         |        |                                   | 14                      | 79, 79               | 2.16, 3.36                           |
|                                                       |                              |                         |        |                                   | 28                      | 60, 60               | 1.86, 1.77                           |
| 12                                                    | Hillsboro, OR, 1997          | Broadcast Foliar        | 1      | 0.73                              | 0.1                     | 80, 80               | <b>38.9, 33.5</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 79, 79               | 4.84, 5.06                           |
|                                                       |                              |                         |        |                                   | 14                      | 74, 75               | 1.89, 2.55                           |
|                                                       |                              |                         |        |                                   | 28                      | 64, 61               | 1.53, 1.89                           |
| 8                                                     | Spearman, TX, 1996           | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 78, 80               | <b>49.5, 39.1</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 76, 76               | 4.70, 4.09                           |
|                                                       |                              |                         |        |                                   | 14                      | 69, 68               | 4.32, 2.95                           |
|                                                       |                              |                         |        |                                   | 28                      | 55, 55               | 1.97, 2.65                           |
| 12                                                    | Halsey, OR, 1996             | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 49, 50               | <b>68.8, 80.8</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 49, 51               | 5.63, 4.69                           |
|                                                       |                              |                         |        |                                   | 14                      | 53, 50               | 3.05, 3.51                           |

| Table 2.3.1. Field Trial Results. <b>Grass Forage</b> |                              |                         |        |                                   |                         |                      |                                      |
|-------------------------------------------------------|------------------------------|-------------------------|--------|-----------------------------------|-------------------------|----------------------|--------------------------------------|
| EPA Region                                            | Location (City, State), Year | Application Information |        |                                   | PHI <sup>b</sup> (days) | Moisture Content (%) | Imazapyr Residues (ppm) <sup>c</sup> |
|                                                       |                              | Method                  | Number | Total Rate (lb ae/A) <sup>a</sup> |                         |                      |                                      |
| 5                                                     | Noblesville, IN, 1997        | Broadcast Foliar        | 1      | 0.76                              | 0.1                     | 67, 67               | <b>96.7, 98.0</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 67, 68               | 6.84, 5.96                           |
|                                                       |                              |                         |        |                                   | 14                      | 62, 63               | 3.10, 3.51                           |
|                                                       |                              |                         |        |                                   | 28                      | 55, 55               | 1.49, 1.69                           |
|                                                       |                              |                         |        |                                   | 56                      | 54, 52               | <0.5, <0.5 <sup>e</sup>              |
| 7                                                     | Grand Island, NE, 1997       | Broadcast Foliar        | 1      | 0.76                              | 0.1                     | 64, 63               | <b>66.4, 62.8</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 61, 61               | 4.38, 4.64                           |
|                                                       |                              |                         |        |                                   | 14                      | 57, 56               | 2.56, 2.63                           |
|                                                       |                              |                         |        |                                   | 28                      | 52, 52               | 0.92, 0.81                           |
| 5                                                     | York, NE, 1996               | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 76, 77               | <b>64.6, 65.7</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 69, 69               | 6.63, 6.04                           |
|                                                       |                              |                         |        |                                   | 14                      | 65, 66               | 4.28, 4.52                           |
|                                                       |                              |                         |        |                                   | 28                      | 45, 49               | 2.34, 2.56                           |
| 5                                                     | Verona, WI, 1996             | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 73, 73               | <b>64.1, 71.0</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 71, 71               | 3.88, 4.46                           |
|                                                       |                              |                         |        |                                   | 14                      | 66, 65               | 1.91, 2.13                           |
|                                                       |                              |                         |        |                                   | 28                      | 60, 60               | 0.70, 0.80                           |
| 5                                                     | Sears, MI, 1996              | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 83, 84               | <b>34.0, 32.7</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 84, 83               | 0.593, 0.807                         |
|                                                       |                              |                         |        |                                   | 14                      | 85, 83               | <0.50, <0.50                         |
|                                                       |                              |                         |        |                                   | 28                      | 68, 66               | <0.50, <0.50                         |
| 9                                                     | Read, Co, 1997               | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 73, 74               | <b>28.1, 26.9</b>                    |
|                                                       |                              |                         |        |                                   | 7                       | 70, 70               | 7.94, 6.50                           |
|                                                       |                              |                         |        |                                   | 14                      | 62, 65               | 4.86, 5.34                           |
|                                                       |                              |                         |        |                                   | 28                      | 58, 59               | 1.76, 1.80                           |

<sup>a</sup> The maximum proposed use rate for imazapyr is 0.75 lb ae/A.

<sup>b</sup> PHI (post harvest interval) = the number of days between application and harvest.

<sup>c</sup> Residues values in **bold** are from samples harvested at the maximum proposed PHI of zero days for grass forage.

<sup>d</sup> Residues values in *italics* are from duplicate analyses of a single sample.

<sup>e</sup> The LOQ is 0.50 ppm

| Table 2.3.2. Field Trial Results. <b>Grass Hay</b> |                              |                         |        |                                   |                         |                      |                                      |
|----------------------------------------------------|------------------------------|-------------------------|--------|-----------------------------------|-------------------------|----------------------|--------------------------------------|
| EPA Region                                         | Location (City, State), Year | Application Information |        |                                   | PHI <sup>b</sup> (days) | Moisture Content (%) | Imazapyr Residues (ppm) <sup>c</sup> |
|                                                    |                              | Method                  | Number | Total Rate (lb ae/A) <sup>a</sup> |                         |                      |                                      |
| 4                                                  | Newport, AR, 1996            | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 8.6, 8.8             | 115, 112 <sup>d</sup>                |
|                                                    |                              |                         |        |                                   | 7                       | 9.9, 10.4            | <b>17.6, 18.4</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 8.5, 8.7             | 8.34, 8.23                           |
|                                                    |                              |                         |        |                                   | 28                      | 20, 20               | 2.04, 2.09                           |
| 2                                                  | Hawkinsville, GA, 1996       | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 29, 23               | 111, 151                             |
|                                                    |                              |                         |        |                                   | 7                       | 14, 19               | <b>13.4, 12.8</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 16, 20               | 9.06, 10.8                           |
|                                                    |                              |                         |        |                                   | 28                      | 17, 18               | 2.25, 2.24                           |
| 6                                                  | Brookshire, TX, 1997         | Broadcast Foliar        | 1      | 0.77                              | 0.1                     | 16, 21               | 149, 129                             |
|                                                    |                              |                         |        |                                   | 7                       | 18, 20               | <b>23.2, 24.2</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 15, 16               | 15.5, 19.6                           |
|                                                    |                              |                         |        |                                   | 28                      | 14, 14               | 7.87, 8.56                           |
| 11                                                 | Payette, ID, 1996            | Broadcast Foliar        | 1      | 0.76                              | 0.1                     | 9.1, 8.9             | 168, 132                             |
|                                                    |                              |                         |        |                                   | 7                       | 7.8, 7.8             | <b>14.6, 9.51</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 9.1, 8.6             | 4.88, 4.51                           |
|                                                    |                              |                         |        |                                   | 28                      | 8.9, 9.0             | 2.56, 2.53                           |
|                                                    |                              |                         |        |                                   | 58                      | 9.6                  | 0.664                                |
| 1                                                  | Germansville, PA, 1996       | Broadcast Foliar        | 1      | 0.78                              | 0.1                     | 21, 27               | 186, 153                             |
|                                                    |                              |                         |        |                                   | 7                       | 40, 47               | <b>22.3, 18.3</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 20, 23               | 10, 11                               |
|                                                    |                              |                         |        |                                   | 28                      | 17, 18               | 4.13, 4.30                           |
| 12                                                 | Hillsboro, OR, 1997          | Broadcast Foliar        | 1      | 0.73                              | 0.1                     | 44, 35               | 121, 164                             |
|                                                    |                              |                         |        |                                   | 7                       | 15, 19               | <b>27.1, 23.7</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 45, 31               | 5.81, 6.61                           |
|                                                    |                              |                         |        |                                   | 28                      | 33, 33               | 3.58, 3.56                           |
| 8                                                  | Spearman, TX, 1996           | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 14, 22               | 189, 196                             |
|                                                    |                              |                         |        |                                   | 7                       | 29, 39               | <b>14.0, 11.2</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 35, 29               | 9.76, 11.1                           |
|                                                    |                              |                         |        |                                   | 28                      | 24, 37               | 3.71, 4.06                           |
| 12                                                 | Halsey, OR, 1996             | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 12, 11               | 139, 139                             |
|                                                    |                              |                         |        |                                   | 7                       | 12, 12               | <b>11.4, 10.4</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 17, 17               | 5.24, 5.30                           |

| Table 2.3.2. Field Trial Results. <b>Grass Hay</b> |                              |                         |        |                                   |                         |                      |                                      |
|----------------------------------------------------|------------------------------|-------------------------|--------|-----------------------------------|-------------------------|----------------------|--------------------------------------|
| EPA Region                                         | Location (City, State), Year | Application Information |        |                                   | PHI <sup>b</sup> (days) | Moisture Content (%) | Imazapyr Residues (ppm) <sup>c</sup> |
|                                                    |                              | Method                  | Number | Total Rate (lb ae/A) <sup>a</sup> |                         |                      |                                      |
| 5                                                  | Noblesville, IN, 1997        | Broadcast Foliar        | 1      | 0.76                              | 0.1                     | 13, 15               | 261, 277                             |
|                                                    |                              |                         |        |                                   | 7                       | 36, 39               | <b>12.2, 11.2</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 34, 26               | 5.61, 6.47                           |
|                                                    |                              |                         |        |                                   | 28                      | 21, 23               | 2.28, 2.42                           |
|                                                    |                              |                         |        |                                   | 56                      | 17, 16               | <0.50 <sup>e</sup> , <0.50           |
| 7                                                  | Grand Island, NE, 1997       | Broadcast Foliar        | 1      | 0.76                              | 0.1                     | 15, 18               | 159, 140                             |
|                                                    |                              |                         |        |                                   | 7                       | 22, 18               | <b>10.0, 10.2</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 15, 19               | 4.97, 5.13                           |
|                                                    |                              |                         |        |                                   | 28                      | 10, 10               | 1.88, 1.91                           |
| 5                                                  | York, NE, 1996               | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 28, 28               | 75, 88                               |
|                                                    |                              |                         |        |                                   | 7                       | 19, 18               | <b>19.6, 16.5</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 22, 21               | 9.98, 9.83                           |
|                                                    |                              |                         |        |                                   | 28                      | 13, 13               | 4.59, 4.43                           |
| 5                                                  | Verona, WI, 1996             | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 32, 25               | 164, 197                             |
|                                                    |                              |                         |        |                                   | 7                       | 24, 23               | <b>11.6, 12.5</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 25, 30               | 3.89, 3.70                           |
|                                                    |                              |                         |        |                                   | 28                      | 21, 23               | 1.92, 1.65                           |
| 5                                                  | Sears, MI, 1996              | Broadcast Foliar        | 1      | 0.74                              | 0.1                     | 64, 65               | 65.1, 64.7                           |
|                                                    |                              |                         |        |                                   | 7                       | 46, 49               | <b>0.88, 2.11</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 53, 55               | 0.84, 0.51                           |
|                                                    |                              |                         |        |                                   | 28                      | 29, 31               | 0.56, <0.50                          |
| 9                                                  | Read, CO, 1997               | Broadcast Foliar        | 1      | 0.75                              | 0.1                     | 34, 26               | 64.5, 77.5                           |
|                                                    |                              |                         |        |                                   | 7                       | 21, 20               | <b>21.7, 21.5</b>                    |
|                                                    |                              |                         |        |                                   | 14                      | 20, 26               | 12.3, 12.1                           |
|                                                    |                              |                         |        |                                   | 28                      | 16, 18               | 3.43, 3.99                           |

<sup>a</sup> The maximum proposed use rate for imazapyr is 0.75 lb ae/A.

<sup>b</sup> PHI (post harvest interval) = the number of days between application and harvest.

<sup>c</sup> Residues values in **bold** are from samples harvested at the maximum proposed PHI of 7 days for grass hay.

<sup>d</sup> Residues values in *italics* are from duplicate analyses of a single sample.

<sup>e</sup> The LOQ is 0.50 ppm.

| Table 2.4. Summary of Residue Data in/on Grass from Crop Field Trials with Imazapyr. |                              |                             |                         |                               |             |             |                   |             |
|--------------------------------------------------------------------------------------|------------------------------|-----------------------------|-------------------------|-------------------------------|-------------|-------------|-------------------|-------------|
| Grass Matrix                                                                         | Total Applic. Rate (lb ae/A) | Applic. Method <sup>a</sup> | PHI (days)              | Imazapyr Residue Levels (ppm) |             |             |                   |             |
|                                                                                      |                              |                             |                         | Minimum                       | Maximum     | HAFT        | Mean <sup>b</sup> | Std. Dev.   |
| Forage                                                                               | 0.73-0.78                    | Broadcast Foliar            | <b>0.1</b> <sup>c</sup> | <b>26.9</b>                   | <b>98.0</b> | <b>97.0</b> | <b>53.6</b>       | <b>19.5</b> |
|                                                                                      |                              |                             | 7                       | 0.59                          | 12.2        | 11.2        | 5.83              | 2.51        |
|                                                                                      |                              |                             | 14                      | <0.50                         | 10.6        | 10.5        | 3.63              | 2.34        |
|                                                                                      |                              |                             | 28                      | <0.50                         | 6.41        | 6.25        | 1.80              | 1.44        |
| Hay                                                                                  | 0.73-0.78                    | Broadcast Foliar            | 0.1                     | 64.5                          | 277         | 269         | 142               | 53.5        |
|                                                                                      |                              |                             | <b>7</b>                | <b>0.88</b>                   | <b>27.1</b> | <b>25.4</b> | <b>15.1</b>       | <b>6.41</b> |
|                                                                                      |                              |                             | 14                      | 0.51                          | 19.6        | 17.6        | 7.90              | 4.22        |
|                                                                                      |                              |                             | 28                      | 0.50                          | 8.56        | 8.22        | 3.19              | 1.86        |

<sup>a</sup> A single application of imazapyr (2 lb ae/gal AS) was made for all tests.

<sup>b</sup> N = 28, except at the 28-day sampling interval (N=26).

<sup>c</sup> Data for the proposed PHIs for forage (0-day) and hay (7-day) are **bolded**.

### 3. Discussion

#### 3.1. Methods

The number of crop field trials and geographic representation of the residue data on grass are adequate.

Total frozen ( $\leq -10$  C) storage intervals for grass forage and hay samples were 11-24 months. When submitted, supporting storage stability data for residues in/on field corn forage and fodder may be used to support the grass field trials.

Based on the concurrent method recoveries, the CE/UV method (BASF Method M 3023) is adequate for collecting data on imazapyr residues in/on grass forage and hay. Concurrent method recoveries were all within the acceptable 70-120% range, except one forage (127%) and two hay (62 and 132%) samples, and average method recoveries were  $85 \pm 10\%$  for forage and  $87 \pm 12\%$  for hay. Residues were also  $<0.50$  ppm ( $<LOQ$ ) in/on all control samples, except two hay samples with apparent imazapyr residues of 1.27 and 0.548 ppm. The method LOQ for imazapyr is 0.5 ppm in/on grass forage and hay. Adequate sample calculations and chromatograms were provided.

#### 3.2. Results

In a total of 14 field trials conducted throughout the U.S. during 1996 and 1997, imazapyr (2 lb/gal AS) was applied as a single broadcast foliar application to established grass (bermuda, tall fescue, bluegrass, and brome) at 0.73-0.78 lb ae/A. Grass forage and hay samples were harvested 0.1, 7, 14, and 28 days after application, and the hay samples were allowed to field dry for 1-17 days after cutting.

Residues of imazapyr were 27-98 ppm in/on 28 grass forage samples harvested immediately following application (0.1 day), which is the proposed PHI. Residues in/on forage declined steadily at subsequent sampling intervals to

0.59-12.2 ppm by 7 DAT (n=28), <0.5-10.6 ppm by 14 DAT(n=28), and <0.5-6.25 ppm by 28 DAT (n=26).

Residues of imazapyr were 65-277 ppm in/on 28 hay samples harvested immediately following application, and declined to 0.88-27.1 ppm in/on 28 hay samples harvested at 7 DAT, which is the proposed PHI. Residues in/on hay continued to decline at later sampling intervals: 0.51-19.6 ppm by 14 DAT (n=28) and <0.5-8.56 ppm by 28 DAT (n=26).

No unusual weather conditions were noted at any of the field trial sites in this study.

The grass field trial data are adequate, pending the submission of acceptable supporting storage stability data on corn forage and fodder, which will be used to support the grass field trials.

#### **4. Deficiencies**

Data are required supporting the stability of imazapyr in/on grass forage and hay for frozen storage intervals of up to 24 months. The registrant has indicated that data are available indicating that imazapyr is stable in frozen corn forage and fodder for up to 27 months (D234429, PP#6F04641, N. Dodd, 3/21/97); these data can be translated to support the grass field trials. However, the final report for these data has not yet been received or reviewed.

Also, in seven of the fourteen trial reports, no mention was made of which spray adjuvant was used or in what quantity. This information should be provided to ensure that the field trials were conducted in accord with the study protocol and that the residue data collected reflects the highest residue levels that may be expected when following label directions.

#### **5. References**

DP Barcode: D234429  
Subject: PP#6F04641 - Imazapyr on Field Corn. Amendment dated 3/18/97.  
From: N. Dodd  
To: D. McCall  
Dated: 3/21/97  
MRID(s): None

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

*David Soderberg*

**Petition Number:** PP#0F06166

**DP Barcode:** D275561

**Citation:** 45119719 Bixler, T., Fletcher, J., Khunachak, A., Naumann, L. (2000) Freezer Storage Stability of Imazapyr Residues in Cattle Tissue, Milk, and Fish: Lab Project Nos: RES 99-035, RES 99-031, RES 99-064.01. Unpublished study prepared by ABC Labs, Inc., Maxim Tech., Inc., and American Cyanamid Co. 102 p.

**Sponsor:** BASF (formerly American Cyanamid Company)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

Control samples of homogenized cattle muscle, liver, and milk were fortified with 0.1 ppm of imazapyr, then stored frozen for up to 6 or 8 months, with analysis at 0, 3, and 6-8 months. Control samples of fish muscle were homogenized and fortified with 0.5 ppm of imazapyr, then stored frozen for up to 21 months, with analysis at 0, 3, 6, 12, 18, and 21 months. Residues of imazapyr were stable for up to 6 months in frozen milk, 8 months in frozen (< -15 C) cattle tissue, and up to 21 months in frozen fish muscle.

This storage stability study is acceptable.

### GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. There were no deviations from regulatory requirements that would impact the study results or their interpretation.

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

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This storage stability study is acceptable.

## GLP Compliance

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## 1. Materials and Methods

### 1.1. Test Substance: Imazapyr

#### Active Ingredient

Common Name: Imazapyr

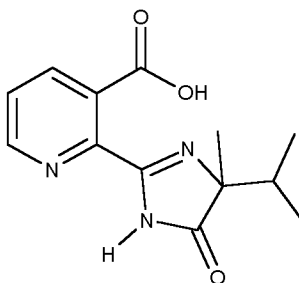
IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid

CAS Number: 81334-34-1

Company Name: AC 243997 or CL 243997

Other Synonyms: Arsenal Herbicide



### 1.2. Methods

Control samples of homogenized cattle muscle and liver tissue (20 g) and bovine milk (20 mL) were fortified with 0.1 ppm of imazapyr; fish muscle tissue samples were homogenized and fortified with 0.5 ppm of imazapyr. Cattle and fish samples were placed in a freezer (< -15 C). The milk freezer temperature was not reported. For each matrix, duplicate stored samples from each interval were analyzed along with one control sample and one freshly fortified sample. Information on storage conditions and sampling intervals is provided in Table 2.1.1.

Samples were analyzed for residues of imazapyr using the CE/UV methods (BASF Methods M 3184 for cattle tissues, M 3075 for milk, and M 3066 for fish).

#### BASF Method M 3066

Briefly, residues are extracted with acidic acetone/water (1:4, v/v), concentrated, redissolved in methylene chloride, and cleaned up using SPE. The final extract is analyzed by CE with a UV detector set at 240 nm and quantified by direct comparison of peak heights of the sample to those of external standards. The reported LOQ is 0.05 ppm.

#### BASF Method M 3184

Briefly, residues are extracted with acidic acetone/water (1:4, v/v), concentrated, redissolved in methylene chloride, and cleaned up using SPE. The final extract is analyzed by CE with a UV detector set at 240 nm and quantitated by direct comparison of peak heights of the sample to those of external standards. The reported LOQ is 0.05 ppm.

### BASF Method M 3075

Briefly, residues are extracted with acidic water (10 ml of 1 M HCL added to 20 ml milk), concentrated, redissolved in methylene chloride, and cleaned up using SPE. The final extract is analyzed by CE with a UV detector set at 240 nm and quantitated by direct comparison of peak heights of the sample to those of external standards. The reported LOQ is 0.01 ppm.

These methods are the same as the data collection methods.

## 2. Results

### 2.1. Stability in the Commodity

| Table 2.1.1. Frozen Storage Stability of Imazapyr in Animal Matrices |                   |                     |                         |                               |                                        |                                         |
|----------------------------------------------------------------------|-------------------|---------------------|-------------------------|-------------------------------|----------------------------------------|-----------------------------------------|
| Matrix                                                               | Spike Level (ppm) | Storage Temp (°C)   | Storage Period (months) | Concurrent Spike Recovery (%) | Apparent Recovery in Stored Sample (%) | Corrected Recovery in Stored Sample (%) |
| Cattle Muscle                                                        | 0.1               | < -15               | 0                       | 86                            | 84, 84                                 | 98, 98                                  |
|                                                                      |                   |                     | 3                       | 84                            | 80, 83                                 | 95, 99                                  |
|                                                                      |                   |                     | 8                       | 76                            | 80, 82                                 | 105, 108                                |
| Cattle Liver                                                         | 0.1               | < -15               | 0                       | 83                            | 81, 81                                 | 98, 98                                  |
|                                                                      |                   |                     | 3                       | 80                            | 82, 81                                 | 103, 101                                |
|                                                                      |                   |                     | 8                       | 75                            | 77, 77                                 | 103, 103                                |
| Milk                                                                 | 0.1               | Frozen <sup>a</sup> | 0                       | 86                            | 87, 85                                 | 101, 99                                 |
|                                                                      |                   |                     | 3                       | 76                            | 73, 75                                 | 96, 99                                  |
|                                                                      |                   |                     | 6                       | 95                            | 84, 79                                 | 88, 83                                  |
| Fish Muscle                                                          | 0.5               | < -15               | 0                       | 83                            | 80, 81                                 | 96, 98                                  |
|                                                                      |                   |                     | 3                       | 78                            | 82, 73                                 | 105, 94                                 |
|                                                                      |                   |                     | 6                       | 86                            | 88, 89                                 | 102, 103                                |
|                                                                      |                   |                     | 12                      | 79                            | 84, 83                                 | 106, 105                                |
|                                                                      |                   |                     | 18                      | 79                            | 87, 86                                 | 110, 109                                |
|                                                                      |                   |                     | 21                      | 70                            | 77, 76                                 | 110, 109                                |

<sup>a</sup> Milk samples were stored in a freezer at an unspecified temperature.

## 3. Discussion

Control samples of homogenized cattle muscle, liver, and milk were fortified with 0.1 ppm of imazapyr, then stored frozen for up to 6 or 8 months, with interim analyses at 0 and 3 months. Control samples of fish muscle were homogenized and fortified with 0.5 ppm of imazapyr, then stored frozen for up to 21 months, with interim analyses at 0, 3, 6, 12, and 18 months. Residues of imazapyr were stable for up to 6 months in frozen milk, 8 months in frozen (< -15 C) cattle tissue, and up to 21 months in frozen fish muscle.

## 4. Deficiencies

No deficiencies were noted that would impact the acceptability of this storage stability study. However, the petitioner should specify the temperature at which the milk samples were stored while in frozen storage.

## **5. References**

None

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

*David Soderberg*

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**Sponsor:** BASF (formerly American Cyanamid Co.)

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### Executive Summary

A series of independent laboratory validation (ILV) trials were conducted on the following potential enforcement methods: (i) a capillary electrophoresis (CE)/UV detection method (BASF Method M 3023.01) for determining imazapyr in grass forage and hay; (ii) a CE/UV method (BASF Method M 3184) for determining imazapyr in cattle tissues; (iii) a CE/UV method (BASF Method M 3075) for determining imazapyr in milk; (iv) a CE/UV method (BASF Method M 3223) for determining imazapyr in milk fat; and (v) a CE/UV method (BASF Method M 3066) for determining imazapyr in fish and crayfish. In addition, three of these CE/UV methods (M 3075, M 3184, and M 3066) were radiovalidated using samples from the goat and oyster metabolism studies.

Each of these CE/UV methods was successfully validated by an independent laboratory. The average recovery of imazapyr from grass forage and hay fortified at 0.5-50.0 ppm was  $84 \pm 2.4\%$  (n=16) using CE/UV Method M 3023. The average recoveries of imazapyr from cattle tissues fortified at 0.05-0.50 ppm were  $80 \pm 4.8\%$  from muscle (n=6),  $80 \pm 4.4\%$  from liver (n=6),  $79 \pm 5.4\%$  from kidneys (n=6), and  $82 \pm 6.2\%$  from fat (n=6) using CE/UV Method M 3184. The average recovery of imazapyr from milk fortified at 0.01-0.50 ppm was  $90 \pm 5.6\%$  (n=6) using CE/UV Method M 3075, and the average recovery of imazapyr from milk fat fortified at 0.01-0.10 ppm was  $78 \pm 9.5\%$  (n=6) using CE/UV Method M 3223. The average recoveries of imazapyr from fish

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Each of these CE/UV methods was successfully validated by an independent laboratory. The average recovery of imazapyr from grass forage and hay fortified at 0.5-50.0 ppm was  $84 \pm 2.4\%$  (n=16) using CE/UV Method M 3023. The average recoveries of imazapyr from cattle tissues fortified at 0.05-0.50 ppm were  $80 \pm 4.8\%$  from muscle (n=6),  $80 \pm 4.4\%$  from liver (n=6),  $79 \pm 5.4\%$  from kidneys (n=6), and  $82 \pm 6.2\%$  from fat (n=6) using CE/UV Method M 3184. The average recovery of imazapyr from milk fortified at 0.01-0.50 ppm was  $90 \pm 5.6\%$  (n=6) using CE/UV Method M 3075, and the average recovery of imazapyr from milk fat fortified at 0.01-0.10 ppm was  $78 \pm 9.5\%$  (n=6) using CE/UV Method M 3223. The average recoveries of imazapyr from fish

and shellfish fortified at 0.05-0.50 ppm were  $84 \pm 4.1\%$  from fish fillets (n=6) and  $84 \pm 11\%$  from crayfish meat (n=6) using CE/UV Method M 3066, which is essentially identical to Method M 3184.

The validated LOQs for imazapyr are 0.5 ppm in/on grass forage and hay, 0.05 ppm in cattle tissues, fish and shellfish, and 0.01 ppm in milk and milk fat.

