IMAZAPIC (266)

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EXPLANATION

Imazapic is an imidazolinone herbicide developed for the control of grasses and broadleaf weeds in a variety of crops. Like all other imidazolinone herbicides, its mode of action is the inhibition of acetohydroxy acid synthase (AHAS or ALS) which catalyses the production of three branched chain amino acids, valine, leucine, and isoleucine, required for protein synthesis and cell growth.

Imazapic has been registered in a number of countries and was included in the Codex Priority List in 2009 as a new compound for evaluation by the current JMPR.

The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, supervised trials, processing and farm animal feeding.

IDENTITY

ISO common name: Imazapic

Chemical name

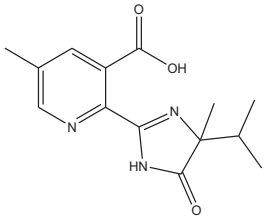
IUPAC: 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methylnicotinic acid

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

CAS Registry No.: 104098-48-8

CIPAC No.: None yet

Structural formula:



Molecular formula: $C_{14}H_{17}N_3O_3$

Molecular weight: 275.3

PHYSICAL AND CHEMICAL PROPERTIES

Pure active ingredient

Property	Results	Reference
Appearance	White powdered solid	IA-301-002 Cortes D. *et al*., 1993a
Odour:	Odourless (room temperature)	A-301-002 Cortes D. *et al*., 1993a
Melting point:	206 °C	2012/1010149 Kroehl T. 2012a
Boiling point:	Decomposes prior to boiling	2012/1010149 Kroehl T. 2012a
Temperature of decomposition	ca. 210 °C	2012/1010149 Kroehl T. 2012a
Solubility in water	In distilled water 1.92 g/L (15 °C), 2.23 g/L (25 °C) 2.63 g/L (35 °C).	IA-311-001 Mangels G. 1986b
Vapour pressure:	ca. 2.7 x 10^{-9} Pa (20 °C) ca. 7.5 x 10^{-9} Pa (25 °C) (Extrapolation from measurements at 150°C to 190 °C) < 1.3 × 10^{-5} Pa (1 x 10^{-7} mmHg) (25 °C) (Triplicate test system using gas saturation method)	2012/1010149 Kroehl T. 2012a IA-306-001 Mangels G., 1986a
Volatility, Henry's Law Constant at	1.6 x 10^{-6} Pa m^3/mole (Calculation by dividing vapour pressure by water solubility)	
Octanol-water partition coefficient at (logPow)	0.054 (Average logPow uncorrected for dissociation using initial concentration of 0.88, 0.42, 0.06 and 0.04 mg/L in n-octanol) No significant effect of pH (4-10) on log_{Pow}.	IA-315-003 Morelli D. 1993a
Dissociation in water	pKa: 3.9 (calculation from potentiostatic titration) pKa: 3.1 and 11.4 (by spectrophotometry)	IA-322-003 Mangels G. 1986c

Technical material

Property	Results	Reference

Appearance:	White fine powdery solid	2012/1010149 Kroehl T. 2012a
Odour	Odourless at room temperature	2012/1010149 Kroehl T. 2012a
Relative density:	Tapped bulk density, 0.38 g/ml (24 °C) Untapped bulk density, 0.24 g/ml (24 °C)	IA-301-002 Cortes D. *et al*.1993a
Solvent solubility at 25 °C:	mg/mL g/100 mL of solvent* Hexane 0.0084 -- Toluene 1.48 0.135 Methanol 50.9 5.07 Dichloromethane 89.2 9.58 Acetone 18.9 1.93 Acetonitrile 12.9 1.33 Dimethyl sulfoxide 310 41.8 Deionized water 2.15 0.215 pH 5 Buffer 36.0 3.69 pH 7 Buffer 479 68.1 pH 9 Buffer 518 76.5 * corrected for solution density	IA-310-001 Teeter D. 1992a
Hydrolysis:	Stable in pH 5, 7 and 9 buffers (25°C)	IA-322-004 Mangels G. 1992a
Photolysis:	Rapid photolysis in sterilized water with the following six photo-degradation products, each of which accounted for 10% of the dose: - Carbon dioxide - 5-methyl-3-pyridinecarboxylic acid -2-[(l-carbamoyl-1,2-dimethylpropyl) carbamoyl]-5- methyl nicotinic acid (CL 290210) - 5-ethyl-2,3-pyridine dicarboxylic acid - 2-carbamoyl-5-methyl-nicotinic acid - 2-carbamoyl-5-methyl-3-nicotinic acid Several other photoproducts were present at less than 10% of the dose. DT_{50} under continuous irradiation: 0.30 day (7.2 h) at pH 5 0.25 day (6.0 h) at pH 7 0.26 day (6.24 h) at pH 9	IA-630-002 Ta, 1994a
Dissociation in water:	pKa: 2.0, 3.6 and 11.1 (by UV spectrophotometry).	IA-322-002 Melcer M. 1993a

Formulations

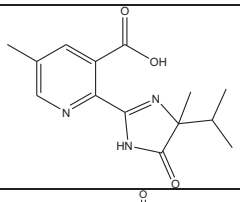
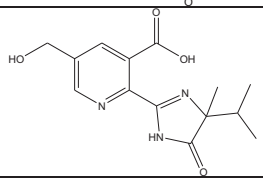
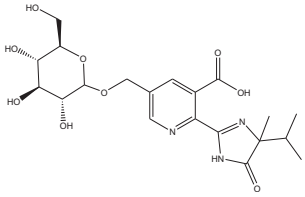
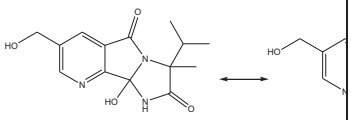
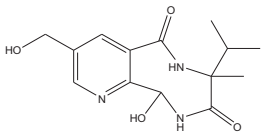
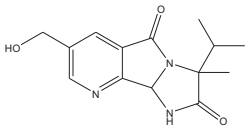
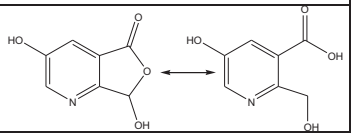
- Soluble concentrate (SL) formulations containing 25, 90 or 240 g ai/L
- Emulsifiable concentrate (EC) formulations containing 22 or 240 g ai/L.
- Water dispersible granule (WG) formulations containing 175, 525 or 700 g ai/kg.

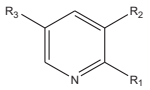
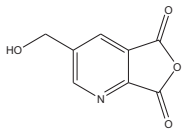
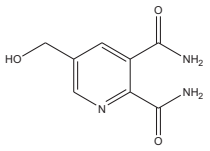
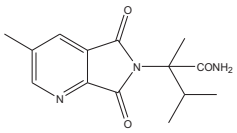
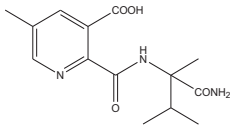
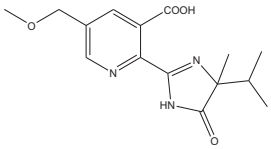
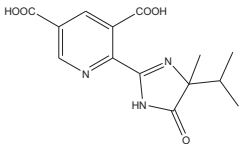
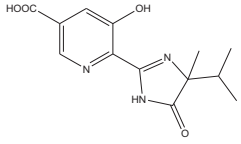
Except 240 SL and 700 WG, each of these formulations contain one or two other active ingredient(s).

METABOLISM AND ENVIRONMENTAL FATE

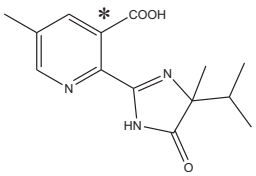
The following links code number and structure or description of the compound appearing in the various metabolism and environmental fate studies.

Structure of compounds appearing in metabolism and environmental fate studies

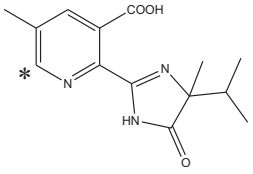
Code (MW)	IUPAC chemical name	Structure	Found in
Imazapic (275.3)	2-(4-isopropyl-4-methyl-5-oxo-imidazolin-2-yl)-5-methyl-nicotinic acid		Livestock (goat, hen), rat, Plants (soya bean, sugar cane, peanut, Bermuda grass) Soil
CL263284 M715H001 (M5) (291)	2-(4-isopropyl-4-methyl-5-oxo-imidazolin-2-yl)-5-hydroxymethyl-nicotinic acid		Livestock (goat) rat) Plants (soya bean, sugar cane, peanut, Bermuda grass) Soil
CL189215 M715H002 (M4) (453)	5-[(β-glucopyranosyloxy) methyl]-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-nicotinic acid		Plants (soya bean, sugar cane, peanut, Bermuda grass)
M6-D/M6-H (291)	3-(hydroxymethyl)-7-isopropyl-7-methyl-6H-pyrido[2,3-f][1,4]diazocine-5,8,10-trione		Plant (Bermuda grass)
M6-J2 (293)	10-hydroxy-3-(hydroxyl-methyl)-7-isopropyl-7-methyl-9,10-dihydro-6H-pyrido[2,3-f][1,4]diazocine-5,8- dione		Plant (Bermuda grass)
M6-J3 (275)	-		Plant (Bermuda grass)
M2-B (167)	Left: 3,7-dihydroxy-7H-furo [3,4-b]pyridin-5-one Right: 5-hydroxy-2-		Plant (Bermuda grass)

Code (MW)	IUPAC chemical name	Structure	Found in
	(hydroxymethyl)pyridine-3-carboxylic acid		
M1-C (183)	[1] 5-hydroxypyridine-2,3-dicarboxylic acid [2] 3-hydroxypyridine-2,5-dicarboxylic acid [3] 2-hydroxypyridine-3,5-dicarboxylic acid	 R1=COOH, R2 = COOH, R3 = OH or R1=COOH, R2 = OH, R3 = COOH or R1=OH, R2 = COOH, R3 = COOH	Plant (Bermuda grass)
M2-A (179)	3-(hydroxymethyl)furo [3,4-b]pyridine-5,7-dione		Plant (Bermuda grass)
M1-B (195)	5-(hydroxymethyl)-pyridine-2,3-dicarboxamide		Plant (Bermuda grass)
CL 303459 (275)	2,3-dimethyl-2-(3-methyl-5,7-dioxo-pyrrolo[3,4-b]pyridin-6-yl)butanamide		Rat
CL 290610 (292)	2-[(1 -carbamoyl-1,2-dimethylpropyl) carbamoyl]-5-methyl-nicotinic acid		Rat
CL 299263	2-(4-isopropyl-4-methyl-5-oxo-imidazolin-2-yl)-5-methoxymethyl nicotinic acid		Soil
CL 312622	2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-3,5-pyridine-dicarboxylic acid		Soil
CL 354825	5-carboxy-3-hydroxypyridine imidazolinone		Soil

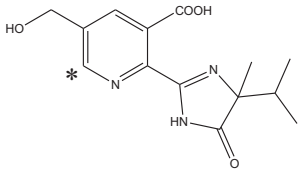
The metabolism and distribution of imazapic in animals and plants and the fate of imazapic in the environment were investigated using the following $^{14}C/^{13}C$-radiolabelled compounds.



[pyridine-3-^{14}C]-imazapic



[pyridine-6-^{14}C]-imazapic



[pyridine-6-^{14}C]-CL263284

In the descriptions below, the application rates are all in acid equivalents.

Animal metabolism

The Meeting received information on the results of studies on lactating goats and laying hens which were fed isotope-labelled imazapic or CL263284.

Metabolism studies on laboratory animals including rats were reviewed in the framework of toxicological evaluation by the current JMPR and the relevant information is summarized below.

Rat

A metabolism study using rats was evaluated and described in detail by the WHO Panel. Radiolabelled imazapic administered by oral gavage was rapidly and extensively absorbed, minimally metabolized, and excreted primarily in the urine after single dose of 10 or 1000 mg/kg bw, or repeated low dose of 10 mg/kg bw per day over 14 days to rats. Biliary excretion was minimal. The majority of radioactivity was excreted as the unchanged parent compound within the first 6 hours post-dosing. Less than 2% of the administered dose (AD) was detected in the carcass with trace amounts detected in blood, kidneys and liver of the high dose group; however, radiolabelled test substance was not detected in any other organs. There was no evidence of accumulation. Imazapic and its metabolites were not excreted in expired air. Parent compound accounted for 94% of the AD in the urine and 2.3% of the AD in the faeces. The metabolites produced from oxidation, reduction and hydrolysis, including CL 263284, CL 280442 and several other unidentified metabolites, accounted for a total of approx. 6% AD in the urine and faeces.

Lactating goats

Imazapic

A lactating goat was orally given single encapsulated doses of [pyridine-6-^{14}C]- and [pyridine-6-^{13}C]-imazapic daily for 5 consecutive days at a dose of 254 mg/day, equivalent to 175 ppm of test material in the diet, based on the actual food consumption during the treatment period (Sharp and Thalacker, 1999a). A control goat was given placebo capsules for 5 days. Urine and faeces samples were collected daily. Milk samples were collected twice daily. The animals were sacrificed approximately 23 hours after the last dose, and liver, kidneys, fat, bile, muscle, blood, and gastrointestinal (GI) tract and its contents were collected. All samples, except the bile and GI tract and its contents, were analysed for radioactivity content.

Samples were analysed by LSC after combustion. Selected tissue (liver, kidney, muscle and fat) and milk extracts and ^{14}C-sample eluents were analysed using HPLC and TLC to determine the metabolic profile. The limits of detection were 0.001–0.004 mg/kg with sample sizes of 0.2–5 g. Kidney, liver, muscle and selected milk samples were extracted and analysed for ^{14}C-imazapic, CL 263284 (imazapic alcohol metabolite) and CL 312622 (5-acid imidazolinone metabolite). Fat (omental and renal) was not extracted and analysed because the concentration of radioactive residues was < 0.010 mg/kg

Among 90.7% of the total administered radioactivity (TAR) recovered, urine contained 81.7% and faeces 6.7% of TAR with cage wash and wipe accounted 1.2% and 1.2% respectively of

TAR. This indicate that urinary excretion is the major route of elimination from body. Approximately 75% of each daily dose was excreted in the urine in the 24-hours following each dose administration.

From milk 0.03% TAR was recovered and from the edible tissues and blood collected contained 0.01% TAR.

The radioactive residues in the milk from pm milking, the first collection of milk after each capsule administration ranged from 0.045 mg eq/kg (Day 1) to 0.078 mg eq/kg (Day 5), expressed in mg/kg-equivalents of imazapic. The radioactive residues in the milk from am milking, the second milk collection after each capsule administration, ranged from 0.012 to 0.014 mg eq/kg, lower than those in the milk from pm milking. The calculated daily radioactivity residue levels in milk ranged from 0.026 mg eq/kg (Day 1) to 0.037 mg eq/kg (Day 5). The total amount of radioactivity in milk ranged from 53.4 to 83.9 µg eq per day.

Among the tissues collected and analysed, the kidney contained 0.275 mg/kg, liver 0.033 mg/kg, muscle 0.010 mg/kg, blood 0.086 mg/kg and fat 0.003 mg/kg, expressed in mg/kg-equivalents of imazapic.

The results of the TRR in lactating goats are presented in the following table.

Table 1 Total radioactive residues (TRR) in goat treated with ^{14}C-imazapic.

Sample	TRR (mg/kg)*	Imazapic	
		mg/kg	% TRR
Milk (48 h p.m.)	0.058	0.039	66.8
Milk (120 h p.m.)	0.078	0.051	65.0
Kidney	0.275	0.234	85.0
Liver	0.033	0.016	49.1
Muscle	0.010	0.003	33.8

* expressed in mg/kg-equivalents of imazapic.

Parent ^{14}C-imazapic was the only major residue detected in tissues and milk.

In another study (Kao, 1993a), lactating goats were orally treated with a mixture of [pyridine-6-^{13}C]- and [pyridine-6-^{14}C]-imazapic. Feeding level for each of two goats was equivalent to 2.0 or 11.8 ppm in the diet daily for 7 consecutive days. One goat was used as a control. Samples of blood, milk and excreta were collected daily and either refrigerated or stored frozen. After seven days of dosing, the goats were sacrificed and the tissues (kidney, liver, muscle, and fat) were collected approximately 20 hours after the last dose. Tissue samples were stored frozen.

Samples were analysed by LSC either after combustion or by direct analysis. The validated detection limit of the radioassay method was approximately 0.01 mg/kg for milk, blood, and all tissues except fat with a validated sensitivity of 0.02 mg/kg. Extracts of the kidney and faeces and aliquots of urine were analysed by reverse phase radio-HPLC with two different phases used for analysis of the kidney.

Elimination of radioactive residues in the urine accounted for 67.2% and 94.0% of the TAR for the low and high doses, respectively. The radioactivity excreted in the faeces represented 7.0% and 9.6% of the TAR for the low and high doses, respectively.

The TRR in the daily collected blood and milk samples were < 0.01 mg eq/kg at both dose levels. The TRR in liver and muscle were < 0.01 mg eq/kg and < 0.02 mg eq/kg in fat regardless of the dose level. The TRR in the kidney was < 0.01 mg eq/kg from the goat dosed at 2.0 ppm and 0.05 mg eq/kg from the goat dosed at 11.8 ppm.

93% of TRR in kidney of the high-dose goat were extracted with acetonitrile and methanol. Analysis of the extract by reverse phase HPLC showed imazapic at 30% TRR (0.02 mg/kg), CL 263284 (hydroxymethyl imazapic) at 8% TRR (< 0.01 mg/g) and unknown A at 7% TRR(< 0.01 mg/kg). An unresolved peak near the solvent front comprised 33% TRR (0.02 mg/kg).

Analysis of the kidney extract by reverse phase HPLC using two different HPLC phases with comparison to reference standards confirmed the identification of the components.

HPLC analysis of the urine showed that radioactive residue was essentially unmetabolized imazapic. HPLC analysis of extracts of faeces showed the presence of imazapic (58% TRR) and the lesser amount of hydroxymethylated metabolite CL 263284 (9% of TRR).

CL 263284

A study (Kao, 1994a) was conducted using the [pyridine-6-^{14}C]-radiolabelled hydroxymethyl imazapic (CL 263284) fed in capsule to lactating goats daily for seven days. Feeding levels for each of two goats were equivalent to 2.33 or 14.5 ppm of feed. One goat was used as a control. Samples of blood, milk and excreta were collected daily. After seven days of dosing, the goats were sacrificed, and the tissues kidney, liver, muscle and fat were collected 20 hours after the last dose. CL 283284 was eliminated via urine (15–18% of the applied dose) and faeces (82% and 68% of the applied dose at low dose and high dose respectively). The TRR in the daily blood and milk samples and those in liver, muscle, and omental fat were all less than 0.01 mg eq/kg, for both feeding levels. The TRR in kidney was < 0.01 mg eq/kg for low dose and 0.03 mg eq/kg for high dose. Analysis of the extract of kidney from the high dose goat showed that 9% (< 0.01 mg/kg) of the extracted TRR was CL 263284, and the remaining residue was predominantly a labile component M1 (78% TRR, 0.02 mg/kg), possibly a salt of CL 263284, which converted to CL 263284 on exposure to aqueous buffer.

Laying hens

Laying hens were orally dosed with a mixture of [pyridine-6-^{13}C]- and [pyridine-6-^{14}C]-imazapic in capsules (Gatterdam, 1993a). Feeding levels for three groups, each containing 8 hens, were 0, 2.1, or 11.4 ppm in feed daily for 7 consecutive days.

Eggs were collected twice daily and, after compositing, were refrigerated. After 7 days of dosing, the hens were sacrificed and blood and tissues (liver, kidney, muscle and skin with adhering fat) were collected approximately 22 hours after the last dose. Tissue samples were composited for each group and stored frozen at -20 °C.

Samples were analysed by LSC either after combustion or by direct analysis The validated limit of detection of the radioassay method was approximately 0.01 mg/kg for eggs, blood, and all tissues.

TRR in the all egg samples and all tissue and blood samples were less than 0.01 mg eq/kg. Total recovery of radioactivity in excreta was 90.6% and 95.2% for the low and high dose, respectively.

Study 2 (Afzal, 1994a)

A study was conducted on laying hens with the [pyridine-6-^{14}C]-radiolabelled hydroxymethyl imazapic (CL 263284). Hens were dosed orally, with feeding levels for each group at 2.14 or 10.9 ppm in diet daily for seven days. After seven days, the hens were sacrificed and the tissues (liver, kidney, muscle and skin with adhering fat) were collected for analysis approximately 22 hours after the final dose. Total recovery of radioactivity in excreta was 85.3% and 88.6% for the low and high doses, respectively. Residues in all tissues, liver, kidney, muscle, skin with adhering fat, blood and eggs, were less than 0.01 mg eq/kg. In summary, orally administered CL 263284, the hydroxymethyl metabolite of imazapic was mainly eliminated from the hen through excreta. No detectable (< 0.01 mg eq/kg) radioactive CL 263284-derived residues were found in eggs or edible tissues.

Proposed metabolic pathway of imazapic in animals

A proposed metabolic pathway for imazapic in rat and the lactating goats is presented in Figure 1. The majority of orally dosed imazapic was eliminated unchanged, mostly via urine (goats) and excreta (hens). The radioactive residues in edible tissues, milk or eggs were very low. In lactating goats, residues remaining in kidney were mostly unchanged parent with a minor amount of CL263284. In laying hens the studies with oral administration of either imazapic or its hydroxymethyl metabolite

did not result in detectable residues in any tissues or eggs. In the case of rats, imazapic was also primarily eliminated rapidly, unchanged, via urine. A small amount of the total dose excreted via faeces, consisted mostly of unchanged parent with minor amounts of metabolites CL 303459, CL 263284 and CL 290610 together with even lower amounts of several unknown radioactive components. Some amount of imazapic remaining in the body of animals did not go through extensive metabolism with minor oxidation to produce hydroxylated metabolite and in the case of rats, another metabolism to produce two minor metabolites through possibly hydrolysis.

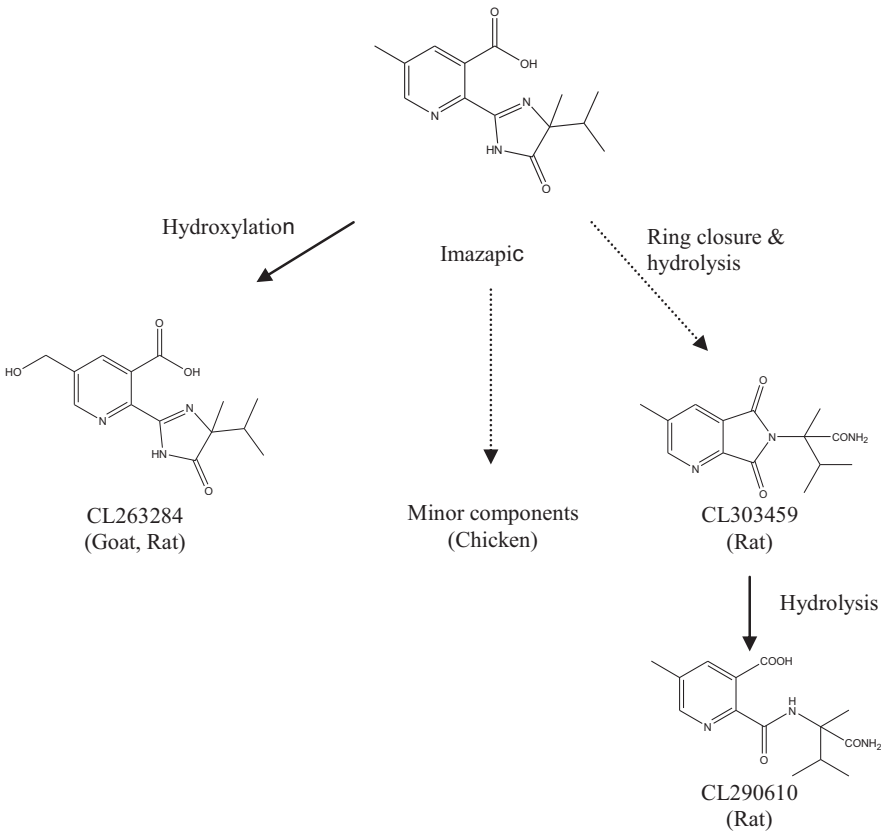


Figure 1 Proposed metabolic pathway of imazapic in animals

Plant metabolism

The Meeting received information on the fate of imazapic in sugar cane, peanuts, Bermuda grass and transgenic soya beans.

Sugar cane

Study 1 (Mangels, 1988a)

A metabolism study was conducted to investigate [pyridine-6-^{14}C]-imazapic in sugar cane variety CP 65-357. [^{14}C]-labelled imazapic was mixed with unlabelled imazapic and applied to the soil surface as a pre-emergence treatment at a rate approximating 150 g/ha by spraying 200 mL of the

aqueous solution of the compound onto the soil. The test was carried out under natural climatic conditions using one test plot of 0.6 × 3.1 m size and one control plot.

At harvest, approximately 13 months after pre-emergence treatment, mature sugar canes were cut above the soil and the outer leaves were removed by hand. The stalks were cut into thirds and each third was cut into pieces of 15–20 cm length.

All plant samples were frozen after collection and shipped to the analytical laboratory over dry ice. Twelve pieces from each section were combined and ground to a fine consistency in the presence of solid CO_2. The radioactive residues present in the sugar canes were determined by combustion and liquid scintillation counting (LCS).

Radioassays of the stalk samples indicated that there were no detectable ^{14}C residues present in the harvest samples (< 0.005 mg/kg).

Study 2 (Grosshans, 2009a)

In another study, the metabolism of [pyridine-6-^{14}C]-imazapic was investigated in sugar cane grown in plastic boxes (0.365 m × 0.56 m) filled with a sandy loam soil. The cultivation of the crop and the plant uptake part of the study was conducted in a greenhouse. A calculated amount of ^{14}C-imazapic was dissolved in blank formulation solution (DG formulation with adjuvant BAS 160 00 S in water). This resulted in a nominal target rate of 245 g ai/ha (corresponding to spray volumes of approximately 220 L/ha). The formulation was applied as a pre-emergence treatment using an automatic spray track system.

Sugar cane plants were sampled at 63, 96, 151 and 236 DAT and adult plants were split into leaves and stalks.

Frozen samples (sugar cane forage, leaves or stalks) were mixed with dry ice and homogenized using a Stephan mill. Weighed homogenized subsamples were extracted three times with methanol and twice with water. The methanol extracts of the three steps were combined and adjusted to a defined volume. Aliquots of the combined extracts were measured by Liquid Scintillation Counting (LSC). The residue was further extracted in the same way with appropriate volumes of water (twice). The aqueous extracts were also combined, adjusted to a defined volume, and aliquots of the combined aqueous extracts were radioassayed by LSC as well. Based on the sum of radioactive counts found in the methanol and aqueous extracts, the ERR is calculated. The residue remaining after methanol and water extraction of each sample was air dried, and the weight of the remaining sample was determined. The samples were homogenized using an analytical mill prior to combustion analysis of aliquots for the determination of the PES. The PES after methanol and water extraction of sugar cane samples accounted only for low concentrations, therefore no further processing was attempted.

Additional extraction procedures were carried out to get further information about the extractability of radioactive residues. Solvents or solvent mixtures were used which are applied in residue analytical methods: A three step extraction method with methanol / 25 mM HCl (6/4, v/v; Method SOP-PA.0288); and a three step extraction method with methanol / acetone / water (1/1/1, v/v/v; Method M 2253.01).

The calculated total radioactive residues of the growing sugar cane forage sampled 63 DAT of pre-emergence treatment with ^{14}C-imazapic accounted for 0.0358 mg/kg (Table 2). The TRR of forage sampled 96 DAT was lower with 0.0161 mg/kg. On 151 DAT, small plants were sampled as forage material (TRR 0.0222 mg/kg). The leaves (TRR) and stalks (TRR) of full grown plants sampled 15 DAT contained TRR of 0.0242 and 0.0086 mg/kg respectively. At the last sampling date of 236 DAT, leaves and stalks of the adult plants contained residues at 0.0151 mg/kg and 0.0039 mg/kg, respectively.

Table 2 TRRs in sugar cane samples after pre-emergence treatment with [pyridine-6-^{14}C]-imazapic

Matrix	Days after Treatment	TRRs in Treated Sugar Cane Matrices	
		Determined by Direct Combustion (mg eq/kg)*	Sum of Extracted Radioactive Residue and PES (mg eq/kg)*
Forage	63	0.0424	0.0358
Forage	96	0.0169	0.0161
Forage [a]	151	0.0220	0.0222
Leaves [b]	151	0.0266	0.0242
Stalks [b]	151	0.0075	0.0086
Leaves [b]	236	0.0162	0.0151
Stalks [b]	236	0.0048	0.0039

PES = post extraction solid (after solvent extraction)

* Expressed in mg-imazapic equivalents/kg.

[a] young small plants

[b] adult plants

The extraction profiles of radioactive residues from sugar cane leaves and stalks are summarized in Table 3.

High percentage (82.0–84.4% TRR) of the radioactive residues in forage (63, 96 and 151 DAT) was extracted, most of which (77.1 to 80.0%) extracted with methanol. The additional water extraction released only 3.2 to 4.4% TRR. Most of the extracted radioactive residues were water-soluble (48.1 to 79.0% TRR). The residual radioactive residues accounted for 15.6 to 19.7% TRR (0.0032 to 0.0064 mg eq/kg).

Equaly high percentage (78.1–83.6% TRR) of radioactive residues in leaves (151 and 236 DAT) were extracted, most of which extracted with methanol (72.3 to 78.6%). The additional water extraction released only 5.1 to 5.8% TRR. Most of the extracted radioactive residues were water-soluble (71.5 to 75.8% TRR). The residual radioactive residues accounted for 16.4 and 21.9% TRR (0.0033 and 0.0040 mg eq/kg).

Slightly lower but still quite high percentage (63.4–81.9% TRR) of radioactive residues in stalks (151 and 236 DAT) was extracted, most of which extracted with methanol (59.2 to 78.4%). The additional water extraction released 3.4 to 4.2% TRR. Most of the extracted radioactive residues were water-soluble (63.3 to 65.2% TRR). The residual radioactive residues accounted for 18.1 and 36.6% TRR (0.0014 and 0.0016 mg eq/kg).