Adequate radiovalidation data were also submitted for the proposed CE/UV methods (M 3066, M 3075, and M 3185) and demonstrated the efficiency of the methods in extracting residues from aged samples. The submitted CE/UV methods (BASF Methods M 3023.01, M 3184, M 3075, M 3223, and 3066) are adequate for determination of imazapyr in various plant and animal matrices, and will be forwarded to the EPA's Analytical Chemistry Branch (ACB) for petition method validation (PMV) trials.

## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations were noted that would impact the acceptability the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Test Substances

| Table 1.1.1. List of Analytes Tested with CE/UV methods (BASF Methods M 3023.01, M 3184, M 3075, M 3223, and M 3066). |                                                                 |                                                                                             |             |               |                      |
|-----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------|---------------|----------------------|
| Common Name:                                                                                                          | IUPAC Name:                                                     | CAS Name:                                                                                   | CAS Number: | Company Name: | Other Synonyms:      |
| Imazapyr                                                                                                              | 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid | 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid | 81334-34-1  | Imazapyr      | CL 243997, AC 243997 |

| Table 1.1.2. Matrices and Imazapyr Fortification Levels Tested with CE/UV methods (BASF Methods M 3023, M 3184, M 3075, M 3223, and M 3066). |              |             |                               |
|----------------------------------------------------------------------------------------------------------------------------------------------|--------------|-------------|-------------------------------|
| Method                                                                                                                                       | Matrix       | Matrix Form | Imazapyr fortification levels |
| M 3023                                                                                                                                       | Grass        | Forage      | 0.5-50.0                      |
|                                                                                                                                              |              | Hay         | 0.5-50.0                      |
| M 3184                                                                                                                                       | Cattle       | Muscle      | 0.05-0.50                     |
|                                                                                                                                              |              | Liver       | 0.05-0.50                     |
|                                                                                                                                              |              | Kidney      | 0.05-0.50                     |
|                                                                                                                                              |              | Fat         | 0.05-0.50                     |
| M 3075                                                                                                                                       | Dairy Cattle | Milk        | 0.01-0.50                     |
| M 3223                                                                                                                                       | Dairy Cattle | Milkfat     | 0.01-0.10                     |
| M 3066                                                                                                                                       | Fish         | Muscle      | 0.05-0.50                     |
|                                                                                                                                              | Crayfish     |             | 0.05-0.50                     |

## 1.2. Methods

A series of ILV trials were conducted on the following potential enforcement methods: (i) a capillary electrophoresis (CE)/UV detection method (BASF Method M 3023.01) for determining imazapyr in grass forage and hay; (ii) a CE/UV method (BASF Method M 3184) for determining imazapyr in cattle tissues; (iii) a CE/UV method (BASF Method M 3075) for determining imazapyr in milk; (iv) a CE/UV method (BASF Method M 3223) for determining imazapyr in milk fat; and (v) a CE/UV method (BASF Method M 3066) for determining imazapyr in fish and crayfish.

In addition, three of these CE/UV methods (M 3075, M 3184, and M 3066) were also radiovalidated using samples from the goat and oyster metabolism studies. Each of these methods is described separately below.

### BASF Method M 3023.01

For this method, residues are extracted with acidic acetone:water (1:3, v/v), partitioned into methylene chloride, and cleaned up using solid phase extraction (SPE). Residue are loaded onto a benzenesulfonic acid strong cation exchange (SCX) SPE cartridge, which is then rinsed with acetone and methanol. Residues are then eluted with KCl-saturated methanol, concentrated to dryness, and redissolved in 1% formic acid. Residues are then loaded onto a RP102 SPE cartridge and eluted with methanol. The final fraction is concentrated to dryness, and residues are redissolved in water and analyzed by CE with a UV detector set at 240 nm. Residues are quantified by direct comparison of peak heights of the sample to those of external standards. The reported LOQ for imazapyr in/on grass forage and hay is 0.5 ppm, and the LOD is approximately 0.05 ppm. This method also includes directions for a confirmatory analysis by LC/MS, in which residues of imazapyr are determined by scanning for the m/z 262 ion.

For the ILV trial, which was conducted by ABC Laboratories (Columbia, MO), homogenized control samples of grass forage and hay fortified with imazapyr dissolved in acetone at 0.5, 1.0, 5.0, and 50.0 ppm. Equilibration time was not reported.

#### BASF Method M 3184

For this method, residues are extracted with acidic acetone:water (1:3, v/v), centrifuged and filtered through Celite. Residues are then partitioned into methylene chloride, and cleaned up using SPE. Residue are loaded onto a SCX SPE cartridge, which is then rinsed with acetone and methanol. Residues are then eluted with KCl-saturated methanol, concentrated to dryness, and redissolved in acidic water (pH 2). Residues are then loaded onto a C<sub>18</sub> SPE cartridge, which is washed with water and hexane, and residues are eluted with methylene chloride. The final fraction is concentrated to dryness, and residues are redissolved in water and analyzed by CE with a UV detector set at 265 nm. Residues are quantified by direct comparison of peak heights of the sample to those of external standards. The reported LOQ for imazapyr in cattle meat, meat byproducts, and fat is 0.05 ppm, and the LOD is approximately 0.005 ppm. This method also includes directions for a confirmatory analysis by LC/MS, in which residues of imazapyr are determined by scanning for the m/z 262 ion.

For the ILV trial, which was conducted by Maxim Technologies (Middleport, NY), homogenized control samples of cattle muscle, liver, kidney, and fat were fortified with imazapyr dissolved in acetone at 0.05, 0.25, and 0.50 ppm. Equilibration time was not reported.

#### BASF Method M 3075

For this method, milk is diluted with 1M HCl (2:1) and centrifuged. The resulting supernatant is filtered through Celite and diluted (1:1) with 1M K<sub>3</sub>PO<sub>4</sub>·H<sub>2</sub>O. Residues are then partitioned into methylene chloride, and cleaned up using SPE. Residue are loaded onto a SCX SPE cartridge, which is then rinsed with acetone and methanol. Residues are then eluted with KCl-saturated methanol, concentrated to dryness, and redissolved in acidic water (pH 2). Residues are then loaded onto a C<sub>18</sub> SPE cartridge, which is washed with water and hexane, and residues are eluted with methylene chloride. The final fraction is concentrated to dryness, and residues are redissolved in water and analyzed by CE with a UV detector set at 240 nm. Residues are quantified by direct comparison of peak heights of the sample to those of external standards. The reported LOQ for imazapyr in milk is 0.01 ppm, and the LOD is approximately 0.001 ppm. This method also includes directions for a confirmatory analysis by LC/MS, in which residues of imazapyr are determined by scanning for the m/z 262 ion.

For the ILV trial, which was conducted by ABC Laboratories (Columbia, MO), control samples of homogenized milk were fortified with imazapyr dissolved in acetone at 0.01, 0.10, and 0.50 ppm. Equilibration time was not reported.

#### BASF Method M 3223

For this method, milk fat is melted, extracted with water:acetonitrile (ACN):hexane (2:5:10, v/v/v), and filtered. The resulting filtrate is allowed to separate into ACN/water and hexane fractions, discarding the hexane phase. The ACN/water fraction is then partitioned with hexane:methyl isobutyl ketone (1:1) with a phosphate buffer, draining off the aqueous phase. The organic fraction is then repartitioned with 0.2% phosphate buffer, and the resulting aqueous fraction is added to the initial aqueous fraction. The aqueous phase is acidified and residues are partitioned into methylene chloride. Residues are then concentrated to dryness, redissolved in methanol and

loaded onto a SCX SPE cartridge, which is then rinsed with methanol. Residues are then eluted with KCl-saturated methanol, concentrated to dryness, and redissolved in acidic water (pH 2). Residues are then loaded onto a C<sub>18</sub> SPE cartridge, which is washed with water and hexane, and residues are eluted with methanol. The final fraction is concentrated to dryness, and residues are redissolved in water and analyzed by CE with a UV detector set at 265 nm. Residues are quantified by direct comparison of peak heights of the sample to those of external standards. The reported LOQ for imazapyr in milk fat is 0.01 ppm, and the LOD is approximately 0.001 ppm. This method also includes directions for a confirmatory analysis by LC/MS, in which residues of imazapyr are determined by scanning for the m/z 262 ion.

For the ILV trial, which was conducted by Maxim Technologies (Middleport, NY), control samples of homogenized milk fat were fortified with imazapyr dissolved in acetone at 0.01, 0.05, and 0.10 ppm. Equilibration time was not reported.

### BASF Method M 3066

For this method is essentially the same as Method 3184 above. Residues are extracted with acidic acetone:water (1:3, v/v), centrifuged, and filtered through Celite. Residues are then partitioned into methylene chloride, and cleaned up using SPE. Residues are loaded onto a SCX SPE cartridge, which is then rinsed with acetone and methanol. Residues are then eluted with KCl-saturated methanol, concentrated to dryness, and redissolved in acidic water (pH 2). Residue are then loaded onto a C<sub>18</sub> SPE cartridge, which is washed with water and hexane, and residues are eluted with methylene chloride. The final fraction is concentrated to dryness, and residues are redissolved in water and analyzed by CE with a UV detector set at 240 nm. Residues are quantified by direct comparison of peak heights of the sample to those of external standards. The reported LOQ for imazapyr in fish and shellfish meat is 0.05 ppm, and the LOD is approximately 0.005 ppm. This method also includes directions for a confirmatory analysis by LC/MS, in which residues of imazapyr are determined by scanning for the m/z 262 ion.

For the ILV trial, which was conducted by Maxim Technologies (Middleport, NY), control samples of fish fillets and crayfish tail meat were fortified with imazapyr dissolved in acetone at 0.05, 0.25, and 0.50 ppm. Equilibration time was not reported.

## **2. Results**

### **2.1. Stability of Reference Materials**

Reference standards were stored in a refrigerator at 5 ± 4 C. Stability of the standards was not reported.

### **2.2. Method Characteristics**

#### **2.2.1. Chromatography**

The migration time for imazapyr through the various CE/UV methods was approximately 18.5 minutes in Method M 3023.01, 11.7 minutes in Methods M 3184, M 3223, and M 3066, and 4.9 minutes in Method M 3075. All methods produced discrete peaks for imazapyr. There was no co-extracted material that may interfere with analysis or that may carry over into sequential chromatograms. Adequate representative

chromatograms and calculation examples were provided. For the LC/MS confirmatory analyses, the retention time for imazapyr is approximately 3.5 minutes in Method M 3075, 4.2 minutes in Method M 3023, M 3184, and M 3066, and 5.7 minutes in Method M 3223.

#### 2.2.2. Linearity

The linearity of the methods was verified; however, the coefficient of determination ( $r^2$ ) and the range over which the linearity was evaluated was not reported.

#### 2.2.3. Specificity

The methods employ UV detection, which has adequate specificity. There were no peaks at the retention time(s) of interest in control samples. In addition, the CE/UV method includes directions for confirmatory analysis of imazapyr using LC/MS analysis and monitoring for the m/z 262 ion.

#### 2.2.4. Method Limits

The validated LOQs for imazapyr are 0.5 ppm in/on grass forage and hay (Method M 3023), 0.05 ppm in cattle tissues (Method M 3184), fish and shellfish (Method M 3066), and 0.01 ppm in milk and milk fat (Methods M 3075 and M 3223). The reported LODs for imazapyr are as follows: 0.05 ppm in/on grass forage and hay, 0.005 ppm in cattle tissues, fish and shellfish, and 0.001 ppm in milk and milk fat.

## 2.2.5. Independent Laboratory Validation Analyte Recoveries

BASF Method 3023.01

The first method trial was successful, and the average recovery of imazapyr was  $84 \pm 2.4\%$  from both grass forage and hay (Table 2.2.5.1). There was no communication between the independent analytical laboratory (ABC Laboratories, Inc.) and the study sponsor during the ILV trial. A set of ten samples required 18 person hours for extraction and preparation, with an additional 12 hours was required for the analysis and calculations, for a total of 30 hours. No difficulties were experienced during the conduct of the ILV, and no changes were made by the analytical laboratory.

| Table 2.2.5.1 Independent Laboratory Validation. of the CE/UV method (BASF Method M 3023) for determining Imazapyr in grass forage and hay. |                           |                |                        |
|---------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|----------------|------------------------|
| Crop/ Matrix                                                                                                                                | Fortification Level (ppm) | Recoveries (%) | Mean Recovery $\pm$ SD |
| Grass Forage                                                                                                                                | 0.5                       | 81, 85         | $84 \pm 2.3$           |
|                                                                                                                                             | 1.0                       | 82, 82         |                        |
|                                                                                                                                             | 5.0                       | 86, 86         |                        |
|                                                                                                                                             | 50.0                      | 85, 88         |                        |
| Grass Hay                                                                                                                                   | 0.5                       | 81, 85         | $84 \pm 2.4$           |
|                                                                                                                                             | 1.0                       | 85, 82         |                        |
|                                                                                                                                             | 5.0                       | 87, 81         |                        |
|                                                                                                                                             | 50.0                      | 86, 86         |                        |

BASF Method 3184

The first method trial was successful, and the average recovery of imazapyr was  $80 \pm 4.8\%$  from muscle,  $80 \pm 4.4\%$  from liver,  $79 \pm 5.4\%$  from kidneys, and  $82 \pm 6.2\%$  from fat (Table 2.2.5.2). There was no communication between the analytical laboratory and the study sponsor during the ILV trial. A set of eight samples required 8 person hours for extraction and preparation, with auto injection and analysis occurring overnight. No difficulties were experienced during the conduct of the ILV, and only three changes were made by the independent analytical laboratory, Maxim Technonogies, Inc. The following minor changes were made to the BASF Method: Celite 521 (from Aldrich) was used instead of Celite 545 AW (from Supelco). Both products are acid washed, but the particle size of Celite 521 is smaller ( $2 \mu\text{m}$ ) than that of Celite 545 AW ( $17 \mu\text{m}$ ). Also, the nitrogen evaporator was set at 35-40 C instead of ambient temperature, and some CE parameters were slightly modified. The laboratory noted the following critical step in the analysis: during the methylene chloride step, the mixture should remain undisturbed for 5 minutes to allow for complete phase separation.

BASF Method 3075

The first method trial was successful, and the average recovery of imazapyr was  $90 \pm 5.6\%$  from milk (Table 2.2.5.2). There was no communication between the analytical laboratory (ABC Laboratories, Inc.) and the study sponsor during the ILV trial. A set of eight samples required 12 person hours for extraction and preparation, and

another 8 hours for CE/UV analysis of eight samples and nine standards. No difficulties were experienced during the conduct of the ILV, and no changes were made by the analytical laboratory.

### BASF Method 3223

The independent analytical laboratory (Maxim Technologies, Inc.) reported that the first method trial was unsuccessful due to low recoveries, but the recoveries were not reported. Prior to beginning the second trial, the method was updated by the sponsor. During the second trial, low recoveries were again noted in the first set of samples each fortification (67-73%), and the laboratory again notified the sponsor. After discovering that the pH meter in the laboratory was malfunctioning, the analytical laboratory ran another set of each fortification and achieved adequate recoveries (81-91%). Including both sets from the second trial, the average recovery of imazapyr was  $78 \pm 9.5\%$  from milk fat (Table 2.2.5.2). A set of eight samples required 8 person hours for extraction and preparation, with auto injection and analysis occurring overnight. Only one minor modification to the method was noted by Maxim: some CE parameters were slightly modified.

| Table 2.2.5.2 Independent Laboratory Validation of the CE/UV methods (BASF Methods M 3184, M 3075, and M 3223) for determining Imazapyr in Livestock Tissues and Milk. |                           |                      |                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|----------------------|--------------------|
| Animal Matrix                                                                                                                                                          | Fortification Level (ppm) | Recoveries (%)       | Mean Recovery ± SD |
| BASF Method 3184                                                                                                                                                       |                           |                      |                    |
| Cattle Muscle                                                                                                                                                          | 0.05                      | 71, 78               | 80 ± 4.8           |
|                                                                                                                                                                        | 0.25                      | 80, 81               |                    |
|                                                                                                                                                                        | 0.50                      | 82, 85               |                    |
| Cattle Liver                                                                                                                                                           | 0.05                      | 72, 78               | 80 ± 4.4           |
|                                                                                                                                                                        | 0.25                      | 85, 79               |                    |
|                                                                                                                                                                        | 0.50                      | 82, 81               |                    |
| Cattle Kidney                                                                                                                                                          | 0.05                      | 71, 77               | 79 ± 5.4           |
|                                                                                                                                                                        | 0.25                      | 78, 86               |                    |
|                                                                                                                                                                        | 0.50                      | 80, 84               |                    |
| Cattle Fat                                                                                                                                                             | 0.05                      | 71, 78               | 82 ± 6.2           |
|                                                                                                                                                                        | 0.25                      | 87, 87               |                    |
|                                                                                                                                                                        | 0.50                      | 84, 84               |                    |
| BASF Method M 3075                                                                                                                                                     |                           |                      |                    |
| Milk                                                                                                                                                                   | 0.01                      | 85, 81               | 90 ± 5.6           |
|                                                                                                                                                                        | 0.10                      | 92, 96               |                    |
|                                                                                                                                                                        | 0.50                      | 93, 92               |                    |
| BASF Method M 3223                                                                                                                                                     |                           |                      |                    |
| Milk Fat                                                                                                                                                               | 0.01                      | 67 <sup>a</sup> , 81 | 78 ± 9.5           |
|                                                                                                                                                                        | 0.05                      | 73 <sup>a</sup> , 86 |                    |
|                                                                                                                                                                        | 0.10                      | 70 <sup>a</sup> , 91 |                    |

<sup>a</sup> Low recoveries were initially obtained due to malfunctioning pH meter.

BASF Method 3066

The first method trial was successful, and the average recovery of imazapyr was  $86 \pm 4.1\%$  from fish fillets and  $84 \pm 11\%$  from crayfish tails. (Table 2.2.5.3). There was no communication between the analytical laboratory and the study sponsor during the ILV trial. A set of eight samples required 8 person hours for extraction and preparation, with auto injection and analysis occurring overnight. No difficulties were experienced during the conduct of the ILV, and only two changes were made by the independent analytical laboratory, Maxim Technologies, Inc. The following minor changes were made to the BASF Method: Celite 521 (from Aldrich) was used instead of Celite 545 AW (from Supelco). Both products are acid washed, but the particle size of Celite 521 is smaller ( $2 \mu\text{m}$ ) than that of Celite 545 AW ( $17 \mu\text{m}$ ). Also, the nitrogen evaporator waterbath was set at room temperature. The laboratory noted the following critical step in the analysis: during the methylene chloride step, the mixture should remain undisturbed for 5 minutes to allow for complete phase separation.

| Table 2.2.5.3 Independent Laboratory Validation of the CE/UV method (BASF Method M 3066) for determining Imazapyr in fish and shellfish. |                           |                |                        |
|------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|----------------|------------------------|
| Animal Matrix                                                                                                                            | Fortification Level (ppm) | Recoveries (%) | Mean Recovery $\pm$ SD |
| Fish Muscle                                                                                                                              | 0.05                      | 79, 85         | $86 \pm 4.1$           |
|                                                                                                                                          | 0.25                      | 84, 90         |                        |
|                                                                                                                                          | 0.50                      | 86, 90         |                        |
| Crayfish Tails                                                                                                                           | 0.05                      | 72, 72         | $84 \pm 11$            |
|                                                                                                                                          | 0.25                      | 82, 92         |                        |
|                                                                                                                                          | 0.50                      | 90, 97         |                        |

## 2.2.6. Radiovalidation

The petitioner provided radiovalidation data for the proposed CE/UV methods for determining imazapyr residues in milk (Method M 3075) and livestock tissues (Method M 3185), demonstrating the efficiency of the methods in extracting residues in aged samples. Samples of goat milk and kidney bearing  $^{14}\text{C}$ -residues were obtained from the metabolism study (MRID 45119716).

In the metabolism study,  $^{14}\text{C}$ -residues in milk were extracted sequentially with ACN and ACN:water (5:1, v/v), and hexane, centrifuging after each extraction. The solvent extracts were combined and partitioned, resulting in a hexane fraction and an ACN/water fraction. The ACN/water fraction was concentrated to remove the ACN and partitioned first with hexane and then with ethyl acetate.  $^{14}\text{C}$ -Residues of imazapyr in the aqueous and ethyl acetate fractions were determined by HPLC.  $^{14}\text{C}$ -Residues in kidney were extracted with acetone:water (3:1, v/v), concentrated, redissolved in methanol, and analyzed by HPLC with LSC of collected fractions.

For the RAM extractions, milk and kidney samples were extracted according to procedures described above for Methods M 3075 and M 3184, respectively. The extractability was determined by radioassay (LSC) of the extract fractions. The milk and kidney extracts were then partitioned as specified in Methods M 3075 and M 3184, and residues of  $^{14}\text{C}$ -imazapyr were determined by HPLC analysis and LSC of collected fractions.

Methods M 3075 and M 3184 extracted 98 and 100% of the radioactivity from the milk and kidney samples, respectively (Table 2.2.6.1). The levels of imazapyr determined in samples extracted as in the metabolism study

and by the proposed enforcement methods were similar. Residues of [ $^{14}\text{C}$ ]imazapyr in milk and kidneys were 0.01 and 0.061 ppm, respectively, according to the metabolism study, and were 0.011 and 0.065 ppm, respectively, using procedures from the proposed enforcement methods.

| Table 2.2.6.1. Radiovalidation recoveries of [ $^{14}\text{C}$ ]imazapyr from radiolabeled goat milk and kidney samples from the metabolism study using procedures from the proposed enforcement methods. |                  |                    |      |                                      |       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|--------------------|------|--------------------------------------|-------|
| Commodity                                                                                                                                                                                                 | Sample TRR (ppm) | Extractability (%) |      | Imazapyr residues (ppm) <sup>a</sup> |       |
|                                                                                                                                                                                                           |                  | Metabolism         | RAM  | Metabolism                           | RAM   |
| Goat Milk                                                                                                                                                                                                 | 0.014            | 80.3               | 97.9 | 0.010                                | 0.011 |
| Goat Kidney                                                                                                                                                                                               | 0.074            | 93.5               | 100  | 0.061                                | 0.065 |

<sup>a</sup> Residues of imazapyr were determined by HPLC and LSC of collected fractions.

The petitioner also provided radiovalidation data for the proposed CE/UV method for determining imazapyr residues in fish and shellfish (Method M 3066), demonstrating the efficiency of the methods in extracting residues in aged samples. Two samples of oysters bearing  $^{14}\text{C}$ -residues were obtained from the shellfish  $^{14}\text{C}$ -bioaccumulation study (MRID 45119709). Only TRR levels in oyster tissues were determined in the bioaccumulation study;  $^{14}\text{C}$ -residues in oyster tissues were not extracted or characterized due to low levels of radioactivity.

For the radiovalidation, oyster tissue samples were extracted according to procedures described above for Method M 3066. The extractability was determined by radioassay (LSC) of the extract fractions. Actual residues of imazapyr were determined using CE/UV.

Method M 3066 extracted 92-95% of the TRR from the two oyster samples (Table 2.2.6.2), and CE/UV analysis determined that [ $^{14}\text{C}$ ]imazapyr accounted for 87 and 78 ppb in the two samples (81-89% of the TRR).

| Table 2.2.6.2. Radiovalidation recoveries of [ $^{14}\text{C}$ ]imazapyr from radiolabeled oyster samples using procedures from the proposed CE/UV enforcement method (Method M 3066). |           |                    |                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|--------------------|--------------------------------------|
| Commodity                                                                                                                                                                              | TRR (ppb) | Extractability (%) | Imazapyr residues (ppb) <sup>a</sup> |
| Oyster Soft Tissues                                                                                                                                                                    | 97.6      | 95                 | 86.8                                 |
|                                                                                                                                                                                        | 96.8      | 92                 | 78.4                                 |

<sup>a</sup> Residues of imazapyr in oyster tissues were determined by CE/UV.

### 3. Discussion

#### 3.1. Method Recovery

The submitted ILV trials for the CE/UV methods (BASF Methods M 3023.01, M 3184, M 3075, M 3223, and 3066) for determination of imazapyr in various matrices are adequate. Method validation recoveries were all within the acceptable 70-120% range, with the exception of one recovery of 67% from milk fat that was due to an equipment malfunction.

The average recovery of imazapyr from grass forage and hay fortified at 0.5-50.0 ppm was  $84 \pm 2.4\%$  (n=16) using CE/UV Method M 3023. The average recoveries of imazapyr from cattle tissues fortified at 0.05-0.50 ppm were  $80 \pm 4.8\%$  from muscle (n=6),  $80 \pm 4.4\%$  from liver (n=6),  $79 \pm 5.4\%$  from kidneys (n=6), and  $82 \pm 6.2\%$  from fat (n=6) using CE/UV Method M 3184. The average recovery of imazapyr from milk fortified at 0.01-0.50 ppm was  $90 \pm 5.6\%$  (n=6) using CE/UV Method M 3075, and the average recovery of imazapyr from milk fat fortified at 0.01-0.10 ppm was  $78 \pm 9.5\%$  (n=6) using CE/UV Method M 3223. The average recoveries of imazapyr from fish and shellfish fortified at 0.05-0.50 ppm were  $84 \pm 4.1\%$  from fish fillets (n=6) and  $84 \pm 11\%$  from crayfish meat (n=6) using CE/UV Method M 3066, which is essentially identical to Method M 3184.

The validated LOQs for imazapyr are 0.5 ppm in/on grass forage and hay, 0.05 ppm in cattle tissues, fish and shellfish, and 0.01 ppm in milk and milk fat.

#### 3.2. Method Efficiency

The submitted radiovalidation data for the proposed CE/UV methods (M 3066, M 3075, and M 3185) are adequate and demonstrated the efficiency of the methods in extracting residues from aged samples. CE/UV Methods M 3075 and M 3184 extracted 98 and 100% of the TRR from the milk and kidney samples, respectively, and Method M 3066 extracted 92-95% of the TRR from the two oyster samples. For the milk and kidney samples, residues recovered by the two methods (0.011 ppm in milk and 0.065 ppm in kidneys) were in close agreement with residue values determined in the metabolism study (0.01 ppm in milk and 0.061 ppm in kidneys). For the oyster samples, Method M 3066 detected imazapyr at 0.087 and 0.078 ppm (81-89% of the TRR).

The submitted CE/UV methods (BASF Methods M 3023.01, M 3184, M 3075, M 3223, and 3066) are adequate for determination of imazapyr in various matrices, and will be forwarded to ACB for PMV trials.

A similar CE/UV method (Method M 2657) was previously submitted in conjunction with the petition for use of imazapyr on field corn (PP#6F04641). Method M 2657 has undergone both a successful ILV trial (Barcode D230175, N. Dodd, 2/11/97) and a PMV trial (Barcode D287190, N. Dodd, 8/25/97).

### 4. Deficiencies

None

### 5. References

DP Barcode: D236238

Subject: PP#6F04641 - Imazapyr on Field Corn. Review of Analytical Method M 2657 and Independent Lab Validation for M 2567.