Table 3 Extracted radioactive residues from sugar cane samples after pre-emergence treatment with [pyridine-6-^{14}C]-imazapic

(a) Distribution of radioactive residues among methanol extracts, aqueous extracts, total extracted radioactive residue and PES of various matrices

Matrix	DAT	TRR Sum of Extracted Residue and PES	Distribution of Radioactive Residues							
			Combined Methanol Extract		Combined Aqueous Extract		Total Extracted Radioactive Residue		PES	
		(mg/kg)*	(mg/kg)*	(%TRR)	(mg/kg)*	(%TRR)	(mg/kg)*	(%TRR)	(mg/kg)*	(%TRR)
Forage	63	0.0358	0.0282	78.7	0.0012	3.3	0.0294	82.0	0.0064	18.0
Forage	96	0.0161	0.0124	77.1	0.0005	3.2	0.0129	80.3	0.0032	19.7
Forage [1]	151	0.0222	0.0177	80.0	0.0010	4.4	0.0187	84.4	0.0035	15.6
Leaves [2]	151	0.0242	0.0190	78.6	0.0012	5.1	0.0202	83.6	0.0040	16.4
Stalks [2]	151	0.0086	0.0067	78.4	0.0003	3.4	0.0070	81.9	0.0016	18.1
Leaves [2]	236	0.0151	0.0109	72.3	0.0009	5.8	0.0118	78.1	0.0033	21.9
Stalks [2]	236	0.0039	0.0023	59.2	0.0002	4.2	0.0025	63.4	0.0014	36.6

DAT=days after treatment

PES=post extraction solid

* Expressed in mg-imazapic equivalents/kg.

(b) Partition of methanol extracts into organic solvent and water (See Table 3a above)

Matrix	DAT	Organosoluble				Water Soluble (water phase)	
		Dichloromethane Extract (alkaline conditions)		Ethyl Acetate Extract (acidic conditions)			
		(mg/kg)*	(%TRR)	(mg/kg)*	(%TRR)	(mg/kg)*	(%TRR)
Forage	63	0.0038	10.7	0.0085	23.7	0.0172	48.1
Forage	96	0.006	3.8	ne	ne	0.0123	76.7
Forage [a]	151	0.0022	9.8	ne	ne	0.0175	79.0
Leaves [b]	151	0.0021	8.5	ne	ne	0.0183	75.8
Stalks [b]	151	0.0014	16.0	ne	ne	0.0054	63.3
Leaves [b]	236	n.m.	n.m.	ne	ne	0.0108	71.5
Stalks [b]	236	n.m.	n.m.	ne	ne	0.0025	65.2

n.m.= not measured by LSC

ne = not extracted with ethyl acetate.

*= Expressed in mg-imazapic equivalents/kg.

[a] Young small plants

[b] Adult plants

The identification of metabolites was based on HPLC analyses of the concentrated extracts and/or clean-up fractions either via solvent partition or fractionation. The parent compound and the metabolites were assigned by comparison of the retention times with synthesized and certified reference compounds. All the quantification of the parent compound and its metabolites was based on the HPLC analyses of the concentrated extracts or clean-up fractions obtained from the individual matrices using two different HPLC methods with radio-detection. The results of identification and characterization are shown in Table 4.

Imazapic was found in all samples investigated (5.7 to 41.3% TRR, 0.0005 to 0.0058 mg/kg). In addition, the hydroxymethyl metabolite CL263284 was detected in all samples investigated (10.1 to 44.5% TRR, 0.0005 to 0.0139 mg eq/kg). Its glucoside CL189215 was found only in forage and leaves (2.7 to 8.0% TRR, 0.0005 to 0.0019 mg/kg) and could not be identified in stalks. Some HPLC peaks in the chromatograms could be characterized by their chromatographic behavior.

In samples taken up to 151 DAT, the degree of identification was 55.1 to 69.0% of the TRR. Additional 14.7 to 30.5% TRR could be characterized for its chromatographic, SPE fractionation, partition or extraction behavior.

Since the extractability of the 236 DAT stalk and leaves samples was lower, only 21.3 and 34.7% TRR, respectively, only known metabolites could be identified. Additional 29.9 and 29.7% TRR could be characterized in stalks and leaves, respectively.

Table 4 Characterization and identification of radioactive residues in sugar cane samples after pre-emergence treatment with [pyridine-6-^{14}C]-imazapic

Component	Forage 63 DAT mg/kg (% TRR)	Forage 96 DAT mg/kg (% TRR)	Forage 151 DAT mg/kg (% TRR)	Leaves 151 DAT mg/kg (% TRR)	Stalks 151 DAT mg/kg (% TRR)	Leaves 236 DAT mg/kg (% TRR)	Stalks 236 DAT mg/kg (% TRR)
Imazapic	0.0058 (16.4)	0.0051 (31.4)	0.0040 (18.0)	0.0027 (11.0)	0.0026 (41.3)	0.0010 (5.7)	0.0005 (11.2)
CL263284	0.0139 (38.7)	0.0061 (37.6)	0.0094 (42.5)	0.0108 (44.5)	0.0010 (15.8)	0.0045 (26.3)	0.0005 (10.1)
CL189215	nd	nd	0.0011 (5.1)	0.0019 (8.0)	nd	0.0005 (2.7)	nd
Total identified and characterized [a]	0.0306 (85.6)	0.0135 (83.7)	0.0206 (93.2)	0.0217 (89.4)	0.0051 (82.2)	0.0111 (64.4)	0.0023 (51.1)

DAT = Days after last treatment

nd = not detected

[a] in extracted radioactive residues (ERR)

The TRR in adult sugar cane parts were low (leaves 0.024 mg eq/kg and 0.015 mg eq/kg and stalks 0.009 and 0.004 mg eq/kg for the 151 DAT and 236 DAT, respectively). Most of the radioactive residues were extracted with methanol, and only minor portions subsequently with water. The major radioactive components in the extracts were identified as unchanged parent, the 5-hydroxymethyl metabolite CL263284 and its glucose conjugate CL189215 (in forage and leaves only).The glucose conjugate CL189215 was not present in sugar cane stalks.

Peanut

A metabolism study was conducted with [pyridine-6-^{14}C]-imazapic in peanuts of variety NC7 (Wu, 1993a). The plants were kept in outdoor test plots. Both untreated and treated plots had total area of 1.2 × 2.4 m of sandy loam soil. Radiolabelled imazapic in a 2ASU formulation was applied to peanut plants at a rate of approximately 72 g ai/ha 30 days post-emergence. Pre-harvest (green plant) samples were collected at 0, 31, and 61 DAT. At harvest (131 DAT) hay and peanuts were collected. Soil samples were taken prior to and just after application and at harvest.

Samples were frozen after collection and shipped to the analytical laboratory over dry ice. Each mature harvest sample was separated into hay, hulls, and nutmeat. Green plants sampled at 0, 31, and 61 DAT and hay were first cut into small pieces and then homogenized in dry ice and/or liquid nitrogen. Nutmeat and hull were also ground in dry ice and/or liquid nitrogen. Levels of radioactivity in soil and plant matrices were determined by combusting subsamples in a sample oxidizer. All combustions were performed in triplicate.

Crop samples were sequentially extracted with methanol:water:acetone (1:1:1) and methanol:water (80:20) containing 2% hydrochloric acid except for nutmeat, which was extracted only with the former. HPLC of the peanut green plant, hay, hull, and nutmeat extracts was performed on a Supelco C_{18} reversed-phase column using an acetonitrile and phosphate buffer gradient system.

The TRR in all plant parts were determined by combustion. Additionally, the TRR was calculated by summing of the ERR extracted with methanol:acetone:water (1:1:1, v/v/v) and the PES. A summary of the TRR values in peanut plant matrices collected at various time intervals is shown in Table 5.

The TRR in the peanut plant declined significantly from 4.23 mg eq/kg at 0 DAT in green plants to 0.216 mg eq/kg (hay). The TRR in nutmeat at 131 DAT was low at 0.022 mg eq/kg (nutmeat)

Table 5 TRR in peanut plant samples after 30 days post-emergence treatment with [pyridine-6-^{14}C]-imazapic

Matrix	Days after Treatment	TRRs in Treated Sugar Cane Matrices	
		Determined by Direct Combustion (mg eq/kg)*	Sum of Extracted Radioactive Residue and PES (mg eq/kg)*
Plant	0	4.230	4.756
Plant	31	0.067	0.071
Plant	61	0.094	0.085
Hay	131	0.216	0.197
Hull	131	0.086	0.089
Nutmeat	131	0.022	0.016

PES =post extraction solid (after solvent extraction)

* Expressed in mg-imazapic equivalents/kg.

The extraction profiles of radioactive residues from peanut plant samples are summarized in Table 6.

The radioactive residue in peanut plant samples (green plant, hay, hull, and nutmeat) was initially extracted with methanol:water:acetone (1:1:1, v/v/v). Table 9 shows that 76–96% of the TRR in peanut samples was extracted with aqueous methanol:acetone. In order to get sufficient solid residue after solvent extraction for further process of the unextracted radioactivity, fresh peanut samples, with the exception of nutmeat, were extracted with methanol:water:acetone (1:1:1). The resultant residue after solvent extraction containing 6–20% of the TRR was then further processed by extraction with methanol:water (80:20) containing 2% hydrochloric acid. An additional 3–8% of the TRR was extracted into the acid methanol:water mixture. In the case of the green plant (0 DAT), the residue after solvent extraction with 2% HCl-methanol:water was subjected to a third extraction by using methanol:water (80:20) containing 0.5 M sodium hydroxide. An additional 30% of the unextracted fraction (1% of TRR) was soluble in the base methanol:water mixture. Following extraction of the hay with acid:methanol:water, the resulting residue was treated with *Aspergillus* cellulase at 37 °C for 72 hours which solublized about 36% of ^{14}C-residues in the marc (4% of TRR). The results suggest the presence of glycosidic linkage of the residues with endocons in the peanut hay.

Table 6 Extracted radioactive residues from peanut plant samples after 30days post-emergence treatment with [pyridine-6-^{14}C]-imazapic

Matrix	DAT	TRR Sum of Extracted Radioactive Residue and PES	Extracted Radioactive Residue (extracted with methanol:acetone:H_2O (1:1:1, v/v/v))		PES	
		(mg eq/kg)*	(mg eq/kg)*	(%TRR)	(mg eq/kg)*	(%TRR)
Plant	0	4.756	4.561	95.9	0.195	4.1
Plant	31	0.071	0.058	81.2	0.013	18.8
Plant	61	0.085	0.076	88.9	0.009	11.1
Hay	131	0.197	0.156	79.2	0.041	20.8
Hull	131	0.089	0.073	81.9	0.016	18.1
Nutmeat	131	0.016	0.012	76.4	0.004	23.6

DAT=days after treatment

PES =post extraction solid (after solvent extraction)

* Expressed in mg-imazapic equivalents/kg.

The nature of the radioactive components in the extracts of peanut samples was characterized first by matching the HPLC profile with the reference compounds. The identification of metabolites was then accomplished by isolating them from peanut plant (61 DAT) and harvest peanut hay. Structural characterization of the isolated metabolites was confirmed by mass spectral analysis. The results of identification and quantification are shown in Table 7.

The [^{14}C]-Imazapic accounted for 76% TRR in the green plant at 0 DAT, 2% in the plant at 31 DAT, 3% in the plant at 61 DAT, 3% in the hay, 2% in the hull, and 1% in the nutmeat. Residues of imazapic in all samples, except at 0 DAT, were < 0.01 mg/kg. This indicates imazapic was significantly metabolized.

The metabolite CL263284 accounted for 1% of the TRR (0.048 mg/kg) in the plant at 0 DAT, 12% (< 0.01 mg/kg) in the plant at 31 DAT, 12% (0.010 mg/kg) in the plant at 61 DAT, 28% (0.055 mg/kg) in the hay, 28% (0.025 mg/kg) in the hull, and 8% (0.001 mg/kg) in the nutmeat, showing increase over time.

The metabolite CL189215, which is glycoside of CL263284, accounted for 2% of the TRR (0.095 mg/kg) in the plant at 0 DAT, 32% (0.023 mg/kg) in the plant at 31 DAT, 46% (0.039 mg/kg) in the plant at 61 DAT, 16% (0.032 mg/kg) in the hay, 36% (0.032 mg/kg) in the hull, and 35% (0.006 mg/kg) in the nutmeat.

No other component of the extracted ^{14}C-residue from the immature plant and harvest samples exceeded 10% TRR as determined by HPLC. The concentration was all less than 0.01 mg eq/kg, with the exception of one component (0.014 mg eq/kg) detected in the hay.

Table 7 Characterization and identification of radioactive residues in peanut plant samples after 30 days post-emergence treatment with [pyridine-6-^{14}C]-imazapic

Component	Plant (0 DAT)		Plant (31 DAT)		Plant (61 DAT)		Hay (131 DAT)		Hull (131DAT)		Nutmeat (131DAT)	
	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)
Imazapic	3.615	76	0.001	2	0.003	3	0.006	3	0.002	2	< 0.001	1
CL 189215	0.095	2	0.023	32	0.039	46	0.032	16	0.032	36	0.006	35
CL 263284	0.048	1	0.009	12	0.010	12	0.055	28	0.025	28	0.001	8
Unknown	-	< 1	0.004	6	0.005	6	0.014	7	0.002	2	< 0.001	1

DAT=days after treatment

Bermuda grass

A metabolism study was conducted to investigate metabolism of [pyridine-6-^{14}C]-imazapic in Bermuda grass (Fung, 1999a). The Bermuda grass was treated when approximately 51 cm tall (62 days after sprigging) with a blank formulation for the control plot or a single post-emergence application of [^{14}C]-imazapic at an actual rate of approximately 203 g ai/ha and a spray volume of 300 L/ha for the treated plot. The field portion of the study was conducted in outdoor test plots. Both treated and untreated plots had total area of 0.9 × 4.6 m of loam soil. Bermuda grass forage samples were collected within three hours after application (0 DAT) and at 15, 32, and 49 DAT. Bermuda grass foliage for hay samples was cut at 68 days after treatment.

Samples were frozen after collection and shipped to the analytical laboratory over dry ice. Bermuda grass samples were cut with a pair of shears into small pieces (approximately 5 cm long) and ground to fine powder in two steps with dry ice. The TRR in all plant parts were determined by combustion. Additionally, the Bermuda grass samples were each extracted sequentially with an aqueous methanol/acetone mixture followed by sequential extraction with acidified aqueous methanol, aqueous acid, and aqueous base. The post-extraction solids were treated sequentially with enzymes, 10 N NaOH reflux, and 6 N HCl reflux to attempt releasing of the bound residues. The aqueous methanol/acetone extract, the combined sequential extracts and the post-extraction solids wash were each analysed by HPLC on a reversed phase C_{18} column.

A summary of the TRR in Bermuda grass samples collected at various time intervals is shown in Table 8. The increased residue concentration in the hay compared to 49 DAT forage is attributed to the dehydration of the grass.

Table 8 TRR in Bermuda grass samples after post-emergence treatment with [pyridine-6-^{14}C]-imazapic

Matrix	Days after Treatment	TRRs in Treated Sugar Cane Matrices	
		Determined by Direct Combustion (mg eq/kg)*	Sum of Extracted Radioactive Residue and PES (mg eq/kg)*
Forage	0	8.25	8.29
Forage	15	4.58	3.92
Forage	32	2.73	2.42
Forage	49	0.77	0.78
Hay	68	0.92	0.83

PES =post extraction solid (after solvent extraction)

* Expressed in mg-imazapic equivalents/kg.

The extraction profiles of the radioactive residues from Bermuda grass are summarized in Table 9.

The incurred residues extracted by the neutral aqueous methanol/acetone solvent decreased from 98% for the 0 DAT sample, to the range of 69% to 51% for the 15 to 49 DAT samples, and about 35% for the hay sample at 68 DAT. A significant amount of the radioactivity ranging from

12.6% TRR in the 15 DAT sample to 27% TRR in the 68 DAT hay was extracted with dilute acid and base. Thus, the total extracted radioactive residues were 84% to 101% of the TRR in forage, and 74% of the TRR in hay.

The remaining radioactivity in the residues after solvent extraction (PES) accounted for about 2–3% of the TRR in the 15 and 32 DAT samples, 10% of the TRR in the 49 DAT sample, and 31% of the TRR in the 68 DAT sample. Treatment of the PES with enzymes cellulase and pepsin released only a very small amount of the bound residues (< 5% TRR) suggesting that the unextracted residues in the PES did not contain glycoside or peptide bonds. Following enzymatic treatment, the resulting solids were subjected to base (NaOH) and acid (HCl) hydrolyses under reflux. The acid and base hydrolyses steps also released a very small amount of radioactive residues: < 1% TRR in the 15 and 32 DAT samples, 4.3% TRR in the 49 DAT sample, and 8.0% TRR in the 68 DAT sample. The results suggest that portions of the radiolabelled carbon have been incorporated into undefined plant constituents.

Table 9 Extracted radioactive residues from Bermuda grass samples after post-emergence treatment with [pyridine-6-^{14}C]-imazapic

Matrix	Days after Treatment	TRR	Distribution of Radioactive Residues									
			MeOH:Acetone: H2O Extract		Extracts [a] (MeOH:HCl:H_2O, 1 N HCl, 1 N NaOH)		Rinse of PES by Methanol:Water (1:1)		Extracted Radioactive Residue		PES after Rinse	
		(mg/kg)*	(mg/kg) *	(%TRR)	(mg/kg) *	(%TRR)	(mg/kg) *	(%TRR)	(mg/kg) *	(%TRR)	(mg/kg) *	(%TRR)
Forage	0	8.29	8.05	97.6	0.22	2.7	0.02	0.3	8.29	100.6	< 0.01	0.1
Forage	15	3.92	3.14	68.6	0.58	12.6	0.14	3.0	3.86	84.2	0.12	2.7
Forage	32	2.42	1.70	62.1	0.43	15.6	0.20	7.2	2.33	84.9	0.06	2.3
Forage	49	0.78	0.39	51.2	0.21	27.3	0.11	13.7	0.71	92.2	0.08	10.2
Hay	68	0.83	0.32	34.8	0.25	27.0	0.11	12.4	0.68	74.2	0.29	31.2

TRR=calculated sum of the extracted residues (MAH, MHH and PES wash), different solubilizates from the PES and the final residue (values not given in this table)

PES =post extraction solid (after solvent extraction)

[a] combined sequential extracts of (1) methanol: HCl: H_2O, (2) 1 N HCl and (3) 1 N NaOH

* Expressed in mg-imazapic equivalents/kg.

The chemical structure of the parent compound and its putative Bermuda grass metabolites were confirmed by the characteristic ions detected in the Liquid Chromatography - Electrospray Positive Ion Mass Spectrometry (LC-ESP/PIMS) of the purified metabolites and, where available, by authentic reference standard compounds.

When the aqueous methanol/acetone extract, the combined sequential extracts and the post-extraction solids wash were each analysed by HPLC on a reversed phase C_{18} column, there were 11 discernible regions or peaks, designated as M1 (the most polar) through M10 (the least polar), detected on the radiochromatograms. The chromatographic patterns were compared with those obtained from the known reference compounds including parent compound imazapic, hydroxymethyl analog CL 263284, glucose conjugate CL 189215, 3,5-dicarboxylic acid analog CL 312622, CL 290610 and CL 397695. Chromatography of the isolates of some of the regions or peaks by HPLC on Hypercarb graphitic column and other solid support showed that these peaks were comprised of multiple components as illustrated below:

The enzymes, acid, and base hydrolyzates of the samples from Bermuda grass hay and forage contained radioactivity which was too low to conduct HPLC analysis.

The metabolites detected in Bermuda grass forage and hay are summarized in Table 10.

Imazapic was extensively metabolized in Bermuda grass and accounted for only about 6% of the TRR at 15 DAT. Quantitatively, CL 263284 and CL 189215 were the major Bermuda grass metabolites of imazapic. Although M1 accounted for up to 26.8% of TRR in the grass samples at various time intervals, it was comprised of at least twelve groups of multi-radioactive components.

The 0 DAT (about three hours after application) sample extracts showed a main chromatographic peak that coincided with the standard of the parent compound (89.3% of TRR). In addition, several radioactive residue components including hydroxymethyl metabolite CL 263284 (0.7% TRR) and its glucose conjugate CL 189215 (1.0% TRR) and minor residue fractions M2, M3, M3A, M6, and M8 through M10 of varying quantities were detected. The proportions of these radioactive residue fractions/or metabolites changed with time.

At 15 DAT, the hydroxymethyl metabolite CL 263284 became the single most prominent residue component (30.2% TRR) and the quantity was maintained at > 20% TRR at 32 and 49 DAT, then declined to 8.4% TRR at 68 DAT. The increase of CL 263284 and its glucoside conjugate CL 189215 contents in the grass sample coincided with the decline of the parent compound from about 89% of the TRR at three hours after application to 6% TRR at 15 DAT, 3% TRR at 49 and 2% TRR at 68 DAT.

In addition to the CL 263284 metabolite, a significant amount of highly polar metabolites in the M1 fraction were formed at 15 DAT (20.6% TRR) and at 49 and 68 DAT (~27% TRR). Among at least 12 groups of multi-radioactive components of M1 fraction, groups M1-B and M1-C were present at about 0.1 mg/kg at 15 DAT. Their chemical structures are shown to be multi-substituted pyridine derivatives. M1-B was characterized as 5-hydroxymethyl-nicotinamide derivative and additionally M2-A 5-hydroxymethylquinolic acid anhydride. Some other minor factions were also identified.

Table 10 Characterization and identification of radioactive residues in Bermuda grass samples after post-emergence treatment with [pyridine-6-^{14}C]-imazapic

Components	Forage (0 DAT)		Forage (15 DAT)		Forage (32 DAT)		Forage (49 DAT)		Hay (68 DAT)	
	(mg/kg)*	(%TRR)**	(mg/kg	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR0
Imazapic (M7)	7.37	89.3	0.27	5.9	0.08	3.1	0.02	2.6	0.02	2.3
M1 (>12 groups of multi-components)	0.24	3.0	0.95	20.6	0.67	24.2	0.20	26.8	0.25	26.9
M2 (M2-A + M2-B)	0.10	1.3	0.29	6.4	0.13	4.9	0.04	4.9	0.02	2.6
M3 (> 5 components)	0.04	0.5	0.18	4.0	0.19	6.9	0.02	3.0	0.02	2.5
M3A (~ 28 components)	0.03	0.4	0.10	2.2	0.11	4.0	0.05	7.1	0.04	4.0
CL189215 (M4)	0.08	1.0	0.17	3.9	0.14	5.3	0.08	9.2	0.08	9.2
CL263284 (M5)	0.06	0.7	1.38	30.2	0.60	22.0	0.16	20.5	0.08	8.4
M6 (>12 components)	0.08	0.9	0.18	4.0	0.12	4.4	0.05	7.1	0.04	4.8
M8 (> 6 components)	0.13	1.5	0.13	2.7	0.12	4.4	0.02	2.3	0.04	4.6
M9 (>18 components)	0.05	0.6	0.08	1.8	0.07	2.7	0.02	3.2	0.03	3.0
M10 (>16 components)	0.05	0.6	0.07	1.6	0.07	2.6	0.03	3.7	0.03	2.8
Total	8.23	99.8	3.80	83.3	2.30	84.5	0.69	90.4	0.65	71.1

* The values of mg/kg for individual metabolites (M1 to M10) were the sum of mg/kg values obtained from the aqueous methanol-acetone extract, the combined sequential extracts and the post-extraction solids wash except that in case of < 0.01 mg/kg in one of the extract, the sum of mg/kg values was calculated from the sum of % TRR times TRR in mg/kg in the grass or hay.

** The values of % TRR for individual metabolites (M1 to M10) were the sum of % TRR values obtained from the aqueous methanol/acetone extract, the combined sequential extracts and the post-extraction solids wash.

Transgenic soya beans

A metabolism study was conducted in 2011 to investigate metabolism of [pyridine-3-^{14}C]-imazapic in transgenic soya bean variety BPS-CV127-9 in which the csr1-2 gene encoding an altered AtAHASL protein was inserted to make the host tolerant to imidazolinone herbicides (Dohn and Estigoy, 2012a). [^{14}C]-imazapic in DG formulation was sprayed once at BBCH growth stage 65 with an application rate of 80.1 g/ha to the above-ground portion of soya beans plants. This application rate is equivalent to 4.6 times the maximum anticipated label rate. Soya bean forage was harvested approximately one hour after application and hay was harvested 35 days after treatment. Soya bean straw, pods, and seeds were harvested when mature, 97 days after treatment.

Samples were frozen after collection and shipped to the analytical laboratory over dry ice. They were ground to a fine consistency in the presence of solid CO_2 using a food processors. The moisture content of hay and seed was measured by drying subsamples at 105 °C. Portions (25 g of forage, hay, straw, and pods, 50.7 g of soya bean seed) of the processed samples were subjected to sequential solvent extraction. Samples were extracted sequentially by 100% methanol (two times), followed by 100% water and methanol:water:1N HCl (60:39:1,v:v:v). The radioactive residue remaining in the PES samples was measured by combustion analysis and LSC counting. The TRR were calculated as the sum of extracted radioactivity (ERR) and radioactivity recovered in the PES.

The TRR of soya bean forage was 0.689 mg eq/kg, soya bean hay 0.241 mgeq/kg, soya bean seed 0.014 mg eq/kg, soya bean straw 0.092 mg eq/kg and soya bean pod 0.042 mg eq/kg as shown in Table 11. The extraction procedure released 77.8% to 98.4% of the TRR.

Table 11 TRR in transgenic soya bean samples following foliar application of [pyridine-3-^{14}C]-imazapic

Matrix	Days after Treatment	TRRs in Treated Soya Bean Matrices	
		Determined by Direct Combustion (mg eq/kg)*	Sum of Extracted Radioactive residue and PES (mg eq/kg)*
Forage	0	0.689	0.673
Hay	35	0.241	0.240
Seeds	97	0.014	0.015
Straw	97	0.092	0.088
Pods	97	0.042	0.045

PES = post extraction solid (after solvent extraction)

* Expressed in mg-imazapic equivalents/kg.

The extraction profiles of radioactive residues from soya bean forage, hay, straw, hull and seed are summarized in Table 12. As a consequence of low residual radioactive residues (PES), no further processing was conducted on PES.

The residue extracted from forage 0 DAT was 0.662 mg eq/kg (98.4% of the TRR calculated to be 0.673 mg eq/kg). The methanol extract contained 92.1% of the radioactive residues (0.620 mg eq/kg), the aqueous fraction 5.2% (0.035 mg eq/kg), and the acidic methanol 1.1% of the radioactivity (0.007 mg eq/kg). The PES contained residue of 0.011 mg eq/kg (1.6% of the TRR).

The residue extracted from hay 35 DAT was 0.221 mg eq/kg (92.1% of the TRR calculated to be 0.270 mg eq/kg). The methanol extracts contained 76.7% of the radioactive residues (0.184 mg eq/kg) and the aqueous fraction 12.9% (0.031 mg eq/kg). The acidic methanol extracted 2.5% of the radioactivity (0.006 mg eq/kg) and was not further analysed. The PES contained residue of 0.019 mg eq/kg (7.9% of TRR).

The residue extracted from seeds 97 DAT was 0.013 mg eq/kg (86.7% of the TRR calculated to be 0.015 mg eq/kg). The methanol extracts contained 46.7% of the radioactive residues (0.007 mg eq/kg) and the aqueous fraction 26.7% (0.004 mg eq/kg). The acidic methanol extracted 13.3% of the radioactivity (0.002 mg eq/kg). The PES had 0.002 mg eq/kg of radioactive residue (13.3% of TRR).

The residue extracted from straw 97 DAT was 0.072 mg eq/kg (81.8% of the TRR calculated to be 0.088 mg eq/kg). The methanol extracts contained 59.1% of the radioactive residues (0.052 mg eq/kg) and the aqueous fraction 17.0% (0.015 mg eq/kg). The acidic methanol extracted 5.7% of the radioactivity (0.005 mg eq/kg). The PES had 0.016 mg eq/kg of radioactive residue (18.2% of TRR).

The residue extracted from pods 97 DAT was 0.035 mg eq/kg (77.8% of the TRR calculated to be 0.045 mg eq/kg). The methanol extracts contained 33.3% of the radioactive residues (0.015 mg eq/kg) and the aqueous fraction 24.4% (0.011 mg eq/kg). The acidic methanol extracted 20.0% of the radioactivity (0.009 mg eq/kg). The PES had 0.010 mg eq/kg of radioactive residue (22.2% of TRR).

Table 12 Extracted radioactive residues from transgenic soya bean samples following foliar application of [pyridine-3-^{14}C]-imazapic

Matrix	Days after Treatment	TRR Sum of ERR and PES	Distribution of Radioactive Residues									
			Combined Methanol Extract		Combined Aqueous Extract		Acidic Methanol Extract		Extracted Radioactive Residue		PES	
		(mg/kg)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)
Forage	0	0.673	0.620	92.1	0.035	5.2	0.007	1.1	0.662	98.4	0.011	1.6
Hay	35	0.240	0.184	76.7	0.031	12.9	0.006	2.5	0.221	92.1	0.019	7.9
Seeds	97	0.015	0.007	46.7	0.004	26.7	0.002	13.3	0.013	86.7	0.002	13.3
Straw	97	0.088	0.052	59.1	0.015	17.0	0.005	5.7	0.072	81.8	0.016	18.2
Pods	97	0.045	0.015	33.3	0.011	24.4	0.009	20.0	0.035	77.8	0.010	22.2

PES = post extraction solid (after solvent extraction)

(mg/kg): Expressed in mg-imazapic equivalents/kg.

The methanol and water extracts of the forage and hay samples were combined and, after concentration, characterized by reverse phase HPLC. The radioactive residue detected in the acidic extract of soya bean forage and hay was insignificant and no further analyses were performed on these extracts. Representative portions (20% of each extract) of methanol, aqueous and acidic methanol extracts of the soya bean straw, soya bean pods and soya bean seeds were combined and liquid/liquid extracted with hexane. The hexane and the aqueous/methanol phases were assayed by LSC. No radioactive residue was detected in the hexane portions and no further analysis was done on these fractions. The aqueous/methanol portions were concentrated and characterized by reverse phase HPLC. Imazapic and the metabolites CL263284 and CL189215 were identified by co-chromatography of the extracts with reference standards. The results of identification and quantification are shown in Table 13.

A mixture of the methanol and water extracts of forage (0 DAT) containing 0.655 mg eq/kg of residue was analysed. The primary component of the residue was imazapic (0.605 mg/kg, 89.9% of the TRR) with other minor components that did not individually exceed 3.4% TRR. The total identified and characterized residues were 97.3% of the TRR.

The most abundant component of the residue in a mixture of the methanol and water extracts of hay (35 DAT) was imazapic (0.087 mg/kg, 36.3% of the TRR). Imazapic was metabolized to a large number of components including a low amount of a component with the retention time matching that of the analytical standard CL189215, the glucoside of hydroxymethyl imazapic (CL263284) (0.006 mg/kg, 2.5% of the TRR). The total identified and characterized residues were 90.4% of the TRR.

The analysis of a mixture of methanol, water and acidic extracts of seeds (97 DAT) indicated that imazapic was metabolized to a large number of components. Imazapic comprised 0.0030 mg/kg (20.0% of the TRR), and imazapic metabolites CL189215, the glucoside, and CL263284, hydroxymethyl imazapic, comprised 4.7% TRR (0.0007 mg/kg) and 0.7%TRR (0.0001 mg/kg), respectively. A significant radioactive peak (0.0034 mg eq/kg, 22.7% TRR) was very polar and consisted of multiple components by analysis using a Hypercarb method. Multiple minor components were detected at very small concentrations (≤ 0.0014 mg eq/kg). The total identified and characterized residues were 88.7% of the TRR.

Imazapic was the most abundant component of the residue at 0.013 mg/kg (14.8% of the TRR) in a mixture of methanol, water and acidic extracts of straw (97 DAT). The imazapic metabolites CL189215 and CL263284 comprised 6.8% TRR (0.006 mg/kg) and 2.3%TRR (0.002 mg/kg), respectively. The amount of polar radioactive residue (0.004 mg/kg, 4.5% TRR) was less than that seen in the seed and pod. Multiple minor components indicated that metabolism of imazapic was complex and extensive. The total identified and characterized residues were 84.1% of the TRR.

Imazapic comprised 0.004 mg/kg (8.9% of the TRR), and imazapic metabolites CL189215, the glucoside, and CL263284, hydroxymethyl, comprised 4.4% TRR (0.002 mg/kg) and 2.2% TRR (0.001 mg/kg), respectively in a mixture of methanol, water and acidic extracts of pods (97 DAT). As in the seed, significant radioactive residue (0.007 mg/kg, 15.6% TRR) was polar. Multiple minor degradates were present as seen in the straw and bean samples. The total identified and characterized residues were 80.0% of the TRR.

Table 13 Characterization and identification of radioactive residues in transgenic soya bean samples following foliar application of [pyridine-3-^{14}C]-imazapic

Components	Forage 0 DAT		Hay 15 DAT		Seeds 32 DAT*		Straw 49 DAT*		Pods 68 DAT*	
	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)
Imazapic	0.605	89.9	0.087	36.3	0.0030	20.0	0.013	14.8	0.004	8.9
CL189215	ND		0.006	2.5	0.0007	4.7	0.006	6.8	0.002	4.4
CL263284	ND		ND		0.0001	0.7	0.002	2.3	0.001	2.2
M5	ND		ND		0.0004	2.7	ND		0.002	4.4
M6	ND		0.004	1.7	0.0009	6.0	0.005	5.7	0.001	2.2
M18-19	ND		0.004	1.7	0.0001	0.7	0.007	8.0	0.003	6.7
M23	ND		ND		ND		0.005	5.7	ND	
M25-26	ND		0.008	3.3	0.0003	2.0	0.002	2.3	0.001	2.2
M35	ND		0.006	2.5	0.0003	2.0	ND		ND	
M36	ND		0.022	9.2	ND		0.009	10.2	ND	
M37	ND		0.004	1.7	ND		ND		ND	
M39-40	ND		0.0018	7.5	ND		0.006	6.8	0.001	2.2
M42	0.005	0.7	ND		ND		ND		ND	
M45-46	0.007	1.0	0.007	2.9	0.0007	4.7	0.004	4.5	0.001	2.2
M52	0.016	2.4	ND		ND		ND		ND	
M53/M63	0.023	3.4	ND		0.0003	2.0	ND		ND	
Total of the above	0.655	97.3	0.176	73.3	0.0102	68.0	0.057	64.8	0.023	51.1
Other characterized components at concentration 0.003 mg/kg	None		0.015	6.3	none		0.003	3.4	none	
Other characterized components at concentration ≤0.002mg/kg	None		0.026	10.8	0.003	20.0	0.014	15.9	0.013	28.9
Total identified and characterized	0.655	97.3	0.217	90.4	0.0133	88.7	0.074	84.1	0.037	80.0
Acidic methanol extraction	0.007	1.0	0.006	2.5	Included in the above		Included in the above		Included in the above	

* The extracts (methanol + aqueous + acidic) for these samples were combined and analysed by HPLC

ND = not detected

No phytotoxicity was observed in this study.

Despite extensive metabolism, imazapic was the main component in all soya bean matrices. The concentration of imazapic declined from 0.605 mg/kg in forage collected approximately 1 hour

after application to 0.087 mg/kg in the hay sample collected 35 days after application, and then to 0.013 mg/kg in the straw sample collected 97 days after application. Imazapic measured as a percentage of the TRR declined from 89.9% of the TRR in forage to 36.3% of the TRR in hay, and then to 14.8% of the TRR in straw. The imazapic concentration was 0.0030 mg/kg (20.0% of the TRR) in seed, and 0.004 mg/kg (8.9% of the TRR) in pods.

Proposed metabolic pathway of imazapic in plants

[^{14}C]-Imazapic used for pre-emergence treatment of sugar cane plant or post-emergence treatment of peanut, Bermuda grass plants or transgenic soya bean, was rapidly metabolized over time with significant decline of imazapic concentration and increase of the concentrations of the 5-hydroxymethyl metabolite CL263284 and its glucoside CL189215. There were very low radioactive residues remaining in the soya bean seeds, peanut seeds and sugar cane stalks at the time of harvest. Except for Bermuda grass, the predominant residue was either imazapic, CL263284 or CL189215 with varying ratios. A number of more polar components were found from the extracts of soya bean and Bermuda grass matrices. Such polar components found from the Bermuda grass extracts include nicotinic acid derivatives, 5-hydroxymethylniconinamide derivative and 5-hydroxymethylquinolia acid anhydride, potentially oxidized from the hydroxymethyl metabolite.

Imazapic was very rapidly metabolized in plants by oxidative hydroxylation of the 5-methyl substituent to form a 5-hydroxymethyl metabolite CL263284. Subsequently, the hydroxymethyl metabolite was either rapidly conjugated with glucose to produce a prominent metabolite component CL 189215 or further metabolized to form more polar components.

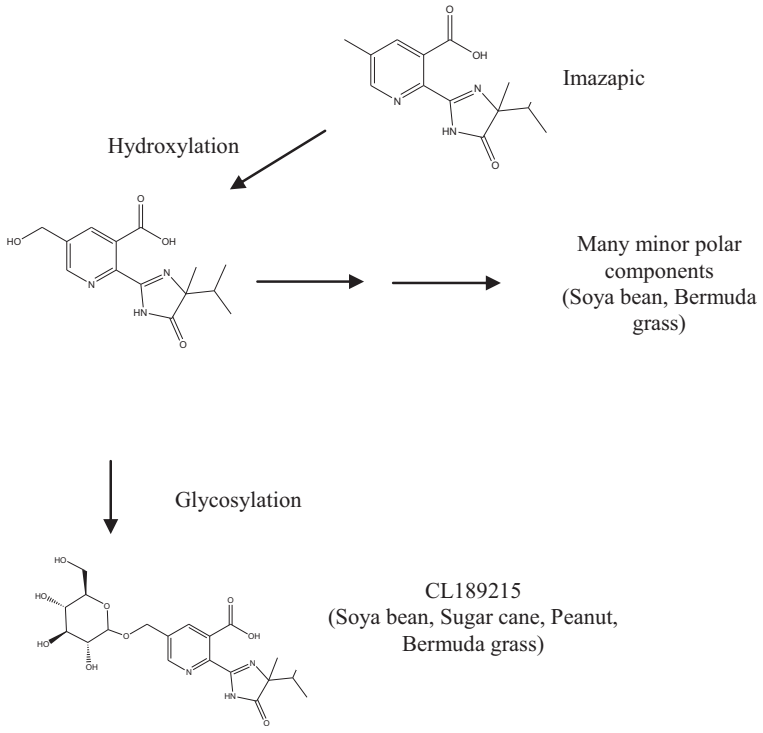


Figure 2 Proposed metabolic pathway of imazapic in plants

Environmental fate in soil

The Meeting received information on aerobic soil metabolism, photodegradation on soil, anaerobic aquatic metabolism, hydrolysis, photodegradation in water and residues in succeeding crops. Since imazapic is a herbicide with ground or aerial spay, aerobic degradation in soil, soil photolysis (some

included in the Physical and Chemical Properties Section), hydrolysis rate and products (included in the Physical and Chemical Properties Section) and rotational crop were relevant to the current evaluation.

Aerobic soil metabolism

The characteristics of soils used in the aerobic soil metabolism studies are summarized in Table 14.

Table 14 Characteristics of soils used in laboratory studies

Study reference	Location	Type	% Sand	% Silt	% Clay	Bulk Density (dry soil; g/cc)	CEC (MEQ/100g)	% Moisture at 1/3 bar	% Organic Matter	pH
Madsen, 1993 a	Princeton	Sandy Loam	60	30	10	1.26	8.2	17.2	1.3	5.2
Ta, 1997	Princeton	Sandy Loam	62	25	13	-	9.1	14.1	1.5	6.8 [b]
Ta, 1994b	Princeton	Sandy Loam	55	29	16	-	5.1	14.7	0.6	5.4 [b]

- = not reported

[a] IA-620-003 and IA-630-001

[b] pH in $CaCl_2$

Study 1 (Madsen, 1993a)

The aerobic soil metabolism of ^{14}C-Imazapic was studied in Princeton sandy loam soil. [Pyridine-6-^{14}C]-imazapic was dissolved in acetonitrile and applied to the soil at the rate equivalent to 0.14 kg/ha and kept under aerobic conditions in the dark. Water was added to the soil to bring the moisture of the soil to 75–80% of field capacity. At intervals of 0, 7, 14 31, 62, 91, 122, 183, 274, and 365 days after soil treatment, the potassium hydroxide traps were analysed for $^{14}CO_2$ by LSC. At the same intervals, the ethylene glycol and sulphuric acid traps were assayed for the ^{14}C-volatile materials.

The soil was extracted three times with 0.5N aqueous sodium hydroxide and the humic acid was removed by acid precipitation. The aqueous fulvic acid fraction was partitioned with methylene chloride. The humic acid fraction was rinsed with ethyl acetate and the rinse was combined with the methylene chloride partition. The methylene chloride partition was then poured through sodium sulfate. The sodium sulfate was then rinsed with methylene chloride. The extract was then evaporated to dryness. The residue was dissolved in methanol:water (1:1 v:v) and aliquots were analysed by LSC. The extracted soil was finally combusted to determine the bound residues. The solvent extractions were concentrated and analysed by HPLC.

After 12 months of incubation approximately 0.9% of the applied dose was mineralized to $^{14}CO_2$. No other radiolabelled volatile compounds were detected, which indicates that volatilization of the parent compound and other metabolites did not occur. Approximately 70.8% of the applied imazapic remained intact after 12 months. Carbon dioxide was the only major metabolite detected which derived from ^{14}C-imazapic. No significant degradation of imazapic occurred during 12 months of incubation. The unextracted radioactivity varied from 1.8 to 8.0% of the total applied dose during the 365 days of incubation (Table 15).

Table 15 Material balance for aerobic soil metabolism of [pyridine-6-^{14}C]-imazapic

DAT	% of Applied radioactivity										
	Total extracted	Soil extract I	Imazapic	Humic acid	Soil extract II	Imazapic	Unextracted residue	KOH	H_2SO_4	Ethylene glycol	Total
0	94.3	94.3	84.1	4.7	-	-	5.7	0.0	0.0	0.0	100.0
7	92.7	92.7	81.6	6.7	-	-	7.3	0.3	0.0	0.0	100.3
14	100.3	100.3	77.4	8.3	-	-	7.3	0.3	0.0	0.0	107.9
31	100.9	100.9	77.0	5.4	-	-	3.6	0.4	0.0	0.0	104.9
62	83.7	83.7	71.5	10.8	-	-	5.8	0.4	0.0	0.0	90.0

DAT	% of Applied radioactivity										
	Total extracted	Soil extract I	Imazapic	Humic acid	Soil extract II	Imazapic	Unextracted residue	KOH	H_2SO_4	Ethylene glycol	Total
91	81.3	81.3	76.8	5.0	-	-	8.0	0.6	0.0	0.0	89.9
122	91.9	79.5	71.2	8.3	12.4	6.2	4.3	0.6	0.0	0.0	96.8
183	96.2	86.3	60.6	9.2	9.9	4.9	5.5	0.8	0.0	0.0	102.5
274	85.2	85.2	71.8	6.7	-	-	4.7	0.8	0.0	0.0	90.7
365	96.0	83.5	62.4	12.9	12.5	8.4	1.8	0.9	0.0	0.0	98.7
										Mean:	98.1

DAT=days after treatment

Imazapic was found to be very persistent in soil under an aerobic condition, with about 70% remaining after 1 year.

Study 2 (Ta, 1997a)

The aerobic soil metabolism of [pyridine-6-^{14}C]-imazapic and its metabolites, CL263284, CL312622 and CL299263 was studied in Princeton Sassafras sandy loam soil. Each of the radiolabelled compounds listed above was dissolved in water and each solution was applied separately to the soil at the rate equivalent to 0.14 kg/ha and kept under aerobic conditions at 25 °C in the dark. Water was added to the soil to bring the moisture of the soil to 75% of 0.33 bar. At intervals of 0, 3, 7, 14, 21, 28, 60, 90 and 120 days after treatment with radiolabelled imazapic, CL 263284, CL 312622 and CL 299263, the sodium hydroxide traps were analysed for $^{14}CO_2$ by LSC. At the same intervals, the extracted foam plugs and ethylene glycol traps were assayed for the ^{14}C-volatile materials. For each compound the soil was extracted three times with 0.5 N aqueous sodium hydroxide. The extracted radioactive residues was filtered through C-18 OH then eluted with methanol:water (1:1 v/v). The solvent was concentrated and analysed by HPLC and HPLC/MS. The extracted soil was combusted to determine the bound residues. No samples were taken at 3 and 7 days for CL 299263 and at 60 days for CL 312622. One sterile soil sample from each incubation group at 28 and 60 days after incubation were analysed as described above.

At 120 days after incubation under non-sterile conditions, approximately 11, 30, 43, and 41% of the applied dose was recovered as $^{14}CO_2$ from ^{14}C-imazapic, CL 299263, CL 263284, and CL 312622, respectively (Table 16). No volatile compounds were detected in the glycol traps or in the foam plugs from any of the compounds tested. Under sterile conditions, no CO_2 or volatiles were detected in any traps. At 120 days after incubation under non-sterile condition approximately 81% of the parent was still present. Several metabolites less than 5%TAR such as CL 312622 (1.7%) and CL 354825 (4.1%) were also generated. Degradate CL 263284 was formed in a small amount but as data showed its instability, it was not included in the Table.

Approximately 80, 10, 0 and 4% of the applied dose of imazapic, CL 299263, CL 263284, and CL 312622, respectively, remained after 120 days. Under sterile conditions, the total recoveries of radioactivity ranged from approximately 92–95, 99–104, 99–104, and 86–90% of the applied dose of imazapic, CL 299263, CL 263284 and CL 312622, respectively.

Table 16 Material balance and ratio of degradates found in aerobic soil metabolism of [pyridine-6-^{14}C]-imazapic and its metabolites.

% of Total Applied Radioactivity							
Treatment with ^{14}C-imazapic							
DAT	Imazapic	CL312622	CL354825	Others	CO_2	Unextracted	Total
0	96.0	0.6	0.1	3.4	0.0	0.7	101
3	91.6	0.9	0.5	4.2	1.6	1.9	101
7	86.7	1.4	1.8	4.9	3.2	3.0	101
14	87.5	1.7	1.5	3.5	4.4	3.4	102
21	81.4	2.3	1.9	3.8	5.1	3.8	99
28	82.4	1.9	1.6	4.1	6.1	4.5	101
60	81.4	1.9	2.9	3.6	7.4	5.5	102
90	81.8	2.0	3.1	2.7	9.2	5.4	104
120	80.5	1.7	4.1	3.2	10.9	6.1	106

% of Total Applied Radioactivity							
Treatment with ^{14}C-imazapic							
DAT	Imazapic	CL312622	CL354825	Others	CO_2	Unextracted	Total
Sterile							
28	88.8	0.6	0.0	2.8	0.0	2.7	95
60	84.3	1.5	0.6	2.4	0.0	3.1	92
Treatment with ^{14}C-CL299263							
DAT	CL299263	CL312622	CL354825	Others	CO_2	Unextracted	Total
0	89.6	3.2	1.0	2.4	0.0	2.1	98
14	35.9	43.6	7.2	4.5	2.5	5.5	99
28	18.2	41.1	15.0	9.4	7.1	5.8	97
60	15.3	26.1	24.0	8.4	15.6	8.6	98
90	9.5	6.7	33.6	7.9	25.4	9.1	93
120	10.1	5.0	34.0	6.4	29.8	10.2	96
Sterile							
28	96.2	3.7	0.0	2.5	0.0	2.4	104
60	89.2	3.4	0.0	2.6	0.0	3.5	99
Treatment with ^{14}C-CL263284							
DAT	CL263284	CL312622	CL354825	Others	CO_2	Unextracted	Total
0	87.0	7.7	0.2	3.6	0.0	0.9	99
3	0.8	88.0	1.0	5.4	1.6	1.8	99
7	0.9	82.2	3.3	6.5	3.2	2.4	99
14	0.0	77.2	5.8	6.2	4.9	3.4	98
21	0.0	65.7	10.5	9.2	7.9	4.0	97
28	0.0	60.5	12.6	9.5	9.7	5.4	98
60	0.0	31.0	24.7	16.4	21.2	7.3	101
90	0.0	10.5	30.2	13.6	32.2	7.8	95
120	0.0	6.8	31.7	12.9	42.8	8.0	102
Sterile							
28	88.6	4.9	0.0	7.6	0.0	2.7	104
60	82.8	5.4	0.0	7.6	0.0	3.4	99
Treatment with ^{14}C-CL312622							
DAT	CL312622	CL354825		Others	CO_2	Unextracted	Total
0	98.5	0.7		3.7	0.0	1.1	104
3	93.1	1.5		3.8	0.4	1.7	101
7	88.7	5.0		2.5	1.9	2.7	101
14	75.9	7.7		3.8	4.5	3.8	96
28	57.6	15.1		5.9	10.0	6.0	95
90	14.9	35.3		7.6	29.5	9.7	97
120	3.6	37.9		6.9	40.6	9.4	99
Sterile							
28	89.0	0.0		0.0	0.0	1.0	91
60	78.8	0.0		0.0	6.2	1.0	87

DAT=days after treatment

The data were evaluated by plotting the remaining percentage of imazapic, CL299263, CL263284, and CL312622 versus time and the half-lives were estimated assuming a first order kinetic. The half-life of imazapic, CL 299263, CL 263284, and CL 312622 were 133, 12, < 0.5 and 36 days, respectively. For all four compounds studied the unextracted radioactivity varied from 0.7 to 10.2% of the total applied dose during the 120 days of incubation.

Under sterile conditions, all of the compounds tested remained intact after 2 months of incubation and there was no detectable level of evolved CO_2 trapped in NaOH. The results indicate that microbial activity is required for the degradation of imidazolinone herbicides in soil.

Photodegradation on soil surface

The soil photolysis of [pyridine-6-^{14}C]-Imazapic was studied in Princeton Sassafras sandy loam soil (Ta, 1994b). The ^{14}C-Imazapic was dissolved in water and applied to the soil at the equivalent rate of about 0.08 kg/ha and exposed continuously for up to 30 days by light from a xenon-arc lamp which had been filtered to remove wavelengths shorter than 290 nm. Control samples were maintained in the

dark. The temperature was maintained at 25°C during the course of the study. The output of the lamp was set to 0.35 w/m^2 at 340 nm, which is comparable to mid-autumn sunlight in Princeton, NJ, USA. At intervals of 0, 1, 3, 7, 14, 21 and 30 days after treatment of soil with ^{14}C-imazapic the soil samples were extracted two times with 0.5 M aqueous sodium hydroxide. The NaOH extracts were combined and the pH was adjusted to 1.7 with HCl, and then filtrated through celite. The clear filtrates were then subjected to solid phase extraction using C18-OH three times with methanol followed by three washings with acidified (pH 1. 7) Millipore water. The radioactivity was eluted from the cartridges with methanol/water (1/1 v/v). The extract was concentrated and analysed by TLC and HPLC. The soil were dried and then assayed by combustion to determine the bound residues.

A mass balance and the ratio of degradates at each sampling point is shown in Tables 17 and 18. One major product was formed, the diacid CL312622 which accounted for up to 11% of the applied dose. Several minor photoproducts formed, each of which accounted for less than 6.2% of applied dose. The control samples were stable, with less than 4% degradation, throughout the course of the study.

Table 17 Mass balance of photodegradation of imazapic

Days	% of Total Applied Dose					
	Irradiated			Control		
	Extracted	Unextracted	Total	Extracted	Unextracted	Total
0	100.8	0.5	101.2	100.8	0.5	101.2
1	97.0	2.3	99.2	98.6	0.9	99.4
3	94.8	3.2	98.0	101.6	1.3	102.8
7	93.0	2.7	95.7	95.6	1.5	97.0
14	90.7	3.2	93.9	94.5	1.8	96.3
21	92.1	3.0	95.2	96.7	2.7	99.3
30	90.2	3.3	93.5	94.9	2.8	97.7

Table 18 Ratio of photodegradation products

Days	% of Total Extract			
	Irradiated		Control	
	Imazapic	CL312622	Imazapic	CL312622
0	94.0	2.1	94.0	2.1
1	89.5	3.5	94.9	1.0
3	83.3	5.9	93.7	1.9
7	84.3	5.2	92.9	2.5
14	80.2	8.7	92.6	3.1
21	78.4	9.6	92.1	3.5
30	74.8	11.0	89.0	4.2

The decline of imazapic can be described by first-order kinetics. The half-life was calculated as 106 days.

Hydrolysis

[Pyridine-6-^{14}C]-Imazapic was dissolved in acetonitrile and applied to the water + soil at a nominal rate of 0.125 µg/g soil (equivalent to about 0.14 kg/ha) and kept under anaerobic conditions with nitrogen gas in the dark at 25 °C (Madsen, 1993b). At intervals of 0, 7, 14, 31, 60, 91, 121, 182, 274 and 366 days after treatment, the potassium hydroxide traps were analysed for $^{14}CO_2$ by LSC. At the same intervals, the ethylene glycol and sulphuric acid traps were assayed for the ^{14}C-volatile materials.

The analysis of the water was accomplished by centrifuging the samples and decanting the water. The entire water sample's pH was adjusted to approximately 1.7 with 6 N HCl then filtered through the C_{18} cartridge. The C_{18} cartridge was eluted with methanol:water (1:1 v:v). The concentrated samples were then analysed by LSC and HPLC-MS. The soil was extracted three times with 0.5 N aqueous sodium hydroxide and the humic acid was removed by acid precipitation. The aqueous fulvic acid fraction was partitioned with methylene chloride. The humic acid fraction was

rinsed with ethyl acetate and combining the rinse with the methylene chloride partition. The methylene chloride partition was then poured through sodium sulfate. The sodium sulfate was then rinsed with methylene chloride. The extract was then evaporated to dryness. The residue was dissolved in methanol:water (1:1, v:v) and aliquots were analysed by LSC. The extracted soil was finally combusted to determine the bound residues. The solvent extractions were concentrated and analysed by HPLC-MS.

After 366 days of anaerobic aquatic incubation, 0.5% of the applied dose was mineralized to $^{14}CO_2$. No other radiolabelled volatile compounds were collected. Volatilization of the parent compound and other metabolites did not occur. The measured concentration of imazapic decreased from 92.1% (35.7% in the soil extract and 56.4% in the water) at day 0 to 83.0% (54.3% in the soil extract and 28.7% in the water) of the applied dose after 366 days of incubation (Table 2.3/9). This indicates that imazapic is stable against hydrolysis.

Proposed degradation pathway of imazapic in soil under aerobic condition

Imazapic degrades in soil under aerobic conditions, leading to eventual mineralization. Three metabolites were identified as CL263284, CL312622 and CL354825. The rapid degradation of CL 299263 in soil under aerobic conditions proceeds via formation of the diacid metabolite, CL312622, the hydroxy acid, CL354825 and by mineralization to CO_2. Degradation of imazapic, although much slower than that of CL 299263, gave the same metabolites, and ultimately CO_2. It is concluded that the routes of degradation for the two imidazolinones proceed through common intermediates. Since the hydroxy methyl derivative, CL263284, was rapidly transformed in the same fashion, this is proposed as an intermediate in the metabolism of imazapic.

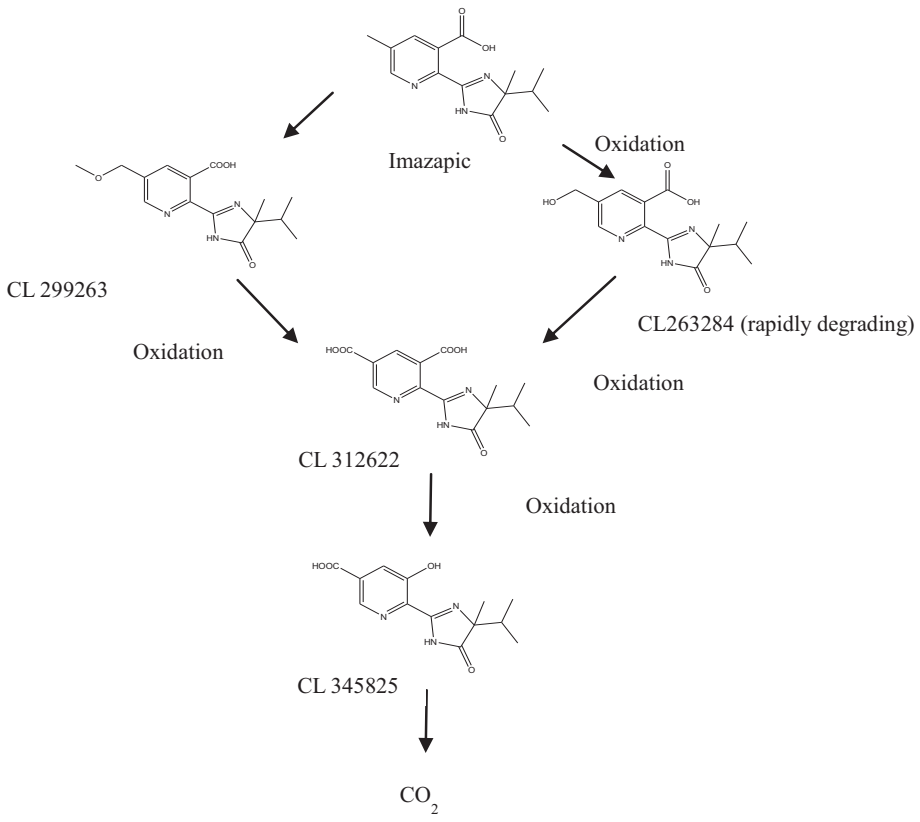


Figure 3 Proposed degradation pathway of imazapic in soil under aerobic condition

Metabolism and Distribution in Succeeding Crops

Confined Rotational Crop Study

The metabolism and distribution of imazapic in succeeding crops were investigated using [pyridine-6-^{14}C]- or [pyridine-6-^{13}C]-imazapic (Afzal, 1993a).

The field phase of the study was conducted in Madera California from July 1991 through December 1992. Three field plots (labelled A – C, 0.9 × 3.7 m, 0.9 × 5.5 m and 0.9 × 7.3 m, respectively) were treated with ^{14}C-labelled imazapic at the rate equivalent to approximately 772 g ai/ha. Ninety days (90 Plant back interval (PBI)) and 120 days (120 PBI) after treatment, Plot A was planted with barley in subplots 1 and 2, respectively. At 270 days after treatment (270 PBI), Plot B was planted with barley, cotton and maize in subplots 1, 2 and 3, respectively. Similarly, 300 days after treatment (300 PBI), Plot C was planted with cotton, maize, lettuce and carrots in subplots 1 to 4, respectively. Three test plots D–F were used to grow the corresponding control crops. Soil core samples were taken prior to treatment, immediately following treatment, at planting and at crop sampling (mid-maturity and harvest). All cores were 46 cm deep except immediately after treatment where they were 30 cm.

Samples were frozen after collection and shipped to the analytical laboratory over dry ice. All unprocessed samples from the field were stored frozen (ca. -20 °C) until the time of analysis. All crops sampled at mid-maturity were composited for analysis on a whole plant basis except for carrots where only the root was composited. Samples taken at crop maturity were separated and the following crop parts were analysed: (a) barley straw and grain (removed from seed head); (b) cotton seed and linters (separated from seed); (c) maize fodder (dried stalk + cob + leaves + husks) and grain (removed from cob), (d) carrot root and (e) whole lettuce plants.

Fifty to 100 g of crop sample were homogenized with a mixture of high purity grade water:methanol:acetone (1:1:1). The sample to solvent ratio used for extraction was 1/10 (w/v). The supernatant was filtered through qualitative filter paper. The homogenization process was repeated once more and the supernatant was collected. The supernatants from the first and second extraction were pooled and triplicate aliquots were used for radioactivity measurement by LSC. Residual radioactive residues (PES) after drying were weighed and combusted in triplicate aliquots. The PES in barley straw (120 PBI and 270 PBI planting) and grain contained residues > 0.01 mg/kg. Therefore, they were digested with 2% HCl in MeOH:H_2O (80:20) overnight and homogenized twice with a polytron homogenizer using a tissue to solvent ratio of approximately 1/10 (w/v). The supernatants were pooled and triplicate aliquots were used for radioactivity measurement by LSC. The final residues were air-dried and combusted in triplicate aliquots to determine the amount of unextracted radioactivity.

Selected soil samples at application, planting and different crop sampling intervals containing radioactive residue of > 0.01 mg/kg were extracted twice for approximately 1 hour with water:methanol:acetone (1:1:1) using a mechanical shaker. Celite (30%) was added to aid filtration and the extracts was filtered and pooled. Triplicate aliquots were used for radioactivity measurement by LSC. The PES were air-dried and triplicate aliquots combusted. Soil samples containing residues > 0.01 mg/kg were subjected to mild acid hydrolysis with 2% HCl (80:20 MeOH:H_2O 1/10 w/v) using a mechanical shaker for 1 h. This procedure was repeated once, and the resulting supernatants were pooled and analysed in triplicates by LSC. The final residues were air-dried and combusted in triplicate aliquots.

HPLC of the soil and crop extracts was performed on a Supelco C_{18} reversed phase column using an acetonitrile and phosphate buffer gradient system.

The TRR in all crop and soil samples were determined by combustion. A summary of the TRR values in rotational crop matrices collected at different plant back intervals is shown in Table 19 and those in soild samples in Table 20.

The TRR in barley and maize samples show the tendency to be higher in samples obtained from longer plant back interval. The TRR in crop samples were quite low with the highest in 270

DAT barley straw at 0.070 mg eq/kg. The TRR in edible portions of rotational crops were lower with the highest in 270 PBI barley grain at 0.045 mg eq/kg. The TRR in 270 PBI lettuce and carrots were < 0.01 mg eq/kg.

Table 19. TRR in rotational crop matrices after soil application of [pyridine-6-^{14}C]-imazapic

Matrix	Plant back interval	TRR Determined by Direct Combustion (mg eq/kg)*
Barley plant	90	< 0.004
	120	0.009
	270	0.023
Barley straw	90	0.013
	120	0.056
	270	0.070
Barley grain	90	0.014
	120	0.030
	270	0.045
Maize forage	270	0.010
	300	0.016
Maize fodder	270	0.019
	300	0.028
Maize grain	270	0.007
	300	0.008
Cotton seed	270	0.017
	300	0.009
Cotton linters	270	0.015
	300	0.009
Lettuce	270	0.006
Carrots	270	< 0.004

* Expressed in mg-imazapic equivalents/kg.