From: N. Dodd

To: D. McCall

Dated: 2/11/97

MRID(s) None

DP Barcode: D236238

Subject: PP#6F04641 - Imazapyr on Field Corn. Evaluation of Method Trial Report dated 5/19/97.

From: N. Dodd

To: P. Errico

Dated: 8/25/97

MRID(s) None

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

*David Soderberg*

**DP Barcode:** D275561

**Petition:** PP#0F06166

**Citation:** 45119717 Mallipudi, N. (2000) Imazapyr (AC 243997): Confined Rotational Crop Study with (carbon 14) Labeled AC 243997: Lab Project Number: MET 00-003: M97P997NC1: 0527. Unpublished study prepared by American Cyanamid Company and American Agricultural Services, Inc. 338 p.

**Sponsor:** BASF (formerly American Cyanamid Co.)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

In a field study, [<sup>14</sup>C-6-pyridinyl] imazapyr was applied to a sandy loam soil at a rate of 0.79 lb ae/A. Rotational crops of carrots, lettuce, and wheat were planted at approximately 12 and 18 months post-treatment, and the appropriate commodities were collected from each crop at both plant-back intervals (PBIs). Total radioactive residues (TRR) were generally low in all commodities from both PBIs. TRRs were <0.005 ppm (<LOD) in carrots (tops and roots) and lettuce from the ~12-month PBI, carrot roots from the 18-month PBI, and wheat forage from the 12- and ~18-month PBIs, and were 0.005-0.009 ppm in lettuce and carrot tops from the 18-month PBI, and wheat hay, grain, and straw from the 12-month PBI. The only commodities with TRRs >0.01 ppm were wheat hay (0.018 ppm), grain (0.016 ppm), and straw (0.015 ppm) from the ~18-month PBI.

Based on the limited identification of <sup>14</sup>C-residues in this study, the petitioner proposes that imazapyr was extensively metabolized in rotational crops by hydrolysis of the imidazolinoyl ring, and cyclization of the carboxyl group with the imidazolinoyl nitrogen.

Although supporting storage stability data were not provided, and metabolite identities were not confirmed using a second analytical method, the submitted confined rotational crop study is adequate.

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

**DP Barcode:** D275561

**Petition:** PP#0F06166

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Based on the limited identification of <sup>14</sup>C-residues in this study, the petitioner proposes that imazapyr was extensively metabolized in rotational crops by hydrolysis of the imidazolinoyl ring, and cyclization of the carboxyl group with the imidazolinoyl nitrogen.

Although supporting storage stability data were not provided, and metabolite identities were not confirmed using a second analytical method, the submitted confined rotational crop study is adequate.

The proposed rotational crop restriction for the use of imazapyr on grasslands requires completion of a successful field bioassay at least 12 months after imazapyr application. The TRRs in rotational crops at both the ~12 and 18 month PBIs were low ( $<0.02$  ppm), and HPLC analyses indicate that any residues of concern in crops at these PBI intervals would be  $<0.005$  ppm. In addition, while the use rate in the current study (0.79 lb ae/A) is equivalent to the maximum proposed rate for spot treatments, the total amount applied per full acre is limited to 0.075 lb ae/A, since the spot treatment is only permitted on a maximum of one tenth (1/10) of a given acre.

### **GLP Compliance**

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. There were no deviations from regulatory requirements that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Test Substance

#### Active Ingredient

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid

CAS Number: 81334-34-1

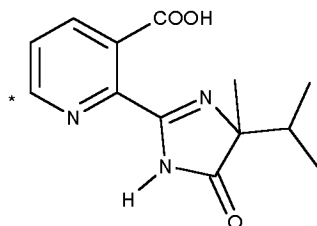
Company Name: AC 243997, CL 243997

Other Synonyms: Arsenal Herbicide

Location of <sup>14</sup>C-Label: <sup>14</sup>C-labeled at the 6 position of the pyridine ring [<sup>14</sup>C-6-PYR]

Radiochemical Purity: 96.9% by HPLC

Specific Activity: For application, the test substance was isotopically diluted with a mixture of <sup>13</sup>C-labeled (6-pyridine) and non-radiolabeled imazapyr to a final specific activity of 8.97  $\mu$ Ci/mg (19,913 dpm/ $\mu$ g).



### 1.2. Crop and Cultural Information

Types and Varieties of Crops: The following rotational crops were used: carrot (Danvers 126), lettuce, (black-seeded Simpson), winter wheat (Fidel IR) and spring wheat (96J001).

Soil: Sandy loam (60% sand, 22% silt, 18% clay, and 1.2% organic matter; pH 5.7; bulk density 1.36 g/cm<sup>3</sup>; and CEC of 7.8 meq/100 g)

Test plots: A single treated and control test plot (3' x 28') were located in an outdoor fenced area (American Agricultural Services, Inc., Lucama, NC) with each plot enclosed in a plastic wrapped wood frame dug into the ground within 2" of the soil surface. Each plot was divided into six subplots for planting each rotational crop.

Primary crops: Five months after treatment, the plots were initially planted with imazapyr-resistant annual weeds (sicklepod, hemp sesbania, chickweed, henbit and red sorrel) which were maintained until the designated plant-back interval (PBI).

Conditions: Prior to application of the test material and planting the rotational crops, the soil was tilled to approximately 3". The crops were maintained outdoors under ambient environmental conditions, with additional watering as necessary. No unusual environmental conditions were observed during the study. The report noted that carrot and lettuce plants at the initial 330-day PBI were stunted compared to the controls.

### 1.3. Application Information

Type of Application: Broadcast application to bare soil. Application was made after enclosing each plot in a two-foot tall plastic sheeting fence.

Application Matrix: The radiolabeled test substance formulated as the end-use product for application by dissolving in water with isopropylamine.

Volume: 20 gal/A

Application Rate: 0.79 lb ae/A

Number of Applications: One (10/21/97)

Plant-back Intervals (PBI): Carrot and lettuce were planted twice at 330 and 540 days after treatment (DAT), winter wheat was planted at 359 DAT, and spring wheat was planted at 520 DAT.

### 1.4. Harvest/Post-harvest Procedures

Samples of wheat forage were collected 155 (winter) or 54 (spring) days after planting (DAP), and hay samples were collected 223 DAP (winter) or 70 DAP (spring). Mature samples of lettuce and wheat were collected by cutting at soil level. Mature carrot samples were pulled from the ground and separated into roots and tops. Mature samples of rotational crops were harvested at the following intervals: lettuce leaf (61 and 83 DAP), carrot roots and tops (97 and 106 DAP), winter wheat grain and straw (237 DAP), and spring wheat grain and straw (103 DAP). Samples were frozen (-29 to -10 C) soon after collection or drying (wheat hay only), then shipped frozen overnight to American Cyanamid, where samples were stored frozen (-37 to -4 C) until analysis. All samples were homogenized with dry ice before analysis. A summary of storage conditions is presented in Table 1.4.1.

The petitioner did not address storage stability issues except to state that the samples were extractable and appeared to be stable throughout the experimental period. Crop samples were stored frozen for <1-5.7 months prior to TRR determinations and for 1.5-9.2 months prior to extraction for analysis. Sample extracts were stored at frozen for <2 months.

| Table 1.4.1. Summary of Storage Conditions |                          |                           |                       |                        |
|--------------------------------------------|--------------------------|---------------------------|-----------------------|------------------------|
| Matrix                                     | Storage Temperature (°C) | Storage intervals (days)  |                       |                        |
|                                            |                          | Harvest to Combustion/LSC | Harvest to Extraction | Extraction to Analysis |
| Lettuce                                    | -37 to -4                | 16-25                     | 54-97                 | 37-43                  |
| Carrot roots                               |                          | 100-173                   | NA                    | NA                     |
| Carrot tops                                |                          | 38-141                    | 100-173               | 3                      |
| Wheat forage                               |                          | 25-92                     | NA                    | NA                     |
| Wheat hay                                  |                          | 9-24                      | 270-281               | 2                      |
| Wheat grain                                |                          | 17-45                     | 45-163                | 23-42                  |
| Wheat straw                                |                          | 18-52                     | 54-139                | 52-56                  |

<sup>a</sup> NA = Not Analyzed

## 1.5. Analytical Methods

### 1.5.1. Total Radioactive Residues (TRR)

Total radioactive residues (TRR) in rotated crop samples were determined by combustion followed by liquid scintillation counting (LSC). These levels are summarized in Table 2.1 for the various crop matrices analyzed. Radioactivity in rinses and extracts was determined by LSC and radioactivity in nonextractable residues was determined by combustion/LSC. For the initial radioassays, the petitioner stated that the LOD was 0.005 ppm.

### 1.5.2. Extraction and Fractionation of $^{14}\text{C}$ -residues.

Rotational crop samples were extracted with acetone:water (1:1, v/v) and the resulting extract was concentrated and analyzed by reverse-phase HPLC. The remaining solids were then extracted with acetone:water (1:1) containing 2% HCl, and the solubilized  $^{14}\text{C}$ -residues were concentrated and analyzed by HPLC. (Tables 2.2.1-2.2.3).

Post-extraction solids (PES) from lettuce, wheat grain and straw were then extracted with acetone:water (1:1) containing 3% HCl and sequentially hydrolyzed using 6N HCl and 6N NaOH at reflux for 4 hours. Solubilized  $^{14}\text{C}$ -residues in the acidic extract and hydrolysates were radioassayed by LSC, but were not further characterized due to low levels of radioactivity.

### Identification of $^{14}\text{C}$ -residues.

$^{14}\text{C}$ -Residues in solvent fractions were profiled and quantified by HPLC using a reverse phase  $\text{C}_{18}$  column with a mobile phase gradient of water to methanol (each containing 1% acetic acid), equipped with a diode array detector (254 nm). Radioactivity in eluant fractions was determined by LSC. Reference standards were also analyzed by HPLC analysis under the same conditions as the test samples. The limit of detection by HPLC was 0.001 ppm. Parent and metabolite fractions were identified by the their HPLC retention times.

A major polar unknown fraction occurred in each commodity that eluted near the solvent front. This unknown was isolated from lettuce, carrot tops, and wheat grain and straw for further analysis. The isolated unknown was subjected to enzymatic, acid and based hydrolyses. For the acid hydrolysis, the unknown fraction was heated (90-95 C) with 6 N HCl for five hours, cooled, neutralized, and reanalyzed by HPLC. For the base hydrolysis, another aliquot was mixed with 6 N NaOH and heated (40-60 C) for 40 minutes, then cooled, neutralized, and reanalyzed by HPLC. Separate aliquots of this fraction were also incubated (~37 C) with either  $\beta$ -glucosidase, cellulase, or pepsin plus cellulase in a 0.2 M sodium acetate buffer (pH 5) for 1 day, then concentrated and analyzed by HPLC.

## 2. Results

| Table 2.1. Total Radioactive Residues in Rotational Crops planted following a single soil application of [ <sup>14</sup> C-PYR]Imazapyr at 0.79 lb ae/A. |        |                            |                                        |                                  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|--------|----------------------------|----------------------------------------|----------------------------------|
| Crop                                                                                                                                                     | Matrix | Plant-Back Interval (days) | Harvest interval (days after planting) | Total Radioactive Residues (ppm) |
| Lettuce                                                                                                                                                  | Leaves | 330 days                   | 61                                     | <0.005 <sup>a</sup>              |
|                                                                                                                                                          |        | 540 days                   | 83                                     | 0.009                            |
| Carrot                                                                                                                                                   | Tops   | 330 days                   | 97                                     | <0.005                           |
|                                                                                                                                                          |        | 540 days                   | 106                                    | 0.007                            |
|                                                                                                                                                          | Roots  | 330 days                   | 97                                     | <0.005                           |
|                                                                                                                                                          |        | 540 days                   | 106                                    | <0.005                           |
| Winter Wheat                                                                                                                                             | Forage | 359 days                   | 155                                    | <0.005                           |
|                                                                                                                                                          | Hay    |                            | 223                                    | 0.005                            |
|                                                                                                                                                          | Grain  |                            | 237                                    | 0.007                            |
|                                                                                                                                                          | Straw  |                            | 237                                    | 0.006                            |
| Spring Wheat                                                                                                                                             | Forage | 520 days                   | 54                                     | <0.005                           |
|                                                                                                                                                          | Hay    |                            | 70                                     | 0.018                            |
|                                                                                                                                                          | Grain  |                            | 103                                    | 0.016                            |
|                                                                                                                                                          | Straw  |                            | 103                                    | 0.015                            |

<sup>a</sup> LOD = 0.005 ppm for combustion/LSC.

| Table 2.2.1 Extraction, Characterization, and Identification of $^{14}\text{C}$ -Residues in rotational <b>Carrot and Lettuce Tops</b> planted following a single soil application of [ $^{14}\text{C}$ ]Imazapyr at 0.79 lb ae/A. |       |        |                                                                                                                                                                                                                                                                                                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                        | % TRR | ppm    | Characterization/identification                                                                                                                                                                                                                                                                                                                           |
| <b>Carrot Tops, 540-day PBI<sup>a</sup> (TRR = 0.007 ppm)</b>                                                                                                                                                                      |       |        |                                                                                                                                                                                                                                                                                                                                                           |
| Acetone:water                                                                                                                                                                                                                      | 49.8  | 0.003  | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 4.6% TRR <0.001 ppm <sup>b</sup><br>CL 247087 2.1% TRR <0.001 ppm<br>CL 252974 3.6% TRR <0.001 ppm<br>CL 17226 1.9% TRR <0.001 ppm<br>CL 119060 2.5% TRR <0.001 ppm<br>Polar Unknown 12.3% TRR 0.001 ppm<br>A total of 14 additional unknown peaks were detected totaling 19.4% TRR (0.001 ppm). |
| Acetone:water (2% HCl)                                                                                                                                                                                                             | 7.7   | 0.001  | Not further analyzed                                                                                                                                                                                                                                                                                                                                      |
| Residual solids                                                                                                                                                                                                                    | 45.2  | 0.003  | Not further analyzed                                                                                                                                                                                                                                                                                                                                      |
| <b>Lettuce, 540-day PBI<sup>a</sup> (TRR = 0.009 ppm)</b>                                                                                                                                                                          |       |        |                                                                                                                                                                                                                                                                                                                                                           |
| Acetone:water                                                                                                                                                                                                                      | 51.1  | 0.005  | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 4.7% TRR <0.001 ppm<br>CL 247087 2.2% TRR <0.001 ppm<br>CL 252974 1.9% TRR <0.001 ppm<br>CL 17226 1.2% TRR <0.001 ppm<br>CL 119060 0.7% TRR <0.001 ppm<br>Polar Unknown 12.3% TRR 0.001 ppm<br>A total of 13 additional unknown peaks were detected totaling 23.8% TRR (0.002 ppm).              |
| Acetone:water (2% HCl)                                                                                                                                                                                                             | 9.9   | 0.001  | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 0.2% TRR <0.001 ppm<br>CL 247087 0.4% TRR <0.001 ppm<br>CL 252974 0.2% TRR <0.001 ppm<br>CL 17226 0.4% TRR <0.001 ppm<br>Polar Unknown 5.2% TRR <0.001 ppm<br>A total of 10 additional unknown peaks were detected totaling 3.3% TRR (0.001 ppm).                                                |
| PES                                                                                                                                                                                                                                | 48.1  | 0.004  | Extracted with acetone:water (1:1) with 3% HCl, and then sequentially hydrolyzed by refluxing in 6N HCl and 6N NaOH for 4 hours each time                                                                                                                                                                                                                 |
| Acetone:water (3% HCl)                                                                                                                                                                                                             | 7.6   | 0.001  | Not further analyzed.                                                                                                                                                                                                                                                                                                                                     |
| 6 N HCl                                                                                                                                                                                                                            | 13.2  | 0.001  | Not further analyzed.                                                                                                                                                                                                                                                                                                                                     |
| 6 N NaOH                                                                                                                                                                                                                           | 3.1   | <0.001 | Not further analyzed                                                                                                                                                                                                                                                                                                                                      |
| Residual solids                                                                                                                                                                                                                    | 22.4  | 0.002  | Not further analyzed                                                                                                                                                                                                                                                                                                                                      |

<sup>a</sup> PBI=plant-back interval. Lettuce and carrots were also planted at a 330 day PBI; however, TRR in these samples were <0.005 ppm and were not further analyzed.

| Table 2.2.2 Extraction, Characterization, and Identification of $^{14}\text{C}$ -Residues in rotational <b>Winter Wheat</b> planted following a single soil application of [ $^{14}\text{C}$ ]Imazapyr at 0.79 lb ae/A. <sup>a</sup> |       |        |                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                          | % TRR | ppm    | Characterization/identification                                                                                                                                                                                                                                                                                                                          |
| <b>Wheat Hay, 359-day PBI (TRR = 0.005 ppm)</b>                                                                                                                                                                                      |       |        |                                                                                                                                                                                                                                                                                                                                                          |
| Acetone:water                                                                                                                                                                                                                        | 23.6  | 0.001  | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 2.0% TRR <0.001 ppm <sup>b</sup><br>CL 247087 0.3% TRR <0.001 ppm<br>CL 252974 0.7% TRR <0.001 ppm<br>CL 17226 0.7% TRR <0.001 ppm<br>CL 119060 1.5% TRR <0.001 ppm<br>Polar Unknown 8.1% TRR <0.001 ppm<br>A total of 9 additional unknown peaks were detected totaling 13.9% TRR (0.001 ppm). |
| Acetone:water (2% HCl)                                                                                                                                                                                                               | 7.0   | <0.001 | Not further analyzed                                                                                                                                                                                                                                                                                                                                     |
| Residual solids                                                                                                                                                                                                                      | 71.2  | 0.004  | Not further analyzed                                                                                                                                                                                                                                                                                                                                     |
| <b>Wheat Grain, 359-day PBI (TRR = 0.007 ppm)</b>                                                                                                                                                                                    |       |        |                                                                                                                                                                                                                                                                                                                                                          |
| Acetone:water                                                                                                                                                                                                                        | 31.0  | 0.002  | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 1.1% TRR <0.001 ppm<br>CL 247087 1.1% TRR <0.001 ppm<br>CL 252974 1.4% TRR <0.001 ppm<br>CL 17226 0.8% TRR <0.001 ppm<br>CL 119060 1.1% TRR <0.001 ppm<br>Polar Unknown 6.2% TRR <0.001 ppm<br>A total of 16 additional unknown peaks were detected totaling 18.3% TRR (0.001 ppm).             |
| Acetone:water (2% HCl)                                                                                                                                                                                                               | 15.0  | 0.001  | Not further analyzed.                                                                                                                                                                                                                                                                                                                                    |
| PES                                                                                                                                                                                                                                  | 80.1  | 0.006  | Sequentially hydrolyzed by refluxing in 6N HCl and 6N NaOH for 4 hours each time.                                                                                                                                                                                                                                                                        |
| 6 N HCl                                                                                                                                                                                                                              | 12.7  | 0.001  | Not further analyzed                                                                                                                                                                                                                                                                                                                                     |
| 6 N NaOH                                                                                                                                                                                                                             | 18.7  | 0.001  | Not further analyzed                                                                                                                                                                                                                                                                                                                                     |
| Residual solids                                                                                                                                                                                                                      | 19.3  | 0.001  | Not further analyzed                                                                                                                                                                                                                                                                                                                                     |

<sup>a</sup> TRR in wheat forage samples were <0.005 ppm and were not further isolated or characterized.

| Table 2.2.3 Extraction, Characterization, and Identification of <sup>14</sup> C-Residues in rotational <b>Spring Wheat</b> planted following a single soil application of [ <sup>14</sup> C]Imazapyr at 0.79 lb ae/A. <sup>a</sup> |       |       |                                                                                                                                                                                                                                                                                                                                                        |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                        | % TRR | ppm   | Characterization/identification                                                                                                                                                                                                                                                                                                                        |
| <b>Wheat Hay, 520-day PBI (TRR = 0.018 ppm)</b>                                                                                                                                                                                    |       |       |                                                                                                                                                                                                                                                                                                                                                        |
| Acetone:water                                                                                                                                                                                                                      | 37.8  | 0.007 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 9.9% TRR 0.002 ppm<br>CL 247087 1.5% TRR <0.001 ppm <sup>b</sup><br>CL 252974 1.5% TRR <0.001 ppm<br>CL 17226 0.7% TRR <0.001 ppm<br>CL 119060 1.4% TRR <0.001 ppm<br>Polar Unknown 7.6% TRR 0.001 ppm<br>A total of 9 additional unknown peaks were detected totaling 15.9% TRR (0.003 ppm). |
| Acetone:water (2% HCl)                                                                                                                                                                                                             | 10.9  | 0.002 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 1.1% TRR <0.001 ppm<br>CL 247087 0.6% TRR <0.001 ppm<br>CL 252974 0.3% TRR <0.001 ppm<br>CL 17226 0.4% TRR <0.001 ppm<br>CL 119060 0.3% TRR <0.001 ppm<br>Polar Unknown 1.5% TRR <0.001 ppm<br>A total of 12 additional unknown peaks were detected totaling 5.8% TRR (0.001 ppm).            |
| Residual solids                                                                                                                                                                                                                    | 51.1  | 0.009 | Not further analyzed                                                                                                                                                                                                                                                                                                                                   |
| <b>Wheat Straw, 520-day PBI (TRR = 0.015 ppm)</b>                                                                                                                                                                                  |       |       |                                                                                                                                                                                                                                                                                                                                                        |
| Acetone:water                                                                                                                                                                                                                      | 28.0  | 0.004 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 7.8% TRR <0.001 ppm<br>CL 247087 1.8% TRR <0.001 ppm<br>CL 252974 1.8% TRR <0.001 ppm<br>CL 17226 0.6% TRR <0.001 ppm<br>CL 119060 0.6% TRR <0.001 ppm<br>Polar Unknown 2.6% TRR <0.001 ppm<br>A total of 16 additional unknown peaks were detected totaling 13.0% TRR (0.002 ppm).           |
| Acetone:water (2% HCl)                                                                                                                                                                                                             | 5.5   | 0.001 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 0.3% TRR <0.001 ppm<br>CL 247087 0.4% TRR <0.001 ppm<br>CL 252974 0.2% TRR <0.001 ppm<br>CL 17226 0.2% TRR <0.001 ppm<br>CL 119060 0.2% TRR <0.001 ppm<br>Polar Unknown 0.5% TRR <0.001 ppm<br>A total of 12 additional unknown peaks were detected totaling 2.9% TRR (<0.001 ppm).           |
| PES                                                                                                                                                                                                                                | 67.2  | 0.010 | Extracted with acetone:water (1:1) with 3% HCl, and then sequentially hydrolyzed by refluxing in 6N HCl and 6N NaOH for 4 hours each time                                                                                                                                                                                                              |
| Acetone:water (3% HCl)                                                                                                                                                                                                             | 15.0  | 0.002 | Not further analyzed.                                                                                                                                                                                                                                                                                                                                  |
| 6 N HCl                                                                                                                                                                                                                            | 15.1  | 0.002 | Not further analyzed.                                                                                                                                                                                                                                                                                                                                  |
| 6 N NaOH                                                                                                                                                                                                                           | 12.1  | 0.002 | Not further analyzed                                                                                                                                                                                                                                                                                                                                   |
| Residual solids                                                                                                                                                                                                                    | 19.2  | 0.003 | Not further analyzed                                                                                                                                                                                                                                                                                                                                   |

| Table 2.2.3 Extraction, Characterization, and Identification of $^{14}\text{C}$ -Residues in rotational <b>Spring Wheat</b> planted following a single soil application of [ $^{14}\text{C}$ ]Imazapyr at 0.79 lb ae/A. <sup>a</sup> |       |       |                                                                                                                                                                                                                                                                                                                                             |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                          | % TRR | ppm   | Characterization/identification                                                                                                                                                                                                                                                                                                             |
| <b>Wheat Grain, 520-day PBI (TRR = 0.016 ppm)</b>                                                                                                                                                                                    |       |       |                                                                                                                                                                                                                                                                                                                                             |
| Acetone:water                                                                                                                                                                                                                        | 47.9  | 0.008 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 19.1% TRR 0.003 ppm<br>CL 247087 1.4% TRR <0.001 ppm<br>CL 252974 1.1% TRR <0.001 ppm<br>CL 17226 1.1% TRR <0.001 ppm<br>CL 119060 1.1% TRR <0.001 ppm<br>Polar Unknown 4.6% TRR 0.001 ppm<br>A total of 16 additional unknown peaks were detected totaling 18.6% TRR (0.003 ppm). |
| Acetone:water (2% HCl)                                                                                                                                                                                                               | 5.0   | 0.001 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 0.4% TRR <0.001 ppm<br>CL 247087 0.1% TRR <0.001 ppm<br>CL 252974 0.3% TRR <0.001 ppm<br>CL 17226 0.3% TRR <0.001 ppm<br>CL 119060 0.2% TRR <0.001 ppm<br>Polar Unknown 0.4% TRR <0.001 ppm<br>A total of 15 additional unknown peaks were detected totaling 3.2% TRR (0.001 ppm). |
| Acetone:water (3% HCl)                                                                                                                                                                                                               | 9.8   | 0.002 | Not further analyzed.                                                                                                                                                                                                                                                                                                                       |
| 6 N HCl                                                                                                                                                                                                                              | 13.2  | 0.002 | Not further analyzed.                                                                                                                                                                                                                                                                                                                       |
| 6 N NaOH                                                                                                                                                                                                                             | 8.4   | 0.001 | Not further analyzed.                                                                                                                                                                                                                                                                                                                       |
| Residual solids                                                                                                                                                                                                                      | 12.7  | 0.002 | Not further analyzed.                                                                                                                                                                                                                                                                                                                       |

<sup>a</sup> TRR in wheat forage samples were <0.005 ppm and were not further isolated or characterized.