Radioactive residues remained largely confined to the top 0–8 cm soil horizon throughout the course of the study. At 0 DAT, the TRR in soil of 0–8 cm depth ranged from 0.026 to 0.095 mg/kg while the TRR in deeper soil layers were mostly < 0.004 mg/kg. The TRR in soil of 0–8 cm depth declined to the range of < 0.04 to 0.078 mg/kg at the different plant back intervals.

Table 20 TRR in soil samples after soil application of [pyridine-6-^{14}C]-imazapic

Days after Treatment	TRR Determined by Direct Combustion (mg eq/kg)* at Various Soil Depth			
	0-8 cm	8-30 cm	30-46 cm	46-61 cm
Plot A				
-1	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
0	0.057 / 0.029	< 0.004 / < 0.004	< 0.004 / < 0.004	na
90	0.044 / 0.078	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
120	0.023 / 0.019	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
244	0.019 / 0.008	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
262	0.021 / 0.027	0.006 / 0.010	0.005 / < 0.004	< 0.004 / < 0.004
297	0.010 / 0.013	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
308	0.015 / 0.032	0.007 / 0.013	0.004 / 0.005	< 0.004 / < 0.004
Plot B				
-1	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
0	0.065 / 0.095	< 0.004 / < 0.004	< 0.004 / < 0.004	Not analysed.
270	0.018 / 0.049	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004

Days after Treatment	TRR Determined by Direct Combustion (mg eq/kg)* at Various Soil Depth			
	0-8 cm	8-30 cm	30-46 cm	46-61 cm
332	0.015 / 0.012	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
332	0.012 / 0.013	< 0.004 / 0.007	< 0.004 / < 0.004	0.005 / < 0.004
364	< 0.004 / 0.012	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
399	0.009 / 0.016	0.004 / 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
406	0.009 / 0.007	0.005 / 0.005	< 0.004 / < 0.004	< 0.004 / < 0.004
493	0.012 / 0.019	0.006 / 0.010	0.006 / 0.009	< 0.004 / < 0.004
Plot C				
-1	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
0	0.026 / 0.046	< 0.004 / < 0.004	< 0.004 / < 0.004	Not analysed
300	0.006 / 0.025	< 0.004 / < 0.004	0.005 / < 0.004	0.007 / < 0.004
351	0.011 / 0.014	0.005 / 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
361	0.006 / 0.014	0.006 / 0.006	0.007 / 0.007	< 0.004 / < 0.004
364	0.015 / 0.010	0.007 / 0.008	< 0.004 / < 0.004	< 0.004 / < 0.004
374	0.005 / 0.005	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
375	0.009 / 0.012	0.005 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
436	0.008 / 0.008	< 0.004 / < 0.004	< 0.004 / < 0.004	< 0.004 / < 0.004
442	0.005 / 0.011	0.005 / 0.005	0.005 / < 0.004	< 0.004 / < 0.004
493	0.008 / 0.007	0.006 / < 0.004	0.005 / < 0.004	< 0.004 / < 0.004

* Expressed in mg-imazapic equivalents/kg.

The extraction profile of radioactive residues from rotational crop matrices is summarized in Table 21 and that from soil samples in Table 22.

The efficiency of extraction of radioactive residues in crop samples was 56.5–90.8% with a mixture of methanol:water:acetone (1:1:1). The unextracted portion of the TRR in selected crops was subjected to mild hydrolysis with 2% HCl in MeOH/H_2O (80:20) which released an additional 9–18% of the radioactivity. The remaining unextracted residues (PES) accounted for ≤ 0.01 mg/kg in all crops.

Table 21 Extracted radioactive residues from rotational crops after soil application of [pyridine-6-^{14}C]-imazapic

Matrix	Plant back interval	TRR [a] (mg eq/kg)*	Solvent extract [b] (mg eq/kg)*	(%TRR)	2% HCl [c] (mg eq/kg)*	(%TRR)	PES (mg eq/kg)*	(%TRR)
Barley forage	90	< 0.004	No extraction attempted due to low TRR.					
	120	0.009	No extraction attempted due to low TRR.					
	270	0.023	0.018	79.2	-	-	0.005	20.8
Barley straw	90	0.013q	0.007	56.5	-	-	0.006	43.5
	120	0.056	0.041	72.4	0.005	8.92	0.010	18.7
	270	0.070	0.053	76.4	0.007	10.0	0.010	13.6
Barley grain	90	0.014	0.009	66.8	-	-	0.005	33.2
	120	0.030	0.023	75.2	-	-	0.007	24.8
	270	0.045	0.033	73.8	0.004	8.89	0.008	17.3
Maize forage	270	0.010	No extraction attempted due to low TRR.					
	300	0.016	0.015	90.8	-	-	0.001	9.2
Maize fodder	270	0.019	0.014	76.0	-	-	0.005	24.0
	300	0.028	0.018	63.5	0.005	17.9	0.005	18.6
Maize grain	270	0.007	No extraction attempted due to low TRR.					

Matrix	Plant back interval	TRR [a] (mg eq/kg)*	Solvent extract [b] (mg eq/kg)*	(%TRR)	2% HCl [c] (mg eq/kg)*	(%TRR)	PES (mg eq/kg)*	(%TRR)
	300	0.008	No extraction attempted due to low TRR.					
Cotton seed	270	0.017	0.013	74.0	-	-	0.004	26.0
	300	0.009	No extraction attempted due to low TRR.					
Cotton linters	270	0.015	0.011	70.5	-	-	0.004	29.5
	300	0.009	No extraction attempted due to low TRR.					
Lettuce	270	0.006	No extraction attempted due to low TRR.					
Carrots	270	< 0.004	No extraction attempted due to low TRR.					

PES = post extraction solid (after solvent extraction)

[a] Sum of extracts and PES, if extraction was performed, otherwise TRR combusted. Expressed in mg-imazapic equivalents/kg

[b] extracted with methanol:acetone:H_2O (1:1:1)

[c] extracted with 2% HCl (methanol:H2O, 80:20)

[*] Expressed in mg-imazapic equivalents/kg.

Extracted residues from soil using a mixture of methanol:water:acetone (1:1:1) declined with time from 83% of TRR (0.054 mg eq/kg) at 0 DAT to 38% of TRR (0.007 mg eq/kg) by the last harvest (493 DAT). Exhaustive extraction of soil PES in selected soil samples (90 DAT, 300 DAT and 308 DAT) showed that a majority (34–52% of TRR; 0.013–0.015 mg eq/kg) of the radioactivity was extracted following mild acid hydrolysis with 2% HCl in MeOH/H_2O (80:20). The low residues in soil PES (≤ 0.01 mg/kg) precluded further characterization.

Table 22 Extracted radioactive residues from soil samples after soil application of [pyridine-6-^{14}C]-imazapic

Days after Treatment	Sampling Timing	TRR [a] (mg eq/kg)*	Solvent extract [b] (mg eq/kg)*	(%TRR)	2% HCl [c] (mg eq/kg)*	(%TRR)	PES (mg eq/kg)*	(%TRR)
0	Application	0.065	0.054	82.7	-		0.011	17.3
90	90 PBI Planting	0.044	0.023	52.1	0.015	34.1	0.006	13.8
120	120 PBI Planting	0.019	0.010	53.6	-		0.009	46.4
270	270 PBI Planting	0.018	0.005	28.2	-		0.013	71.8
297	90 PBI Planting Barley Harvest	0.013	0.007	50.4	-		0.006	49.6
300	300 PBI Planting	0.025	0.005	21.5	0.013	52.0	0.007	26.5
308	120 PBI Planting Barley Harvest	0.032	0.010	32.5	0.015	46.9	0.007	20.6
332	270 PBI Planting Barley Mid Harvest	0.015	0.007	47.8	-		0.008	52.2
364	300 PBI Planting Maize Mid Harvest	0.015	0.006	35.4	-		0.009	64.6
399	270 PBI Planting Barley Harvest	0.016	0.004	27.1	-		0.012	72.9
406	270 PBI Planting Maize Harvest	< 0.010	No extraction attempted due to low TRR.					
436	300 PBI Planting Carrot Harvest	< 0.010	No extraction attempted due to low TRR.					
442	300 PBI	0.010	No extraction attempted due to low TRR.					

Days after Treatment	Sampling Timing	TRR [a] (mg eq/kg)*	Solvent extract [b] (mg eq/kg)*	(%TRR)	2% HCl [c] (mg eq/kg)*	(%TRR)	PES (mg eq/kg)*	(%TRR)
	Planting Maize Harvest							
493	270 PBI Planting Cotton Harvest	0.019	0.007	37.5			0.012	62.5
493	300 PBI Planting Cotton Harvest	< 0.010	No extraction attempted due to low TRR.					

PES = post extraction solid (after solvent extraction)

[a] Sum of extracts and PES, if extraction was performed, otherwise TRR combusted. Expressed in mg-imazapic/kg.

[b] extracted with methanol:acetone:H_2O (1:1:1)

[c] extracted with 2% HCl (methanol:H_2O 80:20)

[*] Expressed in mg-imazapic equivalents/kg.

Structural characterization of imazapic in 0 DAT soil extract was performed by thermospray LC-MS. HPLC chromatograms obtained from crop sample extracts suggested the presence of CL 263284 and CL 189215 based on the retention time of reference compounds. The identity of CL 189215 was confirmed both by retention time comparison with a synthetic reference compound and -glucosidase hydrolysis. Because of the low concentrations of CL 189215 and CL 263284 (0.005-0.031 mg/kg combined) no spectroscopic identification was possible. All other components of the crop residues were < 0.01 mg/kg.

Imazapic was the principal component of the residue in all extracted soil fractions at all planting (49–74%; 0.012–0.048 mg eq/kg) and crop sampling intervals (16–49%; 0.003–0.005 mg eq/kg). The identity of the parent in soil was confirmed by mass spectrometry. Furthermore, the radioactivity released from the PES following hydrolysis with 2% HCI in MeOH/H_2O (80:20) indicated that a majority of the radiocarbon accounted for the unaltered parent. One polar unknown component was detected at all intervals, however, its concentration was in all cases < 0.01 mg/kg. All other radioactive components were < 5% of the TRR and were insignificant.

The extracted ^{14}C-residues from crops were used to determine the metabolic profile of imazapic following HPLC analysis. The two major metabolites detected in crop extracts were the hydroxymethyl derivative of parent, CL 263284, and the corresponding glucoside, CL 189215. Since the two components were not completely resolved in either of the gradient HPLC methods, their concentrations in crop extracts are reported as a sum of the two components. Characterization of the residue components were performed on HPLC by comparing the retention time of each component with known reference compounds.

Barley straw: The unaltered parent imazapic was a minor component of TRR (< 10%) for 120 DAT and 270 DAT planting intervals. The two major radioactive components corresponded to CL 263284 and CL 189215, collectively accounting for 37 and 44% of TRR (0.021 and 0.031 mg /kg) for 120 PBI and 270 PBI, respectively. One minor component designated as a polar unknown metabolite was detected accounting for 2.7% and 4.2% of TRR (0.002 and 0.003 mg eq/kg) at the two planting intervals.

Barley grain: Imazapic was the major component of the TRR (23%; 0.007 mg/kg) for 120 PBI and depleted to 9.6% of the TRR (0.004 mg eq/kg) for 270 PBI. CL 263284 and CL 187215 collectively constituted 18% and 33% of the TRR (0.006 and 0.015 mg/kg) for 120 and 270 PBI, respectively. The minor polar unknown metabolite accounted for 1.5–4.2% of TRR (< 0.001–0.002 mg eq/kg) at the two intervals. The radiocarbon released from barley straw PES and grain PES after hydrolysis with 2% HCI in MeOH:H_2O (80:20) was not amenable to HPLC or TLC characterization.

Maize forage and fodder: The major components of the radioactive residue were CL 263284 and CL 189215. Collectively they accounted for 31% of the TRR (0.006 mg/kg) in fodder for 270 day

PBI; 28% of the TRR (0.005 mg/kg) in forage for 300 day PBI and 28% of the TRR (0.008 mg/kg) in fodder for 300 day PBI. The parent was a minor component accounting for 0.001 mg/kg or less in these samples. The polar metabolite in all cases was < 0.01 mg/kg.

Cotton: The major components of the organosoluble residue corresponded to CL 263284 and CL 189215. Imazapic constituted 23% (0.004 mg/kg) and 17% (0.003 mg/kg) of the TRR in cotton seed and linters for 270 PBI interval, respectively. The minor polar component was insignificant accounting for <10% (0.001 mg/kg) of the TRR in cotton seed and linters for the 270 PBI.

Lettuce and carrots planted 300 DAT showed no significant residues (0.006 and < 0.004 mg eq/kg), respectively.

The metabolites found in rotational crop matrices (barley and maize) are summarized in Table 23.

Table 23 Metabolites found in barley and maize matrices after soil application of [pyridine-6-^{14}C]-imazapic

Barley												
Component	Straw (90 PBI)		Grain (90 PBI)		Straw (120 PBI)		Grain (120 PBI)		Straw (270 PBI)		Grain (270 PBI)	
	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)
Imazapic	Not analysed		Not analysed.		0.003	5.5	0.007	23	0.004	5.4	0.004	9.6
CL 189215 + CL 263284					0.021	37	0.006	18	0.031	44	0.015	33
Polar unknown					0.002	2.7	< 0.001	1.5	0.003	4.2	0.002	4.2
Maize												
Component	Forage (270 PBI)		Fodder (270 PBI)		Grain (270 PBI)		Forage (300 PBI)		Fodder (300 PBI)		Grain (300 PBI)	
	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)	(mg/kg)	(%TRR)
Imazapic	Not analysed		< 0.001	2.5	Not analysed		0.001	7.3	< 0.001	3.5	Not analysed	
CL 189215 + CL 263284			0.006	31			0.005	28	0.008	28		
Polar unknown			0.002	8.0			0.007	41	< 0.001	5.7		

While the major component in soil was imazapic, the principal components of the residue in barley, maize and cotton were CL 263284 and CL 189215, the hydroxymethyl derivative of imazapic and the corresponding glucose conjugate, respectively. The metabolic pathway in rotational crops is proposed to be the hydroxylation of the methyl group on the pyridine ring to the hydroxymethyl group followed by conjugation with glucose, similar to the metabolic pathway of most of plants.

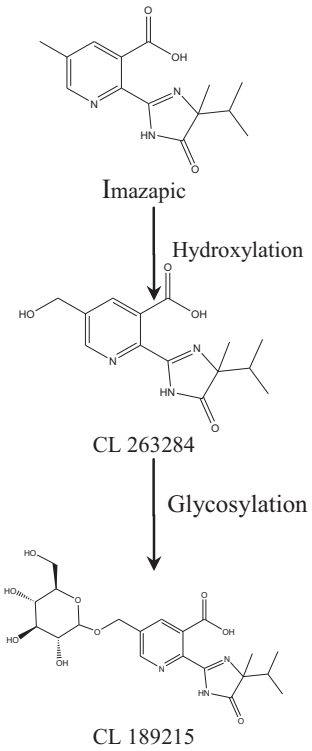


Figure 4 Proposed metabolic pathway in rotational crops (barley, maize)

RESIDUE ANALYSIS

Analytical methods

The Meeting received descriptions and validation data (including concurrent recovery data) for analytical methods for residues of imazapic and its metabolites CL263284 and CL189215.

Analytical methods for plant matrices

The descriptions of the analytical methods used in supervised residue trials are shown below.

All of these methods showed good linearity with a correlation coefficient ≥ 0.99. There were no interferences at the analyte concentration higher than 30% of LOQ. Additional information on method performance is described later.

Method SOP-PA.0288

Analyte:	Imazapic, CL263284 and CL189215
Matrix:	Sugar cane (cane, sugar, molasses) and soya bean (seed, oil, hull, flake, meal toasted meal)
LOQ:	0.01 mg/kg for each analyte.
Description:	Residues are extracted from sugar cane or sugar using methanol:water:1M HCl (60:39:1, v/v/v) with shaking and centrifugation as necessary. and an aliquot is taken and diluted with 0.1% formic acid in water: 0.1% formic acid in methanol (50:50, v/v). Residues in molasses are extracted in the same manner and an aliquot is taken and diluted with 0.1% formic acid in water:0.1% formic acid in methanol (50:50, v/v). The final extracts are filtered and the determination is performed by LC-MS/MS (for quantification: imazapic, 276.2 → 231.2; CL263284, 292.2 → 247.3; CL189215, 454.2 → 292.4 and for confirmation: imazapic, 276.2 → 163.2; CL263284, 292.2 → 179.1;

CL189215, 454.2 → 179.1). Mobile phase solution A, 0.1% formic acid in water; and mobile phase solution B, 1% formic acid in methanol (same for other HPLC separation).
Reference: Leite, 2008a; Leite and Souza, 2009a; Resende and Takahashi, 2010a;

Method SOP-PA.0249

Analyte: Imazapic
Matrix: Rice grain and soya bean (seed)
LOQ: 0.05 mg/kg
Description: Residues are extracted from samples using methanol:water:1M HCl (60:39:1, v/v/v) in a homogenizer. An aliquot is taken, the volume reduced and diluted with water. The pH is adjusted to 2.1 with 1 M HCl solution. An aliquot is partitioned with dichloromethane. An aliquot of the organic phase is evaporated to dryness and the residue dissolved with 0.1% formic acid in water:0.1% formic acid in methanol (50:50, v/v). The determination is performed by LC-MS/MS (for quantification: imazapic, 276.2 → 231.2; CL263284, 292.2 → 247.3; CL189215, 454.2 → 292.4 and for confirmation: imazapic, 276.2 → 163.2; CL263284, 292.2 → 179.1; CL189215, 454.2 → 179.1).
Reference: Borges, 2003a; Leite, 2005a; Borges, 2004a; Borges, 2004b; Borges, 2004c

Method LAADL R0001.01

Analyte: Imazapic
Matrix: Maize (grain)
LOQ: 0.1 mg/kg
Description: Imazapic is extracted from maize with acidic aqueous methanol using an ultrasonic extractor. The analyte is partitioned with methylene chloride from aqueous acid solution. The methylene chloride is evaporated and reconstituted in 50% methanol in pH 2.5 water for cation exchange clean-up. Imazapic is eluted with a saturated KCl in methanol solution, partitioned with methylene chloride, evaporated to dryness and diluted in Milli-Q water for HPLC analysis. The final determination is performed by HPLC-UV with UV detection at 254 nm.
Reference: Steling, 1998a

Method SOP-PA.0231

Analyte: Imazapic and CL263284
Matrix: Rice (grain)
LOQ: 0.05 mg/kg for each analyte.
Description: Based on method R0001.01. Residues of imazapic are extracted with methanol:water:1M HCl (60:39:1, v/v/v) and the solution is filtered over Celite. The extract is concentrated, and after addition of lead acetate and water, the pH is adjusted to 6.2 with NaOH. Following centrifugation and addition of water, the pH is adjusted to 2.1 with HCl. The solution is solvent-extracted with dichloromethane, and the dichloromethane phase is evaporated to dryness. The residue is dissolved in a solution of methanol:water (1:1, v/v) and cleaned up with an SCX cartridge rinsed with methanol. The residue is eluted from the cartridge with KCl-saturated methanol, evaporated to dryness and dissolved in water at pH 2.5. The solution is again solvent-extracted with dichloromethane, the dichloromethane phase evaporated to dryness and the residue dissolved in water. The final analysis is performed with HPLC with UV detection at 254 nm.
Reference: Dantas C., 2003a; Steling C., 2001a; Steling C., 2001b; Steling C., 2001c;

Method M2253.01

Analyte: Imazapic and CL263284
Matrix: Wheat (grain, forage, stubble), chickpea (seed, forage), barley (grain, forage), peanut (nutmeat, hull)
LOQ: 0.1 mg/kg for each analyte.
Description: Residues of imazapic and CL263,284 are extracted from the sample in acetone:methanol:water (1:1:1). Organic layers are removed and samples are centrifuged after addition of 10% lead acetate. The aqueous sample is then cleaned up, using resin AG 1-X8 anion exchange, partitioning with methylene chloride and subsequent benzene sulfonic acid (SCX) cation exchange solid phase extraction (SPE). Determination is performed by HPLC with UV detection and external standards.

Reference: Minoura, 1995a; Minoura, 1995b; Minoura, 1995c; Minoura, 1995d; Minoura, 1995e; Minoura, 1995f; Minoura, 1995g; Nejad H., 1993a; Nejad and Cenni, 1997b

Method M3114

Analyte: Imazapic, CL263284 and CL189215
Matrix: Bermuda grass (forage, hay), big bluestem grass (forage, hay), grass mixture (forage, hay)
LOQ: 0.5 mg/kg for each analyte.
Description: Residues are extracted from grass samples with methanol:water:1 MHCl (60:39:1, v/v/v). The extract is cleaned up, using precipitation, centrifugation and solid phase extraction techniques. Determination is performed by capillary electrophoresis equipped with a high sensitivity cell and UV detection at 240 nm and external standards
Reference: Duan and Nejad, 1999a; Nejad, 1999a

Method M2599

Analyte: Imazapic, CL263284 and CL189215
Matrix: Peanut (hay)
LOQ: 0.1 mg/kg for each analyte.
Description: Residues are extracted from the sample with methanol:water:1 M HCl (60:39:1, v/v/v). The extract is then cleaned up, using precipitation, centrifugation and solid phase extraction techniques. Determination is performed by capillary electrophoresis equipped with a high sensitivity cell and UV detection at 240 nm and external standards.
Reference: Nejad H., 1998a

Method L741/1

Analyte: Imazapic
Matrix: Rape (seed, foliage, straw)
LOQ: 0.05 mg/kg for each analyte.
Description: The extract is partitioned with dichloromethane after adjusting the pH to 2 with HCl. Subsequently, the extract is evaporated to dryness and the residue dissolved in methanol/water (1:1, v/v). Following SCX cartridge clean-up, separation and quantification of imazapic is accomplished by reverse phase HPLC with UV detection at 254 nm.
Reference: Anonymous, 1998a

Method M2379

Analyte: Imazapic, CL263284 and CL189215
Matrix: Peanut (nutmeat, hull)
LOQ: 0.1 mg/kg for each analyte.
Description: Residues are extracted from samples using methanol:water: 1M HCl (60:39:1, v/v/v). The extract is then cleaned up, using precipitation, centrifugation and solid phase extraction techniques. Determination is performed by capillary electrophoresis equipped with a high sensitivity cell and UV detection at 240 nm and external standards.
Reference: Nejad H., 1995a; Nejad H., 1994a; Nejad H., 1994b; Safarpour M., 1994a

Method 2463

Analyte: Imazapic, CL263284 and CL189215
Matrix: Wheat (grain, forage, straw, hay)
LOQ: 0.1 mg/kg for each analyte.
Description: Residues are extracted from samples using methanol:water: 1M HCl (60:39:1, v/v/v) in a homogenizer. An aliquot is taken and diluted with 0.1% formic acid in water: 0.1% formic acid in methanol (50:50, v/v). The final extracts are filtered and the determination is performed by LC-MS/MS (for quantification: imazapic, 276.2 → 231.2; CL263284, 292.2 → 247.3; CL189215, 454.2 → 292.4 and for confirmation: imazapic, 276.2 → 163.2; CL263284, 292.2 → 179.1; CL189215, 454.2 → 179.1).
Reference: Nejad and Cenni, 1995a; Nejad and Cenni, 1996a

Method SOP-PA.0288

The recoveries from sugar cane matrices or soya bean matrices (fortification range, 0.01-1.0 mg/kg) were within the acceptable range for all fortification levels and matrices tested (Table 25). Good linearity was observed in the range of 0.100 to 2.00 ng/mL for sugar cane matrices and 0.020 to 2.00 ng/mL for soya bean matrices. The repeatability relative standard deviations for all commodities and all fortification levels were below 15%.

Table 25 Recovery results obtained for the analysis of sugar cane matrices and soya bean matrices with Method SOP-PA.0288

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification			Transition 2** for confirmation		
				Mean (%)	SD (%)	RSD (%)	Mean (%)	SD (%)	RSD (%)
Sugar cane matrices									
Sugar cane	Imazapic	5	0.01	108	4.5	4.1	108	4.5	4.1
		5	0.10	90	4.8	5.3	95	11	12
		5	1.0	104	5.5	5.3	104	5.5	5.3
	CL263284	5	0.01	108	4.5	4.1	108	4.5	4.1
		5	0.10	91	3.7	4.1	99	1.3	1.4
		5	1.0	110	0.0	0.0	106	5.5	5.2
	CL189215	5	0.01	102	8.0	7.8	99	7.2	7.3
		5	0.10	95	10	11	95	4.3	4.6
		5	1.0	108	4.5	4.1	106	5.5	5.2
Sugar	Imazapic	5	0.01	102	4.5	4.4	97	9.0	9.3
		5	0.10	98	4.5	4.6	101	9.3	9.3
		5	1.0	92	6.8	7.4	90	4.1	4.6
	CL263284	5	0.01	97	13	13	101	7.1	6.9
		5	0.10	94	3.5	3.7	95	4.9	5.2
		5	1.0	101	6.1	6.0	101	5.8	5.7
	CL189215	5	0.01	90	13	14	92	6.1	6.6
		5	0.10	92	4.5	4.9	89	6.0	6.7
		5	1.0	89	6.2	7.0	88	5.1	5.7
Molasses	Imazapic	5	0.01	102	4.5	4.4	100	6.7	6.7
		5	0.10	94	4.3	4.6	91	1.3	1.4
		5	1.0	98	1.9	1.9	97	1.9	2.0
	CL263284	5	0.01	97	10	10	94	10	11
		5	0.10	84	3.0	3.6	85	4.5	5.3
		5	1.0	100	0.0	0.0	100	0.0	0.0
	CL189215	5	0.01	99	6.9	6.9	89	4.8	5.3
		5	0.10	87	6.4	7.3	91	3.5	3.9
		5	1.0	97	3.4	3.5	103	6.4	6.2
Soya bean matrices									
Seeds	Imazapic	5	0.01	96	4	5	94	7	7
		5	1.0	93	1	1	93	1	1
	CL263284	5	0.01	102	5	5	85	4	5
		5	1.0	100	2	2	100	2	2
	CL189215	5	0.01	88	4	4	-	-	-
		5	1.0	102	2	2	-	-	-
Oil	Imazapic	5	0.01	92	4	5	101	5	5
		5	1.0	101	2	2	100	3	3
	CL263284	5	0.01	83	2	2	82	2	2
		5	1.0	95	3	3	93	5	5
	CL189215	5	0.01	93	3	3	90	8	9
		5	1.0	101	9	9	96	8	8
Flaked soya bean	Imazapic	5	0.01	103	5	4	101	3	3
		5	1.0	97	3	3	99	4	4
	CL263284	5	0.01	112	4	4	102	5	5
		5	1.0	102	3	3	100	4	4

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification			Transition 2** for confirmation		
				Mean (%)	SD (%)	RSD (%)	Mean (%)	SD (%)	RSD (%)
	CL189215	5 5	0.01 1.0	106 99	2 4	1 4	85 98	7 3	8 3
Meal	Imazapic	5 5	0.01 1.0	94 92	4 3	4 4	99 92	5 3	5 4
	CL263284	5 5	0.01 1.0	100 82	8 1	8 1	75 82	4 5	5 6
	CL189215	5 5	0.01 1.0	108 84	8 4	7 5	- -	- -	- -
Toasted meal	Imazapic	5 5	0.01 1.0	97 85	9 3	9 4	107 85	7 4	7 4
	CL263284	5 5	0.01 1.0	89 76	10 3	12 4	95 76	8 2	9 3
	CL189215	5 5	0.01 1.0	105 77	5 3	5 4	- -	- -	- -

* imazapic: 276 → 231; CL263284: 292 → 247; CL189215: 454 → 292

** imazapic: 276 → 163; CL263284: 292 → 179; CL189215: 454 → 179

An independent laboratory validation of Method SOP-PA.0288 was conducted for the determination of imazapic, CL 263284 and CL 189215 in sugar cane matrices (Grosshans, 2009b) and soya bean matrices (Robaugh, 2012a). Fortification levels used are 0.01 and 0.1 mg/kg for sugar cane matrices (Table 26) and 0.01 and 0.5 mg/kg for soya bean matrices (Table 27).

The recoveries were within the acceptable range for all fortification levels and matrices tested. Good linearity was observed in the range of 0.100 to 2.00 ng/mL for sugar cane matrices and 5.00 to 200 ng/g for soya bean matrices. The repeatability relative standard deviations for all sugar cane matrices at all fortification levels were below 20% using matrix-matched standards, except for metabolite CL189215 in molasses at a fortification level of 0.01 mg/kg, where the RSD was 25%; and those for all soya bean matrices were below 30% a fortification level of 0.01 mg/kg and below 10% at the other fortification levels, using matrix-matched standards.

Table 26 Recovery results obtained during independent laboratory validation of Method SOP-PA.0288 for sugar cane matrices

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification			Transition 2** for confirmation		
				Mean (%)	SD (%)	RSD (%)	Mean (%)	SD (%)	RSD (%)
1st set of analysis									
Sugar cane	Imazapic	5 5	0.01 0.10	83 81	6.7 1.8	8.1 2.2	87 82	11 1.8	12 2.2
	CL263284	5 5	0.01 0.10	30 44	1.2 1.8	3.9 4.2	31 44	2.2 1.5	7.0 3.5
	CL189215	5 5	0.01 0.10	63 62	8.6 2.2	14 3.6	54 60	11 2.3	21 3.8
Sugar	Imazapic	5 5	0.01 0.10	90 93	3.9 2.4	4.4 2.6	87 96	3.1 1.4	3.6 1.5
	CL263284	5 5	0.01 0.10	47 71	1.6 0.8	3.4 1.2	47 70	2.6 1.7	5.5 2.4
	CL189215	5 5	0.01 0.10	89 104	3.3 2.8	3.7 2.7	82 104	7.4 2.9	9.0 2.8
Molasses	Imazapic	5 5	0.01 0.10	57 70	6.8 1.7	12 2.5	48 71	9.3 0.7	19 0.9
	CL263284	5 5	0.01 0.10	28 40	1.3 1.0	4.8 2.4	26 39	5.1 0.7	20 1.7

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification			Transition 2** for confirmation		
				Mean (%)	SD (%)	RSD (%)	Mean (%)	SD (%)	RSD (%)
	CL189215	5 5	0.01 0.10	-[a] 53	-[a] 13	-[a] 24	45 46	23 4.5	51 9.7
2nd set of analysis using matrix-matched standards									
Sugar cane	Imazapic	5 5	0.01 0.10	105 90	5.5 1.9	5.3 2.2	108 90	3.8 4.2	3.5 4.7
	CL263284	5 5	0.01 0.10	109 100	3.9 2.6	3.6 2.6	109 99	2.5 1.6	2.3 1.6
	CL189215	5 5	0.01 0.10	105 103	11 3.1	10 3.1	91 105	5.6 4.5	6.2 4.3
Sugar	Imazapic	5 5	0.01 0.10	105 98	3.4 1.9	3.2 2.0	106 100	2.0 3.2	1.9 3.2
	CL263284	5 5	0.01 0.10	103 101	3.6 2.2	3.5 2.2	103 100	2.1 3.1	2.0 3.1
	CL189215	5 5	0.01 0.10	106 103	12 2.3	11 2.3	106 101	4.5 1.4	4.3 1.4
Molasses	Imazapic	5 5	0.01 0.10	115 93	4.3 1.6	3.8 1.8	111 92	3.8 3.6	3.4 3.9
	CL263284	5 5	0.01 0.10	115 92	3.8 1.8	3.3 1.9	113 94	11 1.8	10 1.9
	CL189215	5 5	0.01 0.10	106 102	27 5.0	25 4.9	117 92	17 5.9	15 6.5

* imazapic: 276 → 231; CL263284: 292 → 247; CL189215: 454 → 292; used for quantification

** imazapic: 276 → 163; CL263284: 292 → 179; CL189215: 454 → 179; used for confirmation

[a] due to low sensitivity, no peaks above 3 fold signal to noise ratio detectable

Table 27 Recovery results obtained during independent laboratory validation of Method SOP-PA.0288 for soya bean matrices

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification		
				Mean (%)	SD (%)	RSD (%)
Seed	Imazapic	5 5	0.01 0.5	90 93	10 5	11 5
	CL263284	5 5	0.01 0.5	91 90	14 4	16 4
	CL189215	5 5	0.01 0.5	80 91	15 7	19 7
Oil	Imazapic	5 5	0.01 0.1	99 109	3 1	3 3
	CL263284	5 5	0.01 0.1	98 89	5 5	5 6
	CL189215	5 5	0.01 0.1	114 110	5 2	4 2
Hulls	Imazapic	8 5	0.01 2.0	78 99	11 4	14 4
	CL263284	8 5	0.01 2.0	92 93	19 6	21 6
	CL189215	5 5	0.01 2.0	83 85	4 3	5 3

* imazapic: 276 → 231; CL263284: 292 → 247; CL189215: 454 → 292

Method SOP-PA.0249

The recoveries from rice grain (Borges, 2004a, c) and in soya bean seed (Borges, 2004a, c) at fortification levels of 0.05 and 5.0 mg/kg were within the acceptable range (Tables 28 and 29). Good linearity was observed in the range of 0.500 to 10.0 ng/mL for rice grain and soya bean seed. The repeatability relative standard deviations for all commodities and all fortification levels were below 10%.

Table 28 Recovery results obtained for the analysis of imazapic in rice grain with Method SOP-PA.0249

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification			Transition 2** for confirmation		
				Mean (%)	SD (%)	RSD (%)	Mean (%)	SD (%)	RSD (%)
Grain	Imazapic	15 15	0.05 5.0	94 105	4.7 6.6	5.0 6.3	94 105	5.0 6.8	5.4 6.5

* imazapic: 276.0 → 231.0
** imazapic: 276.0 → 163.0

Table 29 Recovery results obtained or the analysis of imazapic in rice grain with Method SOP-PA.0249

Matrix	Analyte	No.	Fortification Level (mg/kg)	Transition 1* for quantification			Transition 2** for confirmation		
				Mean (%)	SD (%)	RSD (%)	Mean (%)	SD (%)	RSD (%)
Seed	Imazapic	5 5	0.05 5.0	95 96	3.6 6.5	3.8 6.8	95 97	3.3 5.9	3.5 6.1

* imazapic: 276.0 → 231.0
** imazapic: 276.0 → 163.0

Method LAADL R0001.01

Although the method was not subjected to formal validation, the method showed recoveries of 92 and 96% (n=1) at the fortification levels of 0.1 and 0.2 mg/kg.

Method SOP-PA.0231

The recoveries of imazapic from rice grain at fortification levels of 0.05 and 0.5 mg/kg were within the acceptable range (Table 30). Good linearity was observed in the range of 5.0 to 100 ng/mL (2003/3001442) or 5.0 to 50 ng/mL (2001/3001704, -05 and -06). The repeatability relative standard deviations for all fortification levels were below 15%.

Table 30 Recovery results obtained for analysis of imazapic in rice grain with Method SOP-PA.0231

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)
Grain (2003/3001442)	Imazapic	2 2	0.05 0.50	96 93
Grain (2001/3001704) (2001/3001705) (2001/3001706)	Imazapic	2 2	0.05 0.51	94 88

Method 2253.01

The recoveries of imazapic and CL263284 from chick-pea matrices (Minoura, 1995b and 1995e), barley matrices (Minura, 1995c and 1995f), wheat matrices (Minoura, 1995a, 1995d and 1995g), and

peanut matrices (Nejad, 1993a)(Table 31) at fortification levels from 0.10 to 0.50 mg/kg (wheat stubble up to 5.0 mg/kg) were all within the acceptable range (Table 31).

Table 31 Recovery results obtained for analysis of imazapic and CL 263284 in various plant matrices with Method 2253.01

Matrix		Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Crop	Portion						
Chickpea	Seed	Imazapic	2 2	0.10 0.50	84 74		
		Total	4	0.10-0.50	79	5.9	7.4
		CL263284	2 2	0.10 0.50	75 69		
		Total	4	0.10-0.50	72	3.5	4.9
	Forage	Imazapic	2 2	0.10 0.50	72 69		
		Total	4	0.10-0.50	70	1.8	2.6
		CL263284	2 2	0.10 0.50	82 73		
		Total	4	0.10-0.50	77	5.8	7.5
Wheat	Grain	Imazapic	2 2	0.10 0.50	74 90		
		Total	4	0.10-0.50	82	9.5	12
		CL263284	2 2	0.10 0.50	69 85		
		Total	4	0.10-0.50	77	9.5	12
	Forage	Imazapic	2 2	0.10 0.50	103 87		
		Total	4	0.10-0.50	95	11	12
		CL263284	2 2	0.10 0.50	90 82		
		Total	4	0.10-0.50	86	8.5	9.9
	Stubble	Imazapic	2 2 2 2	0.10 0.50 1.0 5.0	93 98 100 94		
		Total	8	0.10-5.0	96	4.3	4.5
		CL263284	2 2 2 2	0.10 0.50 1.0 5.0	78 79 95 91		
		Total	8	0.10-5.0	86	7.9	9.3
Barley	Grain	Imazapic	2 2	0.10 0.50	80 86		
		Total	4	0.10-0.50	83	5.5	6.6
		CL263284	2 2	0.10 0.50	73 83		
		Total	4	0.10-0.50	78	6.4	8.3
	Forage	Imazapic	2 2	0.10 0.50	87 103		
		Total	4	0.10-0.50	95	9.5	10
		CL263284	2 2	0.10 0.50	92 78		
		Total	4	0.10-0.50	85	12	14

Matrix		Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Crop	Portion						
Peanut	Nutmeat	Imazapic	3 3 3 3	0.10 0.50 1.0 5.0	85 82 85 83	8.9 11 6.4 7.7	10 13 7.5 9.2
		CL263284	3 3 3 3	0.10 0.50 1.0 5.0	79 86 82 83	2.8 5.5 4.9 5.4	3.5 6.4 6.0 6.5
	Hull	Imazapic	3 3 3 3	0.10 0.50 1.0 5.0	78 85 70 76	3.2 4.3 2.8 3.5	4.1 5.0 4.0 4.5
		CL263284	3 3 3 3	0.10 0.50 1.0 5.0	110 98 87 87	11 9.8 2.9 4.9	10 10 3.4 5.6

An independent laboratory validation of Method M 2253.01 was conducted for the determination of imazapic and CL 263284 in peanut hull and peanut meat at fortification levels of 0.10 and 0.50 mg/kg (Nejad and Cenni, 1997b)(Table 32). The recoveries were within the acceptable range for all fortification levels. The repeatability relative standard deviations for both commodities over all fortification levels were below 20%.

Table 32 Recovery results obtained during independent laboratory validation of Method M2253.01 for peanut matrices

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Peanut hull	Imazapic	2 2	0.10 0.50	100 93		
	Total	4	0.10-0.50	97	10	10
	CL263284	2 2	0.10 0.50	100 85		
	Total	4	0.10-0.50	92	10.0	10
Peanut meat	Imazapic	2 2	0.10 0.50	86 76		
	Total	4	0.10-0.50	81	9.1	11
	CL263284	2 2	0.10 0.50	89 89		
	Total	4	0.10-0.50	89	2.4	2.7

Method M3114

An independent laboratory validation of Method M 3114 was conducted for the determination of imazapic, CL 263284 and CL 189215 in grass at fortification levels of 0.50–50 mg/kg (Duan and Nejad, 1999a)(Table 32).

The recoveries of these analytes from grass matrices were within the acceptable range. The repeatability standard deviations were below 15%.

Table 32 Recovery results obtained during independent laboratory validation of Method M3114 for grass matrices

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Bermuda grass forage	Imazapic	2 2 2	0.50 1.0 50	85 80 87		
	Total	6	0.50-50	84	4.9	5.8
	CL263284	2 2 2	0.50 1.0 50	87 84 90		
	Total	6	0.50-50	87	5.2	6.0
	CL189215	2 2 2	0.50 1.0 50	89 84 90		
	Total	6	0.50-50	87	4.3	4.9
Bermuda grass hay	Imazapic	2 2 2	0.50 1.0 50	76 81 65		
	Total	6	0.50-50	74	8.1	11
	CL263284	2 2 2	0.50 1.0 50	78 84 68		
	Total	6	0.50-50	76	8.4	11
	CL189215	2 2 2	0.50 1.0 50	78 85 65		
	Total	6	0.50-50	76	10	14
Big Bluestem grass forage	Imazapic	2 2 2	0.50 1.0 50	80 84 73		
	Total	6	0.50-50	79	5.6	7.1
	CL263284	2 2 2	0.50 1.0 50	77 83 71		
	Total	6	0.50-50	77	6.1	8.0
	CL189215	2 2 2	0.50 1.0 50	81 85 74		
	Total	6	0.50-50	80	5.8	7.2
Big Bluestem grass hay	Imazapic	2 2 2	0.50 1.0 50	73 73 71		
	Total	6	0.50-50	72	2.0	2.8
	CL263284	2 2 2	0.50 1.0 50	69 84 72		
	Total	6	0.50-50	75	7.0	9.4
	CL189215	2 2 2	0.50 1.0 50	71 84 72		
	Total	6	0.50-50	76	6.7	8.9
Grass mixture forage	Imazapic	2 2 2	0.50 1.0 50	77 91 84		
	Total	6	0.50-50	84	7.4	8.9
	CL263284	2 2 2	0.50 1.0 50	82 93 91		
	Total	6	0.50-50	89	6.0	6.8

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
	CL189215	2 2 2	0.50 1.0 50	80 93 84		
	Total	6	0.50-50	85	6.9	8.1
Grass mixture hay	Imazapic	2 2 2	0.50 1.0 50	84 78 77		
	Total	6	0.50-50	80	5.0	6.3
	CL263284	2 2 2	0.50 1.0 50	84 77 76		
	Total	6	0.50-50	79	5.9	7.5
	CL189215	2 2 2	0.50 1.0 50	90 83 83		
	Total	6	0.50-50	85	5.5	6.5

Method M2599

An independent laboratory validation of Method M 3114 was conducted for the determination of imazapic, CL 263284 and CL 189215 in peanut hay at fortification levels of 0.10–0.40 mg/kg (Duan and Nejad, 1998a)(Table 33). The recoveries were within the acceptable range. The repeatability standard deviations were below 15%.

Table 33 Recovery results obtained during independent laboratory validation of Method M2599 for peanut hay

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Peanut hay	Imazapic	2 2 2	0.10 0.20 0.40	73 72 73		
	Total	6	0.10-0.40	73	4.9	6.8
	CL263284	2 2 2	0.10 0.20 0.40	82 81 84		
	Total	6	0.10-0.40	82	3.4	4.2
	CL189215	2 2 2	0.10 0.20 0.40	76 72 75		
	Total	6	0.10-0.40	74	3.8	5.2

Method L 741/1

The recoveries of imazapic from rape matrices at fortification levels of 0.05 and 0.5 mg/kg were within the acceptable range (Table 34). Good linearity was observed in the range of 0.5 to 100 ng/mL. The repeatability relative standard deviations for all fortification levels were below 15%

Table 34 Recovery results obtained for analysis of imazapic in rape seed matrices with Method L 741/1

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Foliage Straw Grain	Imazapic	2 2	0.05 0.5	92 81		
	Total	4	0.05-0.5	86	8	10

M 2379

The recoveries of imazapic and CL263284 from peanut matrices at fortification levels from 0.10 to 0.50 mg/kg were within the acceptable range (Table 35). The repeatability standard deviations were below 15%.

Table 35 Recovery results obtained for analysis of imazapic, CL263284 and CL189215 in peanut matrices with Method M 2379

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Peanut meat	Imazapic	2 2 2	0.10 0.20 0.50	80 83 90		
	Total	6	0.10-0.50	90	4.4	4.9
	CL263284	2 2 2	0.10 0.20 0.50	93 89 93		
	Total	6	0.10-0.50	93	6.7	7.2
	CL189215	2 2 2	0.10 0.20 0.50	75 75 84		
	Total	6	0.10-0.50	83	7.1	8.5
Peanut hull	Imazapic	2 2 2	0.10 0.20 0.50	91 91 89		
	Total	6	0.10-0.50	84	6.5	7.8
	CL263284	2 2 2	0.10 0.20 0.50	99 91 89		
	Total	6	0.10-0.50	91	4.1	4.5
	CL189215	2 2 2	0.10 0.20 0.50	87 84 79		
	Total	6	0.10-0.50	78	4.7	6.0

The extraction efficiency was investigated using peanut hay obtained 131 days after the treatment of plants with ^{14}C-labelled imazapic (Nejad, 1994b). Two separate samples of peanut hulls containing ^{14}C-residues were extracted with methanol:water:1N hydrochloric acid (60:39:1, v/v/v). Triplicate 30 mL aliquots from the control and each of the ^{14}C-treated sample extracts were evaporated and peanut residue was dissolved in a total of 5 mL methanol, sonicated and centrifuged. Triplicate 0.5 mL of each subsample were combusted using an oxidizer and subjected to liquid scintillation counting (LSC). Aliquots of approximately 250 mg of unextracted peanut hulls from control and treated plants were also combusted and subjected to LSC.

The average percentage of extracted radioactive residues were 73% (range 71–76%) compared with the radioassay result.

An independent laboratory validation of Method M 2379 was conducted for the determination of imazapic, CL 263284 and CL 189215 in peanut matrices at fortification levels of 0.10–0.50 mg/kg (Safarpour, 1994a)(Table 36). The recoveries were within the acceptable range. The repeatability standard deviations were below 20%.

Table 36 Recovery results obtained during independent laboratory validation of Method M 2379 for peanut matrices

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Peanut meat	Imazapic	2 2 2	0.10 0.20 0.50	89 88 85		
	Total	6	0.10-0.50	87	8.1	9.3
	CL263284	2 2 2	0.10 0.20 0.50	89 98 96		
	Total	6	0.10-0.50	94	6.1	6.5
	CL189215	2 2 2	0.10 0.20 0.50	74 68 80		
	Total	6	0.10-0.50	73	11.5	16
Peanut hull	Imazapic	2 2 2	0.10 0.20 0.50	77 102 92		
	Total	6	0.10-0.50	90	11.3	13
	CL263284	2 2 2	0.10 0.20 0.50	77 105 95		
	Total	6	0.10-0.50	92	13.1	14
	CL189215	2 2 2	0.10 0.20 0.50	60 77 76		
	Total	6	0.10-0.50	71	8.7	12

Method M 2463

An independent laboratory validation of Method M 2463 was conducted for the determination of imazapic, CL 263284 and CL 189215 in wheat matrices at fortification levels of 0.10–0.50 mg/kg (Nejad and Cenni, 1995a)(Table 37). The recoveries were within the acceptable range. The repeatability standard deviations were below 15%.

Table 37 Recovery results obtained during independent laboratory validation of Method M 2463 for wheat matrices

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Straw	Imazapic	2 2 2	0.10 0.20 0.50	95 86 92		
	Total	6	0.10-0.50	91	7	8
	CL263284	2 2 2	0.10 0.20 0.50	91 87 97		
	Total	6	0.10-0.50	91	6	7

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
	CL189215	2 2 2	0.10 0.20 0.50	77 75 80		
	Total	6	0.10-0.50	77	4	5
Hay	Imazapic	2 2 2	0.10 0.20 0.50	80 81 82		
	Total	6	0.10-0.50	81	10	13
	CL263284	2 2 2	0.10 0.20 0.50	81 84 86		
	Total	6	0.10-0.50	84	10	11
	CL189215	2 2 2	0.10 0.20 0.50	81 81 80		
	Total	6	0.10-0.50	80	4	5
Forage	Imazapic	2 2 2	0.10 0.20 0.50	74 86 88		
	Total	6	0.10-0.50	82	8	9
	CL263284	2 2 2	0.10 0.20 0.50	75 89 91		
	Total	6	0.10-0.50	85	8	9
	CL189215	2 2 2	0.10 0.20 0.50	76 88 86		
	Total	6	0.10-0.50	83	6	7
Grain	Imazapic	2 2 2	0.10 0.20 0.50	85 85 89		
	Total	6	0.10-0.50	86	3	4
	CL263284	2 2 2	0.10 0.20 0.50	81 83 90		
	Total	6	0.10-0.50	84	4	5
	CL189215	2 2 2	0.10 0.20 0.50	80 83 82		
	Total	6	0.10-0.50	82	2	2

The extraction efficiency was investigated using barley straw from the confined rotational crop study (Nejad and Cenni, 1996a). Two separate samples of barley straw containing ^{14}C-residues were extracted with methanol:water:1N hydrochloric acid (60:39:1, v/v/v). Duplicate 3 mL aliquots from the control and each of the ^{14}C-containing sample extracts were assayed by LSC. The post-extraction solids were collected and combusted and also subjected to LSC.

The average percentage of extracted radioactive residues was 67% compared with the radioassay results.

Analytical methods for animal matrices

The descriptions of the analytical methods related to the current review are shown below.

All of these methods showed good linearity with a correlation coefficient ≥ 0.99. There were no interferences at the analyte concentration higher than 30% of LOQ. Additional information on method performance is described later.

Method M 3188

Analyte: Imazapic and CL263284
Matrix: Milks
LOQ: 0.01 mg/kg for each analyte.
Description: Residues are extracted from the sample with acidic water. Precipitation, centrifugation and solid phase extraction (SPE) techniques are used for sample clean-up. Measurement of imazapic and CL263284 is accomplished by capillary electrophoresis (CE) equipped with a high sensitivity flow cell and a UV detector set at 240 nm. Results are calculated as imazapic and CL 263284 by the direct comparison of the peak heights in the sample to those of external standards. Confirmation of residues > 0.01 mg/kg for each compound is provided by LC/MS of the final extract.
Reference: Sweeney and Nejad, 1998a

Method M 3222

Analyte: Imazapic and CL263284
Matrix: Tissues
LOQ: 0.05 mg/kg for each analyte.
Description: Residues are extracted from the sample with acidic methanol water. Precipitation, centrifugation and solid phase extraction (SPE) techniques are used for sample clean-up. Measurement of imazapic and CL 263284 is accomplished by capillary electrophoresis (CE) equipped with a high sensitivity flow cell and a UV detector set at 240 nm. Results are calculated as imazapic and CL 263284 by the direct comparison of the peak heights in the sample to those of external standards.
Reference: Sweeney and Nejad, 1998b

Method M 3223

Analyte: Imazapic and CL263284
Matrix: Milk fat and tissue fat
LOQ: 0.01 mg/kg in milk and 0.05 mg/kg in tissue fat for each analyte.
Description: Residues are extracted from bovine milk fat and tissue fat with acidic acetonitrile in hexane. Filtration, solvent partitioning and solid phase extraction (SPE) techniques are used for sample clean-up. Measurement of imazapic and CL263284 is accomplished by HPLC/MS (positive ion electrospray ionization tandem mass spectrometry). Results are calculated as imazapic and CL263284 by the direct comparison of the peak area in the sample to those of external standards.
Reference: Boner and Nejad, 1999a

M 3188

An independent laboratory validation of Method M 3188 was conducted for the determination of imazapic and CL 263284 in cattle milk at fortification levels of 0.01–1.0 mg/kg (Sweeney and Nejad, 1998a)(Table 38). The recoveries were within the acceptable range. The repeatability standard deviations were below 15%.

Table 38 Recovery results obtained during independent laboratory validation of Method M 3188 for milk

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Bovine milk	Imazapic	2 3 2	0.01 0.02 1.0	89 95 91		
	Total	7	0.01-1.0	92	3	4

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
	CL 263284	2 3 2	0.01 0.02 1.0	87 92 91		
	Total	7	0.01-1.0	90	3	3

M 3222

An independent laboratory validation of Method M 3222 was conducted for the determination of imazapic and CL 263284 in cattle tissues at fortification levels of 0.5–1.0 mg/kg (Sweeney and Nejad, 1998a)(Table 39). The recoveries were within the acceptable range. The repeatability standard deviations were below 15%.

Table 39 Recovery results obtained during independent laboratory validation of Method M 222 for tissues

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Bovine muscle	Imazapic	2 2 2	0.05 0.10 1.0	74 74 81		
	Total	6	0.05-1.0	76	4	5
	CL263284	2 2 2	0.05 0.10 1.0	89 77 77		
	Total	6	0.05-1.0	81	6	8
Bovine kidney	Imazapic	3 2 2	0.05 0.10 1.0	79 79 85	1	1
	Total	7	0.05-1.0	81	5	6
	CL263284	3 2 2	0.05 0.10 1.0	88 81 84	7	8
	Total	7	0.05-1.0	85	6	7
Bovine liver	Imazapic	2 2 2	0.05 0.10 1.00	79 83 82		
	Total	6	0.05-1.00	81	2	3
	CL263284	2 2 2	0.05 0.10 1.0	78 83 78		
	Total	6	0.05-1.0	80	4	4

The extraction efficiency was investigated using goat milk and kidney from metabolism study (Nejad, 1999b). Two separate samples each of treated milk and kidney tissue containing ^{14}C-residues were analysed for imazapic and CL 263284 by Method M 3188 (milk) and M 3222 (kidney) respectively. With both methods, quantification of residues is made by capillary electrophoresis and UV detection. For milk samples, triplicate 1 mL aliquots of extracted and unextracted milk were assayed by using LSC. For the kidney samples, prior to extraction, the samples were combusted to determine total radioactive residue (TRR). The respective extracts were counted for determination of extraction efficiency.

The extraction procedure used in method M 3188 (milk) yielded a total extractability of 94% for milk (based on total ^{14}C recovery) using 22.1 mL of a 6 N HCl:water:10% lead acetate (0.1:20:2, v/v/v) extraction.

The extraction procedure used in method M 3222 (kidney) yielded a total extractability of 88% in kidney (based on total carbon-14 recovery) using 150 mL of an HCl:water:methanol (1.5:58.5:90, v/v/v) extraction and a 5-minute extraction time.

CE analysis of the treated milk indicated an average of 0.060 mg/kg imazapic compared to 0.051 mg/kg using HPLC radioassay. CE analysis of the treated kidney indicated an average of 0.271 mg/kg imazapic compared to 0.234 mg/kg using HPLC radioassay. No detectable residues for CL 263284 were found in milk or kidney.

M 3223

An independent laboratory validation of Method M 3223 was conducted for the determination of imazapic and CL 263284 in milk fat and tissue fat at fortification levels of 0.01–0.2 or 0.05–1.0 mg/kg respectively (Sweeney and Nejad, 1998a)(Table 40). The recoveries were within the acceptable range. The repeatability standard deviations were below 15%.

Table 40 Recovery results obtained during independent laboratory validation of Method M 3223 for fat

Matrix	Analyte	No.	Fortification Level (mg/kg)	Mean (%)	SD (%)	RSD (%)
Bovine milk fat	Imazapic	2 2 2	0.01 0.02 0.20	106 102 104		
	Total	6	0.01-0.20	104	8	8
	CL263284	2 2 2	0.01 0.02 0.20	100 104 92		
	Total	6	0.01-0.20	99	11	11
Bovine tissue fat	Imazapic	2 2 2	0.05 0.10 1.00	91 94 104		
	Total	6	0.05-1.00	96	12	12
	CL263284	2 2 2	0.05 0.10 1.00	100 88 97		
	Total	6	0.05-1.00	95	8	8

Stability of pesticide residues in stored analytical samples

The Meeting received information on freezer storage stability of imazapic and two of its metabolites in various plant and animal commodities.

Frozen samples were homogenized and fortified with test compound. The fortified homogenate was stored in deep freezer. After each specified period, a portion of sample was analysed for test compound. The stability results are expressed as average percentage of the nominal fortification and are not corrected for the procedural recoveries. In order to account for possible variations over the time investigated, the mean procedural recovery results are given in addition.

Table 41 summarized the storage conditions, storage periods and percent remaining after each period

Table 41 Stability of imazapic in frozen plant matrices

Matrix.	Storage period (month)	Imazapic % remaining	Procedural recovery	CL 263284 % remaining	Procedural recovery	CL 189215 % remaining	Procedural recovery
Soya bean matrices fortified at 0.1 mg/kg, stored at <-20 °C and analysed with Method SOP-PA.0288 (Leite, Alves, 2011a)							
Seed	0	105	-	107	-	107	-
	1	101	103	101	103	102	108
	2	100	97	92	99	93	106
	3	93	91	104	103	105	107
	7	69	81	75	89	97	103
	10	134	148	111	123	124	124
Laminated bean	0	93	-	95	-	90	-
	1	94	104	98	108	95	111
	3	93	95	93	104	88	89
Defatted meal	0	94	-	101	-	92	-
	1	102	110	105	107	107	126
	3	90	93	107	97	97	100
Toasted	0	103	-	101	-	98	-
Defatted meal	1	115	101	105	107	111	126
	3	93	99	107	97	100	90
Oil	0	110	-	111	-	106	-
	1	95	92	86	102	83	100
	3	109	104	100	110	108	116
Wheat matrices fortified at 0.5 mg/kg, stored at <-10 °C and analysed with Method M 2463 (Nejad H., 1999a)							
Grain	14	91	90	91	91	83	90
	18	89	108	102	104	82	98
	24	102	100	112	106	85	103
Straw	14	87	88	92	91	79	78
	18	86	93	93	98	80	89
	24	92	87	92	92	87	77
Hay	14	84	90	82	92	83	92
	18	74	92	75	89	77	95
	24	92	103	84	108	88	108
Forage	14	85	88	79	89	79	85
	18	86	90	97	87	84	95
	24	71	85	70	87	71	90
Sugar cane matrices fortified at 0.1 mg/kg, stored at ≤-20 °C and analysed with Method SOP-PA.0288 (Leite and Goncalves, 2010a)							
Cane	0 d	101	-	108	-		
	31 d	84	89	97	91		
	62 d	84	93	97	97		
	91 d	89	92	96	94		
	185 d	80	85	74	79		
	276 d	72	84	73	75		
	367 d	85	95	100	93		
	731 d	70	86	74	89		
Peanut matrices fortified at 1 mg/kg, stored at -25 to -5°C Method M 2379 (nutmeat & hull) or M 2599 (hay) (Nutmeat and hull: Nejad and Xu, 2000a)(Hay: Nejad and Xu, 2000b)							
Nutmeat	1	84	96	87	98	80	87
	5	81	86	83	92	84	91
	6	74	81	76	85	76	86
	12	70	89	75	93	78	96
	18	71	81	62	84	66	83
	24	80	78	85	81	82	77
Hull	1	89	79	87	78	81	78
	5	111	101	122	113	84	79
	6	84	90	86	88	97	96
	12	74	83	81	84	84	86
	18	83	82	68	85	74	85
	24	80	95	83	93	83	96

Matrix.	Storage period (month)	Imazapic		CL 263284		CL 189215	
		% remaining	Procedural recovery	% remaining	Procedural recovery	% remaining	Procedural recovery
Hay	0	81	77	110	87	100	83
	3	73	84	83	88	82	83
	6	61	86	84	93	78	90
	12	53	77	68	85	63	81
	18	64	92	80	100	74	98
	24	75	87	103	96	100	96

Table 42 Stability of imazapic in frozen animal matrices

Matrix	Storage period (month)	Imazapic		CL 263284	
		% remaining	Procedural recovery	% remaining	Procedural recovery
Cattle milk fortified at 1 mg/kg, stored frozen (no information on the temperature) and analysed with Method M 3188 (Naumann and Nejad, 1999a)					
Cattle milk	1	89	87	87	88
	3	95	97	95	97
	6	88	90	92	90
Cattle tissues fortified at 1 mg/kg, stored frozen (no information on the temperature) and analysed with Method M 3222 (Sweeney and Nejad, 1999a)					
Muscle	1	84	84	66	72
	3	78	83	80	76
	5	80	80	82	78
	8	79	79	75	77
Kidney	1	85	87	75	82
	3	87	90	83	86
	5	86	90	85	93
	8	77	82	76	78
Liver	1	81	80	78	72
	3	82	88	82	86
	5	79	82	89	81
	8	78	77	81	76

The stability of imazapic and ist metabolites CL 263,284 and CL 189,215 was also investigated in standard calibration solutions diluted in 0.1% formic acid in water: 1% formic acid in methanol (1:1, v/v) and stored at 5±3 °C in the dark. The standard solutions contained 0.400 ng/mL per analyte. After 32 days, fresh standard calibration solutions were diluted by the above solution mixture, and the results obtained for the stored standard solutions were compared to the results obtained for the fresh standard solutions.

The storage stability of standard calibration solutions containing imazapic and its metabolites in 0.1% formic acid in water: 1% formic acid in methanol (1:1, v/v) was demonstrated for a period of 32 days when stored at 5±3 °C in the dark.

USE PATTERNS

Imazapic is used to control broad leaf and grassy weeds. It is formulated as a liquid or granular product either as a solo product or in combination with other active substances for use on pulses, cereal grains, grasses for sugar, oilseeds, and straw, forage and fodder of cereal grains. It is registered in a number of countries for crops with pre-emergence and post emergence applications.

The authorized uses relevant to the supervised trials data submitted to the current Meeting is summarized in Table 43.