| Metabolite or Fraction <sup>a</sup> | Carrot Top<br>540-DAT<br>(0.007 ppm) <sup>b</sup> |            | Lettuce<br>540-DAT<br>(0.009 ppm) |            | W. Wheat <sup>c</sup> Hay<br>359-DAT<br>(0.005 ppm) |            | W. Wheat Grain<br>359-DAT<br>(0.007 ppm) |            | S. Wheat <sup>c</sup> Hay<br>520-DAT<br>(0.018 ppm) |        | S. Wheat Straw<br>520-DAT<br>(0.015 ppm) |        | S. Wheat Grain<br>520-DAT<br>(0.016 ppm) |        |
|-------------------------------------|---------------------------------------------------|------------|-----------------------------------|------------|-----------------------------------------------------|------------|------------------------------------------|------------|-----------------------------------------------------|--------|------------------------------------------|--------|------------------------------------------|--------|
|                                     | %TRR                                              | ppm        | %TRR                              | ppm        | %TRR                                                | ppm        | %TRR                                     | ppm        | %TRR                                                | ppm    | %TRR                                     | ppm    | %TRR                                     | ppm    |
| AC 243997 (parent)                  | 4.6                                               | <0.00<br>1 | 4.9                               | <0.00<br>1 | 2.0                                                 | <0.00<br>1 | 1.1                                      | <0.00<br>1 | 11.0                                                | 0.002  | 8.1                                      | 0.001  | 19.5                                     | 0.003  |
| CL 247087                           | 2.1                                               | <0.00<br>1 | 2.6                               | <0.00<br>1 | 0.3                                                 | <0.00<br>1 | 1.1                                      | <0.00<br>1 | 2.1                                                 | <0.001 | 2.2                                      | <0.001 | 1.5                                      | <0.001 |
| CL 252974                           | 3.6                                               | <0.00<br>1 | 2.1                               | <0.00<br>1 | 0.7                                                 | <0.00<br>1 | 1.4                                      | <0.00<br>1 | 1.8                                                 | <0.001 | 2.0                                      | <0.001 | 1.4                                      | <0.001 |
| CL 17226                            | 1.9                                               | <0.00<br>1 | 1.6                               | <0.00<br>1 | 0.7                                                 | <0.00<br>1 | 0.8                                      | <0.00<br>1 | 1.1                                                 | <0.001 | 0.8                                      | <0.001 | 1.4                                      | <0.001 |
| CL 119060                           | 2.5                                               | <0.00<br>1 | 0.7                               | <0.00<br>1 | 1.5                                                 | <0.00<br>1 | 1.1                                      | <0.00<br>1 | 1.7                                                 | <0.001 | 0.8                                      | <0.001 | 1.3                                      | <0.001 |
| Total Identified (TI)               | 14.7                                              | 0.001      | 11.9                              | 0.001      | 5.2                                                 | <0.00<br>1 | 5.5                                      | <0.00<br>1 | 17.7                                                | 0.003  | 13.9                                     | 0.002  | 25.1                                     | 0.004  |
| Polar Unknown <sup>d</sup>          | 12.2                                              | 0.001      | 17.5                              | 0.002      | 8.1                                                 | <0.00<br>1 | 6.2                                      | <0.00<br>1 | 9.1                                                 | 0.002  | 3.1                                      | <0.001 | 5.0                                      | <0.001 |
| Total Minor Unknowns <sup>e</sup>   | 19.4                                              | 0.001      | 27.1                              | 0.002      | 13.9                                                | <0.00<br>1 | 18.3                                     | 0.001      | 21.7                                                | 0.004  | 15.9                                     | 0.002  | 21.8                                     | 0.003  |
| Acidic solvent extract <sup>f</sup> | 7.7                                               | 0.001      | 7.6                               | 0.001      | 7.0                                                 | <0.00<br>7 | 15.0                                     | 0.001      | NA                                                  | --     | 15.0                                     | 0.002  | 9.8                                      | 0.002  |
| Acid hydrolysates                   | NA                                                | --         | 13.2                              | 0.001      | NA                                                  | --         | 12.7                                     | 0.001      | NA                                                  | --     | 15.1                                     | 0.002  | 13.2                                     | 0.002  |
| Base hydrolysates                   | NA                                                | --         | 3.1                               | <0.00<br>1 | NA                                                  | --         | 18.7                                     | 0.001      | NA                                                  | --     | 12.1                                     | 0.002  | 8.4                                      | 0.001  |
| Total Characterized (TC)            | 39.3                                              | 0.003      | 68.5                              | 0.006      | 29.0                                                | 0.001      | 70.9                                     | 0.005      | 30.8                                                | 0.006  | 61.2                                     | 0.009  | 58.2                                     | 0.009  |
| Total Bound (TB)                    | 45.2                                              | 0.003      | 22.4                              | 0.002      | 71.2                                                | 0.004      | 19.3                                     | 0.001      | 51.1                                                | 0.009  | 19.2                                     | 0.003  | 12.7                                     | 0.002  |
| % Mass Balance                      | 99.2                                              |            | 102.8                             |            | 105.4                                               |            | 95.7                                     |            | 99.6                                                |        | 94.3                                     |        | 96.0                                     |        |

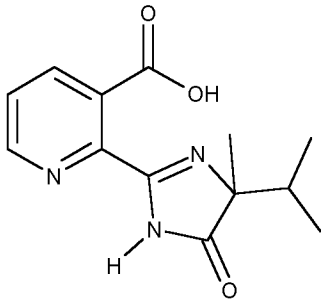
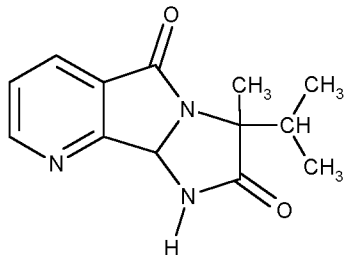
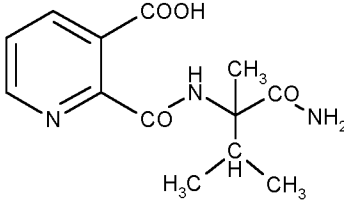
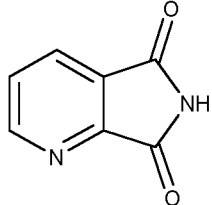
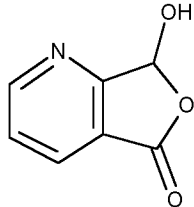
<sup>a</sup> Metabolite names and structures are presented in Table 2.4.<sup>b</sup> TRR for each matrix is listed in parentheses.<sup>c</sup> W. wheat = winter wheat, S. wheat = spring wheat

- <sup>d</sup> This unknown fraction was isolated from several matrices and subjected to enzymatic, acid, and base hydrolyses, and then analyzed by HPLC; no appreciable changes were apparent in the profile following treatments.
- <sup>e</sup> Comprised of a total of 9-16 unknown peaks for each commodity; each unknown was present at <0.001 ppm.
- <sup>f</sup> Acetone:water (2% or 3% HCl) fractions that were not analyzed by HPLC.

NA = not applicable

Total characterized (TC) is the sum of all unidentified, extractable residues.

% Mass Balance = TI (%TRR) + TC (%TRR) + TB (%TRR)

| Table 2.4. Metabolites of Imazapyr in Rotational Crops <sup>a</sup> |                                                                                                                                                      |                                                                                       |
|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Common Name/<br>Metabolite Identifier                               | Chemical Name <sup>b</sup>                                                                                                                           | Structure                                                                             |
| Imazapyr<br><br>(AC 243997; CL 243997)                              | 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1 <i>H</i> -imidazol-2-yl]-3-pyridinecarboxylic acid                                                 |    |
| CL 247087                                                           | 1,9b- $\alpha,\beta$ -dihydro-3 $\alpha$ -isopropyl-3-methyl]-5 <i>H</i> -imidazo [1',2':1,2] pyrrolo[3,4- <i>b</i> ]pyridine-2(3 <i>H</i> ),5-dione |    |
| CL 252974                                                           | 2-[(1-carbamoyl-1,2-dimethylpropyl)-carbamoyl]-nicotinic acid                                                                                        |   |
| CL 17226                                                            | Quinolinimide                                                                                                                                        |  |
| CL 119060                                                           | 7-hydroxyfuro[3,4- <i>b</i> ]pyridin-5(7 <i>H</i> )-one                                                                                              |  |

<sup>a</sup> Each compound was tentatively identified in each rotational crop commodity based on HPLC retention times. However, metabolite identities were not confirmed using a second analytical method due to low levels of metabolites.

<sup>b</sup> The CA name was used when available.

### 3. Discussion

#### 3.1. Methods

[<sup>14</sup>C-6-pyridinyl] imazapyr was applied to a bare sandy loam soil at a rate of 0.79 lb ae/A (the maximum proposed rate for grasslands is 0.75 lb ae/treated A). A cover of annual weeds was maintained on the field plots until planting of the rotational crops. Rotational crops of carrots and lettuce were planted at both 330 and 540 DAT (~11 and ~18 months), winter wheat was planted at 359 DAT (~12 months), and spring wheat was planted at 520 DAT (17 months). The appropriate RACs were collected from each crop at each PBI at typical harvest intervals. The petitioner noted that carrot and lettuce plants from the 330-day PBI were stunted compared to the controls, but adequate samples were still obtained. Samples were stored frozen for ~1-9 months prior to extraction for analysis; however, no supporting storage stability data are provided.

Samples of carrot tops and lettuce (330-day PBI) and carrot roots and wheat forage (both PBIs) were not extracted because of low TRR levels (<0.005 ppm). For the remaining samples, solvent extraction released 31-59% of the TRR, and acid and base hydrolyses released an additional 16-31% of the TRR from lettuce and wheat grain and straw. Radioactivity remaining in the residual solids accounted for 13-71% of the TRR, but amounted to ≤0.009 ppm. The recovery of radioactivity from each rotational crop commodity was 94.3-105.4% of the TRR.

Residues in solvent extracts were profiled and quantified by HPLC analysis, comparing solvent peaks with the retention time of reference standards. The identities of the metabolite fractions were not confirmed using a second method; however, parent was detected at ≤0.003 ppm in all crops and the other metabolite fractions each accounted for <0.001 ppm.

The methods used to conduct the field study were adequate, and considering the low levels of radioactivity in the RAC samples the analytical methods used were also adequate.

#### 3.2. Results

Following a single application of [<sup>14</sup>C-PYR]imazapyr to a bare sandy loam soil at 0.79 lb ae/A, TRRs were generally low in all commodities from both PBIs. TRRs were <0.005 ppm (<LOD) in carrots (tops and roots) and lettuce from the 11-month PBI, carrot roots from the 18-month PBI, and wheat forage from the 12- and 17-month PBIs. TRRs were detectable, but below 0.01 ppm, in lettuce (0.009 ppm) and carrot tops (0.007 ppm) from the 18-month PBI, and wheat hay (0.005 ppm), grain (0.007 ppm), and straw (0.006 ppm) from the 12-month PBI. The only commodities with TRRs >0.01 ppm were wheat hay (0.018 ppm), grain (0.016 ppm), and straw (0.015 ppm) from the 17-month PBI.

Based on HPLC retention times, 5.2-25.1% of the TRR was tentatively identified in lettuce, carrot tops, and wheat hay, grain, and straw. The parent, imazapyr, was the major residue identified in rotational commodities, accounting for 1.1-19.5% TRR (≤0.003 ppm). The metabolites CL 247087, CL 252974, CL17226, and CL 119060 were all present as minor components, each at 0.3-3.6% TRR (<0.001 ppm).

HPLC analysis of the solvent extracts also detected numerous (10-17) unknowns. These unknowns were minor components ( $<0.001$  ppm) of the TRR, with the exception of one unknown polar fraction that eluted near the solvent front and accounted for 3.1-17.5% of the TRR ( $\leq 0.002$  ppm) in rotational commodities. This component was isolated from lettuce, carrots tops, and wheat grain and straw, and subjected to acid and base hydrolysis, and treatment with cellulase,  $\beta$ -glucosidase, and pepsin + cellulase. None of these treatments had a substantial impact on the polar fraction, as the major peak remained near the solvent front. However, numerous minor peaks did appear in the treated fraction.

Although supporting storage stability data were not provided, and metabolite identities were not confirmed using a second analytical method, the submitted confined rotational crop study is adequate. The proposed rotational crop restriction for the use of imazapyr on grasslands is 12 months, and the TRRs in rotational crops at both the ~12 and 18 month PBIs were low ( $<0.02$  ppm). HPLC analyses indicate that any residues of concern in crops at these PBI intervals would be  $<0.005$  ppm. In addition, while the use rate in the current study (0.79 lb ae/A) is equivalent to the maximum proposed rate for spot treatments, the total amount applied per acre is limited to 0.075 lb ae/A. Therefore, average exposure of rotational crops to imazapyr is also likely to be substantially lower than 0.75 lb ae/A.

Based on the limited identification of  $^{14}\text{C}$ -residues in this study, the petitioner proposes that imazapyr was extensively metabolized in rotational crops by hydrolysis of the imidazolinoyl ring, and cyclization of the carboxyl group with the imidazolinoyl nitrogen (Figure 1).

#### 4. Deficiencies

Two substantial deficiencies were noted in this confined rotational crop study.

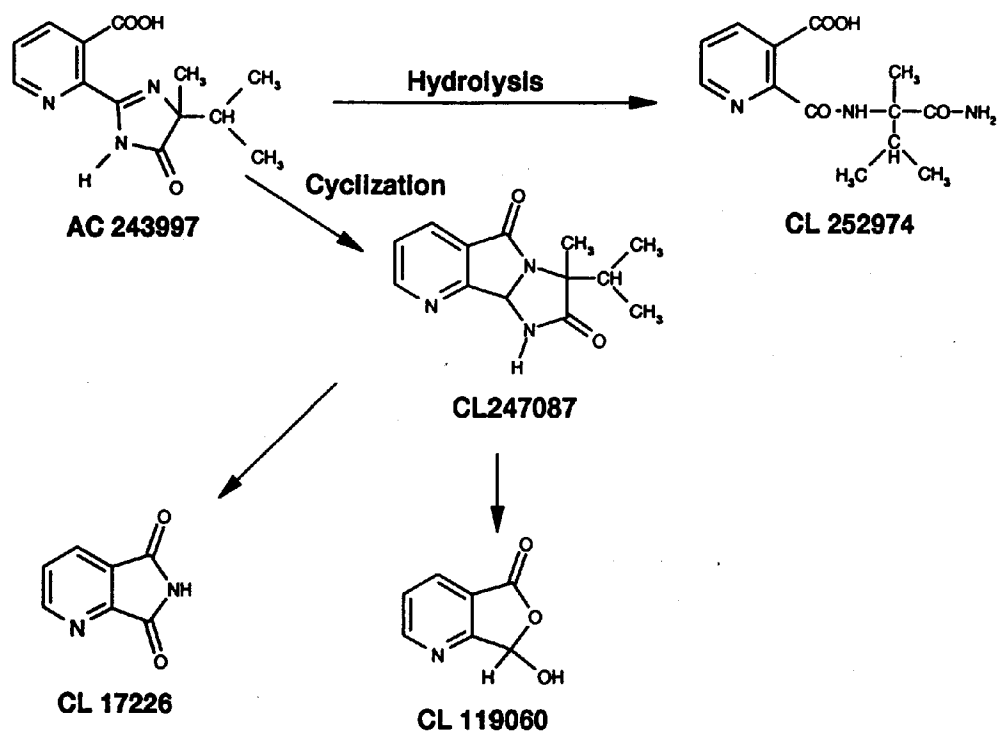
- Samples were stored frozen for up to 9 months prior to extraction for analysis; however, no supporting storage stability data are provided.
- The identification of parent and the four metabolites is based only on HPLC retention times. None of the metabolite identities were confirmed using a second analytical method.

However, considering that TRRs in the rotational crops at both the ~12 and 18 month PBIs were low ( $<0.02$  ppm), and that any residues of concern in crops at these PBI intervals would be  $<0.005$  ppm, the above deficiencies do not preclude using this study for assessing the need for rotational crop tolerances.

#### 5. References

None.

Figure 1. Proposed Metabolic Fate of Imazapyr in Rotational Crops.



**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist *William H. Donovan*  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** David Soderberg, Chemist *David Soderberg*  
RRB3/HED (7509C)

**DP Barcode:** D275561

**Petition:** PP#0F06166

**Citation:** 45119716 Tsaltz, C. (2000) AC 243997: Metabolism of [<sup>14</sup>C]-CL 243997 in the Lactating Goat: Laboratory Project No.: MET 00-002. Unpublished study prepared by American Cyanamid Co. 160 p. (OPPTS 860.1300)

**Sponsor:** BASF (formerly American Cyanamid Co.)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

A single lactating goat was dosed orally once a day for 7 consecutive days via gelatin capsules with [imidazole-5-<sup>14</sup>C]imazapyr at a dose of 90.6 mg ae/day, equivalent to 46.8 ppm in the diet. Urine, feces, and milk were collected daily and kidney samples were collected at sacrifice, ~21 hours after administering the final dose.

A total of 93.3% of the dosed radioactivity was excreted in the urine (58.7% dose) and feces (34.4% dose), and another 0.04% of the dose was excreted in the milk. Radioactivity remaining in the kidneys at sacrifice accounted for <0.01% of the dose. Total radioactive residues (TRR) were 0.014-0.016 ppm in milk and 0.074 ppm in kidneys. Radioactive residues in milk plateaued by Day-1 at 0.015 ppm.

Analyses of solvent extracts (82.0-93.5% TRR) identified parent imazapyr (AC 243997) as the major extractable <sup>14</sup>C-residue, and 3-5 unknown HPLC regions and/or peaks. Parent compound accounted for 65.6% of the TRR in 7-Day milk and 81.9% of the TRR in kidney. Minor unknown regions and/or peaks accounted for <6.5% TRR.

Based on an earlier goat metabolism study (D222027, PP#6F04641, N. Dodd, 6/26/96) involving use of pyridine-ring labeled imazapyr, the qualitative nature of the residue in ruminants is

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## Executive Summary

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Based on an earlier goat metabolism study (D222027, PP#6F04641, N. Dodd, 6/26/96) involving use of pyridine-ring labeled imazapyr, the qualitative nature of the residue in ruminants is

adequately understood. The previously reviewed study sufficiently characterized and identified detectable residues in extracts of milk and kidney by TLC and HPLC. Imazapyr *per se* was the sole radioactive component identified, comprising ~50% of TRR in milk and ~95% of TRR in kidney.

The distribution and identification of imazapyr residues found in various goat tissues in the current goat study using imidazole-ring labeled imazapyr are mostly consistent with the previous goat study, which made use of pyridine-ring labeled imazapyr (MRID 43861504, PP#6F04641, 6/26/96, N. Dodd). In the current goat metabolism study, only milk and kidney were analyzed and the dose rate was slightly higher than the previous study (47 vs 43 ppm). The study was conducted to generate residues in the kidney and milk to be used to conduct an extraction efficiency/accountability study (currently under review). For this stated purpose, the submitted goat metabolism study is adequate.

## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. There were no deviations from regulatory requirements that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Test Substance

#### Active Ingredient

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid

CAS Number: 81334-34-1

Company Codes: AC 243997, CL 243997

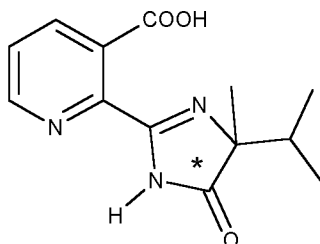
Other Synonyms: Arsenal Herbicide

Purity of Non-labeled Material: 99.6%

Location of Isotopic Label: <sup>14</sup>C-labeled at the 5 position of the imidazole ring

Radiochemical Purity: ≥96.9% (determined by HPLC)

Specific Activity: 6.31 μCi/mg (14,008 dpm/μg)



\* designates <sup>14</sup>C-label

## 1.2. Test Animals and Site

Species/breed: Goat/ breed not specified

Age: < 5 years

Gender: Female

Number: Two goats (1 control and 1 treated)

Test location: Fort Dodge Animal Health, Cyanamid Agricultural Research Center,  
Princeton, NJ

Housing: Goats were housed in individual metabolism cages suitable for the separate  
collection of urine and feces.

Diet and Water: tap water and feed (goat chow, Purina Mills, Inc.), and mixed grass hay  
were provided (*ad libitum* water, 2000 g hay and 1100 g feed provided per day).  
Feed intake was measured daily.

Acclimation period: 5 days

Environmental conditions: The test area was maintained at 64-69 F; relative humidity and  
light/dark cycle not reported.

Predosing: Animals were not predosed.

Study Dates: The in-life phase of the study was conducted from 6/4/99-6/16/99, with  
dosing occurring from 6/9/99-6/15/99. The analytical phase of the study was  
conducted from 6/17/99 to 7/21/99 (34 days).

## 1.3. Dosing

Type(s) of Dosing: Intraruminally with a capsule

Dosing Vehicle: Gelatin capsules containing lactose

Dosing Rate: The average dose of [<sup>14</sup>C]imazapyr was 90.6 mg ae/day, which is  
equivalent to 46.84 ppm in the diet based on the average daily feed consumption of  
1934 g.

Number of Doses: Once a day following afternoon milking

Duration of Dosing: 7 days

## 1.4. Sample Collection Procedures

Beginning 24 hours before the first treatment, urine and feces were collected daily. Milk was  
collected twice a day in the morning and afternoon and the samples were pooled. Milk and  
samples were stored separately in a refrigerator and feces samples were frozen (temperatures not  
specified). The animals were stunned using a captive bolt pistol and sacrificed by exsanguination  
20-21 hours after receiving the final dose on Day 7. Kidney samples were collected from each  
goat, homogenized with dry ice, mixed with water, and then frozen. Feces and kidney samples  
were stored frozen for 3-41 days and milk and urine samples were refrigerated for 8-13 days prior  
to analysis.

#### 1.4. Analytical Methods

Total Radioactive Residues (TRR). For determination of TRR, homogenized samples were radioassayed in triplicate either directly by liquid scintillation counting (milk and urine) or by combustion/LSC (excreta and kidney). Urine and feces samples were not further analyzed. The limits of quantitation (LOQ) for the radioassays were 0.006 ppm in milk and urine, and 0.007 ppm in kidney and feces; the limits of detection (LOD) were not reported.

Milk. The pooled milk sample (Day 7 only) was extracted sequentially with acetonitrile (ACN), ACN:water (5:1, v/v), and hexane, centrifuging after each extraction. The solvent extracts were combined and partitioned, resulting in a hexane fraction and an ACN/water fraction. The ACN/water fraction was concentrated to remove the ACN and partitioned first with hexane and then with ethyl acetate (EtOAc). The resulting hexane fraction was combined with the initial hexane extract and radioassayed. The remaining aqueous and EtOAc fractions were analyzed by HPLC.

Kidney. The kidney homogenate was extracted three times with acetone:water (3:1, v/v). The extracts were combined, concentrated, and redissolved in methanol. The solvent extracted <sup>14</sup>C-residues were then analyzed by LSC and HPLC. The remaining kidney post-extraction solid (PES) fraction was combusted but not analyzed.

Identification of <sup>14</sup>C-residues. <sup>14</sup>C-Residues in fractions from milk (Day 7) and kidney were profiled and quantified by HPLC using a C<sub>18</sub> column with a mobile phase gradient of ACN to water (each containing 1% tetrafluoroacetic acid ). The HPLC was equipped with UV detector, and radioactivity in eluted fractions was determined by LSC.

## 2. Results

| Table 2.1. Total Radioactive Residues in milk, kidney, and excreta from a single goat following oral dosing of [ <sup>14</sup> C] Imazapyr for 7 days at a rate of 90.6 mg ae/day, equivalent to 46.8 ppm in the diet. |                                  |                                 |           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|---------------------------------|-----------|
| Matrix                                                                                                                                                                                                                 | Sampling Interval<br>(Study day) | % of total<br>administered Dose | TRR (ppm) |
| Urine                                                                                                                                                                                                                  | 1                                | 8.60                            | 39.8      |
|                                                                                                                                                                                                                        | 2                                | 8.46                            | 39.6      |
|                                                                                                                                                                                                                        | 3                                | 9.16                            | 44.5      |
|                                                                                                                                                                                                                        | 4                                | 9.13                            | 40.6      |
|                                                                                                                                                                                                                        | 5                                | 8.59                            | 37.0      |
|                                                                                                                                                                                                                        | 6                                | 7.14                            | 33.3      |
|                                                                                                                                                                                                                        | 7                                | 7.64                            | 40.5      |
|                                                                                                                                                                                                                        | Total                            | 58.7                            | NA        |
| Feces                                                                                                                                                                                                                  | 1                                | 4.61                            | 18.6      |
|                                                                                                                                                                                                                        | 2                                | 4.81                            | 21.6      |
|                                                                                                                                                                                                                        | 3                                | 5.78                            | 21.8      |
|                                                                                                                                                                                                                        | 4                                | 5.32                            | 21.5      |
|                                                                                                                                                                                                                        | 5                                | 5.53                            | 19.1      |
|                                                                                                                                                                                                                        | 6                                | 4.60                            | 17.2      |
|                                                                                                                                                                                                                        | 7                                | 3.71                            | 15.3      |
|                                                                                                                                                                                                                        | Total                            | 34.4                            | NA        |
| Milk                                                                                                                                                                                                                   | 1                                | NR                              | 0.015     |
|                                                                                                                                                                                                                        | 2                                |                                 | 0.015     |
|                                                                                                                                                                                                                        | 3                                |                                 | 0.015     |
|                                                                                                                                                                                                                        | 4                                |                                 | 0.016     |
|                                                                                                                                                                                                                        | 5                                |                                 | 0.014     |
|                                                                                                                                                                                                                        | 6                                |                                 | 0.014     |
|                                                                                                                                                                                                                        | 7                                |                                 | 0.014     |
|                                                                                                                                                                                                                        | Total                            | 0.04 <sup>b</sup>               | NA        |
| Kidney                                                                                                                                                                                                                 | 7                                | <0.01 <sup>b</sup>              | 0.074     |

<sup>a</sup> LOQ = 0.006 ppm in milk and urine, and 0.007 ppm in kidney and feces.

<sup>b</sup> Calculated by reviewer.

NR - not reported.

NA - not applicable.

| Table 2.2.1. Extraction, Characterization, and Identification of <sup>14</sup> C-Residues in <b>Milk (Day 7)</b> from a single goat following oral dosing of [ <sup>14</sup> C]Imazapyr for 7 days at a dose equivalent to 46.8 ppm in the diet. |       |        |                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                                      | % TRR | ppm    | Characterization/Identification                                                                                                                        |
| ACN/water                                                                                                                                                                                                                                        | NR    | NR     | ACN/water and hexane extracts were combined and partitioned. The ACN/water fraction was concentrated and partition sequentially with hexane and EtOAc. |
| Hexane                                                                                                                                                                                                                                           | NR    | NR     |                                                                                                                                                        |
| AQ-2                                                                                                                                                                                                                                             | 46.3  | 0.006  | Analyzed by HPLC.<br>CL 243997 (parent) 33.4% TRR 0.005 ppm<br>5 polar unknown regions accounting for 0.4-6.5% of the TRR, totaling 11.3% TRR.         |
| Hexane-2                                                                                                                                                                                                                                         | ND    | <0.001 | Not further analyzed.                                                                                                                                  |
| EtOAc                                                                                                                                                                                                                                            | 35.8  | 0.005  | Analyzed by HPLC.<br>CL 243997 (parent) 32.2% TRR 0.005 ppm<br>5 polar unknown regions accounting for 0.4-1.1% of the TRR, totaling 3.4% TRR.          |
| PES                                                                                                                                                                                                                                              | 18.0  | 0.003  | Not further analyzed.                                                                                                                                  |

NR - not reported.