Table 43 Registered use of imazapic relevant to the residue evaluation by the current Meeting

Country	Formulation Type and g/kg or g/l (Other ai)	F, G, or P	Application				Application rate per treatment		PHI days
			Method	No. per crop and season	Interval days	Timing	Water L/ha	Max Rate kg ai/ha	
Cereal grains: Maize									
Brazil	WG 525* (imazapyr)	F	Ground spraying	1	nr	Post emergence	100-250	0.0525	96
Brazil	WG 525* (imazapyr)	F	Aerial spraying	1	nr	Post emergence	40-50	0.0525	96
Cereal grains: Rice									
Brazil	SL 25* (imazapyr)	F	Ground spraying	1-2	14-21	Post emergence between 4 leaves and 1 tiller of rice	100-250	0.025	60
Brazil	SL 25* (imazapyr)	F	Aerial spraying	1-2	14-21	Post emergence between 4 leaves and 1 tiller of rice	40-50	0.025	60
Brazil	WG 175* (imazapyr)	F	Ground spraying	1-2	-	Post emergence between 2 leaves and 1 tiller of rice	100-200	0.0245	60
Brazil	WG 175* (imazapyr)	F	Aerial spraying	1-2	-	Post emergence between 2 leaves and 1 tiller of rice	40-50	0.0245	60
Cereal grains: Wheat									
Australia	WG 525* (imazapyr)	F	spraying	1	nr	Post emergence 2 to 6 leaf stage	70	0.011	nr
Australia	EC 22* (imazapyr)	F	spraying	1	nr	Post emergence 4 leaf (Z14) to flag leaf (Z37)	50	0.021	nr
Grasses for sugar or syrup production: Sugar cane									
Argentina	WG 700	F	spraying	1	nr	30–45 days before planting	100-150	0.35	392
Australia	SL 240	F	spraying	1	nr	After planting before emergence of cane	200	0.096	nr
Brazil	WG 700	F	spraying	1	nr	30-45 days before planting	200-250	0.245	283
Costa Rica	WG 700	F	spraying	1	nr	Within first 10 days of planting Pre-emergence to weed	250-300	0.175	85
Guatemala	WG 700	F	spraying	1	nr	Within first 10 days of planting Pre-emergence to weed	250-300	0.175	85
Oilseeds: Peanut									
Brazil	WG 700	F	spraying	1	nr	30-45 days before planting	200-250	0.098	70
USA	SL 240	F	spraying	1	nr	Early post emergence	47-94	0.067	90
USA	WG 700	F	spraying	1	nr	Early post emergence	min. 94	0.071	90
Oilseeds: Rape seed									
Australia	WG 525* (imazapyr)	F	spraying	1	nr	Early post emergence 2-6 leaf stage	70	0.0288	nr
Straw, fodder and forage of cereal grains and grasses, except grasses for sugar production (including buckwheat fodder): Bermuda grass, brome grass blue grass, wheat grass, pasture, rangeland, etc.									
USA	SL 240	F	spraying	1	nr	Post emergence (spring & summer, 100% green-up; winter, dormant or full green-up)	18.7-94	0.22	-

nr: not required when used as directed.

* for imidazolinone-tolerant varieties only

RESIDUES RESULTING FROM SUPERVISED RIALS ON CROPS

The Meeting received residue data from supervised field trials conducted on soya bean (including GM soya bean) in Brazil, maize in Brazil, rice in Brazil, wheat in Australia, sugar cane in Argentina, Australia, Brazil, Costa Rica and Guatemala, Peanut in Brazil and the USA, rape seed in Australia and grasses (animal feedstuffs) in the USA.

Application rates and residue concentrations were reported as imazapic. Residue concentrations are recorded unadjusted for recoveries or for residue values in control samples. Where multiple samples were taken from a single plot, individual results are reported, and the calculated average concentration is used for estimation of maximum residue level. Where trials were conducted

in the same location, with the same or similar varieties, same or similar formulations, and same equipment, and at the same or similar timing, they are not regarded as independent and only one result from these trials was chosen for the estimation of a maximum residue level.

Residues from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels and they are underlined.

In many of the following trials, plants resistant to imidazolinone pesticides were used. They are classified into two different categories. The first category is non-GM plants (maize, rape seed, rice, wheat) showing the tolerance due to the mutation(s) of endogenous AHAS gene from conventional breeding technique. and their variety names have "CL" in them. The second category is GM plants (so far soya bean only) to which mutated AHAS gene of Arabidopsis was introduced for imidazolinone tolerance.

Crop Group	Commodity	Table No.
Pulses	Soya beans	Table 44
Cereal grains	Maize	Table 45
	Rice	Table 46
	Wheat	Table 47
Grasses, for sugar or syrup production	Sugar cane	Table 48
Oilseed	Peanut	Table 49
	Rape seed	Table 50
Legume animal feed	Peanut hay	Table 51
Straw, fodder and forage of cereal grains and grasses (including buckwheat fodder)(straws and fodders dry)	Wheat straw and fodder, dry	Table 52
	Hay or fodder (dry) of grasses	Table 53
Miscellaneous fodder and forage crops	Rape seed forage	Table 54

Pulses

Soya beans

During the 2006/2007 growing season, eight field trials were carried out in Brazil to determine the residues levels of imazapic in soya bean after treatment with a WG mix formulation of imazapic and imazapyr. In all trial sites, one trial plot was untreated to provide control samples, and one trial plot received one post-emergence application at a rate of 0.0175 kg imazapic/ha (and 0.0525 kg imazapyr/ha), 60 days before harvest (BBCH 24–75). In three trials, the application was performed 40, 60, 80, 100 and 120 days before harvest, each on a separate plot. Samples were taken 60 days after the application (DALA) in all trials; but in three trials, additional samplings were performed 40, 80, 100 and 120 DALA. The soya bean samples were stored frozen until analysis. Soya bean samples were analysed for imazapic using Method SOP-PA.0249. The limit of quantification was 0.05 mg/kg.

During the 2007/2008 growing season, a field trial was carried out in Brazil to determine the residues levels of imazapic in soya bean after treatment with a mix formulation of imazapic and imazapyr. One trial plot was untreated to provide control samples, and one trial plot received one foliar post-emergence spray application at a rate of 0.0175 kg imazapic/ha (and 0.0525 kg imazapyr/ha), either 40, 60, 80, 100 or 120 days before harvest. Samples were taken 40, 60, 80, 100 or 120 days after the application. The soya bean samples were analysed for imazapic and the two metabolites using Method SOP-PA.0288. The limit of quantification was 0.01 mg/kg for each analyte.

During the 2010 growing season, two field trials were carried out in Brazil to determine the residues levels of imazapic in soya bean after treatment with a mix formulation of imazapic and imazapyr. At both trial sites, one trial plot was untreated to provide control samples, and four trial plots received one foliar post-emergence spray application at a rate of 0.0175 kg imazapic/ha (and 0.0525 kg imazapyr/ha), 20, 40, 60 or 80 days before harvest. Samples of soya bean grain were taken 20, 40, 60 and 80 days after the application. Soya bean samples were analysed for residues using Method SOP-PA.0288.

During the 2011 growing season, five field trials were carried out in Brazil to determine the residues levels of imazapic in transgenic soya bean after treatment with a mix formulation of imazapic and imazapyr. At all trial sites, one trial plot was untreated to provide control samples, and one trial plot received one post-emergence application at a rate of 0.0175 kg imazapic/ha (and 0.0525 kg imazapyr/ha), 60 days before harvest (BBCH 66–73). At one trial with five plots, the application was performed 20, 40, 60, 80 and 100 days before harvest. Samples of soya bean grain were taken 60 days after the application (DALA) at all trials; at one trial, additional samplings were performed 20, 40, 80 and 100 DALA, and at one trial aspirated grain fractions were also sampled. Soya bean samples were analysed for residues using Method SOP-PA.0288.

Table 44 Residues of imazapic in imidazolinone-tolerant soya beans from supervised trials conducted in Brazil

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Brazil	Ground spray Aerial spray	0.014-0.018 0.014-0.018	1 1	PHI 60 PHI 60						
2008/1097470 Uberaba Minas Gerais, BRA (EC-CD-BRVA/ 1088-06) (CV 603)	n.r.	0.0175	1 04.03.07 13.02.07 23.01.07 03.01.07 14.12.06	 40 60 80 100 120	 grain grain grain grain grain	 0.19 0.23 < 0.05 < 0.05 < 0.05	 na na na na na	 na na na na na		
2008/1097470 Santo Antonio de Posse Sao Paulo, BRA (EC-CD-BRUA/ 1088-06) (CV 603)	n.r.	0.0175	1 19.02.07 30.01.07 10.01.07 21.12.06 01.12.06	 40 60 80 100 120	 grain grain grain grain grain	 0.08 < 0.05 < 0.05 < 0.05 < 0.05	 na na na na na	 na na na na na		
2008/1097470 Brasilia Distrito Federal do Brasil, (BRA) (EC-R-BRUC/ 1088-06) (CV 603)	n.r.	0.0175	1 09.02.07	 60	 grain	 0.10	 na	 na		
2008/1097470 Santo Antionio de Goias Goias, BRA (EC-R-BRUB/ 1088-06) (CV 603)	n.r.	0.0175	1 05.02.07	 60	 grain	 0.15	 na	 na		
2008/1097470 Uberaba Minas Gerais, BRA (EC-R-BRVA/ 1088-06)	n.r.	0.0175	1 13.02.07	 60	 grain	 0.25	 na	 na		

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
(CV 603)										
2008/1097470 Santo Antionio de Goias Goias, BRA (EC-CD-BRUB/ 1088-06) (CV 603)	n.r.	0.0175	1 25.02.07 05.02.07 16.01.07 27.12.06 07.12.06	 40 60 80 100 120	 grain grain grain grain grain	 0.15 0.08 < 0.05 < 0.05 < 0.05	 na na na na na	 na na na na na		
2008/1097470 Santo Antonio de Posse Sao Paulo, BRA (EC-R-BRUA/ 1088-06) (CV 603)	n.r.	0.0175	1 30.01.07	 60	 grain	 < 0.05	 na	 na		
2008/1097470 Londrina Parana, BRA (EC-R-BRTA/ 1088-06) (CV 603)	n.r.	0.0175	1 03.01.07	 60	 grain	 < 0.05	 na	 na		
2010/1010261 Santo Antonio de Posse Sao Paulo, BRA (G080102) (CV 127)	spray	0.0175	1 13.02.08 24.01.08 04.01.08 15.12.07 25.11.07	 40 60 80 100 120	 grain grain grain grain grain	 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.03 < 0.03 < 0.03 < 0.03 < 0.03	
2012/3000423 Castro Parana, BRA (G100579) (BRZ 08 200151)	spray	0.0175	1 22.04.11	 60	 grain	 0.07	 < 0.01	 0.01	 0.09	
2010/1127505 Ponta Grossa Parana, BRA (G100005) (L 08)	spray	0.0175	1 24.03.10 04.03.10 12.02.10 23.01.10	 20 40 60 80	 grain grain grain grain	 < 0.01 0.02 0.07 0.03	 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.01 < 0.01 0.02 0.02	 < 0.03 0.04 0.10 0.06	
2010/1127505 Santo Antonio de Posse Sao Paulo, BRA (G100006) (CV 127)	spray	0.0175	1 13.05.10 23.04.10 04.04.10 14.03.10	 20 40 60 80	 grain grain grain grain	 < 0.01 < 0.01 0.05 0.01	 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.03 < 0.03 0.07 0.03	
2012/3000423 Ponta Grossa Parana, BRA (G100575) (BRZ 08 200151)	spray	0.0175	1 20.05.11 30.04.11 10.04.11 21.03.11 01.03.11	 20 40 60 80 100	 grain grain grain grain grain	 < 0.01 < 0.01 0.05 0.12 < 0.01	 < 0.01 < 0.01 < 0.01 < 0.01 < 0.01	 < 0.01 < 0.01 < 0.01 0.01 < 0.01	 < 0.03 < 0.03 0.07 0.14 < 0.03	
2012/3000423 Senador Canedo Goias, BRA (G100576) (BRZ 5384)	spray	0.0175	1 31.01.11	 60	 grain	 < 0.01	 < 0.01	 < 0.01	 < 0.03	

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
2012/3000423 Anapolis Goias, BRA (G100577) (BRZ 5384)	spray	0.0177	1 04.02.11	60	grain	< 0.01	< 0.01	< 0.01	< 0.03	
2012/3000423 Santo Antonio de Posse Sao Paulo, BRA (G100578) (BRZ 08)		0.0175	1 12.05.11	60	grain	0.23	< 0.01	0.02	0.26	

Cereal Grains

Maize

One maize field trial was conducted in Brazil during the 1997 growing season. The trial consisted of an untreated control plot and two treated plots. The treated plots received a single application of a mix formulation of imazapic and imazapyr at growth stage BBCH 17–19 at a rate of either 0.0525 or 0.105 kg imazapic/ha. The maize samples were taken manually at 96 days after treatment Maize RAC samples were analysed for residues using Method LAADL R0001.01. The limit of quantification was 0.1 mg/kg.

During the 2010/2011 growing season, four field trials were carried out in Brazil to determine the residues of imazapic in imidazolinone tolerant maize after treatment with a mix formulation of imazapic and imazapyr. At all trial sites, one trial plot was untreated to provide control samples, and one trial plot received one post-emergence application at a rate of 0.0525 kg imazapic/ha, 96 days before harvest (BBCH 13-17). At two trials, the application was performed 89, 96 and 103 days before harvest. Samples were taken 96 days after the application at all trials; at two trials, additional samples were obtained 89 and 103 DALA. Maize RAC samples were analysed for residues using Method SOP-PA.0288 with a limit of quantification (LOQ) of 0.01 mg/kg.

Table 45 Residues of imazapic in imidazolinone-tolerant maize from supervised trials conducted in Brazil

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Brazil	Ground spray Aerial spray	0.0525 0.0525	1 1	PHI 96 PHI 96						
# IA-731-007 Goiania Goias, BRA (Cargill / IMI 1)	Spray	0.0525 0.105	1 06.01.97	96	grain	< 0.1 < 0.1	na na	na na		7-9 leaves
# 2011/1104555 Santo Antonio de Posse		0.0525	1 29.07.10 22.07.10	 89 96	 grain grain	 < 0.01 < 0.01	 na na	 na na		 G14 G14
Sao Paulo, BRA (G100192) (Dow 2B710CL)			15.07.10	103	grain	< 0.01	na	na		G14

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# 2011/1104555 Senador Canedo Goias, BRA (G100193) (Clearfield)		0.0525	1 28.07.10 21.07.10 14.07.10	89 96 103	grain grain grain	< 0.01 < 0.01 < 0.01	na na na	na na na		G17 G15 G13
# 2011/1104555 Engenheiro Coelho Sao Paulo, BRA (G100194) (Dow 2B710CL)		0.0525	1 22.09.10	96	grain	< 0.01	na	na		G14
# 2011/1104555 Jaboticabal Sao Paulo, BRA (G100195) (Dow 2B710CL)		0.0525	1 07.10.10	96	grain	< 0.01	na	na		G15

Rice

During the 2000/2001 growing season, one field trial was carried out in Brazil to determine the residues of imazapic in rice grain after treatment with a mix formulation of imazapic and imazapyr. One trial plot was untreated to provide control samples, and one trial plot received two post-emergence spray applications at a rate of 0.0375 kg ai/ha each and a retreatment interval of 14–17 days. Samples were taken at physiological maturity 60, 75 and 90 days after the last application. Rice RAC samples were analysed for residues using Method SOP-PA.0231. The limit of quantification was 0.05 mg/kg.

During the 2000/2001 growing season, one field trial was carried out in Brazil to determine the residues of imazapic in rice grain after treatment with a mix formulation of imazapic and imazapyr. One trial plot was untreated to provide control samples, and two trial plots received two post-emergence spray applications, each at a rate of 0.01875 or 0.0375 kg ai/ha, respectively. The retreatment interval was 17 days. Samples were taken at physiological maturity, 60 days after the last application (DALA), at all plots. Rice RAC samples were analysed for residues using Method SOP-PA.0231.

During the 2000/2001 growing season, another field trial was carried out in Brazil to determine the residues of imazapic in rice grain after treatment with a mix formulation of imazapic and imazapyr. One trial plot was untreated to provide control samples, and two trial plots received two post-emergence spray applications, each at a rate of 0.01875 or 0.0375 kg ai/ha, respectively. The retreatment interval was 13 days. Samples were taken at physiological maturity, 60 days after the last application, at all plots. Rice RAC samples were analysed for residues using Method SOP-PA.0231.

During the 2002/2003 growing season, four field trials were carried out in Brazil to determine the residues of imazapic in rice grain after treatment with a mix formulation of imazapic and imazapyr. One trial plot was untreated to provide control samples. In one trial site, the treated plot received two post-emergence spray applications at a rate of 0.025 kg ai/ha each. In three trials, two separate plots received two post-emergence spray applications, each at a rate of 0.025 or 0.05 kg ai/ha, respectively. The retreatment interval was 17–52 days. Samples were taken at physiological maturity, 60 days after the last application at all plots. In the trial treated with the lower rate only, samples were collected at 50, 55, 65 and 70 DALA in addition. Rice RAC samples were analysed for residues using Method SOP-PA.0249. The limit of quantification was 0.05 mg/kg.

During the 2002/2003 growing season, four field trials were carried out in Brazil to determine the residues of imazapic in rice grain after treatment with a mix formulation of imazapic and imazapyr. One trial plot was untreated to provide control samples. At one trial site, the treated plot received two post-emergence spray applications at a rate of 0.015 kg imazapic/ha each. At three trials, two separate plots received two post-emergence spray applications, each at a rate of 0.015 kg imazapic/ha (+0.045 kg imazapyr/ha) or 0.030 kg imazapic/ha (+0.090 kg imazapyr/ha), respectively. The retreatment interval was 17-52 days. Samples were taken at physiological maturity, 60 days after the last application at all plots. At the trial treated with the lower rate only, samples were collected at 50, 55, 65 and 70 DALA in addition. Rice RAC samples were analysed for residues using Method SOP-PA.0231.

Table 46 Residues of imazapic in imidazolinone-tolerant rice from supervised trials conducted in Brazil

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Brazil	Ground spray Aerial spray	0.0245 0.0245	1 1	PHI 60 PHI 60						
# 2001/3001704 Cachoeirinha Rio Grande do Sul, BRA (CDR/H/2001/617/BRTB) (IRGA 417 CL)	Spray	0.0375	2 05.03.01 16.02.01 02.02.01	 60 75 90	grain	 < 0.05 < 0.05 < 0.05	 na na na	 na na na		post-emerg.
# 2001/3001705 Cachoeirinha Rio Grande do Sul, BRA (R/H/2001/618/BRTB) (IRGA 417 CL)	Spray	0.01875 0.0375	2 15.03.01	60	grain	< 0.05 < 0.05	na na	na na		post-emerg.
# 2001/3001706 Uruguaiana Rio Grande do Sul, BRA (R/H/2001/674/BRTB) (Americana,, (AS 3510))	Spray	0.01875 0.0375	2 07.12.00	60	grain	< 0.05 < 0.05	na na	na na		post-emerg.
# 2004/3000911 Sao Vicente do Sul Rio Grande do Sul, BRA (CD/H/2003/523/BRT) (IRGA 422 CL)	Spray	0.025	2 03.03.03 27.02.03 22.02.03 17.02.03 12.02.03	 50 55 60 65 70	grain	 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	 na na na na na	 na na na na na		 booting booting elongation elongation elongation
# 2004/3000911 Sao Vicente do Sul Rio Grande do Sul, BRA (R/H/2003/524/BRT) (IRGA 422 CL)	Spray	0.025 0.05	2 22.02.03	60	grain	< 0.05 < 0.05	na na	na na		booting

Report-No. Location (trial no.) (Variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# 2004/3000911 Uruguaiana Rio Grande do Sul, BRA (R/H/2003/525/BRT) (XP 701 CL)	Spray	0.025 0.05	2 16.01.03	60	grain	< 0.05 < 0.05	na na	na na		1-2 leaves
# 2004/3000911 Cachoeirinha Rio Grande do Sul, BRA (R/H/2003/526/BRT) (XP 701 CL)	Spray	0.025 0.05	2 19.02.03	60	grain	< 0.05 < 0.05	na na	na na		booting
# 2003/3001442 # 2003/3001441 Sao Vicente do Sul Rio Grande do Sul, BRA (CD/H/2003/519/BRT) (IRGA 422 CL)		0.02975	2 03.03.03 27.02.03 22.02.03 17.02.03 12.02.03	 50 55 60 65 70	grain	 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	 na na na na na	 na na na na na		n.r. flowering infloresc. emerg. booting booting elongation
# 2003/3001442 # 2003/3001441 Sao Vicente do Sul Rio Grande do Sul, BRA (R/H/2003/520/BRT) (Tec XP 701 CL)		0.02975 0.0595	2 22.02.03	60	grain	< 0.05 < 0.05	na na	na na		booting
# 2003/3001442 # 2003/3001441 Cachoeirinha Rio Grande do Sul, BRA (R/H/2003/521/BRT) (XP 701 CL)		0.02975 0.0595	2 19.02.03	60	grain	< 0.05 < 0.05	na na	na na		booting
# 2003/3001442 # 2003/3001441 Uruguaiana Rio Grande do Sul, BRA (R/H/2003/522/BRT) (IRGA 422 CL)		0.02975 0.0595	2 16.01.03	60	grain	< 0.05 < 0.05	na na	na na		1-2 leaves

Wheat

Five wheat field trials were conducted in Australia during the 1997 and 1998 growing seasons. The trials consisted of an untreated control plot and two (3 trials) or three (2 trials) treated plots. The treated plots received a single application of WG formulation of imazapic. Two trials were treated with either 35, 52.5 or 70 g ai/ha. Three trials were treated at a rate of either 21 or 35 g ai/ha. Wheat forage samples were collected 1–4 times, 0–42 days after treatment. Samples of grain and straw were

taken at normal harvest, 93–113 days after the application. The samples were analysed for residues using HPLC. The limit of quantification was 0.05 mg/kg.

Table 47 Residues of imazapic in imidazolinone-tolerant wheat from supervised trials conducted in Australia

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Australia	Spray	0.021	1	-						
# 2000/1023962 Galore New South Wales AUS (573/AU/97/01/SN01)	Spray	0.035 0.0525 0.07	1	96 96 96	grain grain grain	< 0.05 < 0.05 < 0.05	na na na	na na na		post-emerg.
# 2000/1023962 Wongan Hills Western Australia AUS (573/AU/97/01/WA01)		0.035 0.0525 0.07	1	113 113 113	grain grain grain	< 0.05 < 0.05 < 0.05	na na na	na na na na		post-emerg.
# 2000/1023962 Burabadji Western Australia AUS (573/AU/98/08/WA01)		0.021 0.035	1	93 93	grain grain	< 0.05 < 0.05	na na	na na		advanced tillering (Z25)
# 2000/1023962 Manoora South Australia AUS (573/AU/98/08/SA01)		0.021 0.035	1	95 95	grain grain	< 0.05 < 0.05	na na	na na		stem elongation 1-2 nodes

Grasses for sugar or syrup production

Sugar cane

One sugar cane field trial was conducted in Argentina during the 1996/1997 growing season. The trial consisted of an untreated control plot and a treated plot. The treated plot received a single application of a WG formulation of imazapic as pre-treatment at a rate of 350 g ai/ha, 38 days pre-plantation. The sugar cane samples were taken at 392 days after treatment. Sugar cane RAC samples were analysed for residues of BAS 715 H (imazapic) using Method LAADL R0002 with a limit of quantification of 0.05 mg/kg.

Two residue trials on sugar cane were conducted in Guatemala and two in Costa Rica during the growing season 2007-2008. Three test plots were established at each trial site. Treatment 1 was the untreated plot and provided control samples for analysis. For one trial each in Costa Rica and Guatemala, the Treatment 2 plot was designated as a RAC plot (non-processing) and received one pre-plant incorporated application of a WG formulation of imazapic, targeting a rate of 0.245 kg ai/ha. The Treatment 3 plot was designated as a processing plot and received one pre-emergence application of a WG formulation of imazapic targeting the 5× rate of 1.225 kg ai/ha. For the remaining two trials, the Treatment 2 plot was designated as a RAC plot (non-processing) and received one pre-emergence application at a targeted a rate of 0.245 kg ai/ha. The 'Treatment 3' plot was designated as a processing plot and received one pre-emergence application of a WG formulation of imazapic targeting the 5× rate of 1.225 kg ai/ha. At about 150 days after the last application, whole cane samples for RACs were

collected from the 'Treatment 2' and untreated plots at all sites. At about 300 DALA, commercially mature, whole cane samples for RACs and processing samples were collected.

Residues of imazapic in sugarcane RAC were determined using SOP-PA.0288. The limit of quantification (LOQ) of the method was 0.01 mg/kg for imazapic and its two metabolites.

Four residue trials on sugar cane were conducted in Australia during the growing season 1996/97. Three test plots were established on each site, one untreated control plot and two plots treated once with a WG formulation of imazapic, targeting a rate of either 0.144 or 0.288 kg ai/ha. Two trials were treated pre-emergence and two post-emergence at the 'spike' growth stage. The spray volume was 119 l/ha. At 211–217 days after the application, sugar cane foliage and cane samples were collected. Residues of imazapic in sugar cane matrices were determined using Method L 741. The limit of quantification (LOQ) of the method was 0.05 mg/kg.

During 2006 growing season, five field trials were carried out in representative sugar cane growing areas in Brazil to determine the residues levels of imazapic and its two metabolites in sugar cane after treatment with a WG formulation of imazapic. At all trial sites, two trial plots were untreated to provide control samples, and two trial plots each received one pre-plant incorporated application at a rate of 0.245 kg ai/ha. In one untreated and one treated plot at four sites, the whole cane samples were harvested (BBCH 30) at 150 days after the last application. At one trial, the 150 DALA samples were not collected due to the insufficient crop productivity. In one control and one treated plot at all five sites, the mature cane samples were harvested at 360 to 365 days after treatment (BBCH 49). The samples were analysed according to SOP-PA.0288 for the determination of imazapic and its metabolites residues with a limit of quantification (LOQ) of 0.01 mg/kg for each analyte.

Table 48 Residues of imazapic in sugar cane from supervised trials conducted in Argentina, Australia, Brazil, Costa Rica and Guatemala

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Argentina	Spray	0.35	1	PHI 392						
GAP in Australia	Spray	0.096	1	nr						
GAP in Brazil		0.245	1	283						
GAP in Costa Rica	Spray	0.175	1	85						
GAP in Guatemala	Spray	0.175	1	85						
# IA-790-011 Lules Tucuman, ARG (Famailla 8116)	Spray	0.35	1 03.06.96		cane	< 0.05	na	na		PPI
# 1997/1007121 Home Hill North Queensland AUS (173/AU/96/20/QU06)		0.144	1 28.08.96	217	foliage cane	< 0.05 < 0.05	na na	na na		pre-emerg. (post pit)
		0.288			foliage cane	< 0.05 < 0.05	na na	na na		
# 1997/1007121 Bunderberg Queensland, AUS (173/AU/96/21/QU05)		0.144	1 04.09.96	211	foliage cane	< 0.05 < 0.05	na na	na na		post-emerg. ('spike')
		0.288			foliage cane	< 0.05 < 0.05	na na	na na		

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# 1997/1007121 Bunderberg Queensland, AUS (173/AU/96/20/QU11)		0.144 0.288	1 04.09.96	211	foliage cane foliage cane	< 0.05 < 0.05 < 0.05 < 0.05	na na na na	na na na na		pre- emerg.
# 1997/1007121 Bunderberg Queensland, AUS (173/AU/96/20/QU12) (138)		0.144 0.288	1 04.09.96	211	foliage cane foliage cane	< 0.05 < 0.05 < 0.05 < 0.05	na na na na	na na na na		post- emerg. ('spike') (5 cm height)
# 2008/7012758 Carrillos Alajuela, CRI (RCN R06486) (Mex 50)		0.245 1.225	1 20.03.07	153 299 299 299	cane cane cane cane	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.03 < 0.03 < 0.03 < 0.03	PPI pre- emerg.
# 2008/7012758 Tacares Alajuela, CRI (RCN R06487) (Mex 50)		0.245 1.225	1 20.03.07	153 299 299 299	cane cane cane cane	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.03 < 0.03 < 0.03 < 0.03	pre- emerg.
# 2008/7012758 La Gomera Escuintla, GTM (RCN R06488) (CP-731547)		0.245 1.225	1 09.03.07	148 310 310 310	cane cane cane cane	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.03 < 0.03 < 0.03 < 0.03	PPI pre- emerg.
# 2008/7012758 Ceiba Amelia Escuintla, GTM (RCN R06489) (CP-731547)		0.245	1 16.12.06	148	cane	< 0.01	< 0.01	< 0.01	< 0.03	pre- emerg.
# 2009/1094340 Uberlandia Minas Gerais, BRA (EC-R-BRVA/1084-06)		0.245	1 20.12.06	150 360-365	cane cane	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.03 < 0.03	0
# 2009/1094340 Campo Alegre Goias, BRA (EC-R-BRVB/1084-06)		0.245	1 20.12.06	360-365	cane	< 0.01	< 0.01	< 0.01	< 0.03	0
# 2009/1094340 Santo Antonio de Posse Sao Paulo, BRA (EC-R-BRUA/1084-06) (SP 801816)		0.245	1 22.11.06	150 360-365	cane cane	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.03 < 0.03	0
# 2009/1094340 Piracicaba Sao Paulo, BRA (EC-R-BRUB/1084-06) (SP 801816)		0.245	1 06.11.06	150 360-365	cane cane	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.03 < 0.03	0

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# 2009/1094340 Santo Antonio de Posse Sao Paulo, BRA (EC-R-BRUC/1084-06) (SP 801816)		0.245	1 22.11.06	150 360-365	cane cane	< 0.01 < 0.01	< 0.01 < 0.01	< 0.01 < 0.01	< 0.03 < 0.03	0

Oilseeds

Peanut

One peanut field trial was conducted in Brazil during the 1996/1997 growing season. The trial consisted of an untreated control plot and two treated plots. The treated plots received a single post-emergence application of a WG formulation of imazapic at a rate of either 0.070 or 0.140 kg ai/ha, 22 days after planting. Peanut RAC samples were analysed for residues using Method 2253.01. The limit of quantification was 0.1 mg/kg.

Four peanut field trials were conducted in Brazil during the 2003/2004 growing season. The trials consisted of an untreated control plot and two (3 trials) or five (1 trial) treated plots. The treated plots received a single application of WG formulation of imazapic. In one trial, the plots were treated with 0.098 kg ai/ha, either 60, 65, 70, 75 or 80 days before harvest. In three trials, the plots were treated at a rate of either 0.098 kg ai/ha or 0.196 kg ai/ha, 70 days before harvest. The peanut samples were collected at 70 days after treatment. In one trial, samples were taken at 60, 65, 70, 75 and 80 days after treatment. Peanut RAC samples were analysed for residues using Method SOP-PA.0249. The limit of quantification was 0.05 mg/kg.