ND - not detected.

| Table 2.2.2. Extraction, Characterization, and Identification of <sup>14</sup> C-Residues in <b>Kidney</b> from a single goat following oral dosing of [ <sup>14</sup> C]Imazapyr for 7 days at a dose equivalent to 46.8 ppm in the diet. |       |       |                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                                | % TRR | ppm   | Characterization/Identification                                                                                                                  |
| Acetone/water                                                                                                                                                                                                                              | 93.5  | 0.069 | Analyzed by HPLC.<br>CL 243997 (parent) 81.9% TRR 0.061 ppm<br>3 polar unknown regions accounting for 1.98-5.73% of the TRR, totaling 11.6% TRR. |
| PES                                                                                                                                                                                                                                        | 6.5   | 0.005 | Not further analyzed.                                                                                                                            |

| Table 2.3. Summary of Characterization and Identification of <sup>14</sup> C-Residues in Milk and Kidney following oral dosing of [ <sup>14</sup> C-imidazole]Imazapyr for 7 days at a dose equivalent to 46.8 ppm in the diet. |                                  |       |                                    |       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|-------|------------------------------------|-------|
| Metabolite or Fraction                                                                                                                                                                                                          | Milk<br>(0.014 ppm) <sup>a</sup> |       | Kidney<br>(0.074 ppm) <sup>a</sup> |       |
|                                                                                                                                                                                                                                 | %TRR                             | ppm   | %TRR                               | ppm   |
| CL 243997 (parent)                                                                                                                                                                                                              | 65.6                             | 0.010 | 81.9                               | 0.061 |
| Minor unknowns <sup>b</sup>                                                                                                                                                                                                     | 14.7                             | 0.002 | 11.6                               | 0.009 |
| Total Bound                                                                                                                                                                                                                     | 18.0                             | 0.003 | 6.5                                | 0.005 |
| % Mass Balance                                                                                                                                                                                                                  | 98.3                             |       | 100.0                              |       |

<sup>a</sup> TRR for each matrix is listed in parentheses.

<sup>b</sup> Comprised of minor unknown HPLC regions and/or HPLC peaks, each accounting ≤6.5% TRR.

### 3. Discussion

#### 3.1. Methods

A single lactating goat was dosed orally once a day for 7 consecutive days via gelatin capsules containing [imidazole-5-<sup>14</sup>C]imazapyr at a dose of 90.6 mg ae/day. This dose is equivalent to 46.8 ppm in the diet, based on an average feed consumption of 1934 g/day. Urine and feces were collected daily, and milk was collected twice a day in the morning and afternoon and the samples were pooled. The goat was sacrificed 20-21 hours after receiving the final dose on Day 7, and kidney samples were collected.

Radioactivity was adequately extracted from milk and kidney samples. Solvent extractions released 82.0-93.5% of the TRR from milk and kidneys. Radioactivity remaining in the final residual solids accounted for 6.5-18.0% of the TRR. Extracted <sup>14</sup>C-residues were then analyzed by HPLC for metabolite quantitation.

Identified components accounted for 65.6% of the TRR in milk and 81.9% of the TRR in kidney. Minor unknown HPLC regions and/or HPLC peaks were identified, each accounting ≤6.5% TRR.

Samples were stored frozen for 3-41 days. No additional storage stability data are required to support this ruminant metabolism study.

The methods used to extract, fractionate, and identify <sup>14</sup>C-residues in milk and tissues of goats were adequate.

#### 3.2. Results

Following 7 days of dosing with [<sup>14</sup>C]Imazapyr at a level equivalent to 46.9 ppm in the diet, 93.3% of the dosed radioactivity was recovered in urine (58.7% dose) and feces (34.4% dose) from the treated goat. Radioactivity excreted in the milk (0.014-0.016 ppm) accounted for 0.04% of the dose, and radioactivity remaining in the kidneys (0.074 ppm) accounted for <0.01% of the dose.

Analyses of solvent extracts (82.0-93.5% TRR) identified parent imazapyr (AC 243997) as the major extractable <sup>14</sup>C-residue, and 3-5 unknown HPLC regions and/or peaks. Parent compound accounted for 65.6% TRR in 7-Day milk and 81.9% TRR in kidney. Minor unknown regions and/or peaks accounted for <6.5% TRR.

Based on previously reviewed data (MRID 43861504, D222027, 6/26/96, N. Dodd), the qualitative nature of the residue in ruminants is adequately understood. Following oral administration of [6-pyridine-<sup>14</sup>C]imazapyr to two goats at 17.7 ppm or 42.5 ppm in the diet for 7 days, the TRR ranged from less than 0.01 ppm (nondetectable) to 0.02 ppm in milk and were 0.08 ppm and 0.11 ppm, respectively, in the kidneys of the low and high dose goats. The study sufficiently characterized and identified detectable residues in extracts of milk and kidney by TLC

and HPLC. Imazapyr *per se* was the sole radioactive component identified, comprising ~50% of TRR in milk and ~95% of TRR in kidney.

The distribution and identification of imazapyr residues found in various goat tissues in the current goat study using imidazole-ring labeled imazapyr are mostly consistent with the previous goat study, which made use of pyridine-ring labeled imazapyr (MRID 43861504, D222027, 6/26/96, N. Dodd). In the current goat metabolism study, only milk and kidney were analyzed and the dose rate was slightly higher than the previous study (47 vs 43 ppm). The study was conducted to generate residues in the kidney and milk to be used to conduct an extraction efficiency/accountability study (currently under review). For this stated purpose, the submitted goat metabolism study is adequate.

#### 4. Deficiencies

No deficiencies were noted in the current goat metabolism study.

#### 5. References

DP Barcode: D222027  
Subject: PP#6F04641. Imazapyr on Field Corn. Review of Analytical Methods and Residue Data. First Use Review.  
From: N. Dodd  
To: D. McCall  
Dated: 6/26/96  
MRID(s): 43861502, 43861503, 43861504, 43861505, 43861506, 43861516, 43861517, 43861518

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist *William H. Donovan*  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** David Soderberg, Chemist *David Soderberg*  
RRB3/HED (7509C)

**DP Barcode:** D275561

**Petition:** PP#0F06166

**Citation:** 45119715 Wu, D. (1997) AC 243997: Metabolism in Grass Under Field  
Conditions: Laboratory Project Nos.: MET 96-007: RPT0084. Unpublished study  
prepared by American Cyanamid Co. 221 p. {OPPTS 860.1300}

**Sponsor:** BASF (formerly American Cyanamid Co.)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

Field-grown bermuda grass was treated with a single broadcast foliar application of [<sup>14</sup>C-6-pyridine]imazapyr at 1.5 lb ae/A. Samples of grass foliage were collected 0, 4, 10, 15, and 21 days after treatment (DAT); hay was not collected.

Total radioactive residues (TRR) were highest at 0 DAT (64.3 ppm) and declined to 17.5-24.9 ppm at 4-15 DAT. TRR levels increased substantially at 21 DAT (47.7 ppm); the petitioner attributed this increase to water loss from senescence and phytotoxicity in the sample.

Based on the metabolite profile, the metabolism of [<sup>14</sup>C]imazapyr in bermuda grass primarily involves esterification of the carboxylic acid of the parent molecule to form CL 240000 and/or CL 247087 (cyclization product), and hydrolysis of the imidazoliny ring to form pyridine dicarboxylic acid (CL 9140).

The submitted bermuda grass metabolism study on imazapyr is adequate.

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

**DP Barcode:** D275561

**Petition:** PP#0F06166

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Total radioactive residues (TRR) were highest at 0 DAT (64.3 ppm) and declined to 17.5-24.9 ppm at 4-15 DAT. TRR levels increased substantially at 21 DAT (47.7 ppm); the petitioner attributed this increase to water loss from senescence and phytotoxicity in the sample.

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## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. There were no deviations from regulatory requirements that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Substances

#### Active Ingredients

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid

CAS Number: 81334-34-1

Company Name: AC 243997

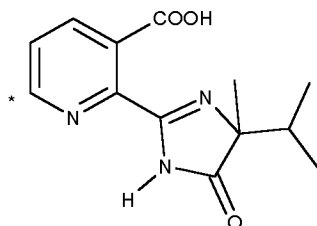
Other Synonyms: Arsenal Herbicide

Purity of Non-labeled Material: 99.5%

Location of Isotopic Label: <sup>14</sup>C-labeled at the sixth position of the pyridine ring [<sup>14</sup>C-6-PYR]

Radiochemical Purity: ≥95% (as determined by HPLC)

Specific Activity: For application, the test substance was isotopically diluted with a mixture of <sup>13</sup>C-labeled (6-pyridine) and non-radiolabeled imazapyr to a final specific activity of 4.08 μCi/mg (9047 dpm/μg).



\* denotes position of <sup>14</sup>C-label

### 1.2. Crop and Site

Type and Variety of Crop: Bermuda Grass (*var.* Hulled Bermuda)

Growth Environment: Grass was seeded into field plots in a sandy loam soil (57-67% sand, 25-33% silt, and 8-12% clay; and a 0.3-0.6% organic matter content with a CEC of 5.5-5.7 meq/100 g soil and a pH of 6.5-7.2) at an ABC Laboratories field station in Madera, CA. Monthly temperatures and precipitation were reported.

Conditions: Plants were grown in field plots using simulated agricultural practices.

### 1.3. Application

Type of Application: Broadcast foliar application.

Application Matrix: Prior to application, the <sup>14</sup>C-test substance was isotopically diluted with a mixture of <sup>13</sup>C-labeled (6-pyridine) and non-radiolabeled imazapyr, formulated as an isopropylamine salt in an aqueous solution (2 AS), and diluted with water.

Application Rate: The radiolabeled test substance was applied to separate plots at ~1.5 lb ae/A; 2x the maximum label rate.

Number and Timing of Application: Single broadcast foliar application to grass

Pre-harvest Intervals: Bermuda grass foliage was harvested at 0, 4, 10, 15, and 21 days after treatment (DAT). Soil samples were also collected prior to treatment, and 0 and 21 DAT.

### 1.4. Harvest/Post-harvest Procedures

Samples of grass foliage were placed in storage at ≤-10 C until extraction, which occurred within 19 days. Extracts were stored in a refrigerator at < 5 C until analysis.

| Table 1.4.1. Summary of Storage Conditions |         |                          |                              |
|--------------------------------------------|---------|--------------------------|------------------------------|
| Crop                                       | RACs    | Storage Temperature (°C) | Duration (days) <sup>a</sup> |
| Bermuda Grass                              | Foliage | ≤-10 C                   | 9-19                         |

<sup>a</sup> Duration of storage until the initial extraction and profiling of <sup>14</sup>C-residues.

### 1.5. Analytical Methods

Bermuda grass samples were homogenized in liquid nitrogen prior to combustion. TRR in plant and soil samples were determined by combustion followed by liquid scintillation counting (LSC). The limit of quantitation (LOQ) for the radioassay was 0.01 ppm.

Grass foliage samples were extracted repeatedly with MeOH:HCl (100:0.75, v/v), and the extracts were combined and concentrated. The <sup>14</sup>C-residues in the concentrated fraction were then profiled and quantified by HPLC. The post-extraction solids fraction number 1 (PES-1) was then air dried and enzymatically hydrolyzed.

The PES-1 fraction was incubated with protease in a phosphate buffer (pH 7.5) at ~37 C for ~24 hours, then separated into an aqueous fraction (AQ-1) and PES-2 fraction. The PES-2 fraction was incubated with cellulase in an acetate buffer (pH 5.0) at ~37 C for ~24 hours. The aqueous fraction (AQ-2) was separated from the solids (PES-3) and combined with AQ-1. The AQ-1-2 fraction was freeze dried to remove the water, then redissolved in MeOH to yield an AQ-1-2 (MeOH) fraction and a precipitate. The AQ-1-2 (MeOH) fraction was then analyzed by HPLC.

The PES-3 fraction was refluxed with 6 *N* HCl for 24 hours, then separated into an aqueous fraction (AQ-3) and a PES-4 fraction. The AQ-3 fraction was then analyzed by HPLC and the PES-4 fraction from the 0, 4, and 21 DAT samples was combusted and analyzed by LSC. The PES-4 fraction from the 10 and 15 DAT samples was hydrolyzed by refluxing in 1 *N* NaOH for 4 hours. The resulting AQ-4 fraction was examined by LSC and the PES-5 fraction was combusted and analyzed by LSC.

#### Identification of <sup>14</sup>C-residues.

<sup>14</sup>C-Residues in solvent fractions were profiled and quantified by HPLC using a Ramona radiometric detector and a UV detector set at 254 nm. Reference standards were also quantified by HPLC analysis under the same conditions as the test samples. Thin-layer chromatography (TLC) was used for purification and qualitative analysis of samples using two solvent systems (isopropanol/ammonium hydroxide, 80:20; and MeOH/water/ethyl acetate, 25:25:50). TLC plates were visualized under UV light (short wavelength) and radioactive residues were detected and quantified using a Fuji Bioimaging Analyzer BAS-1000. The MeOH fraction from 21 DAT bermuda grass was further purified by preparative TLC and HPLC, then analyzed by GC/MS or LC/MS.

## 2. Results

| Table 2.1. Total Radioactive Residues in Bermuda Grass Foliage and Soil following a Single Broadcast Foliar Application of [ <sup>14</sup> C] Imazapyr at 1.5 lb ae/A. |                                      |                            |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|----------------------------|
| Matrix                                                                                                                                                                 | Sampling Interval (DAT) <sup>a</sup> | TRR (ppm)                  |
| Foliage                                                                                                                                                                | 0                                    | 64.290                     |
|                                                                                                                                                                        | 4                                    | 17.501                     |
|                                                                                                                                                                        | 10                                   | 22.024                     |
|                                                                                                                                                                        | 15                                   | 24.891                     |
|                                                                                                                                                                        | 21                                   | 47.673                     |
| Soil (0-3")<br>(3-6")<br>(6-12")<br>(12-18")                                                                                                                           | 0                                    | <0.01 <sup>b</sup> , 0.082 |
|                                                                                                                                                                        |                                      | <0.01, <0.01               |
|                                                                                                                                                                        |                                      | <0.01, <0.01               |
|                                                                                                                                                                        |                                      | <0.01, <0.01               |
| Soil (0-3")<br>(3-6")<br>(6-12")<br>(12-18")                                                                                                                           | 21                                   | 0.122, 0.139               |
|                                                                                                                                                                        |                                      | 0.072, 0.067               |
|                                                                                                                                                                        |                                      | <0.01, 0.095               |
|                                                                                                                                                                        |                                      | <0.01, <0.01               |

<sup>a</sup> DAT = days after treatment.

<sup>b</sup> LOQ = 0.01 ppm

| Table 2.2.1 Extraction, Characterization, and Identification of <sup>14</sup> C-Residues in <b>Bermuda Grass Foliage</b><br>Harvested 0, 10, and 21 Days Following a Single Broadcast Foliar Application of [ <sup>14</sup> C]Imazapyr at<br>1.5 lb ae/A. <sup>a</sup> |       |        |                                                                                                                                                                                                                                                  |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                                                            | % TRR | ppm    | Characterization/identification                                                                                                                                                                                                                  |
| <b>0-DAT (64.29 ppm) <sup>b</sup></b>                                                                                                                                                                                                                                  |       |        |                                                                                                                                                                                                                                                  |
| MeOH/HCl                                                                                                                                                                                                                                                               | 97.29 | 62.548 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 94.74% TRR 60.91 ppm<br>Unknown C 0.70% TRR 0.045 ppm<br>Unknown E 0.64% TRR 0.041 ppm<br>Unknown F 1.22% TRR 0.784 ppm                                                                 |
| PES-1                                                                                                                                                                                                                                                                  | 2.71  | 1.742  | Solids were subjected to sequential enzyme hydrolyses with protease and then cellulase. The resulting hydrolysates were then combined.                                                                                                           |
| AQ-1-2                                                                                                                                                                                                                                                                 | 2.44  | 1.569  | The combined fraction was freeze dried, and the resulting solids were redissolved in MeOH.                                                                                                                                                       |
| AQ-1-2<br>(MeOH)                                                                                                                                                                                                                                                       | 2.37  | 1.524  | Analyzed by HPLC:<br>AC 243997 (parent) 1.99% TRR 1.279 ppm<br>CL 9140 0.08% TRR 0.051 ppm<br>CL 240000 +<br>CL 247087 <sup>c</sup> 0.30% TRR 0.193 ppm                                                                                          |
| Precipitate                                                                                                                                                                                                                                                            | 0.07  | 0.045  | Not further analyzed                                                                                                                                                                                                                             |
| PES-3                                                                                                                                                                                                                                                                  | 0.27  | 0.174  | Acid hydrolyzed by refluxing in 6N HCl for 24 hours.                                                                                                                                                                                             |
| AQ-3                                                                                                                                                                                                                                                                   | 0.24  | 0.154  | Not further analyzed.                                                                                                                                                                                                                            |
| PES-4                                                                                                                                                                                                                                                                  | 0.03  | 0.019  | Not further analyzed                                                                                                                                                                                                                             |
| <b>10-DAT (22.024 ppm) <sup>a</sup></b>                                                                                                                                                                                                                                |       |        |                                                                                                                                                                                                                                                  |
| MeOH/HCl                                                                                                                                                                                                                                                               | 94.52 | 20.817 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 89.14% TRR 19.63 ppm<br>CL 9140 2.19% TRR 0.482 ppm<br>Unknown A 0.42% TRR 0.093 ppm<br>Unknown D 0.87% TRR 0.192 ppm<br>Unknown E 0.36% TRR 0.079 ppm<br>Unknown F 1.54% TRR 0.339 ppm |
| PES-1                                                                                                                                                                                                                                                                  | 5.48  | 1.207  | Solids were subjected to sequential enzyme hydrolyses with protease and then cellulase. The resulting hydrolysates were then combined.                                                                                                           |
| AQ-1-2                                                                                                                                                                                                                                                                 | 2.97  | 0.654  | The combined fraction was freeze dried, and the resulting solids were redissolved in MeOH.                                                                                                                                                       |
| AQ-1-2<br>(MeOH)                                                                                                                                                                                                                                                       | 2.41  | 0.531  | Analyzed by HPLC:<br>AC 243997 (parent) 1.70% TRR 0.374 ppm<br>CL 9140 0.48% TRR 0.106 ppm<br>CL 240000 +<br>CL 247087 <sup>c</sup> 0.20% TRR 0.044 ppm<br>Unknown C 0.03% TRR 0.007 ppm                                                         |
| Precipitate                                                                                                                                                                                                                                                            | 0.56  | 0.123  | Not further analyzed                                                                                                                                                                                                                             |
| PES-3                                                                                                                                                                                                                                                                  | 2.51  | 0.553  | Acid hydrolyzed by refluxing in 6N HCl for 24 hours.                                                                                                                                                                                             |
| AQ-3                                                                                                                                                                                                                                                                   | 1.45  | 0.319  | Analyzed by HPLC:<br>CL 9140 1.45% TRR 0.319 ppm                                                                                                                                                                                                 |

| Table 2.2.1 Extraction, Characterization, and Identification of <sup>14</sup> C-Residues in <b>Bermuda Grass Foliage</b><br>Harvested 0, 10, and 21 Days Following a Single Broadcast Foliar Application of [ <sup>14</sup> C]Imazapyr at<br>1.5 lb ae/A. <sup>a</sup> |       |        |                                                                                                                                                                                                                                                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Fraction ID                                                                                                                                                                                                                                                            | % TRR | ppm    | Characterization/identification                                                                                                                                                                                                                      |
| PES-4                                                                                                                                                                                                                                                                  | 1.06  | 0.233  | Base hydrolyzed by refluxing in 1N NaOH for 4 hours.                                                                                                                                                                                                 |
| AQ-4                                                                                                                                                                                                                                                                   | 1.00  | 0.220  | Not further analyzed                                                                                                                                                                                                                                 |
| PES-5                                                                                                                                                                                                                                                                  | 0.06  | 0.013  | Not further analyzed                                                                                                                                                                                                                                 |
| <b>21-DAT (47.673 ppm) <sup>a</sup></b>                                                                                                                                                                                                                                |       |        |                                                                                                                                                                                                                                                      |
| MeOH/HCl                                                                                                                                                                                                                                                               | 85.76 | 40.884 | Concentrated and analyzed by HPLC:<br>AC 243997 (parent) 75.48% TRR 35.98 ppm<br>CL 9140 7.23% TRR 3.447 ppm<br>Unknown C 0.59% TRR 0.281 ppm<br>CL 240000+<br>CL 247087 <sup>c</sup> 2.47% TRR 1.177 ppm<br>Results confirmed using GC/MS or LC/MS. |
| PES-1                                                                                                                                                                                                                                                                  | 14.24 | 6.789  | Solids were subjected to sequential enzyme hydrolyses with protease and then cellulase. The resulting hydrolysates were then combined.                                                                                                               |
| AQ-1-2                                                                                                                                                                                                                                                                 | 8.93  | 4.257  | The combined fraction was freeze dried, and the resulting solids were redissolved in MeOH.                                                                                                                                                           |
| AQ-1-2<br>(MeOH)                                                                                                                                                                                                                                                       | 7.33  | 3.494  | Analyzed by HPLC:<br>AC 243997 (parent) 4.55% TRR 2.169 ppm<br>CL 9140 1.79% TRR 0.853 ppm<br>CL 240000 +<br>CL 247087 <sup>c</sup> 0.54% TRR 0.257 ppm<br>Unknown C 0.45% TRR 0.215 ppm                                                             |
| Precipitate                                                                                                                                                                                                                                                            | 1.60  | 0.763  | Not further analyzed                                                                                                                                                                                                                                 |
| PES-3                                                                                                                                                                                                                                                                  | 5.31  | 2.531  | Acid hydrolyzed by refluxing in 6N HCl for 24 hours.                                                                                                                                                                                                 |
| AQ-3                                                                                                                                                                                                                                                                   | 3.81  | 1.816  | Not further analyzed                                                                                                                                                                                                                                 |
| PES-4                                                                                                                                                                                                                                                                  | 1.50  | 0.715  | Not further analyzed                                                                                                                                                                                                                                 |

<sup>a</sup> Samples were analyzed from two additional intervals (4 and 15 DAT); the results were similar to the 10 DAT results and therefore are not reported in this table.

<sup>b</sup> TRR for each matrix is listed in parentheses.

<sup>c</sup> CL 240000 and CL 247087 were each 50% of the total, estimated by the petitioner based on re-extraction of the 0- and 21-DAT samples with acetone.

| Metabolite or Fraction <sup>a</sup>       | 0-DAT<br>(64.29 ppm) <sup>b</sup> |        | 4-DAT<br>(17.50 ppm) |       | 10-DAT<br>(22.02 ppm) |       | 15 DAT<br>(24.89 ppm) |       | 21 DAT<br>(47.67 ppm) |       |
|-------------------------------------------|-----------------------------------|--------|----------------------|-------|-----------------------|-------|-----------------------|-------|-----------------------|-------|
|                                           | %TRR                              | ppm    | %TRR                 | ppm   | %TRR                  | ppm   | %TRR                  | ppm   | %TRR                  | ppm   |
| AC 243997 (parent)                        | 96.73                             | 62.19  | 94.40                | 16.52 | 90.84                 | 20.00 | 78.12                 | 19.44 | 80.03                 | 38.15 |
| CL 9140                                   | 0.08                              | 0.051  | 0.25                 | 0.044 | 4.12                  | 0.907 | 4.91                  | 1.22  | 12.83                 | 6.12  |
| CL 240000 + CL 247087 <sup>c</sup>        | 0.30                              | 0.193  | ND                   | --    | 0.2                   | 0.044 | 10.52                 | 2.62  | 3.01                  | 1.43  |
| Total Identified (TI)                     | 97.11                             | 62.434 | 94.65                | 16.56 | 95.16                 | 20.95 | 93.55                 | 23.28 | 95.87                 | 45.70 |
| Minor Discrete Unknowns<br>(each <3% TRR) | 2.56                              | 0.870  | 3.78                 | 0.662 | 3.19                  | 0.703 | 2.21                  | 0.550 | 0.59                  | 0.281 |
| Acid hydrolysates                         | 0.24 <sup>d</sup>                 | 0.154  | 1.07 <sup>d</sup>    | 0.187 | NA                    | NA    | NA                    | NA    | NA                    | NA    |
| Base hydrolysates                         | NA                                | NA     | NA                   | NA    | 1.00                  | 0.220 | 2.80                  | 0.697 | NA                    | NA    |
| Precipitate                               | 0.07                              | 0.045  | 0.22                 | 0.039 | 0.56                  | 0.123 | 0.95                  | 0.236 | 1.60                  | 0.763 |
| Total Characterized (TC)                  | 2.87                              | 1.84   | 5.05                 | 0.885 | 4.75                  | 1.05  | 5.96                  | 1.49  | 2.19                  | 1.04  |
| Total Bound (TB)                          | 0.03                              | 0.019  | 0.27                 | 0.047 | 0.06                  | 0.013 | 0.26                  | 0.065 | 1.50                  | 0.715 |
| % Mass Balance                            | 100.01                            |        | 99.97                |       | 99.97                 |       | 99.77                 |       | 99.56                 |       |

<sup>a</sup> Metabolite names and structures are presented in Table 2.4.

<sup>b</sup> TRR for each matrix is listed in parentheses.

<sup>c</sup> CL 240000 and CL 247087 were each 50% of the total, estimated by the petitioner based on re-extraction of the 0- and 21-DAT samples with acetone.

<sup>d</sup> Fraction not further analyzed, but was assumed to be CL 9140 based on similar peak from 10, 15, and 21 DAT analyses.

ND = not detected

NA = not applicable

TC = Sum of all unidentified, extractable residues

% Mass Balance = TI (%TRR) + TC (%TRR) + TB (%TRR)

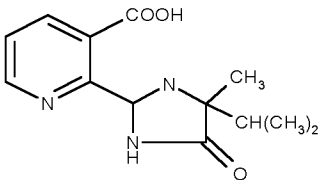
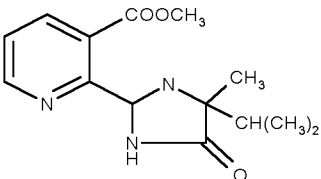
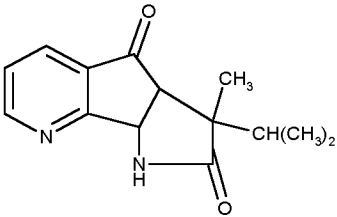
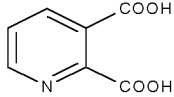
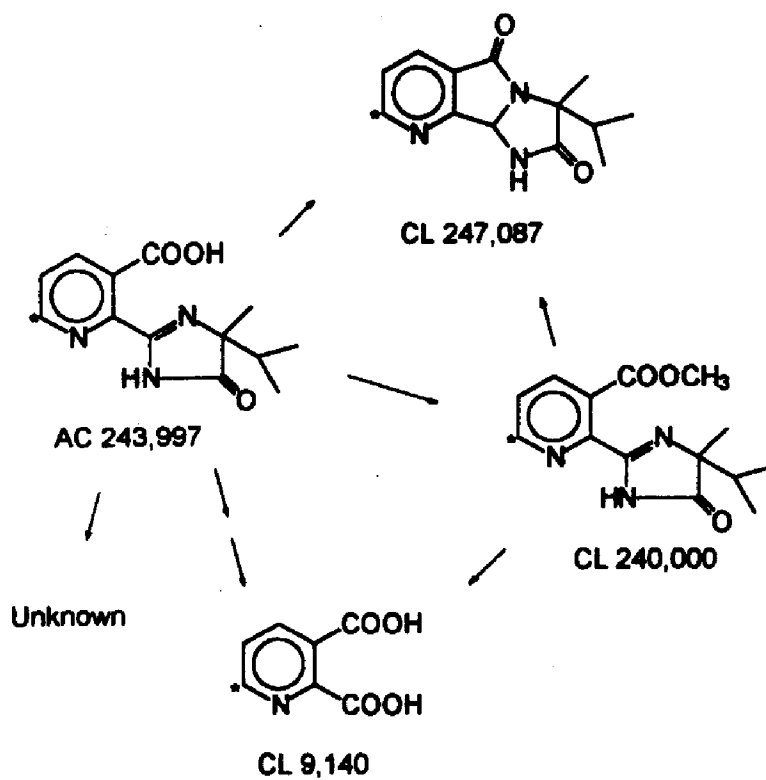
| Table 2.4. Metabolites Identified in Bermuda Grass Foliage following a Broadcast Foliar Application. of Imazapyr. |                                                                                                                                            |                                                                                       |
|-------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Metabolite Identifier                                                                                             | Chemical Name                                                                                                                              |                                                                                       |
| Imazapyr<br>(AC 243997)                                                                                           | 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid                                                                            |    |
| CL 240000                                                                                                         | 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-methyl ester nicotinic acid                                                               |    |
| CL 247087                                                                                                         | 5 <i>H</i> -imidazol[1',2':1,2]pyrrolo [3,4- <i>b</i> ]pyridine-2(3 <i>H</i> ), 5-dione, 1,9 <i>b</i> α(&β)-dihydro-3α-isopropyl-3-methyl] |   |
| CL 9140                                                                                                           | 2,3-pyridinedicarboxylic acid                                                                                                              |  |

Figure 1. Proposed Metabolic Fate of Imazapyr in Field-grown Bermuda Grass.

**Proposed Metabolic Pathways of [<sup>14</sup>C]-AC 243,997 in Bermuda Grass**



### 3. Discussion

#### 3.1. Methods

The methods used to treat the bermuda grass plants were adequate. Field grown grass received a single broadcast foliar application of [<sup>14</sup>C-6-PYR]imazapyr, formulated as an isopropylamine salt, at 1.5 lb ae/A. Samples of grass foliage were collected 0, 4, 10, 15, and 21 DAT; hay was not collected.

Methanolic extraction released 85.8-97.3% of the TRR from all grass foliage samples and the subsequent enzymatic extraction and/or acid and base hydrolyzation released an additional 1.0-8.9% of the TRR. Radioactivity remaining in the PES fractions accounted for <1.5% of the TRR. Isolated metabolites or fractions were characterized by TLC analysis with reference standards, HPLC analysis, GC/MS or LC/MS.

Bermuda grass foliage samples were stored frozen ( $\leq -10$  C) for a maximum of 19 days, therefore storage stability data are not required to support this metabolism study.

The methods used to extract, fractionate, and identify <sup>14</sup>C-residues in bermuda grass foliage were adequate.

#### 3.2. Results

TRR were highest at 0 DAT (64.3 ppm) and declined to 17.5-24.9 ppm by 4-15 DAT. TRR increased substantially at 21 DAT (47.7 ppm); the petitioner attributed this increase to water loss from senescence and phytotoxicity in the sample.

Analyses of solvent extracts identified parent imazapyr (AC 243997) and 3 metabolites, along with numerous minor unknown components. The major extractable <sup>14</sup>C-residue was identified as parent (78.1-96.7% TRR). All other metabolites each accounted for a substantially lower percentage of the TRR: CL 9140 (0.08-12.83% TRR), combined CL 240000 and CL 247087 (0.20-10.52% TRR), and minor discrete unknowns (each <3% TRR).

Based on the metabolite profile, the metabolism of [<sup>14</sup>C]imazapyr in bermuda grass primarily involves esterification of the carboxylic acid of the parent molecule to form CL 240000 and/or CL 247087 (cyclization product), and hydrolysis of the imidazolinyl ring to form CL 9140 (Figure 1).

The submitted bermuda grass metabolism study on imazapyr is adequate.

#### **4. Deficiencies**

Bermuda grass hay samples were not collected; the current Agency guidance requests metabolism data for all RACs. However, as the 21 DAT forage samples exhibited drying due to senescence and phytotoxicity, and the majority of TRR were determined to be parent, data on the metabolism of [<sup>14</sup>C]imazapyr in bermuda grass hay will not be required. No other substantial deficiencies were noted in this grass metabolism study.

#### **5. References**

None

I 2

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist *William H. Donovan*  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** Danette Drew, Chemist *Drew*  
RRB3/HED (7509C)

**DP Barcode:** D275561

**Petition:** PP#0F06166

**Citation:** 45119709 Drottar, K., Swigert, J., Wisk, J. (1996) Uptake, Depuration, and Metabolism of Carbon-14 AC 243997 in Eastern Oyster and Grass Shrimp: Laboratory Project Nos.: 954-93-165; ECO 93-165.01; 954-93-164. Unpublished study prepared by American Cyanamid Co. 228 p. (OPPTS 860.1400)

**Sponsor:** BASF (formerly American Cyanamid Co.)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

Two studies were conducted to examine the potential for bioaccumulation of [pyridine-5-<sup>14</sup>C]imazapyr in shellfish (oysters and grass shrimp). These studies were designed to determine (i) the steady-state bioconcentration factor (BCF) of [<sup>14</sup>C]imazapyr in each species; (ii) uptake and depuration rate constants; and (iii) the metabolism of [<sup>14</sup>C]imazapyr in these animals.

Two replicate populations each of oysters (52 animals/tank) and grass shrimp (410 animals/tank) were exposed to [<sup>14</sup>C]imazapyr at an average concentration of ~0.25 mg a.i./L for 28 days, followed by a 14-day depuration period. Actual average [<sup>14</sup>C]imazapyr concentrations in saltwater during the uptake phase were 0.244 mg a.i./L for oysters and 0.268 mg a.i./L for shrimp, and HPLC analyses of the water samples indicated that [<sup>14</sup>C]imazapyr was stable in the tank water during the tests.

In the oyster study, concentrations of radioactivity in tissues during the uptake phase were low and variable, with the majority of samples having <sup>14</sup>C-residues below the limit of quantitation (LOQ, 0.072 ppm). <sup>14</sup>C-residues in oysters were <0.072-0.078 ppm on Day 7, 0.074-0.120 ppm on Day 14, <0.072-0.101 ppm on Day 21, and 0.073-0.105 ppm on Day 28. Concentrations of radioactivity were <LOQ in all samples collected during the depuration phase and in all control samples.

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

**Through:** Danette Drew, Chemist  
RRB3/HED (7509C)

**DP Barcode:** D275561

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## Executive Summary

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Two replicate populations each of oysters (52 animals/tank) and grass shrimp (410 animals/tank) were exposed to [<sup>14</sup>C]imazapyr at an average concentration of ~0.25 mg a.i./L for 28 days, followed by a 14-day depuration period. Actual average [<sup>14</sup>C]imazapyr concentrations in saltwater during the uptake phase were 0.244 mg a.i./L for oysters and 0.268 mg a.i./L for shrimp, and HPLC analyses of the water samples indicated that [<sup>14</sup>C]imazapyr was stable in the tank water during the tests.

In the oyster study, concentrations of radioactivity in tissues during the uptake phase were low and variable, with the majority of samples having <sup>14</sup>C-residues below the limit of quantitation (LOQ, 0.072 ppm). <sup>14</sup>C-residues in oysters were <0.072-0.078 ppm on Day 7, 0.074-0.120 ppm on Day 14, <0.072-0.101 ppm on Day 21, and 0.073-0.105 ppm on Day 28. Concentrations of radioactivity were <LOQ in all samples collected during the depuration phase and in all control samples.

In the shrimp study, concentrations of radioactivity in tissues were also low and variable, with the majority of samples having residues <LOQ (<0.128 ppm). During the uptake phase, <sup>14</sup>C-residues in shrimp were <0.128 ppm on Days 0, 7, and 14, and were <0.128-0.147 ppm on Day 21 and <0.128-0.160 ppm on Day 28. During the depuration phase, <sup>14</sup>C-residues in shrimp were <0.128-0.137 ppm up to Day 3, ≤0.132 ppm by Day 7, and <0.128 ppm at the later sampling intervals. Concentrations of radioactivity were <LOQ in all control samples.

Due to the low levels of radioactivity in the oyster and shrimp tissues, a BCF value and uptake and depuration rates were not calculated, and <sup>14</sup>C-residues in tissues were not further characterized. Considering that all of the oyster or shrimp tissue samples had concentrations lower than that of the saltwater, [<sup>14</sup>C]imazapyr does not appear to accumulate in shellfish. These studies are adequate for the stated purpose. However, due to the relatively high method LOQs reported for the oyster and shrimp tissues, together with the relatively low residue levels in these tissues and resulting lack of identification/characterization of residues, these studies do not provide insight into the metabolic profile of imazapyr in shellfish.

### **GLP Compliance**

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. There were no deviations from regulatory requirements that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Substance

#### Active Ingredient

Common Name: Imazapyr

IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid

CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid

CAS Number: 81334-34-1

Company Name: AC 243997, CL 243997

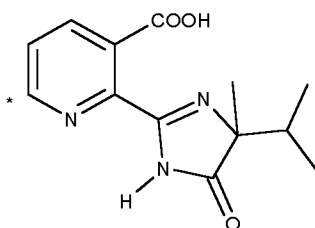
Other Synonyms: Arsenal Herbicide

Purity of Non-labeled Material: >97.5%

Location of Isotopic Label: <sup>14</sup>C-labeled at the sixth position of the pyridine ring

Radiochemical Purity: >96.7% (HPLC)

Specific Activity: ~44 µCi/mg (96,800 dpm/µg). For dosing, a dispensing stock solution was prepared containing ~5.0 mg ae/ml of the test substance isotopically diluted with non-radiolabeled imazapyr and water. The dispensing stock solution was then mixed with water in the dilution system. The resulting concentration of [<sup>14</sup>C]imazapyr was ~0.25 mg ae/L with a specific activity of 2,748 dpm/µg.



\* denotes position of <sup>14</sup>C-label

### 1.2. Test Animals and Site

#### Oyster

Animal/species: Eastern Oyster/ *Crassostrea virginica*

Age: Not reported

Size: 44-56 mm; average of 52 mm

Number: 52 per tank, two treated replicate tanks and one control tank

Test location: Wildlife International Ltd., Easton MD

Housing: housed in a 56 L polyethylene test chamber filled with ~18 L of water. Flow through the tank was equivalent to 16 tank volumes every 24 hours.

Diet: During acclimation, an algal suspension was provided; during the test period, food was obtained from unfiltered sea water (Indian River Inlet, DE).

Acclimation period: 41 days

Environmental conditions: The test water was maintained at  $22 \pm 2$  C; the light/dark cycle was 16 hrs light/8 hrs dark.

Water: Unfiltered sea water diluted with well water to a salinity of 20 parts per thousand, pH 8.1-8.3, and dissolved oxygen of 5.9-7.3 mg/L.

Predosing: Animals were not predosed.

Study Dates: The in-life phase of the study was conducted from 2/16/95-5/10/95, with dosing occurring from 3/29/95-4/25/95. The analytical phase of the study was completed on 9/21/95.

### Shrimp

Animal/species: Grass Shrimp/ *Palaemonetes pugio*

Age: Not reported

Size: 23-31 mm ( $28 \pm 2$  mm); 0.11-0.25 g ( $0.18 \pm 0.029$  g)

Number: 410 shrimp per tank; two treated replicate tanks and one control tank

Test location: Wildlife International Ltd., Easton MD

Housing: housed in a 56 L polyethylene test chamber filled with ~45 L of water. Flow through the tank was equivalent to 6.4 tank volumes every 24 hours.

Diet: brine shrimp, frozen (Kordon, Hayward, CA).

Acclimation period: 48 days

Environmental conditions: The test water was maintained at  $20 \pm 2$  C; the light/dark cycle was 16 hrs light/8 hrs dark.

Water: Unfiltered sea water diluted with well water to a salinity of 20-22 parts per thousand, pH 8.2-8.5, and dissolved oxygen of 4.6-7.5 mg/L.

Predosing: Animals were not predosed.

Study Dates: The in-life phase of the study was conducted from 5/5/93-8/3/93, with dosing occurring from 6/22/93-7/20/93. The analytical phase of the study was completed on 4/18/94.

### 1.3. Dosing

Type(s) of Dosing: Continuous-flow diluter system

Dosing Vehicle: Dose mixed directly with aquatic environment

Dosing Rate: Average dose level was equivalent to ~0.25 mg a.i./L.

Duration of Dosing: Continuous concentration maintained for 28 days, followed by 14 days of depuration (no test substance present)

### 1.4. Sample Collection Procedures

Triplicate water samples were collected from each tank (mid-level) on treatment days -1, 0, 3, 7, 14, 21, and 28, and on days 1, 7, and 14 during the depuration phase.

Tissue samples (soft oyster tissues and whole grass shrimp) were collected on days 0, 7, 14, 21, and 28 of the uptake phase and on days 1, 3, 7, 10, and 14 of the depuration phase. Samples were homogenized and stored frozen (-14 C) until analysis. Actual storage intervals were not reported, however, based on biological termination dates and analytical termination dates, maximum frozen storage intervals were ~4 months for oysters and ~9 months for shrimp.

## 1.4. Analytical Methods

**Total Radioactive Residues (TRR).** Radioactivity in all water samples was determined directly by liquid scintillation counting (LSC). For determination of TRR in animal tissues, homogenized samples were radioassayed in triplicate by tissue solubilization (Soluene 350, 4 hours at 40 C) and LSC. The limits of quantitation (LOQ) for the radioassays were reported to be 0.001 ppm in water (oyster test), 0.026 ppm in water (shrimp test), 0.072 ppm in oyster tissues, and 0.128 ppm in shrimp tissue.

**HPLC analysis of water.** In addition to the LSC analysis, two of the three saltwater samples collected from each tank at each sampling interval were also analyzed for imazapyr using an HPLC/UV method to verify the imazapyr concentration in the saltwater. Saltwater samples were injected directly onto an HPLC system comprised of a Hewlett-Packard Hypersil column with a isocratic mobile phase of 9% acetonitrile, 12% methanol, 78% water, and 1% acetic acid. Residues were detected by UV absorption (240 nm). Imazapyr had a retention time of ~3 minutes. The reported LOQ for imazapyr by this method is 0.05 ppm in saltwater in the oyster test and 0.026 ppm in saltwater from the shrimp test. To validate this method, saltwater samples were fortified with imazapyr at 0.026-0.256 ppm and analyzed by the above HPLC/UV method.

## 2. Results

| Table 2.1. Recovery of Imazapyr from saltwater using HPLC/UV Method for determining Imazapyr in Saltwater |                           |              |                                      |                    |
|-----------------------------------------------------------------------------------------------------------|---------------------------|--------------|--------------------------------------|--------------------|
| Matrix                                                                                                    | Fortification Level (ppm) | # of Samples | Range of Recoveries (%) <sup>a</sup> | Mean Recovery ± SD |
| Oyster study                                                                                              | 0.256                     | 16           | 88-100                               | 96 ± 3.5           |
| Shrimp study                                                                                              | 0.026-0.255               | 43           | 54-96 (6)                            | 80 ± 11            |

<sup>a</sup> The value in parentheses indicates the number of samples with recoveries outside the 70-120% range.

| Table 2.2. Concentration of [ <sup>14</sup> C]Imazapyr in saltwater during treatment and depuration of oysters and grass shrimp. |                                     |                             |                        |                |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|-----------------------------|------------------------|----------------|
| Oyster Test                                                                                                                      |                                     |                             |                        |                |
| Day                                                                                                                              | Concentration (mg a.i./L)           |                             |                        |                |
|                                                                                                                                  | Replicate Tank A                    |                             | Replicate Tank B       |                |
|                                                                                                                                  | LSC                                 | HPLC                        | LSC                    | HPLC           |
| -1                                                                                                                               | 0.249, 0.248, 0.249                 | NA                          | 0.248, 0.247, 0.247    | NA             |
| 0                                                                                                                                | 0.247, 0.248, 0.248                 | 0.259, 0.260                | 0.249, 0.247, 0.251    | 0.265, 0.259   |
| 3                                                                                                                                | 0.242, 0.241, 0.243                 | NA                          | 0.243, 0.243, 0.245    | NA             |
| 7                                                                                                                                | 0.247, 0.234, 0.247                 | 0.259, 0.246                | 0.165, 0.247, 0.248    | 0.173, 0.249   |
| 14                                                                                                                               | 0.256, 0.250, 0.219                 | 0.258, 0.248                | 0.241, 0.250, 0.240    | 0.252, 0.259   |
| 21                                                                                                                               | 0.249, 0.247, 0.247                 | 0.256, 0.260                | 0.247, 0.250, 0.249    | 0.264, 0.261   |
| 28                                                                                                                               | 0.251, 0.253, 0.250                 | 0.263, 0.269                | 0.233, 0.252, 0.253    | 0.250, 0.256   |
| Average ± S.D.                                                                                                                   | 0.246 ± 0.008                       | 0.258 ± 0.007               | 0.242 ± 0.018          | 0.249 ± 0.027  |
| Depuration                                                                                                                       |                                     |                             |                        |                |
| 1                                                                                                                                | <0.001, <0.001, <0.001 <sup>a</sup> | <0.05, <0.05 <sup>a</sup>   | <0.001, <0.001, <0.001 | <0.05, <0.05   |
| 7                                                                                                                                | <0.001, <0.001, <0.001              | <0.05, <0.05                | <0.001, <0.001, <0.001 | <0.05, <0.05   |
| 14                                                                                                                               | <0.001, <0.001, <0.001              | <0.05, <0.05                | <0.001, <0.001, <0.001 | <0.05, <0.05   |
| Shrimp Test                                                                                                                      |                                     |                             |                        |                |
| Day                                                                                                                              | Concentration (mg a.i./L)           |                             |                        |                |
|                                                                                                                                  | Replicate Tank A                    |                             | Replicate Tank B       |                |
|                                                                                                                                  | LSC                                 | HPLC                        | LSC                    | HPLC           |
| -1                                                                                                                               | 0.273, 0.270, 0.269                 | NA                          | 0.269, 0.264, 0.269    | NA             |
| 0                                                                                                                                | 0.243, 0.245, 0.245                 | 0.143, 0.199                | 0.246, 0.246, 0.245    | 0.209, 0.106   |
| 3                                                                                                                                | 0.257, 0.256, 0.255                 | NA                          | 0.258, 0.258, 0.258    | NA             |
| 7                                                                                                                                | 0.284, 0.289, 0.293                 | 0.259, 0.291                | 0.292, 0.287, 0.293    | 0.258, 0.291   |
| 14                                                                                                                               | 0.252, 0.253, 0.255                 | 0.239, 0.241                | 0.253, 0.253, 0.255    | 0.180, 0.258   |
| 21                                                                                                                               | 0.266, 0.266, 0.267                 | 0.264, 0.235                | 0.269, 0.269, 0.266    | 0.265, 0.299   |
| 28                                                                                                                               | 0.299, 0.298, 0.300                 | 0.286, 0.274                | 0.294, 0.298, 0.298    | 0.286, 0.283   |
| Average ± S.D.                                                                                                                   | 0.268 ± 0.019                       | 0.243 ± 0.045               | 0.268 ± 0.018          | 0.243 ± 0.061  |
| Depuration                                                                                                                       |                                     |                             |                        |                |
| 1                                                                                                                                | <0.026, <0.026, <0.026 <sup>a</sup> | <0.026, <0.026 <sup>a</sup> | <0.026, <0.026, <0.026 | <0.026, <0.026 |
| 7                                                                                                                                | <0.026, <0.026, <0.026              | <0.026, <0.026              | <0.026, <0.026, <0.026 | <0.026, <0.026 |
| 14                                                                                                                               | <0.026, <0.026, <0.026              | <0.026, <0.026              | <0.026, <0.026, <0.026 | <0.026, <0.026 |

NA = not applicable.

<sup>a</sup> Reported LOQ for the various analyses.

| Table 2.3. Total Radioactive Residues in oyster muscle and grass shrimp tissues following a continuous dose of [ <sup>14</sup> C-pyridinyl]imazapyr at a concentration of ~0.25 mg a.i./L for 28 days, followed by a 14-day depuration period. |                          |                                              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|----------------------------------------------|
| Animal/Matrix                                                                                                                                                                                                                                  | Sampling Interval (days) | TRR (ppm) <sup>a</sup>                       |
| Oyster<br>(soft tissue)                                                                                                                                                                                                                        | Uptake Phase             |                                              |
|                                                                                                                                                                                                                                                | 0                        | <0.072 <sup>b</sup> , <0.072, <0.072, <0.072 |
|                                                                                                                                                                                                                                                | 7                        | <0.072, 0.078, 0.074, 0.074                  |
|                                                                                                                                                                                                                                                | 14                       | <b>0.120, 0.080, 0.109</b> , 0.074           |
|                                                                                                                                                                                                                                                | 21                       | <0.072, <0.072, <b>0.101</b> , <0.072        |
|                                                                                                                                                                                                                                                | 28                       | <b>0.105, 0.089, 0.082</b> , 0.073           |
|                                                                                                                                                                                                                                                | Depuration Phase         |                                              |
|                                                                                                                                                                                                                                                | 1                        | <0.072, <0.072, <0.072, <0.072               |
|                                                                                                                                                                                                                                                | 3                        | <0.072, <0.072, <0.072, <0.072               |
|                                                                                                                                                                                                                                                | 7                        | <0.072, <0.072, <0.072, <0.072               |
|                                                                                                                                                                                                                                                | 10                       | <0.072, <0.072, <0.072, <0.072               |
|                                                                                                                                                                                                                                                | 14                       | <0.072, <0.072, <0.072, <0.072               |
| Grass Shrimp<br>(whole animal)                                                                                                                                                                                                                 | Uptake Phase             |                                              |
|                                                                                                                                                                                                                                                | 0                        | <0.128 <sup>b</sup> , <0.128, <0.128, <0.128 |
|                                                                                                                                                                                                                                                | 7                        | <0.128, <0.128, <0.128, <0.128               |
|                                                                                                                                                                                                                                                | 14                       | <0.128, <0.128, <0.128, <0.128               |
|                                                                                                                                                                                                                                                | 21                       | 0.147, 0.135, <0.128, 0.141                  |
|                                                                                                                                                                                                                                                | 28                       | <b>0.148, 0.160</b> , <0.128, 0.135          |
|                                                                                                                                                                                                                                                | Depuration Phase         |                                              |
|                                                                                                                                                                                                                                                | 1                        | <0.128, <0.128, <0.128, 0.136                |
|                                                                                                                                                                                                                                                | 3                        | 0.129, 0.132, 0.133, 0.137                   |
|                                                                                                                                                                                                                                                | 7                        | 0.132, <0.128, <0.128, <0.128                |
|                                                                                                                                                                                                                                                | 10                       | <0.128, <0.128, <0.128, <0.128               |
|                                                                                                                                                                                                                                                | 14                       | <0.128, <0.128, <0.128, <0.128               |

<sup>a</sup> Each value is the average of triplicate LSC analyses of the sample. **Bolded** values indicate samples in which all three analyses had residues >LOQ.

<sup>b</sup> The radioassay LOQ is 0.072 ppm in oysters and 0.128 ppm in shrimp.

### 3. Discussion

#### 3.1. Methods

Two replicate populations of both oysters (52 animals/tank) and grass shrimp (410 animals/tank) were dosed with [<sup>14</sup>C]imazapyr at a continuous concentration of ~0.25 mg a.i./L for 28 days, followed by a 14-day depuration period. In the oyster study, the actual average concentration of [<sup>14</sup>C]imazapyr in the saltwater over the dosing period was 0.244 mg a.i./L as determined by LSC and 0.254 mg a.i./L as determined by HPLC/UV. In the shrimp study, the actual average concentration of [<sup>14</sup>C]imazapyr in the saltwater over the dosing period was 0.268 mg a.i./L as determined by LSC and 0.243 mg a.i./L as determined by HPLC/UV.

The LSC methods used to determine radioactivity in saltwater and oyster and shrimp tissues were adequate. The reported LOQs for the LSC analysis of tissues were 0.072 ppm in oysters and 0.128 ppm in shrimp. Samples were stored frozen for less than 4-9 months. No additional storage stability data are required to support this shellfish metabolism study.

#### 3.2. Results

In both the oyster and shrimp studies, the concentration of [<sup>14</sup>C]imazapyr in the saltwater remain consistent throughout the dosing period at ~0.25 mg a.i./L. In addition, the close agreement between the LSC and HPLC/UV analyses indicates that [<sup>14</sup>C]imazapyr was stable in the tank water during the tests. Levels of [<sup>14</sup>C]imazapyr in the water during the depuration phase were <LOQ in both tests.

In the oyster study, concentrations of [<sup>14</sup>C]imazapyr were <LOQ in all control samples and in all treated samples collected during the depuration phase. Concentrations of radioactivity in oyster tissues during the uptake phase were low and variable, with the majority of samples having residues <LOQ (<0.072 ppm). Only seven oyster tissue samples had quantifiable <sup>14</sup>C-residues in all three subsamples. <sup>14</sup>C-Residues in oysters were <0.072-0.078 ppm on Day 7, 0.074-0.120 ppm on Day 14, <0.072-0.101 ppm on Day 21, and 0.073-0.105 ppm on Day 28.

In the shrimp study, concentrations of [<sup>14</sup>C]imazapyr were <LOQ (<0.128 ppm) in all control samples. Concentrations of radioactivity in shrimp tissues during the uptake phase were low and variable, with the majority of samples having residues <LOQ (<0.128 ppm). Only two shrimp samples had quantifiable <sup>14</sup>C-residues in all three subsamples. During the uptake phase, <sup>14</sup>C-residues in shrimp were <0.128 ppm on Days 0, 7, and 14, and were <0.128-0.147 ppm on Day 21 and <0.128-0.160 ppm on Day 28. During the depuration phase, <sup>14</sup>C-residues in shrimp were <0.128-0.137 ppm up to Day 3, ≤0.132 ppm by Day 7, and <0.128 ppm at the later sampling intervals.

Due to the low levels of radioactivity in the oyster and shrimp tissues, a BCF value and uptake and depuration rates were not calculated, and <sup>14</sup>C-residues in tissues were not further characterized. However, considering that all of the oyster or shrimp tissue samples had concentrations lower than in the saltwater, [<sup>14</sup>C]imazapyr does not appear to accumulate in shellfish.

### 4. Deficiencies

These two studies were conducted to generate data on the potential for bioaccumulation of [<sup>14</sup>C]imazapyr residues in oysters and shrimp. These studies are adequate for the stated purpose. However, due to the relatively high method LOQs reported for the oyster and shrimp tissues, together with the relatively low residue levels in these tissues and resulting lack of identification/characterization of residues, these studies do not provide insight into the metabolic profile of imazapyr in shellfish.

## 5. References

None

**Date:** 20-MAR-2003

**EPA Reviewer:** William H. Donovan, Ph.D., Chemist  
Reregistration Branch 3 (RRB3)  
Health Effects Division (HED) (7509C)

*William H. Donovan*

**Through:** David Soderberg, Chemist  
RRB3/HED (7509C)

*David Soderberg*

**Petition Number:** PP#F06166

**DP Barcode:** D275561.

**Citation:** 45119707 Borysewicz, R. (1999) Residues of CL 243997, CL 9140, and CL119060 in Aquatic Field Dissipation and Aquatic Non-Target Organisms for Arsenal Herbicide Applied to Freshwater Ponds: Lab Project Number: RES 99-059: RES 99-060: AR96FL01. Unpublished study prepared by American Cyanamid Company, Centre Analytical Labs Inc., Florida Pesticide Research, and Maxim Technologies, Inc. 681 p.