A total of six peanut field trial was conducted independently in the USA during the 1992 growing season. Each trial consisted of an untreated control plot and two treated plots. The treated plots received a single post-emergence application of an SL formulation of imazapic at a rate of either 70 or 210 g ai/ha, to the 10–15 cm high (vegetative stage) or 15–25 cm high (pre-bloom) peanut plants. The peanut nut and hull samples were taken 95, 102, 104, 105, 117 or 119 days after treatment. Peanut RAC samples were analysed for residues of imazapic and its metabolite CL 263284 using Method M 2253. The limit of quantification was 0.1 mg/kg.

A total of six peanut field trial was conducted in the USA during the 1993 growing season. Each trial consisted of an untreated control plot and one treated plot. The treated plots received a single post-emergence application of an SL formulation of imazapic at a rate of 70 g ai/ha, to the 5 cm high (seedling stage), 7.5–10 cm high (pre-bloom stage) 10–15 cm high (3 weeks post-emergence), or 15–20 cm high (early bloom stage) peanut plants. The peanut nutmeat and hull samples were taken at 75, 95, 105, 116 and 119 days after treatment. Peanut RAC samples were analysed for residues of imazapic and metabolites CL 263,284 and CL 189,215 using Method M 2379. The limit of quantification was 0.1 mg/kg.

Table 49 Residues of imazapic in peanut from supervised trials conducted in Brazil and the USA

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Brazil	Spray	0.098	1	PHI 70						
GAP in USA	Spray	0.071	1	90						
# IA-740-019 Jaboticabal	Spray	0.07	1 11.11.96	70	nut	< 0.1	na	na		post-emerg.

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
Sao Paulo, BRA (Tatu vermelho)		 0.14			 nut	 < 0.1	 na	 na		
# 2005/3001521 Santo Antonio de Posse Sao Paulo, BRA (CD/H/2003/539/BRU) (Tatu vermelho)		0.098 0.098 0.098 0.098 0.098	11/03/03 06/03/03 01/03/03 24/02/03 19/02/03	60 65 70 75 80	nut nut nut nut nut	< 0.05 < 0.05 < 0.05 < 0.05 < 0.05	na na na na na	na na na na na		n.r.
# 2005/3001521 Ponta Grossa Perugia, BRA (R/H/2003/537/BRT) (Tatu vermelho)		0.098 0.196	12/02/03	70 70	nut nut	< 0.05 < 0.05	na na	na na		n.r.
# 2005/3001521 Santo Antonio de Posse Sao Paulo, BRA (R/H/2003/538/BRU) (Tatu vermelho)		0.098 0.196	09/02/03	70 70	nut nut	< 0.05 < 0.05	na na	na na		n.r.
# 2005/3001521 Santo Antonio de Posse Sao Paulo, BRA (EC/R/H/2004/505/ BRU/A1) (Tatu vermelho)		0.098 0.196	15/12/03	70 70	nut nut	< 0.05 < 0.05	na na	na na		post- emerg.
# IA-740-001 Montezuma Macon County GA, USA (GK-7)		0.07 0.21	1 08.07.92	105 105 105 105	hull nutmeat hull nutmeat	< 0.1 < 0.1 < 0.1 < 0.1	< 0.1 < 0.1 < 0.1 < 0.1	na na na na		20-25 cm
# IA-740-002 Malone Jackson County FL, USA (Florunner)		0.07 0.21	1 09.06.92	119 119 119 119	hull nutmeat hull nutmeat	< 0.1 < 0.1 < 0.1 < 0.1	< 0.1 < 0.1 < 0.1 < 0.1	na na na na		10 cm
# IA-740-003 Grangeburg Houston County AL, USA (Florunner)		0.07 0.21	1 11.06.92	117 117 117 117	hull nutmeat hull nutmeat	< 0.1 < 0.1 < 0.1 < 0.1	< 0.1 < 0.1 < 0.1 < 0.1	na na na na		10 cm
# IA-740-004 Caddo County OK, USA (Spanco)		0.07 0.21	1 09.07.92	102 102 102	hull nutmeat hull	< 0.1 < 0.1 < 0.1	< 0.1 < 0.1 < 0.1	na na na		bloom stage, 15-20 cm
# IA-740-005 Jamesville Martin County NC, USA (NC-9)		0.07 0.21	1 25.06.92	104 104 104 104	hull nutmeat hull nutmeat	< 0.1 < 0.1 < 0.1 < 0.1	< 0.1 < 0.1 < 0.1 < 0.1	na na na na		seedling stage, ca. 13 cm

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# IA-740-006		0.07	1	95	hull	< 0.1	< 0.1	na		pre-bloom
			02.07.92	95	nutmeat	< 0.1	< 0.1	na		stage,
Waller County										10-15 cm
TX, USA										
		0.21		95	hull	< 0.1	< 0.1	na		
(Spanish)				95	nutmeat	< 0.1	< 0.1	na		
# IA-740-007		0.07	1	75	hull	< 0.1	< 0.1	< 0.1	< 0.3	pre-bloom
			09.07.93	75	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	stage,
Waller County										7.5-10 cm
TX, USA										
(Spanish)										
# IA-740-008		0.07	1	105	hull	< 0.1	< 0.1	< 0.1	< 0.3	early
Montezuma			07.07.93	105	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	bloom
Macon County										stage,
GA, USA										15-20 cm
		0.21		105	hull	< 0.1	< 0.1	< 0.1	< 0.3	
(GK-7)				105	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	
# IA-740-009		0.07	1	95	hull	< 0.1	< 0.1	< 0.1	< 0.3	early
			12.07.93	95	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	bloom
Caddo County										stage,
OK, USA										15 cm
(Spanco)										
# IA-740-010		0.07	1	116	hull	< 0.1	< 0.1	< 0.1	< 0.3	seedling
Jamesville			18.06.93	116	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	stage,
Martin County										ca. 5 cm
NC, USA										
(NC-9)										
# IA-740-011		0.07	1	119	hull	< 0.1	< 0.1	< 0.1	< 0.3	10-15 cm
Grangeburg			16.06.93	119	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	
Houston County										
AL, USA										
		0.21		119	hull	< 0.1	< 0.1	< 0.1	< 0.3	
(Florunner)				119	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	
# IA-740-012		0.07	1	119	hull	< 0.1	< 0.1	< 0.1	< 0.3	3 weeks
Malone			16.06.93	119	nutmeat	< 0.1	< 0.1	< 0.1	< 0.3	post-
Jackson County										emerg.,
FL, USA										10-15 cm
(Florunner)										

Rapeseed

Six rape seed field trials were conducted in Australia during the 1997 and 1998 growing seasons. The trials consisted of an untreated control plot and two (four trials, 1998) or three (two trials, 1997) treated plots. The treated plots received a single application of a WG formulation of imazapic. In 1997, the plots were treated with either 35, 52.5 or 70 g ai/ha. In 1998, the plots were treated at a rate of either 21 or 35 g ai/ha. The rape seed forage samples were collected 1–5 times, 0–62 days after treatment. Samples of grain were taken once from three trials, 76–95 days after the application. Straw was taken once from two trials, 76-80 days after treatment. Rape seed RAC samples were analysed for residues of imazapic and CL 263222 using Method L741/1. The limit of quantification was 0.05 mg/kg.

Table 50 Residues of imazapic in rape seed from supervised trials conducted in Australia

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Australia	Spray	0.0288	1	nr						
# 1998/1008955 # 1998/1008975 Galore New South Wales AUS (574/AU/97/01/SN01) (IT Canola)		0.035 0.0525 0.07	1 06.09.97	80 80 80	grain grain grain	< 0.05 < 0.05 < 0.05	na na na	na na na		early post- emerg. (PO01)
# 1998/1008955 # 1998/1008975 Beverley Western Australia AUS (574/AU/97/01/WA01) (Canadian IR variety)		0.035 0.0525 0.07	1 18.08.97	95 95 95	grain grain grain	< 0.05 < 0.05 < 0.05	na na na	na na na		post- emerg. (PO1)
# 1998/1008955 # 1998/1008975 Dookie Victoria AUS (574/AU/98/06/SV01) (IT Canola)		0.021 0.035	1 18.09.98	76 76	grain grain	< 0.05 < 0.05	na na	na na		post- emerg. (PO1)

Animal Feeds

Legume animal feed

Peanut hay

A total of six peanut field trials were conducted in the USA during the 1995 growing season. Each trial consisted of an untreated control plot and one treated plot. The treated plots received a single post-emergence application of an SL formulation of imazapic at a rate of 70 g ai/ha except in one trial 84 g ai/ha. Peanut hay samples were taken 90, 95, 98, 100, 102 or 113 days after treatment. Peanut hay samples were analysed for residues of imazapic, CL 263284 and CL 189215 using Method M 2599. The limit of quantification was 0.1 mg/kg.

According to GAP in the USA, grazing and feeding of treated peanut hay to livestock is not allowed.

Table 51 Residues of imazapic in peanut hay from supervised trials conducted in the USA

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in USA		0.071	1	nr						
# IA-740-013 Jamesville Martin County NC, USA (NC-9)		0.07	1 96.07.1995	98	hay	< 0.1	< 0.1	< 0.1	< 0.3	post- emerg.

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# IA-740-014 Hawkinsville Pulaski County GA, USA (Andrew 93)		0.07	1 21.07.95	100	hay	< 0.1	< 0.1	< 0.1	< 0.3	post-emerg.
# IA-740-015 Montezuma Macon County GA, USA (GK07)		0.084	1 13.07.95	95	hay	< 0.1	< 0.1	< 0.1	< 0.3	post-emerg.
# IA-740-016 Lucama Wilson County NC, USA (NC-V11)		0.07	1 25.07.95	113	hay	< 0.1	< 0.1	< 0.1	< 0.3	post-emerg.
# IA-740-017 Groom Armstrong County TX, USA (Spanco)		0.07	1 31.07.95	90	hay	< 0.1	< 0.1	< 0.1	< 0.3	post-emerg.
# IA-740-018 Malone Jackson County FL, USA (GK-7)		0.07	1 29.06.95	102	hay	< 0.1	< 0.1	< 0.1	< 0.3	post-emerg., 20-30 cm

Straw, fodder and forage of cereal grains and grasses, except grasses for sugar production (including buckwheat fodder)

Wheat straw and fodder, dry

According to GAP in Australia, grazing and cutting for stock feed are not allowed for 4 weeks after the application.

Table 52 Residues of imazapic in wheat forage and straw from supervised trials conducted in Australia

Report-No. Location (trial no.)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Australia	Spray	0.021	1	-						
# 2000/1023962 Galore New South Wales AUS (573/AU/97/01/SN01)		0.035	1	5	forage	0.10	na	na		post-emerg.
				19	forage	< 0.05	na	na		
				33	forage	< 0.05	na	na		
				96	straw	< 0.05	na	na		
		0.0525		5	forage	0.17	na	na		
				19	forage	< 0.05	na	na		
				33	forage	< 0.05	na	na		
				96	straw	< 0.05	na	na		
		0.07		5	forage	0.24	na	na		
				19	forage	< 0.05	na	na		
				33	forage	< 0.05	na	na		
				96	straw	< 0.05	na	na		

Report-No. Location (trial no.)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# 2000/1023962 Wongan Hills Western Australia AUS (573/AU/97/01/WA01)		0.035	1	32 113	forage straw	< 0.05 < 0.05	na na	na na		post-emerg.
		0.0525		32 113	forage straw	< 0.05 < 0.05	na na	na na		
		0.07		32 113	forage straw	< 0.05 < 0.05	na na	na na		
# 2000/1023962 Rannock New South Wales AUS (573/AU/98/08/SN01)		0.021	1	1 15 30	forage	0.27 < 0.05 < 0.05	na na na	na na na		post-emerg.
		0.035		1 15 30		0.97 < 0.05 < 0.05	na na na	na na na		
# 2000/1023962 Burabadji Western Australia AUS (573/AU/98/08/WA01)		0.021	1	0 14 28 42 93	forage forage forage forage straw	0.61 < 0.05 < 0.05 < 0.05 < 0.05	na na na na na	na na na na na		advanced tillering (Z25)
		0.035		0 14 28 42 93	forage forage forage forage straw	0.85 < 0.05 < 0.05 < 0.05 < 0.05	na na na na na	na na na na na		
# 2000/1023962 Manoora South Australia AUS (573/AU/98/08/SA01)		0.021	1	0 14 28 42 95	forage forage forage forage straw	0.37 < 0.05 < 0.05 < 0.05 < 0.05	na na na na na	na na na na na		stem elongation 1-2 nodes
		0.035		0 14 28 42 95	forage forage forage forage straw	0.57 0.06 < 0.05 < 0.05 < 0.05	na na na na na	na na na na na		

Hay or fodder (dry) of grasses

The registered use on grass in the USA allows one application at a maximum rate of 0.20 kg ai/ha after grass has reached full (100%) green-up for spring and summer application or grass is dormant before green-up or full green-up for winter application. According to GAP cutting treated area for hay is not allowed within 7 days after treatment. There are grazing restrictions on the US label.

One Bermuda grass (*Cynodon dactylon*) field trial was conducted in the United States during the 1996 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-emergence application of an SL formulation of imazapic at a rate of 0.224 kg ai/ha. Prior to application, the field was observed to contain 85% Bermuda grass. Samples of forage and hay were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment (DALA). Hay samples were allowed to dry after cutting

before sampling. Bermuda grass forage and hay samples were analysed for residues of imatzapic and metabolites CL263284 and CL189215 (CL 189215) using Method M 3114. The limit of quantification was 0.5 mg/kg.

One Bermuda grass field trial was conducted in the United States during the 1996 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-emergence application of an SL formulation of imazapic at a rate of 0.235 kg ai/ha. Samples of forage and hay were cut prior to application (control samples), immediately after application and 7, 14, 28 and 54 days after treatment. Hay samples were allowed to dry for 5–7 days after cutting before sampling.

One Bermuda grass field trial was conducted in the United States during the 1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-broadcast application of an SL formulation of imazapic at a rate of 0.224 kg ai/ha. Samples of forage and hay were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment. Hay samples were allowed to dry for 4-6 days after cutting before sampling.

One Big Bluestem (*Andropogon gerardii*) and Brome grass (*Bromus sp.*) field trial was conducted in the United States during the 1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-broadcast application of an SL formulation of imazapic at a rate of 0.224 kg ai/ha. Samples of forage were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment (DALA). Hay samples were taken after being dried for 4–7 days after cutting.

One common Bermuda grass field trial was conducted in the United States during the 1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-broadcast application of an SL formulation of imazapic at a rate of 0.224 kg ai/ha. Samples of forage and hay were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment. Hay samples were taken after being dried for 5–7 days after cutting.

One Bermuda grass field trial was conducted in the United States during the 1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-broadcast application of SL formulation of imazapic at a rate of 0.224 kg ai/ha. Samples of forage and hay were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment (DALA). Hay samples were taken after being dried on plastic sheets for 1–4 days after cutting.

One Brome grass field trial was conducted in the United States during the 1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-broadcast application of an SL formulation of imazapic at a rate of 0.224 kg ai/ha. Samples of forage were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment (DALA). Hay samples were taken after being dried.

One Little Bluestem and Bluegrass field trial was conducted in the United States during the 1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received a single post-broadcast application of an SL formulation of imazapic at a rate of 0.224 kg ai/ha. Samples of forage were harvested prior to application (control samples), immediately after application and 7, 14, 28 and 56 days after treatment (DALA). Hay samples were taken after being dried after cutting.

One Little Bluestem (*Schizachyrium scoparium*) and Bluegrass (*Poa sp.*) field trial was conducted in the United States during the 1996/1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received two post-broadcast applications of SL formulation of imazapic at rates of 0.157 kg ai/ha (1996) and 0.078 kg ai/ha (1997). Samples of forage were harvested prior to each application (control samples), immediately after each application and 7, 14, 28 and 56 days after the first and the last application (DAFA, DALA). Hay samples were taken after being dried after cutting.

One Big Bluestem grass field trial was conducted in the United States during the 1996/1997 growing season. The trial consisted of an untreated control plot and one treated plot. The treated plot received two post-broadcast applications of an SL formulation of imazapic at rates of 0.157 kg ai/ha (September 1996) and 0.078 kg ai/ha (July 1997). Samples of forage were harvested prior to each application (control samples), immediately after each application and 7 and 14 days after the first application (DAFA) and 7, 14, 28 and 56 days after the last application (DALA). Hay samples were taken after being dried after cutting.

In all the trials, hay samples were placed on a drying rack after harvest to dry. Residue concentrations were not adjusted for moisture content and expressed on as received basis.

Table 53 Residues of imazapic in grass forage and fodder from supervised trials conducted in the USA (* average of the two samples)

Report-No. Location (trial no.)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in USA	Spray	0.22	-	-						
# IA-731-008	Spray	0.224	1	0	forage	15*	< 0.50*	< 0.50*	16	post-
Greenville			28.06.96	0	hay	17*	2.5*	< 0.50*	20	emerg.
Washington County				7	forage	< 0.50*	0.60*	< 0.50*	1.6	Forage
Mississippi, USA				7	hay	0.68*	1.7*	< 0.50*	2.88	0 DALA
				14	forage	< 0.50*	0.96*	< 0.50*	1.96	15, 15
				14	hay	< 0.50*	1.2*	< 0.50*	2.2	Hay
				28	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	14 DALA
				28	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	< 0.5 (2)
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-010		0.235	1	0	forage	15*	0.60*	< 0.50*	16.1	post-
Montezuma			02.08.96	0	hay	25*	10*	0.59*	35.59	emerg.
Macon County				7	forage	< 0.50*	2.8*	< 0.50*	3.8	Forage
Georgia, USA				7	hay	< 0.50*	3.0*	< 0.50*	4.0	0 DALA
				14	forage	< 0.50*	1.9*	< 0.50*	2.9	15, 14
				14	hay	< 0.50*	4.1*	0.75*	5.35	Hay
				28	forage	< 0.50*	1.3*	< 0.50*	2.3	14 DALA
				28	hay	< 0.50*	1.9*	< 0.50*	2.9	< 0.5 (2)
				54	forage	< 0.50*	0.57*	< 0.50*	1.57	
				54	hay	< 0.50*	0.50*	< 0.50*	1.50	
# IA-731-011		0.224	1	0	forage	7.3*	< 0.50*	< 0.50*	8.3	post-
Fort McCoy			21.06.97	0	hay	13*	2.6*	< 0.50*	16.1	emerg.
Marion County				7	forage	< 0.50*	1.6*	< 0.50*	2.6	Forage
Florida, USA				7	hay	< 0.50*	2.1*	< 0.50*	3.1	0 DALA
				14	forage	< 0.50*	1.2*	< 0.50*	2.2	7.2, 7.4
				14	hay	< 0.50*	2.1*	< 0.50*	3.1	Hay
				28	forage	< 0.50*	0.69*	< 0.50*	1.69	14 DALA
				28	hay	< 0.50*	1.4*	< 0.50*	2.4	< 0.5 (2)
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-013		0.224	1	0	forage	22*	< 0.50*	< 0.50*	23	post-
Brookshire			19.06.97	0	hay	36*	12*	0.79*	48.79	emerg.
Waler County				7	forage	0.66*	2.5*	< 0.50*	3.66	Forage
Texas, USA				7	hay	0.54*	2.8*	< 0.50*	3.84	0 DALA
				14	forage	< 0.50*	2.2*	< 0.50*	3.2	22, 21
				14	hay	< 0.50*	3.5*	< 0.50*	4.5	Hay
				28	forage	< 0.50*	1.5*	< 0.50*	2.5	14 DALA
				28	hay	< 0.50*	2.3*	< 0.50*	3.3	0.57. < 0.5
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	

Report-No. Location (trial no.)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# IA-731-014		0.224	1	0	forage	8.8*	< 0.50*	< 0.50*	9.8	post-
Hawkinsville			26.06.97	0	hay	23*	3.2*	< 0.50*	26.7	emerg.
Pulaski County				7	forage	< 0.50*	0.92*	< 0.50*	1.92	Forage
Georgia, USA				7	hay	< 0.50*	1.8*	< 0.50*	2.8	0 DALA
				14	forage	< 0.50*	0.88*	< 0.50*	1.88	10, 7.5
				14	hay	< 0.50*	1.6*	< 0.50*	2.6	Hay
				28	forage	< 0.50*	0.71*	< 0.50*	1.71	14 DALA
				28	hay	< 0.50*	1.2*	< 0.50*	2.2	< 0.5 (2)
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-012		0.224	1	0	forage	12*	< 0.50*	< 0.50*	13	post-
Webster City			03.06.97	0	hay	21*	3.2*	< 0.50*	24.7	emerg.
Hamilton County				7	forage	0.53*	< 0.50*	< 0.50*	1.53	Forage
Iowa, USA				7	hay	0.93*	0.98*	< 0.50*	2.41	0 DALA
				14	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	10, 12
(Bonilla				14	hay	< 0.50*	0.82*	< 0.50*	1.82	Hay
(Big Bluestem))				28	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	14 DALA
(Brome)				28	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	< 0.5 (2)
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-015		0.224	1	0	forage	13*	< 0.50*	< 0.50*	14	post-
Read			15.07.97	0	hay	35*	5.2*	< 0.50*	40.7	emerg.
Delta County				7	forage	1.8*	0.96*	< 0.50*	3.26	Forage
Colorado, USA				7	hay	4.1*	3.2*	0.56*	7.86	0 DALA
				14	forage	0.99*	1.1*	< 0.50*	2.59	14, 12
(Brome)				14	hay	2.3*	2.5*	0.60*	5.4	Hay
				28	forage	< 0.50*	0.61*	< 0.50*	1.61	14 DALA
				28	hay	< 0.50*	1.2*	< 0.50*	2.2	2.2, 2.3
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-016		0.224	1	0	forage	7.5*	< 0.50*	< 0.50*	8.5	post-
Ephrata			02.06.97	0	hay	17*	0.87*	< 0.50*	18.37	emerg.
Grant County				7	forage	< 0.50*	0.52*	< 0.50*	1.52	Forage
Washington, USA				7	hay	< 0.50*	1.4*	< 0.50*	2.4	0 DALA
				14	forage	< 0.50*	0.55*	< 0.50*	1.55	8.5, 6.5
(VNS				14	hay	< 0.50*	1.2*	< 0.50*	2.2	Hay
Bluestem))				28	forage	< 0.50*	0.59*	< 0.50*	1.59	14 DALA
(Kentucky				28	hay	< 0.50*	0.75*	< 0.50*	1.75	< 0.5 (2)
(Blue))				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-017		0.157	1	0	forage	10*	< 0.50*	< 0.50*	11	post-
Ephrata			21.08.96	0	hay	22*	1.7*	< 0.50*	24.2	emerg.
Grant County				7	forage	< 0.50*	1.1*	< 0.50*	2.1	Forage
Washington, USA				7	hay	< 0.50*	1.9*	< 0.50*	2.9	0 DALA
				14	forage	< 0.50*	0.99*	< 0.50*	1.99	11, 9.5
(VNS				14	hay	< 0.50*	1.5*	< 0.50*	2.5	Hay
(Little				28	forage	< 0.50*	0.56*	< 0.50*	1.56	14 DALA
Bluestem))				28	hay	< 0.50*	0.80*	< 0.50*	1.8	< 0.5 (2)
(Kentucky				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
(Blue))				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
		0.157	2	0	forage	2.6*	< 0.50*	< 0.50*	3.6	post-
		0.078	02.06.97	0	hay	5.3*	< 0.50*	< 0.50*	6.3	emerg.

Report-No. Location (trial no.)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
				7	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				7	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				14	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				14	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				28	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				28	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				56	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
# IA-731-018		0.157	1	0	forage	24*	0.56*	< 0.50*	25.06	post-
York			24.09.96	0	hay	48*	3.2*	< 0.50*	51.7	emerg.
York County				7	forage	0.81*	2.9*	4.2*	7.91	Forage
Nebraska, USA				7	hay	1.4*	4.6*	< 0.50*	6.5	0 DALA
				14	forage	0.54*	1.8*	< 0.50*	2.84	24, 24
(Champ				14	hay	0.91*	3.4*	< 0.50*	4.81	Hay
(Big										14 DALA
Bluestem)										0.89, 0.92
		0.157	2	0	forage	7.6*	< 0.50*	< 0.50*	8.6	post-
		0.078	03.07.97	0	hay	15*	4.7*	< 0.50*	20.2	emerg.
				7	forage	< 0.50*	0.57*	< 0.50*	1.57	
				7	hay	< 0.50*	1.6*	< 0.50*	2.6	
				14	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				14	hay	< 0.50*	0.75*	< 0.50*	1.75	
				28	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				28	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				55	forage	< 0.50*	< 0.50*	< 0.50*	< 1.5	
				55	hay	< 0.50*	< 0.50*	< 0.50*	< 1.5	

* average of two samples

Miscellaneous fodder and forage crops

Rape seed forage

Table 54 Residues of imazapic in rape seed forage straw from supervised trials conducted in Australia

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
GAP in Australia		0.0288	1	nr						
# 1998/1008955		0.035	1	5	forage	0.32	na	na		early
# 1998/1008975			06.09.97	19	forage	0.11	na	na		post-
Galore				33	forage	0.06	na	na		emerg.
New South Wales				80	straw	< 0.05	na	na		(PO01)
AUS										
(574/AU/97/01/SN01)		0.0525		5	forage	0.72	na	na		
(IT Canola)				19	forage	0.28	na	na		
				33	forage	0.13	na	na		
				80	straw	< 0.05	na	na		
		0.07		5	forage	0.67	na	na		
				19	forage	0.18	na	na		
				33	forage	0.17	na	na		
				80	straw	< 0.05	na	na		

Report-No. Location (trial no.) (variety)	Application rate			DALA (days)	Portion analysed	Residues (mg/kg)				Timing Remarks
	Method	Rate kg ai/ha	No.			Imazapic	CL263284	CL189215	Sum	
# 1998/1008955		0.035	1	29	forage	0.10	na	na		post-
# 1998/1008975			18.08.97							emerg.
Beverley		0.0525		29	forage	0.20	na	na		(PO1)
Western Australia										
AUS		0.07		29	forage	0.20	na	na		
(574/AU/97/01/WA01)										
(Canadian IR variety)										
# 1998/1008955		0.021	1	19	forage	< 0.05	na	na		post-
# 1998/1008975			05.08.98	33	forage	< 0.05	na	na		emerg.
Rannock				47	forage	< 0.05	na	na		(PO1)
New South Wales				62	forage	< 0.05	na	na		
AUS		0.035		19	forage	< 0.05	na	na		
(574/AU/98/06/SN01)				33	forage	< 0.05	na	na		
(IT Canola)				47	forage	< 0.05	na	na		
				62	forage	< 0.05	na	na		
# 1998/1008955		0.021	1	0	forage	0.46	na	na		post-
# 1998/1008975			24.08.98	14	forage	0.05	na	na		emerg.
Burabadji				28	forage	< 0.05	na	na		(PO1)
Western Australia				42	forage	< 0.05	na	na		(C19-25)
AUS				56	forage	< 0.05	na	na		
(574/AU/98/06/WA01)		0.035		0	forage	1.80	na	na		
(IT Canadian)				14	forage	0.13	na	na		
				28	forage	0.07	na	na		
				42	forage	< 0.05	na	na		
				56	forage	< 0.05	na	na		
# 1998/1008955		0.021	1	9	forage	0.05	na	na		early
# 1998/1008975			24.09.98	20	forage	< 0.05	na	na		flowering
Manoora				35	forage	< 0.05	na	na		(PO1)
South Australia				49	forage	< 0.05	na	na		
AUS		0.035		9	forage	0.07	na	na		
(574/AU/98/06/SA01)				20	forage	< 0.05	na	na		
(IT Canola)				35	forage	< 0.05	na	na		
				49	forage	< 0.05	na	na		
# 1998/1008955		0.021	1	0	forage	0.42	na	na		post-
# 1998/1008975			18.09.98	12	forage	0.13	na	na		emerg.
Dookie				27	forage	< 0.05	na	na		(PO1)
Victoria				42	forage	< 0.05	na	na		
AUS				76	straw	< 0.05	na	na		
(574/AU/98/06/SV01)										
(IT Canola)		0.035		0	forage	0.61	na	na		
				12	forage	0.18	na	na		
				27	forage	0.12	na	na		
				42	forage	< 0.05	na	na		
				76	straw	< 0.05	na	na		

FATE OF RESIDUES IN STORAGE AND PROCESSING

In processing

The Meeting received information on effects of heating in water and processing on imazapic residues in soya bean. An attempt was made to determine processing factors for processing of sugar cane into sugar and related commodities. However, even at the exaggerated rate (5× GAP rate), no residue was

detected in sugar cane samples above the LOQ of 0.01 mg/kg. For this reason no processing was performed.

High temperature hydrolysis

The hydrolysis of [^{14}C]-imazapic was investigated in sterile buffered aqueous solution under a range of hydrolysis conditions simulating processes such as pasteurization, baking/brewing/boiling and sterilization.

[Pyridine-^{14}C]-imazapic was dissolved in the aqueous buffer solutions to give a final concentration of about 10 µg/ml and incubated at 90 °C (pH 4) for 20 minutes, 100 °C (pH 5) for 60 minutes or 120 °C (pH 6) for 20 minutes. All test samples were prepared in sterile amber bottles to eliminate possible photolysis and all sterile buffers were bubbled with nitrogen to avoid effects of oxygen on the test systems. The samples were prepared in duplicates for each test system. For each of conditions, the total radioactivity present was determined at the end of the incubation period after cooling and compared to the theoretical radioactivity before incubation. The total radioactivity in each sample was determined by LSC. Radioactivity in the samples was analysed by HPLC immediately upon collection. Characterization of the radioactivity in the buffered samples after incubation was performed by HPLC-MS/MS.

After incubation at different pH, 101–102% of the applied radioactivity remained. At the end of the incubation periods, the detectable radioactive component was unchanged imazapic only. Imazapic was stable under conditions representing pasteurization and baking/brewing/boiling and sterilization.

Soya beans

Soya bean processing studies were conducted to determine the potential for concentration of residues of imazapic in the processed fractions of soya bean (Resende, 2009a and Leite, 2011a). During the 2008 growing season, two field trials were carried out in Brazil. Soya beans were treated with imazapic. Treated plot received one foliar post-emergence spray application at an exaggerated rate (same rates as the above study), 60 days before harvest. Samples of soya bean grain were taken 60 days after the application. The soya bean seed samples were separately processed using simulated commercial processing procedures into flaked soya bean, oil, meal and toasted meal. Soya bean samples were analysed for residues using Method SOP-PA.0288.

The analytical results indicate that imazapic slightly concentrated in meal. For all other analyte/commodity combinations, the processing factors showed residue decline.