**Sponsor:** BASF (formerly American Cyanamid Company)

This document was prepared by Dynamac Corporation under contract to EPA. It has been reviewed and modified by HED to assure it conforms to HED policy.

### Executive Summary

At two locations in FL and MO during August-September 1996, a single application of imazapyr (2 lb ae/gal AS [aqueous solution]) was made to the banks (4-6 feet) and water's edge (4-6 feet) of a fresh water pond at a broadcast rate of 1.6 lb ae/A (Treatment I). A second pond at each site received a single application of imazapyr (2 lb ae/gal AS) to the banks (4-6 feet) and the entire pond surface at a broadcast rate of 1.6 lb ae/A (Treatment II). Prior to treatment, each pond was stocked with non-target organisms (bluegill, bass, tilapia, catfish, and crayfish), and five sampling stations were established in various areas of each pond. Following treatment, water samples were collected from each station per pond at various intervals from 0 to 42 days after treatment (DAT), and single composite samples of each non-target organism were collected from each pond at intervals from 0 to 42 DAT. Sediment samples were also collected from each sampling station in each pond at various intervals from 0 to ~180 DAT.

Fish and shellfish samples were stored frozen for up to 21 months, an interval which is supported by available storage stability data, and sediment and water samples were stored frozen for up to 27 months. Additional storage stability data are required to support this storage interval. Imazapyr residues were determined using the adequate CE/UV methods BASF M 3001 (water), M 3014 (sediment), and M 3066 (fish and shellfish). The

**Date:** 20-MAR-2003

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Reregistration Branch 3 (RRB3)  
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RRB3/HED (7509C)

**Petition Number:** PP#F06166

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## Executive Summary

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Fish and shellfish samples were stored frozen for up to 21 months, an interval which is supported by available storage stability data, and sediment and water samples were stored frozen for up to 27 months. Additional storage stability data are required to support this storage interval. Imazapyr residues were determined using the adequate CE/UV methods BASF M 3001 (water), M 3014 (sediment), and M 3066 (fish and shellfish). The

method limits of quantitation (LOQ) for imazapyr are 0.001 ppm in water and sediments and 0.05 ppm in fish and shellfish tissues. Residues of the degradate CL 9140 in water were determined using an adequate CE/UV method BASF M 3097, and residues of the degradate CL 119060 were determined using an adequate LC/MS/MS Method BASF M 2672. The LOQ for both degradates in water is 0.002 ppm.

For Treatment I, average imazapyr residues in water peaked at 0.028 ppm by 3 hours post-treatment in the FL test and declined to <0.001 ppm by 36 DAT, and average imazapyr residues in water peaked at 0.029 ppm by 1 hour post-treatment in the MO test and declined to 0.002 ppm by 42 DAT. The highest concentration observed for any one water sample was 0.067 ppm (1 hour) from the FL test and 0.081 ppm (1 hour) from the MO test.

For Treatment II, the average imazapyr residues in water peaked at 0.164 ppm by 12 hours post-treatment in the FL test and declined to <0.001 ppm by 27 DAT, and the average imazapyr residues in water peaked at 0.196 ppm by 1 hour post-treatment in the MO tests, and declined to 0.025 ppm by 42 DAT. The highest residues observed in water samples from Treatment II were 0.186 ppm (12 hours) from the FL test and 0.309 ppm (1 hour) from the MO test. For Treatment II, the registrant calculated the half-life of imazapyr in the pond water to be 3.9 days at FL test site and 15 days at the MO test site. The registrant attributed the increased half-life to higher levels of suspended sediments at the MO site, which reduced light penetration, and decreased the potential for photolytic degradation.

The degradates CL 9140 and CL 119060 were <0.002 ppm (<LOQ) in all water samples from Treatment I collected at both the FL and MO test sites. For Treatment II at the FL site, CL 9140 was detected at 0.002-0.005 ppm at 1-5 DAT and CL 119060 was detected at 0.002-0.006 ppm at 1-5 DAT. For Treatment II at the MO site, CL 9140 was detected in only one sample at 0.003 ppm at 14 DAT, and CL 119060 was not detected (<0.002 ppm) in any samples.

Residues of imazapyr in pond sediments were generally low in both FL tests. For the Treatment I in FL, average imazapyr residues in sediments were 0.003-0.005 ppm from 6 hours to 1 day post-treatment and were  $\leq$ 0.001 ppm at all subsequent intervals. For Treatment II in FL, average imazapyr residues in sediments declined from 0.011 ppm at 6 hours post-treatment to  $\leq$ 0.001 ppm by 22 DAT. Imazapyr residues in sediments from the MO test were more variable and showed little or no decline over time. For the Treatment I in MO, average imazapyr residues in sediments were initially the highest (0.034 ppm) of any treatment; however, average residues at all subsequent intervals (1-180 DAT) were 0.001-0.005 ppm. For Treatment II in MO, average imazapyr residues in sediments averaged 0.009-0.018 ppm at 0.25-180 DAT.

Residues of imazapyr were <0.05 ppm (<LOQ) in all samples of each non-target species from Treatment I at both test sites and from Treatment II at the MO test site. For Treatment II at the FL site, imazapyr residues were detected in bluegill (0.636 ppm), tilapia (0.233 ppm), catfish (0.068 ppm), and crayfish (0.059 ppm) from the 3 hour post-treatment interval. Residues were higher in the mid-level species (bluegill and tilapia), than in the bottom feeding species (catfish and crayfish). At all subsequent sampling intervals (1-42 DAT), imazapyr residues were <0.05 ppm in all species from this test.

The water, fish, and sediment residue data are satisfactory once adequate supporting storage stability data are provided for residues of imazapyr in water and sediments stored for up to 27 months. A storage stability study for residues of imazapyr in frozen water and sediments is ongoing. The currently available data indicate that imazapyr is stable in frozen ( $\leq -10$  C) sediments and pond water for up to 12 months and that the degradates CL 9140 and CL 119040 are stable in frozen water for up to 15 months.

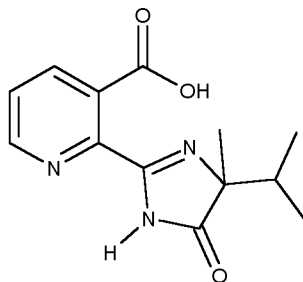
## GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

## 1. Materials and Methods

### 1.1. Test Substances

Common Name: Imazapyr  
IUPAC Name: 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid  
CAS Name: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid  
CAS Number: 81334-34-1  
Company Name: AC 243997 or CL 243997  
Other Synonyms: Arsenal Herbicide  
Formulation: 2 lb ae/gal AS, 22.6% free acid  
EPA Registration No.: 241-346



### 1.2. Trial Information

A total of four tests were conducted at sites in FL and MO (2 test/site) beginning in 1996. At each test site, imazapyr (2 lb ae/gal AS) was applied to two separate ponds. On one of the ponds, imazapyr was applied at a broadcast application rate of 1.6 lb ae/A to the pond banks (4-6 feet from edge) and to the water at the pond margin (4-6 feet from edge). On the other pond, imazapyr was applied at a broadcast application rate of 1.6 lb ae/A to the pond banks (4-6 feet from edge) and to the entire pond surface. Details of the applications are presented in Table 1.2.

#### 1.2.1. Test sites

Location: Oveido, Florida (Seminole County), EPA Region 3  
Pond size: 10,260-10,746 ft<sup>2</sup>, ~0.25 acres, with an average depth of ~2 feet.  
Water: pH 7.5-7.7; turbidity 0.37-2.52 NTU, dissolved oxygen 10 ppm  
Soil: Sand soil (97% sand, 1% silt, 2% clay ) with 2-3.3% organic matter, pH 6.9-7.76

Location: Osage Beach, MO (Miller County), EPA Region 5  
Pond size: 28,368-29700 ft<sup>2</sup>, ~0.7 acres, with an average depth of ~3 feet.

Water: pH 7.5-7.7; turbidity 0.62-0.70 NTU, dissolved oxygen 7-7.2 ppm  
Soil: Silt loam (24-30% sand, 51-63% silt, 13-19% clay ) with 0.9-1.8% organic matter, pH 5.5-6.6

1.2.2. Non-target Organisms. Prior to application, the following non-target organisms were placed in the separate cages in each pond.

Species: Tilapia (*Tilapia aurea*), FL test only  
Age/Size: 2-4 and 4-8 inches

Species: Bluegill (*Lepomis spp.*)  
Age/Size: 2 years old

Species: Catfish (*Ictalurus nebulosus* or *Ictalurus punctatus*)  
Age/Size: 6-8 inches; ~ 1 year old

Species: Bass (*Micropterus salmoides*), MO test only  
Age/Size: 1 year old

Species: Crayfish (*Procambarus alleni* or *Cambarus diogenes*)  
Age/Size: 60-180 days old

| Table 1.2. Field Trial Information. |            |                          |                         |                                      |                |        |                |
|-------------------------------------|------------|--------------------------|-------------------------|--------------------------------------|----------------|--------|----------------|
| Location (City, State)/ Year        | EPA Region | Formulation <sup>a</sup> | Application information |                                      |                |        |                |
|                                     |            |                          | Method                  | Area <sup>b</sup>                    | Rate (lb ai/A) | Number | Volume (gal/A) |
| Oviedo, FL 1996                     | 3          | 2 lb ae/gal AS           | Broadcast               | pond banks and to water at pond edge | 1.6            | 1      | 22             |
|                                     |            |                          | Broadcast               | pond banks and entire pond surface   |                |        | 21             |
| Osage Beach, MO 1996                | 5          | 2 lb ae/gal AS           | Broadcast               | pond banks and to water at pond edge | 1.6            | 1      | 20             |
|                                     |            |                          | Broadcast               | pond banks and entire pond surface   |                |        | 23             |

<sup>a</sup> Application included 0.25% of a non-ionic surfactant

<sup>b</sup> For treatment of pond banks and the water at the pond's edge, applications were made within 4-6 feet of the pond's edge.

### 1.3. Post-harvest Procedures

Five sampling stations were setup in each treated pond for sampling water and pond sediments. Water samples (1 or 4 L) were collected from each station at mid-depth at the target intervals of 0.04, 0.125, 0.25, 0.5, 1, 2, 3, 5, 7, 14, 21, 28, and 42 days after treatment (DAT). Following any rainfall event of  $\geq 0.25$  inches, additional water samples were collected at the two sampling stations nearest the pond's edge.

Two or three pond sediment core samples (~2 or 0-6 inch depth) were collected at each sampling station at target intervals of 0.25, 0.5, 1, 3, 7, 14, 21, 28, 42, 90, and 180 DAT. The cores from each station were then composited into one sample.

A single composite sample (1 lb) of each non-target organism was collected at target intervals of 0 (pretreatment), 0.125, 1, 3, 7, 14, 21, 28, and 42 DAT from each pond. Animals were collected by hand with nets from their respective cages.

All samples were placed in freezer storage (<-6 C) soon after collection and stored a maximum of 28 days until shipment by ACDS freezer truck to Cyanamid Agricultural Research Center (CARC), Princeton, NJ. Fish fillets and crayfish tail tissues were homogenized and all samples were frozen ( $\leq -10$  C) at CARC until shipment on dry ice by Fed Ex or ACDS to Maxim Technologies, Inc. (Middleport, NY) for analysis. Water and sediment samples were shipped frozen to Centre Analytical Labs (State College, PA) for analysis. Maximum frozen storage intervals were 21 months for tissues and sediment and 27 months for pond water; minimum frozen storage intervals were not reported.

#### 1.4. Analytical Methods

Fish and crayfish tissue samples were analyzed for residues of imazapyr using the CE/UV method, BASF Method M 3066, and the analyses were conducted by Maxim Technologies (Middleport, NY). For this method, Residues are extracted with acidic acetone:water (1:3, v/v), centrifuged, filtered, and partitioned into methylene chloride. Residues are then purified using SPX and C<sub>18</sub> solid-phase extraction (SPE) cartridges, and the final fraction is analyzed by CE/UV (240 nm). The reported LOQ for imazapyr in fish and shellfish meat is 0.05 ppm, and the LOD is approximately 0.005 ppm.

Water samples were analyzed by Centre Analytical Labs (State College, PA) for residues of imazapyr using the CE/UV method, BASF Method M 3001. For this method, residues of imazapyr are extracted from acidified water and purified using SPX and C<sub>18</sub> SPE cartridges. Residues are then determined by CE/UV (240 nm). The reported LOQ is 0.001 ppm; the LOD is 0.0002 ppm.

Composited water samples were also analyzed for residues of the degradates, CL 9140 and CL 119060, by Centre Analytical Labs (State College, PA). The CE/UV method, BASF Method M 3097, was used for analysis of CL 9140, and the LC/MS/MS method, BASF Method M 2672, for the analysis of CL 119060. For both methods, residues in acidified water are purified using SPX and C<sub>18</sub> SPE cartridges. Residues of CL 9140 in the final fraction are determined by CE/UV (240 nm), and residues of CL 119060 are determined by LC/MS/MS using the 134 m/z daughter ion. The methods LOQ is 0.002 ppm for both analytes, and the LOD is 0.0004 ppm.

Sediment samples were analyzed by Centre Analytical Labs (State College, PA) for residues of imazapyr using the CE/UV method, BASF Method M 3014. For this method, residues are extracted with 0.5N NaOH, acidified, and partitioned into methylene chloride. Residues are then cleaned up using SPX and C<sub>18</sub> SPE cartridges, and quantified by CE/UV (240 nm). The method LOQ is 0.001 ppm for imazapyr in soil, and the LOD is approximately 0.0001 ppm.

## 2. Results

| Table 2.1. Summary of Concurrent Analytical Method Validation after Fortification with Imazapyr, CL 9140, or CL 119060. |                           |                   |                         |                        |
|-------------------------------------------------------------------------------------------------------------------------|---------------------------|-------------------|-------------------------|------------------------|
| Matrix                                                                                                                  | Fortification Level (ppm) | Number of samples | Range of Recoveries (%) | Mean Recovery $\pm$ SD |
| <b>FLORIDA TEST</b>                                                                                                     |                           |                   |                         |                        |
| <b>Imazapyr (CL 243997)</b>                                                                                             |                           |                   |                         |                        |
| Non-Target Organisms <sup>a</sup>                                                                                       | 0.05-1.00                 | 18                | 72-90                   | 81 $\pm$ 6             |
| Sediment                                                                                                                | 0.001-0.500               | 38                | 60-119 (2) <sup>b</sup> | 84 $\pm$ 11            |
| Pond Water                                                                                                              | 0.001-0.450               | 67                | 45-113 (2)              | 87 $\pm$ 12            |
| <b>CL 119060</b>                                                                                                        |                           |                   |                         |                        |
| Pond Water                                                                                                              | 0.002-0.020               | 10                | 64-85 (3)               | 76 $\pm$ 8             |
| <b>CL 9140</b>                                                                                                          |                           |                   |                         |                        |
| Pond Water                                                                                                              | 0.002-0.020               | 10                | 59-109 (3)              | 85 $\pm$ 18            |
| <b>MISSOURI TEST</b>                                                                                                    |                           |                   |                         |                        |
| <b>Imazapyr (CL 243997)</b>                                                                                             |                           |                   |                         |                        |
| Non-Target Organisms <sup>a</sup>                                                                                       | 0.050-0.500               | 18                | 70-90                   | 83 $\pm$ 6             |
| Sediment                                                                                                                | 0.001-0.500               | 35                | 21-116 (2)              | 86 $\pm$ 17            |
| Pond Water                                                                                                              | 0.001-0.450               | 55                | 68-110 (1)              | 88 $\pm$ 8             |
| <b>CL 119060</b>                                                                                                        |                           |                   |                         |                        |
| Pond Water                                                                                                              | 0.002-0.02                | 38                | 56-99 (6)               | 78 $\pm$ 9             |
| <b>CL 9140</b>                                                                                                          |                           |                   |                         |                        |
| Pond Water                                                                                                              | 0.002-0.02                | 34                | 52-114 (1)              | 95 $\pm$ 11            |

<sup>a</sup> Non-target organisms included bluegill, catfish, and crayfish and tilapia or bass.

<sup>b</sup> Number of recoveries outside the 70-120% range are in parentheses.

| Table 2.2.1. Residues of Imazapyr in <b>Pond Water</b> Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II). |                  |                                                               |               |                                        |               |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|---------------------------------------------------------------|---------------|----------------------------------------|---------------|
| Test Location                                                                                                                                                                                                                                    | DAT <sup>a</sup> | Treatment I                                                   |               | Treatment II                           |               |
|                                                                                                                                                                                                                                                  |                  | Imazapyr Residues (ppm)                                       | Average ± SD  | Imazapyr Residues (ppm)                | Average ± SD  |
| Oveido, FL<br>1996                                                                                                                                                                                                                               | 0.04 (1 hr)      | 0.002, 0.011, <0.001 <sup>b</sup> , 0.045, 0.067 <sup>c</sup> | 0.025 ± 0.029 | 0.106, 0.082, 0.085, 0.097, 0.095      | 0.093 ± 0.010 |
|                                                                                                                                                                                                                                                  | 0.125 (3 hr)     | 0.018, 0.016, 0.012, 0.040, 0.052                             | 0.028 ± 0.017 | 0.076, 0.066, 0.073, 0.079, 0.087      | 0.076 ± 0.008 |
|                                                                                                                                                                                                                                                  | 0.25 (6 hr)      | 0.013, 0.008, 0.023, 0.006, 0.014                             | 0.013 ± 0.007 | 0.070, 0.090, 0.064, 0.072, 0.078      | 0.075 ± 0.010 |
|                                                                                                                                                                                                                                                  | 0.5 (12 hr)      | 0.009, 0.007, 0.009, 0.010, 0.008                             | 0.009 ± 0.001 | 0.178, 0.186, 0.178, 0.158, 0.119      | 0.164 ± 0.027 |
|                                                                                                                                                                                                                                                  | 1                | 0.008, 0.012, 0.009, 0.009, 0.009                             | 0.009 ± 0.002 | 0.061, 0.072, 0.062, 0.059, 0.076      | 0.066 ± 0.008 |
|                                                                                                                                                                                                                                                  | 2                | 0.007, 0.009, 0.008, 0.008, 0.008                             | 0.008 ± 0.001 | 0.053, 0.053, 0.059, 0.059, 0.069      | 0.059 ± 0.007 |
|                                                                                                                                                                                                                                                  | 3                | 0.019, 0.015, 0.018, 0.012, 0.015                             | 0.016 ± 0.003 | 0.069, 0.067, 0.085, 0.070, 0.055      | 0.069 ± 0.011 |
|                                                                                                                                                                                                                                                  | 3 <sup>d</sup>   | 0.009, 0.010                                                  | 0.010         | 0.049, 0.056                           | 0.053         |
|                                                                                                                                                                                                                                                  | 5                | 0.009, 0.015, 0.007, 0.005, 0.005                             | 0.008 ± 0.004 | 0.048, 0.041, 0.029, 0.026, 0.045      | 0.038 ± 0.010 |
|                                                                                                                                                                                                                                                  | 7                | 0.005, 0.005, 0.005, 0.003, <0.001                            | 0.004 ± 0.002 | 0.022, 0.035, 0.028, 0.037, 0.030      | 0.030 ± 0.006 |
|                                                                                                                                                                                                                                                  | 14               | 0.003, 0.004, 0.005, 0.003, 0.003                             | 0.004 ± 0.001 | 0.004, 0.003, 0.005, 0.006, 0.005      | 0.005 ± 0.001 |
|                                                                                                                                                                                                                                                  | 22               | 0.001, 0.003, 0.003, 0.003, 0.002                             | 0.002 ± 0.001 | 0.003, 0.002, 0.003, 0.003, 0.002      | 0.003 ± 0.001 |
|                                                                                                                                                                                                                                                  | 27 <sup>d</sup>  | 0.001, 0.001                                                  | 0.001         | <0.001, <0.001                         | <0.001        |
|                                                                                                                                                                                                                                                  | 29               | 0.001, <0.001, 0.002, 0.001, 0.001                            | 0.001         | <0.001, 0.001, <0.001, 0.001, <0.001   | <0.001        |
|                                                                                                                                                                                                                                                  | 30 <sup>d</sup>  | 0.001, 0.002                                                  | 0.002         | <0.001, <0.001                         | <0.001        |
|                                                                                                                                                                                                                                                  | 36 <sup>d</sup>  | <0.001, <0.001                                                | <0.001        | <0.001, <0.001                         | <0.001        |
|                                                                                                                                                                                                                                                  | 42               | <0.001, <0.001, <0.001, <0.001, <0.001                        | <0.001        | <0.001, <0.001, <0.001, <0.001, <0.001 | <0.001        |

| Table 2.2.1. Residues of Imazapyr in <b>Pond Water</b> Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II). |                  |                                                    |               |                                                      |               |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|----------------------------------------------------|---------------|------------------------------------------------------|---------------|
| Test Location                                                                                                                                                                                                                                    | DAT <sup>a</sup> | Treatment I                                        |               | Treatment II                                         |               |
|                                                                                                                                                                                                                                                  |                  | Imazapyr Residues (ppm)                            | Average ± SD  | Imazapyr Residues (ppm)                              | Average ± SD  |
| Osage Beach, MO 1996                                                                                                                                                                                                                             | 0.04 (1 hr)      | 0.003, 0.007, 0.007, 0.081, 0.048                  | 0.029 ± 0.034 | 0.309, 0.101, 0.218, 0.258, <i>0.095<sup>b</sup></i> | 0.196 ± 0.095 |
|                                                                                                                                                                                                                                                  | 0.125 (3 hr)     | 0.004, 0.005, 0.005, 0.012, 0.016                  | 0.008 ± 0.005 | 0.120, 0.127, 0.151, 0.198, 0.131                    | 0.145 ± 0.032 |
|                                                                                                                                                                                                                                                  | 0.25 (6 hr)      | 0.007, 0.008, 0.008, <i>0.026</i> , 0.008          | 0.011 ± 0.008 | <i>0.110</i> , 0.121, 0.124, 0.189, 0.158            | 0.140 ± 0.033 |
|                                                                                                                                                                                                                                                  | 1                | 0.014, 0.009, 0.011, 0.013, 0.024,                 | 0.014 ± 0.006 | 0.201, 0.151, 0.181, 0.123, 0.166                    | 0.164 ± 0.030 |
|                                                                                                                                                                                                                                                  | 2                | 0.023, 0.014, 0.019, 0.021, 0.021,                 | 0.020 ± 0.003 | 0.190, <i>0.138</i> , 0.169, 0.110, 0.133            | 0.148 ± 0.032 |
|                                                                                                                                                                                                                                                  | 3                | 0.021, 0.015, 0.018, 0.020, 0.012,                 | 0.017 ± 0.004 | 0.192, 0.136, 0.189, 0.085, 0.088                    | 0.138 ± 0.052 |
|                                                                                                                                                                                                                                                  | 5                | 0.014, 0.006, 0.010, 0.006, 0.012,                 | 0.010 ± 0.004 | 0.085, 0.102, 0.127, 0.079, 0.122                    | 0.103 ± 0.021 |
|                                                                                                                                                                                                                                                  | 7                | 0.008, 0.009, 0.007, 0.010, 0.014,                 | 0.010 ± 0.003 | 0.120, 0.115, 0.110, 0.120, 0.121                    | 0.117 ± 0.005 |
|                                                                                                                                                                                                                                                  | 14               | 0.009, 0.007, 0.005, 0.007, 0.006,                 | 0.007 ± 0.001 | 0.064, 0.088, 0.049, 0.069, 0.075                    | 0.069 ± 0.014 |
|                                                                                                                                                                                                                                                  | 21               | 0.003, 0.003, 0.002, 0.003, 0.002,                 | 0.003 ± 0.001 | 0.052, 0.060, 0.057, 0.032, 0.037                    | 0.048 ± 0.012 |
|                                                                                                                                                                                                                                                  | 26 <sup>d</sup>  | 0.001, 0.005                                       | 0.003         | 0.038, 0.023                                         | 0.031         |
|                                                                                                                                                                                                                                                  | 27 <sup>d</sup>  | 0.004, 0.006                                       | 0.005         | 0.037, 0.029                                         | 0.033         |
|                                                                                                                                                                                                                                                  | 28               | 0.003, 0.004, 0.004, 0.005, 0.005,                 | 0.004 ± 0.001 | 0.045, 0.040, 0.033, 0.057, 0.049                    | 0.045 ± 0.009 |
|                                                                                                                                                                                                                                                  | 41 <sup>d</sup>  | 0.003, 0.004                                       | 0.004         | 0.028, 0.026                                         | 0.026         |
|                                                                                                                                                                                                                                                  | 42               | 0.003, 0.003, 0.001, <0.001 <sup>c</sup> , <0.001, | 0.002 ± 0.001 | 0.024, 0.041, 0.018, 0.023, 0.019                    | 0.025 ± 0.009 |

<sup>a</sup> Days after treatment.

<sup>b</sup> The method LOQ is 0.001 ppm for imazapyr in water.

<sup>c</sup> Residue values in *italics* are the average of multiple analyses.

<sup>d</sup> Days on which water samples were collected at the two sampling stations near the pond's edge, following a rainfall of ≥0.25 inches.

| Table 2.2.2. Residues of the degradates CL 9140 and CL 119060 in <b>Pond Water</b> Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II). |                  |                                              |                                |                        |                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|----------------------------------------------|--------------------------------|------------------------|--------------------------|
| Test Location                                                                                                                                                                                                                                                                | DAT <sup>a</sup> | Treatment I                                  |                                | Treatment II           |                          |
|                                                                                                                                                                                                                                                                              |                  | CL 9140 Resiudes (ppm)                       | CL 119060 Residues (ppm)       | CL 9140 Resiudes (ppm) | CL 119060 Residues (ppm) |
| Oviedo, FL 1996                                                                                                                                                                                                                                                              | 0.04 (1 hr)      | <0.002 <sup>b</sup> , <0.002, <0.002, <0.002 | <0.002, <0.002, <0.002, <0.002 | <0.002, <0.002         | <0.002, <0.002           |
|                                                                                                                                                                                                                                                                              | 0.125 (3 hr)     | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | <0.002 <sup>c</sup>    | <0.002 <sup>c</sup>      |
|                                                                                                                                                                                                                                                                              | 1                | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | 0.003 <sup>cd</sup>    | 0.002 <sup>c</sup>       |
|                                                                                                                                                                                                                                                                              | 3 <sup>e</sup>   | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | 0.005 <sup>c</sup>     | 0.003 <sup>c</sup>       |
|                                                                                                                                                                                                                                                                              | 5                | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | 0.002 <sup>b</sup>     | 0.006 <sup>c</sup>       |
|                                                                                                                                                                                                                                                                              | 7                | NA <sup>f</sup>                              | NA                             | <0.002, <0.002         | <0.002, <0.002           |
|                                                                                                                                                                                                                                                                              | 14               | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | <0.002 <sup>c</sup>    | <0.002 <sup>c</sup>      |
|                                                                                                                                                                                                                                                                              | 22               | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | <0.002 <sup>c</sup>    | <0.002 <sup>c</sup>      |
|                                                                                                                                                                                                                                                                              | 27 <sup>e</sup>  | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | <0.002 <sup>c</sup>    | <0.002 <sup>c</sup>      |
|                                                                                                                                                                                                                                                                              | 29               | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | <0.002, <0.002         | <0.002, <0.002           |
|                                                                                                                                                                                                                                                                              | 30 <sup>e</sup>  | <0.002 <sup>c</sup>                          | <0.002 <sup>c</sup>            | <0.002 <sup>c</sup>    | <0.002 <sup>c</sup>      |

| Table 2.2.2. Residues of the degradates CL 9140 and CL 119060 in <b>Pond Water</b> Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II). |                  |                        |                          |                            |                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|------------------------|--------------------------|----------------------------|--------------------------|
| Test Location                                                                                                                                                                                                                                                                | DAT <sup>a</sup> | Treatment I            |                          | Treatment II               |                          |
|                                                                                                                                                                                                                                                                              |                  | CL 9140 Resiudes (ppm) | CL 119060 Residues (ppm) | CL 9140 Resiudes (ppm)     | CL 119060 Residues (ppm) |
| Osage Beach, MO 1996                                                                                                                                                                                                                                                         | 0.04 (1 hr)      | 5 samples, all <0.002  | 5 samples, all <0.002    | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 0.125 (3 hr)     | 5 samples, all <0.002  | 5 samples, all <0.002    | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 0.25 (6 hr)      | 5 samples, all <0.002  | 5 samples, all <0.002    | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 1                | 5 samples, all <0.002  | 5 samples, all <0.002    | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 2                | 5 samples, all <0.002  | 5 samples, all <0.002    | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 3                | 5 samples, all <0.002  | 5 samples, all <0.002    | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 5                | 5 samples, all <0.002  | NA                       | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 7                | <0.002 <sup>b</sup>    | <0.002 <sup>b</sup>      | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 14               | <0.002 <sup>b</sup>    | NA                       | 4 samples at <0.002, 0.003 | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 21               | <0.002 <sup>b</sup>    | <0.002 <sup>b</sup>      | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 26 <sup>e</sup>  | <0.002 <sup>b</sup>    | <0.002 <sup>b</sup>      | <0.002, <0.002             | <0.002, <0.002           |
|                                                                                                                                                                                                                                                                              | 27 <sup>e</sup>  | NA                     | NA                       | <0.002, <0.002             | <0.002, <0.002           |
|                                                                                                                                                                                                                                                                              | 28               | NA                     | NA                       | 5 samples, all <0.002      | 5 samples, all <0.002    |
|                                                                                                                                                                                                                                                                              | 41 <sup>e</sup>  | <0.002 <sup>b</sup>    | <0.002 <sup>b</sup>      | <0.002, <0.002             | <0.002, <0.002           |
|                                                                                                                                                                                                                                                                              | 42               | <0.002 <sup>b</sup>    | <0.002 <sup>b</sup>      | 4 samples, all <0.002      | 3 samples, all <0.002    |

<sup>a</sup> Days after treatment.

<sup>b</sup> The method LOQ is 0.002 ppm for each analyte in water.

<sup>c</sup> Indicates composited sample.

<sup>d</sup> Residue values in *italics* are the average of multiple analyses.

<sup>e</sup> Days on which water samples were collected at the two sampling stations near the pond's edge, following a rainfall of ≥0.25 inches.

<sup>f</sup> NA = sample not analyzed.

| Table 2.3. Residues of Imazapyr in <b>Pond Sediments</b> Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II). |                  |                                           |               |                                          |                |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|-------------------------------------------|---------------|------------------------------------------|----------------|
| Test Location                                                                                                                                                                                                                                      | DAT <sup>a</sup> | Treatment I                               |               | Treatment II                             |                |
|                                                                                                                                                                                                                                                    |                  | Imazapyr Residues (ppm)                   | Average ± S.D | Imazapyr Residues (ppm)                  | Average ± S.D. |
| Oveido, FL<br>1996                                                                                                                                                                                                                                 | 0.25 (6 hr)      | 0.002, 0.002, 0.002, 0.005, 0.003         | 0.003 ± 0.001 | 0.008, 0.013, 0.007, 0.015, 0.014        | 0.011 ± 0.004  |
|                                                                                                                                                                                                                                                    | 0.5 (12 hr)      | 0.002, 0.002, 0.002, 0.002, 0.006         | 0.003 ± 0.002 | 0.011, 0.007, 0.008, 0.005, 0.006        | 0.007 ± 0.002  |
|                                                                                                                                                                                                                                                    | 1                | 0.003, 0.002, 0.002, 0.008, 0.007         | 0.005 ± 0.003 | 0.009, 0.012, 0.013, 0.001, <0.001       | 0.007 ± 0.007  |
|                                                                                                                                                                                                                                                    | 3                | 0.002, <0.001, <0.001, 0.001, <0.001      | 0.001         | 0.013, 0.005, 0.013, <0.001, <0.001      | 0.007 ± 0.006  |
|                                                                                                                                                                                                                                                    | 7                | <0.001, 0.002, 0.001, <0.001, <0.001      | 0.001         | 0.009, 0.005, 0.005, 0.002, 0.002        | 0.005 ± 0.003  |
|                                                                                                                                                                                                                                                    | 14               | <0.001, <0.001, <0.001, <0.001, <0.001    | <0.001        | 0.004, 0.001, 0.002, 0.002, 0.002        | 0.002 ± 0.001  |
|                                                                                                                                                                                                                                                    | 22               | <0.001, <0.001, <0.001, <0.001, <0.001    | <0.001        | <0.001, 0.002, 0.002, <0.001, <0.001     | 0.001 ± 0.001  |
|                                                                                                                                                                                                                                                    | 29               | <0.001, <0.001, <0.001, <0.001, <0.001    | <0.001        | 0.001, 0.001, 0.003, <0.001, <0.001      | 0.001 ± 0.001  |
|                                                                                                                                                                                                                                                    | 42               | <0.001, <0.001, <0.001, <0.001, <0.001    | <0.001        | 0.002, <0.001, 0.002 <0.001, <0.001      | 0.001 ± 0.001  |
|                                                                                                                                                                                                                                                    | 91               | <0.001, <0.001, <0.001, <0.001, <0.001    | <0.001        | <0.001, <0.001, <0.001, <0.001, <0.001   | <0.001         |
|                                                                                                                                                                                                                                                    | 178              | <0.001, <0.001, <0.001, <0.001, <0.001    | <0.001        | <0.001, <0.001, <0.001, <0.001, <0.001   | <0.001         |
| Osage<br>Beach, MO<br>1996                                                                                                                                                                                                                         | 0.25 (6 hr)      | <i>0.005, 0.077, 0.006, 0.037, 0.045,</i> | 0.034 ± 0.030 | 0.009, 0.012, 0.011, 0.013, 0.009        | 0.011 ± 0.002  |
|                                                                                                                                                                                                                                                    | 1                | <0.001, 0.001, 0.001, 0.008, 0.002,       | 0.003 ± 0.003 | 0.009, 0.008, 0.011, 0.013, 0.014        | 0.011 ± 0.003  |
|                                                                                                                                                                                                                                                    | 3                | <0.001, 0.001, <0.001, 0.003, 0.002,      | 0.002 ± 0.001 | 0.005, 0.005, 0.005, 0.015, <i>0.029</i> | 0.012 ± 0.011  |
|                                                                                                                                                                                                                                                    | 7                | 0.002, 0.002, <0.001, 0.003, 0.003,       | 0.002 ± 0.001 | 0.006, 0.005, 0.010, 0.019, 0.025        | 0.013 ± 0.009  |
|                                                                                                                                                                                                                                                    | 14               | 0.002, 0.001, 0.002, 0.003, 0.003,        | 0.002 ± 0.001 | 0.006, 0.006, 0.014, 0.021, 0.031        | 0.016 ± 0.011  |
|                                                                                                                                                                                                                                                    | 21               | 0.002, 0.002, 0.002, 0.004, 0.003,        | 0.003 ± 0.001 | 0.009, 0.031, 0.009, 0.030, <i>0.008</i> | 0.017 ± 0.012  |
|                                                                                                                                                                                                                                                    | 28               | 0.002, 0.002, 0.001, 0.003, 0.003,        | 0.002 ± 0.001 | 0.006, 0.009, 0.015, 0.019, <i>0.040</i> | 0.018 ± 0.013  |
|                                                                                                                                                                                                                                                    | 42               | 0.001, 0.001, 0.001, 0.003, 0.009,        | 0.003 ± 0.003 | 0.004, 0.009, 0.013, 0.007, 0.016        | 0.010 ± 0.005  |
|                                                                                                                                                                                                                                                    | 100              | <0.001, 0.001, <0.001, 0.002, 0.002,      | 0.001 ± 0.001 | 0.008, 0.007, 0.009, 0.011, 0.012        | 0.009 ± 0.002  |
|                                                                                                                                                                                                                                                    | 180              | 0.002, 0.002, 0.002, 0.004, <i>0.013,</i> | 0.005 ± 0.005 | 0.013, 0.008, 0.012, 0.019, 0.020        | 0.014 ± 0.005  |

<sup>a</sup> Days after treatment.

<sup>b</sup> The LOQ is 0.001 ppm for imazapyr in sediments.

<sup>c</sup> Residue values in *italics* are the average of multiple analyses.

| Table 2.4. Residues of Imazapyr in Non-target Organisms Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II). |                             |                                      |         |                 |          |                           |              |         |          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|--------------------------------------|---------|-----------------|----------|---------------------------|--------------|---------|----------|
| Test location/<br>year                                                                                                                                                                                                                            | Sampling Interval<br>(days) | Imazapyr Residues (ppm) <sup>a</sup> |         |                 |          |                           |              |         |          |
|                                                                                                                                                                                                                                                   |                             | Treatment I                          |         |                 |          | Treatment II              |              |         |          |
|                                                                                                                                                                                                                                                   |                             | Bluegill                             | Tilapia | Catfish         | Crayfish | Bluegill                  | Tilapia      | Catfish | Crayfish |
| Oveida, FL<br>1996                                                                                                                                                                                                                                | 0<br>(pretreatment)         | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 0.125 (3 hr)                | <0.05                                | <0.05   | <0.05           | <0.05    | <i>0.636</i> <sup>b</sup> | <i>0.233</i> | 0.068   | 0.059    |
|                                                                                                                                                                                                                                                   | 1                           | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 3                           | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 7                           | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 14                          | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 22                          | <0.05                                | <0.05   | NS <sup>c</sup> | NS       | <0.05                     | <0.05        | <0.05   | NS       |
|                                                                                                                                                                                                                                                   | 29                          | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 42                          | <0.05                                | <0.05   | <0.05           | NS       | <0.05                     | <0.05        | <0.05   | NS       |
| Osage<br>Beach, MO<br>1996                                                                                                                                                                                                                        | 0<br>(pretreatment)         | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 0.125 (3 hr)                | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 1                           | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 3                           | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 7                           | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 14                          | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 22                          | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 29                          | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |
|                                                                                                                                                                                                                                                   | 42                          | <0.05                                | <0.05   | <0.05           | <0.05    | <0.05                     | <0.05        | <0.05   | <0.05    |

<sup>a</sup> The LOQ for imazapyr in fish and shellfish is 0.05 ppm.

<sup>b</sup> Residue values in *italics* are the average of multiple analyses.

<sup>c</sup> NS = not sampled, due to insufficient number of animals.

| Table 2.5. Summary of Imazapyr Residues in Pond Water, Sediments, and Non-target Organisms Following a Single Application of Imazapyr (2 lb ae/gal AS) at 1.6 lb ae/A to either the Pond Banks and Margins (Treatment I) or to the Pond Bank and Entire Pond Surface (Treatment II) in FL and MO Studies. |                                      |         |                              |              |         |                             |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|---------|------------------------------|--------------|---------|-----------------------------|
| Days after Treatment                                                                                                                                                                                                                                                                                      | Imazapyr Residues (ppm) <sup>a</sup> |         |                              |              |         |                             |
|                                                                                                                                                                                                                                                                                                           | Treatment I                          |         |                              | Treatment II |         |                             |
|                                                                                                                                                                                                                                                                                                           | Minimum                              | Maximum | Ave. $\pm$ S.D. <sup>b</sup> | Minimum      | Maximum | Ave $\pm$ S.D. <sup>b</sup> |
| <b>Residues in Pond Water</b>                                                                                                                                                                                                                                                                             |                                      |         |                              |              |         |                             |
| 0.04 (1 hr)                                                                                                                                                                                                                                                                                               | <0.001                               | 0.081   | 0.027 $\pm$ 0.030            | 0.082        | 0.309   | 0.145 $\pm$ 0.084           |
| 0.125 (3 hr)                                                                                                                                                                                                                                                                                              | 0.004                                | 0.052   | 0.018 $\pm$ 0.016            | 0.066        | 0.198   | 0.111 $\pm$ 0.042           |
| 0.25 (6 hr)                                                                                                                                                                                                                                                                                               | 0.006                                | 0.028   | 0.012 $\pm$ 0.007            | 0.064        | 0.189   | 0.108 $\pm$ 0.041           |
| 0.5 (12 hr)                                                                                                                                                                                                                                                                                               | 0.007                                | 0.010   | 0.009 $\pm$ 0.001            | 0.119        | 0.186   | 0.164 $\pm$ 0.027           |
| 1                                                                                                                                                                                                                                                                                                         | 0.008                                | 0.024   | 0.012 $\pm$ 0.005            | 0.059        | 0.201   | 0.115 $\pm$ 0.056           |
| 2                                                                                                                                                                                                                                                                                                         | 0.007                                | 0.023   | 0.014 $\pm$ 0.007            | 0.053        | 0.190   | 0.103 $\pm$ 0.052           |
| 3                                                                                                                                                                                                                                                                                                         | 0.012                                | 0.021   | 0.017 $\pm$ 0.003            | 0.055        | 0.192   | 0.104 $\pm$ 0.051           |
| 5                                                                                                                                                                                                                                                                                                         | 0.005                                | 0.015   | 0.009 $\pm$ 0.004            | 0.026        | 0.127   | 0.070 $\pm$ 0.038           |
| 7                                                                                                                                                                                                                                                                                                         | <0.001                               | 0.014   | 0.007 $\pm$ 0.004            | 0.022        | 0.121   | 0.074 $\pm$ 0.046           |
| 14                                                                                                                                                                                                                                                                                                        | 0.003                                | 0.009   | 0.005 $\pm$ 0.002            | 0.003        | 0.088   | 0.037 $\pm$ 0.035           |
| 21/22                                                                                                                                                                                                                                                                                                     | <0.001                               | 0.003   | 0.003 $\pm$ 0.001            | 0.002        | 0.060   | 0.025 $\pm$ 0.025           |
| 28/29                                                                                                                                                                                                                                                                                                     | <0.001                               | 0.005   | 0.003 $\pm$ 0.002            | <0.001       | 0.057   | 0.023 $\pm$ 0.024           |
| 42                                                                                                                                                                                                                                                                                                        | <0.001                               | 0.003   | 0.001 $\pm$ 0.001            | <0.001       | 0.041   | 0.013 $\pm$ 0.014           |
| <b>Residues in Pond Sediment</b>                                                                                                                                                                                                                                                                          |                                      |         |                              |              |         |                             |
| 0.25                                                                                                                                                                                                                                                                                                      | 0.002                                | 0.077   | 0.018 $\pm$ 0.026            | 0.007        | 0.015   | 0.011 $\pm$ 0.003           |
| 0.5                                                                                                                                                                                                                                                                                                       | 0.002                                | 0.006   | 0.003 $\pm$ 0.002            | 0.005        | 0.011   | 0.007 $\pm$ 0.002           |
| 1                                                                                                                                                                                                                                                                                                         | <0.001                               | 0.008   | 0.004 $\pm$ 0.003            | <0.001       | 0.014   | 0.009 $\pm$ 0.005           |
| 3                                                                                                                                                                                                                                                                                                         | <0.001                               | 0.003   | 0.001 $\pm$ 0.001            | <0.001       | 0.029   | 0.009 $\pm$ 0.009           |
| 7                                                                                                                                                                                                                                                                                                         | <0.001                               | 0.003   | 0.002 $\pm$ 0.001            | 0.002        | 0.025   | 0.009 $\pm$ 0.008           |
| 14                                                                                                                                                                                                                                                                                                        | <0.001                               | 0.003   | 0.002 $\pm$ 0.001            | 0.001        | 0.031   | 0.009 $\pm$ 0.010           |
| 21/22                                                                                                                                                                                                                                                                                                     | <0.001                               | 0.004   | 0.002 $\pm$ 0.001            | <0.001       | 0.031   | 0.009 $\pm$ 0.012           |
| 28/29                                                                                                                                                                                                                                                                                                     | <0.001                               | 0.003   | 0.002 $\pm$ 0.001            | <0.001       | 0.040   | 0.010 $\pm$ 0.012           |
| 42                                                                                                                                                                                                                                                                                                        | <0.001                               | 0.009   | 0.002 $\pm$ 0.003            | <0.001       | 0.016   | 0.006 $\pm$ 0.005           |
| 91/100                                                                                                                                                                                                                                                                                                    | <0.001                               | 0.002   | 0.001                        | <0.001       | 0.012   | 0.005 $\pm$ 0.005           |
| 178/180                                                                                                                                                                                                                                                                                                   | <0.001                               | 0.013   | 0.003 $\pm$ 0.004            | <0.001       | 0.020   | 0.008 $\pm$ 0.008           |
| <b>Residues in Non-Target Organisms <sup>d</sup></b>                                                                                                                                                                                                                                                      |                                      |         |                              |              |         |                             |
| 0                                                                                                                                                                                                                                                                                                         | <0.050                               | <0.050  | <0.050                       | 0.050        | <0.050  | <0.05                       |
| 0.125 <sup>e</sup>                                                                                                                                                                                                                                                                                        | <0.050                               | <0.050  | <0.050                       | <0.050       | 0.636   | 0.150 $\pm$ 0.21            |
| 1-42                                                                                                                                                                                                                                                                                                      | <0.050                               | <0.050  | <0.050                       | <0.050       | <0.050  | <0.050                      |

<sup>a</sup> The LOQ for imazapyr is 0.001 ppm in water and sediments and 0.050 ppm in fish and shellfish tissues.

<sup>b</sup> For water and sediments, N = 10 samples for each matrix, except for at 0.5 days (N = 5). For non-target organisms, a single sample was generally analyzed of each animal at each interval.

<sup>c</sup> The LOQs are 0.001 ppm in water and sediment and 0.05 ppm in non-target organisms.

<sup>d</sup> Non-target organisms included bluegill, bass (MO test only), tilapia (FL test only), catfish, and crayfish.

<sup>e</sup> Residues of imazapyr were only detected at the 0.2 day interval in bluegill (0.636 ppm), tilapia (0.233 ppm), catfish (0.068 ppm), and crayfish (0.059 ppm) from the FL test.

### 3. Discussion

#### 3.1. Methods

The methods used to treat the ponds at the two sites were adequate, and an adequate number of water and non-target organisms samples were collected.

The CE/UV Method M 3001 is adequate for collecting data on residues of imazapyr in water. Concurrent recoveries of imazapyr from water fortified with imazapyr at 0.001-0.450 ppm were generally acceptable. Only 3 of the 122 pond water samples fortified with imazapyr had recoveries below 70% (45, 51, and 68%). Recoveries of imazapyr from water averaged  $87 \pm 12\%$  from the FL tests and  $88 \pm 8\%$  from the MO tests. Apparent residues of imazapyr were  $<0.0001$  ppm in all control samples.

The CE/UV Method M 3014 is adequate for collecting data on residues of imazapyr in sediments. Concurrent recoveries of imazapyr from sediment samples fortified with imazapyr at 0.001-0.500 ppm were generally acceptable. Only 4 of the 73 fortified sediment samples had recoveries below 70% (21, 56, 60, and 65%). Recoveries of imazapyr from sediments averaged  $84 \pm 11\%$  from the FL tests and  $86 \pm 17\%$  from the MO tests. Apparent residues of imazapyr were  $<0.0001$  ppm in all control samples of sediments.

The CE/UV Method M 3097 and the LC/MS/MS Method M 2672 are adequate for collecting data on residues of CL 9140 and CL 119060 in water. Concurrent method recoveries from water fortified with CL 9140 or CL 119060 at 0.002-0.020 ppm were generally within the acceptable range. Nine of the 44 water samples fortified with CL 119060 had recoveries of 56-68%, and 4 of the 48 water samples fortified with CL 9140 had recoveries of 52, 59, 62, and 64%. Average recoveries of CL 9140 were 85 and 95%, and average recoveries of CL 119060 were 76 and 78%. Apparent residues of both analytes were  $<0.0002$  ppm in all control water samples.

The CE/UV Method M 3066 is adequate for collecting data on residues of imazapyr in tissues of fish and shellfish. Recoveries of imazapyr from fish fillets and crayfish tails fortified with imazapyr at 0.05-1.00 ppm were all acceptable (70-90%) and averaged  $81 \pm 6\%$  from the FL tests and  $83 \pm 6\%$  from the MO tests. Apparent residues of imazapyr were  $<0.01$  ppm in all control samples from bluegill (n=13), tilapia (n=7), and bass (n=7), and in nearly all control samples of crayfish (n=13) and catfish (n=12). One control sample of crayfish from the FL test had apparent residues at 0.032 ppm ( $<LOQ$ ), and two control samples of catfish from the FL and MO tests had apparent residues at 0.023 ppm and 0.069 ppm. Adequate sample calculations and chromatograms were provided for all analyses.

Maximum frozen ( $\leq -10$  C) storage intervals were 21 months for tissues of the non-target organisms and pond sediments and 27 months for pond water. A storage stability study (MRID 45119719, currently under review) on fish tissues indicates imazapyr is stable in frozen ( $\leq -10$  C) fish muscle for up to 21 months. An ongoing storage stability study (MRID 45119704, currently under review) on pond water and sediments indicates that imazapyr is stable in frozen ( $\leq -10$  C) sediments and pond water for up to 12 months and that the degradates CL 9140 and CL 119040 are stable in frozen water for up to 15 months.

### 3.2. Results

At two test locations in FL and MO, a single application of imazapyr (2 lb ae/gal AS) was made to the banks (4-6 feet) and water's edge (4-6 feet) of a fresh water pond at a broadcast rate of 1.6 lb ae/A (Treatment I). A second pond at each site received a single application of imazapyr (2 lb ae/gal AS) to the banks (4-6 feet) and the entire pond surface at a broadcast rate of 1.6 lb ae/A (Treatment II). Prior to treatment, each pond was stocked with non-target organisms (bluegill, bass, tilapia, catfish, and crayfish), and five sampling stations were established in various areas of each pond. Following treatment, water samples were collected from each station per pond at various intervals from 0 to 42 DAT, and single composite samples of each non-target organism were collected from each pond at intervals from 0 to 42 DAT. Sediment samples were also collected from each sampling station in each pond at various intervals from 0 to ~180 DAT.

Based on the amount of imazapyr applied and the volume of the treated ponds, the registrant calculated that the theoretical concentration of imazapyr would be 0.075 and 0.261 ppm in the water from Treatments I and II, respectively, at the FL site, and 0.028 and 0.213 ppm in the water from Treatments I and II, respectively, at the MO site.

In the FL tests, average imazapyr concentrations in water peaked at 0.028 ppm by 3 hours post-treatment in Treatment I and declined to <0.001 ppm by 36 DAT. The highest concentration observed for any one water sample from Treatment I was 0.067 ppm (1 hour). For Treatment II in FL, the average imazapyr residues in water peaked at 0.164 ppm by 12 hours post-treatment and declined to <0.001 ppm by 27 DAT. The highest residues observed for any one water sample from Treatment II was 0.186 ppm (12 hours). For Treatment II, the registrant calculated the half-life of imazapyr in the pond water to be 3.9 days.

In the MO tests, average imazapyr concentrations in water peaked at 0.029 ppm by 1 hour post-treatment for Treatment I and declined to 0.002 ppm by 42 DAT. The highest residues observed for any one water sample from Treatment I was 0.081 ppm (1 hour). For Treatment II in MO, the average imazapyr residues in water peaked at 0.196 ppm by 1 hour post-treatment and declined to 0.025 ppm by 42 DAT. The highest residues observed in any one water sample from Treatment II was 0.309 ppm (1 hour). The declines in imazapyr residues in water were slower in the MO tests than the FL tests. For Treatment II at MO, the registrant calculated the half-life of imazapyr in the pond water to be 15 days. The registrant attributed the increased half-life to higher levels of suspended sediments at the MO site, which reduced light penetration, and decreased the potential for photolytic degradation.

The degradates CL 9140 and CL 119060 were <0.002 ppm (<LOQ) in all water samples from Treatment I collected at both the FL and MO test sites. For Treatment II at the FL site, CL 9140 was detected at 0.002-0.005 ppm at 1-5 DAT and CL 119060 was detected at 0.002-0.006 ppm at 1-5 DAT. For Treatment II at the MO site, CL 9140 was detected in only one sample at 0.003 ppm at 14 DAT, and CL 119060 was not detected (<0.002 ppm) in any samples.

Residues of imazapyr in pond sediments were generally low in both FL tests. For the Treatment I in FL, average imazapyr residues in sediments were 0.003-0.005 ppm from 6 hours to 1 day post-treatment and were  $\leq$ 0.001 ppm at all subsequent intervals. For Treatment II in FL, average imazapyr residues in sediments declined from 0.011 ppm at 6 hours post-treatment to  $\leq$ 0.001 ppm by 22 DAT. Imazapyr residues in sediments from the MO test were more variable and showed little or no decline over time. For the Treatment I in MO, average imazapyr residues in sediments were initially the highest (0.034 ppm) of any treatment; however, average residues at all subsequent

intervals (1-180 DAT) were 0.001-0.005 ppm. For Treatment II in MO, average imazapyr residues in sediments averaged 0.009-0.018 ppm at 0.25-180 DAT.

Residues of imazapyr were <0.05 ppm (<LOQ) in all samples of each non-target species from Treatment I at both test sites and from Treatment II at the MO test site. For Treatment II at the FL site, imazapyr residues were detected in bluegill (0.636 ppm), tilapia (0.233 ppm), catfish (0.068 ppm), crayfish (0.059 ppm) from the 3 hour post-treatment interval. Residues were higher in the mid-level species (bluegill and tilapia), than in the bottom feeding species (catfish and crayfish). At all subsequent sampling intervals (1-42 DAT), imazapyr residues were <0.005 ppm in all species from this study.

The water, fish, and sediment residue data are satisfactory once adequate supporting storage stability data are provided for residues of imazapyr in water and sediments stored up to 27 months.

#### **4. Deficiencies**

A storage stability study to support a storage interval of 24 months for CL 9140 and CL 119040 residues in frozen pond water and sediments is ongoing.

#### **5. References**

None