Table 55 Residues of imazapic and its metabolites in processed commodities of soya bean seeds

Trial Location / Trial Number	Processed Commodity	Residues Found (mg/kg parent equiv.)				Processing Factor for Parent Imazapic	Processing Factor for Total Residue [a]
		Imazapic	CL263284	CL189215	Total [a]		
Santo Antonio de Posse, SP, Brazil (G080229) 2008	Seed	< 0.01	< 0.01	< 0.01	< 0.03	-	-
	Flaked soya bean	< 0.01	< 0.01	< 0.01	< 0.03	nd	nd
	Oil	< 0.01	< 0.01	< 0.01	< 0.03	nd	nd
	Meal	< 0.01	< 0.01	< 0.01	< 0.03	nd	nd
	Toasted meal	< 0.01	< 0.01	< 0.01	< 0.03	nd	nd
Londrina, PR, Brazil (G080230) 2008	Seed	0.08	< 0.01	0.03	0.12	-	-
	Flaked s.	0.04	< 0.01	0.01	0.06	0.50	0.50
	Oil	< 0.01	< 0.01	< 0.01	< 0.03	0.13	0.25
	Meal	0.09	< 0.01	0.03	0.13	1.13	1.08
	Toasted meal	0.07	< 0.01	0.02	0.10	0.88	0.83

[a] sum of imazapic, CL263284 and CL189215

nd = not determined since residues in the RAC were below the LOQ

Another soya bean processing studies were conducted to determine the potential for concentration of residues of imazapic in the processed fractions of soya bean (Jones, 2011a). During the 2009 growing season, two field trials were carried out in Brazil. Soya beans were treated with imazapic. Treated plot received one foliar post-emergence spray application at an exaggerated rate of either 0.035 kg imazapic/ha (2×) or 0.0525 kg imazapic/ha (3×), 60 days before harvest. Samples of soya bean seed were taken 60 days after the application. The soya bean seed samples were separately processed using simulated commercial processing procedures into defatted meal, toasted defatted meal, laminated soya bean, oil, meal and hulls.

Soya bean samples were analysed for residues of imazapic and its metabolites using Method SOP-PA.0288.

The analytical results indicate that imazapic slightly concentrated in defatted and toasted defatted meal and CL189215 slightly concentrated in defatted meal. For all other analyte/commodity combinations, the processing factors showed a residue decline.

Table 56 Residues of imazapic and its metabolites in processed commodities of soya bean seeds

Trial Location / Trial Number	Processed Commodity	Residues Found (mg/kg parent equiv.)				Processing Factor for Imazapic	Processing Factor for Total Residue [a]
		Imazapic	CL263284	CL189215	Total [a]		
Santo Antonio de Posse, SP, Brazil (G090003) 2009	Seed	0.07	< 0.01	0.02	0.10	-	-
	Defatted meal	0.09	< 0.01	0.03	0.13	1.29	1.30
	Toasted defatted meal.	0.08	< 0.01	0.02	0.11	1.14	1.10
	Oil	< 0.01	< 0.01	< 0.01	< 0.03	0.14	0.30
	Laminated soya bean.	0.05	< 0.01	< 0.01	0.07	0.71	0.70
	Seed	0.07	< 0.01	0.03	0.11	-	-
	Hulls	0.07	< 0.01	0.01	0.09	1.00	0.81
	Meal	0.07	< 0.01	0.03	0.11	1.00	1.00
Londrina, PR, Brazil (G090004) 2009	Seed	< 0.01	< 0.01	< 0.01	< 0.03	-	-
	Defatted mal	< 0.01	< 0.01	na	(< 0.03)	nd	nd
	Toasted defatted meal.	< 0.01	< 0.01	na	(< 0.03)[#]	nd	nd
	Oil	< 0.01	< 0.01	na	(< 0.03)	nd	nd
	Laminated soya bean.	< 0.01	< 0.01	< 0.01	< 0.03	nd	nd
	Seed	0.02	< 0.01	< 0.01	0.04	-	-
	Hulls	0.02	< 0.01	< 0.01	0.04	1.00	1.00
	Meal	0.02	< 0.01	< 0.01	0.04	1.00	1.00

[a] Sum of imazapic, CL263284 and CL189215

na = not analysed

nd = not determined since residues in the RAC were below the LOQ

Table 57 Summary of processing factors for soya bean processing

Processed commodity	N	Imazapic		Sum of imazapic, CL263284 and CL189215	
		Processing factor	Mean or best estimate	Processing factor	Mean or best estimate
Meal	3	1.00, 1.00, 1.13	1.04	1.00, 1.00, 1.08	1.03
Defatted meal	1	1.29	1.29	1.30	1.30

Processed commodity	N	Imazapic		Sum of imazapic, CL263284 and CL189215	
		Processing factor	Mean or best estimate	Processing factor	Mean or best estimate
Toasted Meal	1	0.88	0.88	0.83	0.83
Toasted Defatted Meal	1	1.14	1.14	1.10	1.10
Oil	2	0.13, 0.14	0.14	0.30, 0.25	0.28
Laminated Soya Bean	1	0.71	0.71	0.70	0.70
Flaked Soya Bean	1	0.50	0.50	0.50	0.50
Hulls	2	1.00, 1.00	1.00	0.81, 1.00	0.91

RESIDUES IN ANIMAL COMMODITIES

Farm animal feeding studies

The Meeting received information on lactating cow feeding study.

Lactating cows

Four groups of lactating Holstein cows, each group containing three animals, were orally dosed with imazapic in gelatin capsules using a balling gun for 28 days following afternoon milking at levels equivalent to 0, 67, 223, and 676 ppm in feed (Leonard, 1999a). Milk samples were collected twice daily during dosing period and afternoon milk and morning milk were pooled. Within 24 hours after the last dose, cows were sacrificed. After sacrifice, loin muscle, omental fat, liver and kidney were collected. All samples were analysed for imazapic and CL 263284 using Methods M 3188 for milk, M 3222 for tissues, and M 3233 for fat.

In the lowest dose group (67 ppm), residues of imazapic in milk reached a plateau after Day 1 and averaged 0.025 mg/kg. In milk fat and kidney, average residues were 0.013 and 0.384 mg/kg, respectively. In muscle, liver and omental fat, average residues were < 0.050 mg/kg.

In the middle dose group (223 ppm), residues of imazapic in milk averaged 0.077 mg/kg. In milk fat and omental fat, average residues were 0.037 and 0.051 mg/kg, respectively. In loin muscle, kidney and liver, average residues were 0.054, 1.57 and 0.082 mg/kg, respectively.

In the highest dose group (676 ppm), residues of imazapic in milk averaged 0.274 mg/kg. In milk fat and omental fat, average residues were 0.127 and 0.051 mg/kg, respectively. In muscle, kidney and liver, average residues were 0.079, 2.71 and 0.192 mg/kg, respectively.

Levels of CL263284 was below the respective LOQ in all matrices and at all dosing levels.

Table 58 Residues of imazapic and CL263284 in whole milk during the feeding study

Day	Mean (mg/kg) (Maximum Individual Residues) (mg/kg)			
	Group A (Control)	Group B (67 ppm)	Group C (223 ppm)	Group D (676 ppm)
Imazapic				
-1	< 0.01	< 0.01 (< 0.01)*	< 0.01 (< 0.01)	< 0.01 (< 0.01)
1	< 0.01	0.025 (0.030)	0.067 (0.090)	0.308 (0.374)
2	< 0.01	0.027 (0.031)	0.081 (0.120)	0.267 (0.316)
3	na	0.027 (0.030)	0.076 (0.103)	0.284 (0.339)
6	< 0.01	0.018 (0.022)	0.076 (0.096)	0.247 (0.289)
10	< 0.01	0.025 (0.029)	0.074 (0.086)	0.244 (0.286)
15	< 0.01	0.030 (0.035)	0.082 (0.093)	0.271 (0.354)

Day	Mean (mg/kg) (Maximum Individual Residues) (mg/kg)			
	Group A (Control)	Group B (67 ppm)	Group C (223 ppm)	Group D (676 ppm)
20	< 0.01	0.025 (0.027)	0.071 (0.080)	0.286 (0.346)
24	< 0.01	0.029 (0.032)	0.089 (0.121)	0.280 (0.317)
27	< 0.01	0.023 (0.026)	0.079 (0.085)	0.281 (0.313)
CL 263284				
-1	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
1	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
2	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
3	na	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
6	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
10	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
15	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
20	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
24	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)
27	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)

na = not analysed

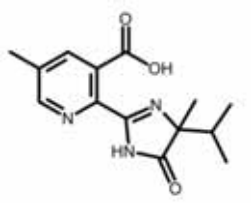
* maximum individual residues

Table 59 Residues of imazapic and CL263284 in tissues and milk fat

Tissue	Mean (mg/kg) Maximum Individual Residues (in parentheses) (mg/kg)			
	Group A (Control)	Group B (67 ppm)	Group C (223 ppm)	Group D (676 ppm)
Imazapic				
Muscle	< 0.05	< 0.05 (< 0.05)	0.054 (0.0626)	0.079 (0.081)
Kidney	< 0.05	0.384 (0.465)	1.567 (2.200)	2.708 (3.750)
Liver	< 0.05	< 0.05 (< 0.05)	0.082 (0.126)	0.192 (0.231)
Fat	< 0.05	< 0.05 (< 0.05)	0.051 (0.054)	0.051 (0.053)
Milk fat	< 0.01	0.013 (0.015)	0.037 (0.043)	0.127 (0.135)
CL 263284				
Muscle	< 0.05	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)
Kidney	< 0.05	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)
Liver	< 0.05	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)
Fat	< 0.05	< 0.05 (< 0.05)	< 0.05 (< 0.05)	< 0.05 (< 0.05)
Milk fat	< 0.01	< 0.01 (< 0.01)	< 0.01 (< 0.01)	< 0.01 (< 0.01)

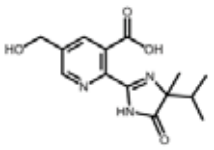
APPRAISAL

Imazapic is an imidazolinone herbicide developed for the control of grass and broadleaf weeds in a variety of crops and registered in a number of countries. It was considered for the first time by the present Meeting.

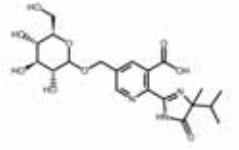


Information on the physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, supervised trials, processing and farm animal feeding was received by the present Meeting.

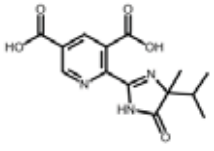
The following abbreviated names were used for the metabolites discussed below.



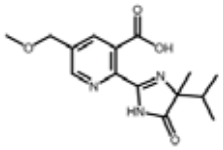
CL263284



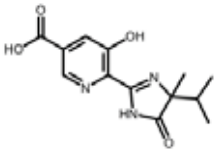
CL189215



CL 312622



CL 299263



CL 354825

Animal metabolism

The Meeting received information on the fate of orally-dosed imazapic in rat, lactating goats and laying hens.

In metabolism studies, total radioactive residues are expressed in mg/kg imazapic acid equivalents unless otherwise stated.

Metabolism of imazapic in rat

Metabolism studies on laboratory animals including rats were reviewed in the framework of toxicological evaluation by the current JMPR.

Metabolism of imazapic in lactating goats

Imazapic

In two studies on lactating goats, [pyridine-6-^{14}C]-imazapic was orally administered at three doses via capsule (equivalent to 2.0, 11.8 and 175 ppm in feed) for 5 or 7 consecutive days. The majority was eliminated in urine (67–94% of TAR) in the form of unchanged imazapic and faeces (7–10% of TAR). Milk contained up to 0.03% of TAR and edible tissues 0.01% of TAR at the highest dose.

The TRR of daily collected milk samples were lower than the LOQ of 0.01 mg eq/kg at the two lower doses.

The goats were sacrificed 20 or 23 hours after the last dose at which time tissue samples were obtained. The highest TRR (0.275 mg eq/kg) was observed in kidney from the highest dose regime, reflecting that urinary excretion was the predominant elimination route. At the two lower doses, except that the TRR of the kidney from goat dosed at 11.8 ppm was 0.05 mg eq/kg, the TRR in milk or tissues were below the respective LOQ of 0.01 (milk) or 0.02 mg eq/kg (muscle).

The analysis of acetonitrile+methanol extract of kidney from the highest dose indicates that imazapic was the major residue at 30% TRR (0.02 mg/kg) with CL263284 at 8% of TRR (< 0.01 mg/kg). As the TRRs in milk and tissues, other than kidney, from any dose levels were too low to characterize any of radioactive residues, extraction of radioactive residues was not attempted for these tissues or milk.

CL 263284

In a study on lactating goats with oral administration of [pyridine-6-^{14}C]- CL 263284 in capsule at doses equivalent to 2.3 or 14.5 ppm in feed for 7 consecutive days, radioactivity was eliminated mostly via faeces (82% TAR for the low dose and 68% TAR for the high dose) and in a lesser amount from urine (15–18% TAR). The TRR of daily milk samples and tissues obtained 20 hours after the last dose were below the LOQ of 0.01 mg/kg except kidney from the high dose goat (0.03 mg eq/kg). About 91% of TRR in the kidney from high dose goat was extracted with methanol and CL 263284 was present at 9% (< 0.01 mg/kg) of the extracted TRR. Component M1 found at significant amounts (78% TRR, 0.02 mg eq/kg) may be an extraction artifact and was converted into CL 263284 on exposure to aqueous buffer.

Metabolism of imazapic in laying hens

In a hen study [Pyridine-6-^{14}C]-imazapic orally administered in capsule to laying hens, at a dose equivalent to 2.1 or 11.4 ppm in feed for 7 consecutive days, was mostly eliminated into excreta (91–95% of TAR). The TRR in all egg and tissue samples were less than the LOQ of 0.01 mg eq/kg.

The [Pyridine-6-^{14}C]-CL263284 orally administered to laying hens at 2.1 or 10.9 ppm in feed for 7 consecutive days was mostly eliminated into excreta and the TRR in all tissues, skin with adhering fat and eggs were below the LOQ of 0.01 mg/kg. No detectable radioactive residues were found in eggs or edible tissues obtained 22 hours after the last dose.

Metabolites of imazapic in goat were similar to those in rat. With rapid excretion, the radioactive residues in edible tissues, milk or eggs were mostly below the LOQ. In lactating goats, residues remaining in the kidney were mostly unchanged parent with a minor amount of CL263284.

Plant metabolism

The Meeting received information on the fate of imazapic in sugar cane, peanuts, Bermuda grass and transgenic soya bean.

When [pyridine-6-^{14}C]-imazapic was applied to the soil surface as a pre-emergent treatment at a rate approximating 0.15 kg ai/ha (within GAP rates) in a small test plot, no radioactive residues were detected above the LOQ of 0.005 mg/kg in the stalk of sugar cane harvested 13 months after the treatment.

When [pyridine-6-^{14}C]-imazapic was applied to the soil surface as a pre-emergent treatment at a rate approximating 0.25 kg ai/ha (within GAP rates) in a greenhouse, the TRR in the adult sugar cane stalk and leaves collected 236 DAT was 0.0039–0.048 mg eq/kg and 0.015–0.016 mg eq/kg respectively.

High percentages (63–84% TRR) of the radioactive residues in forage, leaves and stalks collected at various timings were extracted by methanol. Most of the extracted radioactive residues were water soluble.

Imazapic was found in all samples but declined with time, e.g., in stalks 0.003 mg/kg (41% TRR) at 151 DAT to 0.0005 mg/kg (11% TRR) at 236 DAT. CL263284 was found in all the samples collected at higher concentrations than the parent, but both the concentration and proportion to TRR also declined with time, e.g., in stalk 0.001 mg/kg (16% TRR) at 151 DAT to 0.0005 mg/kg (10% TRR) at 236 DAT. CL189215 was also found at lower concentrations in leaves (0.0005–0.002 mg/kg, 3–8% TRR), but not in stalks.

[Pyridine-6-^{14}C]-imazapic was applied to peanut plants at a rate of 0.072 kg ai/ha (within GAP rates) 30 days post-emergence. The TRR declined significantly from 4.2 mg eq/kg at 0 DAT to 0.094 mg eq/kg at 61 DAT in green plants and 0.22 mg eq/kg in hay collected 131 DAT. The TRR in nutmeat at 131 DAT was low at 0.022 mg eq/kg. A mixture of methanol:water:acetone (1:1:1) extracted 76–96% of the TRR in peanut samples.

The concentration of imazapic sharply declined in plants to 0.006 mg/kg at 61 DAT (2% TRR). The concentration in nutmeat, hull and hay samples collected 131 DAT were even lower (< 0.001–0.006 mg/kg, 1–3% TRR). The concentration of CL263284 also decreased over time to 0.010 mg/kg (12% TRR) at 61 DAT in plants. The concentrations of CL189215 declined over time to 0.039 mg/kg at 61 DAT (46% TRR) in plants. The concentrations of these three compounds were all less than 0.01 mg/kg in nutmeat at 131 DAT. No other component in the extracts from immature or mature samples exceeded 10% of TRR or 0.01 mg/kg.

Bermuda grass was treated post-emergence with [pyridine-6-^{14}C]-imazapic at a rate of 0.2 kg ai/ha (maximum GAP rate for grass in the USA). The TRR in plants decreased over time from 8.3 mg eq/kg at 0 DAT to 0.77 mg eq/kg at 47 DAT and was 0.92 mg eq/kg in hay (68 DAT). From forage and hay samples, 74–100% of radioactive residues were extracted by aqueous solutions with hay sample at the lowest 74%.

The concentration of imazapic declined sharply to 0.02 mg/kg (3% TRR) at 49 DAT. CL263284 concentration showed a sharp increase in the early period to the peak concentration of 1.7 mg/kg (30% TRR) at 15 DAT and then decrease to 0.16 mg/kg (21% TRR) at 49 DAT. It was found in hay at 0.08 mg/kg (8% TRR). CL189215 also increased in concentration in the early stage to the peak concentration of 0.17 mg/kg (4% TRR) at 15 DAT then to 0.08 mg/kg (9% TRR) at 49 DAT. In the hay sample collected 68 DAT, imazapic, CL263284 and CL189215 were present at 0.02, 0.08 and 0.08 mg/kg respectively but all below 10% of TRR.

There were many minor polar components but none exceeded 10% TRR individually.

[Pyridine-3-^{14}C]-imazapic was applied to the above-ground portion of imidazolinone-tolerant soya bean plants (with mutated AtAHASL protein inserted to make the host tolerant to imidazolinone herbicide) at BBCH 65 at an application rate of 0.08 kg ai/ha. The TRR in soya bean seed and straw were 0.014 mg eq/kg and 0.092 mg eq/kg respectively. From forage, hay, seed, straw and pod samples, 78–98% of radioactive residues were extracted by aqueous solutions.

In seeds, imazapic was the most abundant residue at 20% TRR but only 0.003 mg/kg. Imazapic was the most abundant residue in straw and pods but at 0.00013 and 0.004 mg/kg respectively. CL189215 was the second most abundant compound individually but at < 0.01 mg/kg and less than 10% of TRR. CL263284 was also present but less than imazapic or CL189215. There were many minor polar components detected but none exceeded 0.01 mg/kg or 10% of TRR individually.

In the edible portions of treated food crops harvested at maturity, no or little residues of imazapic are expected to be found. In animal feed crops, such as grasses, imazapic, CL 263284 and CL 189215 are expected to be found above the LOQ.

Environmental fate

The Meeting received information on aerobic soil metabolism, photodegradation on the soil surface, hydrolysis and residues in succeeding crops.

Aerobic soil metabolism

About 70% of the applied imazapic (equivalent to 0.14 kg/ha) remained in sandy loam soils after one year of incubation in one study and about 80% remained in soil after 120 days of incubation in the other. Mineralization occurred but at very low percentage.

CL263284 applied to soil degraded to less than 1% of the applied dose in 3 days forming CL312622, CL354825 and carbon dioxide.

AC299263 and CL 312624 applied to soil degraded more gradually both transforming to CL354825 and carbon dioxide.

Degradation of these compounds was significantly less in soil under sterile conditions, indicating microbial action.

Imazapic was found to be persistent in sandy loam soils under anaerobic conditions. However, its metabolites/degradates were more readily degraded in soil.

Photodegradation

^{14}C-Labelled imazapic was applied to a sandy loam soil at a rate equivalent to 0.08 kg/ha (within GAP rates). The total radioactivity in the aqueous sodium hydroxide extract (10% AR) and unextracted fraction (3% AR) was about 7% of the applied dose after 30 days of irradiation by a xenon-arc lamp comparable to mid-autumn sunlight in New Jersey, USA.

The imazapic concentration decreased from 94% of the extracted radioactivity on day 0 to 75% after the 30-day irradiation. A diacid degradate, CL 312622, formed up to 11% of the extracted radioactivity. The DT_{50} was calculated to be 106 days.

Photolysis of imazapic (25 mg/kg) in sterilized water was rapid at pH 5, 7 and 9 under irradiation by a xenon-arc lamp comparable to mid-autumn sunlight in New Jersey, USA, with DT_{50} in a range of 6.0–7.2 hours. Six degradation products, including carbon dioxide were formed, each of which accounted for around 10%.

Imazapic is photodegraded gradually with a half-life around 100 days on the surface of soil and rapidly with half-life of about 6–7 hours in sterilized water.

Hydrolysis

As imazapic can be used in rice production, ^{14}C-Labelled imazapic was applied to water+soil at a nominal rate equivalent to about 0.14 kg/ha (within GAP rates) and kept under anaerobic conditions.

Only 0.5% of the applied dose was mineralized. No other radioactive volatile compounds were collected. No volatilization or degradation occurred.

The concentration of imazapic decreased from 92% (36% in the soil extract and 56% in water) on day 0 to 83% (54% in the soil extract and 29% in the water) of the applied dose after 366 days.

Imazapic was stable for 366 days at 25 °C in water+ soil.

Residues in succeeding crops

A confined study was conducted to examine the nature and level of residues of imazapic in succeeding crops. A single application of [pyridine-6-^{14}C]-imazapic was made on soil in field plots at a nominal rate of 0.72 kg ai/ha, higher than any GAP rates.

At each rotational interval of 90, 120, 270 and 300 days after treatment, barley, maize cotton, lettuce and carrots were sown into the treated soil and harvested at maturity.

Following the application of imazapic to soil, uptake of radioactivity into rotational crops was low (< 0.004–0.070 mg eq/kg). However, the TRR in barley and maize samples show a tendency to be higher in samples obtained from longer plant back intervals. The TRR of lettuce and carrot (both with 20 day plant back intervals (PBI)) were 0.006 and < 0.004 mg eq/kg, respectively.

In barley grain, imazapic was the most abundant residue from the 120 day PBI at 23% TRR (0.007 mg/kg) followed by the sum of CL263284 and CL189215 at 18% TRR (0.006 mg/kg). In the 270 day PBI barley grain, the most abundant residue was the sum of CL263284 and CL189215 at 37% TRR (0.021 mg/kg) followed by imazapic at 10% (0.004 mg/kg). In straw the most abundant residue was the sum of CL263284 and CL189215 at 44% TRR (0.031 mg/kg) for 120 day PBI and

44% TRR (0.031 mg/kg) for 270 day PBI. Imazapic was present in straw at 5.4–5.5% TRR (0.003–0.004 mg/kg).

In the 300 day PBI maize forage and fodder, the sum of CL263284 and CL189215 was the most abundant residue at 28% (0.005 mg/kg) and 28% (0.008 mg/kg) TRR respectively.

Radioactive residues remained mostly in the 0–8 cm depth layer of soil. High percentage (57–91% TRR) of radioactive residues in soil were extracted with aqueous methanol/acetone mixture and 2% HCl. Nine to 43% TRR remained as unextracted residues.

Following the application of imazapic to soil, uptake of radioactivity into rotational crops was low (maximum at 0.070 mg eq/kg). Among registered uses related to the current review, the highest application rate was for sugar cane or grass. In comparison, the application rate used in this confined rotational crop study was about 3 times the GAP rate in Brazil and 7.5 times the GAP rate in Australia for sugar cane or 3.5 times the GAP rate for grass in the USA. This application rate was more than 7.5 times higher than the GAP rate for other crops used in crop rotation. Residues of imazapic in plant portions of rotational crops used as food or feed after approved application are expected to be less than one third of the residues observed in the confined rotational crop study, i.e., around or lower than the lowest LOQ of 0.01 mg/kg.

Methods of analysis

Analytical methods for the determination of residues of imazapic and its metabolites were developed for a wide range of matrices of plant and animal origin.

In general, the methods for data generation employ extraction by homogenization with a mixture of methanol:water:1M HCl (60:39:1) or, in the case of fat, acidic acetonitrile in hexane, clean-up with solid phase extraction or some other techniques, and determination of analytes using LC-MS/MS, HPLC-UV (245 nm) or capillary electrophoresis-UV (240 nm).

A number of specific methods for plant matrices were found suitable for analysis of imazapic, CL263284 and CL189215 with LOQ ranging 0.01–0.1 mg/kg for these analytes except that it was 0.5 mg/kg for grass.

Three methods for animal matrices were found suitable for analysis of imazapic and CL263284 with LOQ of 0.01 (milk and milk fat) and 0.05 (tissues) mg/kg.

No multi-residue methods were submitted.

Stability of residues in stored analytical samples

The stability of imazapic residues during storage of samples frozen at -25 to -5 °C was investigated in a range of plant and animal matrices for which supervised residue trials were submitted.

Compounds tested were imazapic, CL263284 and CL189215. Each compound was spiked to matrices at 0.5 mg/kg.

All of the compounds tested were found to be stable (> 70% remaining) at least during the storage periods tested: 2 years in wheat, sugar cane, peanut and grass matrices; 10 months in soya bean seeds; 3 months in processed soya bean products; 6 months in cattle milk; and 8 months in cattle tissues. These storage periods are longer than the longest storage conditions in trials on respective crops.

Definition of the residue

In animal metabolism, parent imazapic was the predominant residue with CL263284 a minor component at around 10% TRR in goat kidney. In other tissues, milk or eggs, TRR were below the respective LOQs. In a metabolism study in which CL 263284 was administered, TRR in milk and tissues were below the LOQ of 0.01 mg/kg. CL263284 found in goat kidney is also a metabolite in rats and, as such, is covered by the ADI. However, in the livestock feeding study using imazapic, CL 263284 was not detected above the LOQ of 0.01 mg/kg (milk and milk fat) or 0.05 mg/kg (tissues) at

the highest dose of 676 ppm in the feed. Therefore, the Meeting considered that parent imazapic is a suitable residue for enforcement of MRLs and for calculating dietary intake for commodities of animal origin.

With a low $logP_{ow}$ of 0.054 and given the distribution of residues in animal tissues from the animal metabolism studies and animal feeding study, the Meeting considered imazapic residue not fat-soluble.

The plant metabolism studies indicate that no or little residues of imazapic or its metabolites are expected to be found in the edible portions of food crops harvested at maturity. In supervised trials on soya beans, CL 263284 and CL 189215 were found at lower concentrations than the parent, when measured.

In feed crop such as grass, imazapic, CL 263284 and CL 189215 (a glucoside of CL 263284 found in plants and in a very small amount in rats) are expected to be found above the LOQ of 0.5 mg/kg (in the residue trials) as demonstrated in the metabolism study and in residue trials.

The Meeting considered that the parent imazapic was a suitable residue for enforcement of MRLs and for calculating dietary intake for commodities of plant origin.

Based on the above, the Meeting recommended the following residue definition for plant and animal commodities:

Definition of the residue for plant and animal commodities (both for compliance with the MRL and for dietary intake): *Imazapic.*

Residue is not fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for imazapic on conventional and transgenic soya beans, maize, rice, wheat, sugar cane, peanut, grasses and transgenic rape seed.

Soya bean (dry)

A total of 16 supervised trials were conducted on imidazolinone-tolerant soya beans (conventionally bred or transgenic) in different years in Brazil. However, as no GAP for soya bean was available, it was not possible to estimate a maximum residue level.

Maize

A total of five trials were conducted on imidazolinone-tolerant maize in 2010 in Brazil.

The registered use on imidazolinone-tolerant maize in Brazil allows one application at a maximum rate of 0.0525 kg ai/ha with a PHI of 96 days.

Residues of imazapic from trials matching GAP in Brazil were: < 0.01 (4) and < 0.1 mg/kg.

Although the number of trials matching GAP is small, as the metabolism studies indicate that no residues are expected in edible portion of food crops harvested at maturity and the residue trial data on other cereal grains (rice and wheat) indicate that residues were below the respective LOQ at even exaggerated application rates, the Meeting considered that no residues would be expected in maize grain.

The Meeting therefore estimated a maximum residue level and STMR of 0.01* and 0 mg/kg, respectively, for maize.

Rice

A total of 11 trials were conducted on imidazolinone-tolerant rice in Brazil in different years.

The registered use on imidazolinone-tolerant rice in Brazil allows up to two applications at a maximum rate of 0.025 kg ai/ha with a PHI of 60 days.

Residues of imazapic from trials matching GAP in Brazil were: < 0.05 (10) mg/kg. In many trials, 2 × GAP rate was used. In these trials, residues were all < 0.05 mg/kg (9).

The Meeting estimated a maximum residue level and STMR of 0.05 * and 0 mg.kg, respectively, for rice grain.

Wheat

Four trials were conducted for imidazolinone-tolerant wheat in Australia. The registered use of imazapic on wheat in Australia allows one application at a maximum rate of 0.021 kg ai/ha (only for imidazolinone-tolerant wheat) at 4 leaf (Z14) to the commencement of the flag leaf (Z37) stage. No PHI is required. Grazing and cutting for animal feed are not allowed for 4 weeks after application.

Residues of imazapic from trials matching GAP in Australia were: < 0.05 (2) mg/kg.

Although the number of trials matching GAP is small, as residues from trials using higher rate (up to 3.5 × GAP rate) were all < 0.05 mg/kg (total of 4), and the metabolism studies indicate that no residues are expected in edible portions of food crops harvested at maturity, the Meeting considered that no residues would be expected in wheat grain.

The Meeting therefore estimated a maximum residue level and STMR of 0.05* and 0 mg/kg, respectively for wheat.

Sugar cane

A total of 14 trials were conducted on sugar cane in Argentina, Australia, Brazil, Costa Rica and Guatemala.

The registered uses on sugar cane in these countries allow one application at a maximum rate of: 0.35 kg ai/ha with a PHI of 392 days in Argentina, 0.096 kg ai/ha in Australia, 0.245 kg ai/ha with a PHI of 283 days, 0.175 kg ai/ha with a PHI of 85 days in Costa Rica and Guatemala respectively.

The trial data were evaluated against GAP in Brazil. Residues of imazapic in sugar cane from trials matching GAP in Brazil were: < 0.01 (8) and < 0.05 (5) mg/kg. With shorter PHI or at 5 × GAP rate (two trials), residues were < 0.01 mg/kg.

The Meeting estimated a maximum residue level and STMR of 0.01* and 0 mg/kg, respectively, for sugar cane.

Peanut

A total of 17 trials were conducted on peanuts in Brazil (5) and in the USA (12) in different years.

The registered uses on peanut in these countries allow one application at a maximum rate of 0.098 kg ai/ha with a PHI of 70 days in Brazil; and 0.071 kg ai/ha with a PHI of 90 days in the USA.

Residues in peanuts from trials in Brazil matching Brazilian GAP were: < 0.05 (4) and < 0.1 mg/kg. Residues in peanut nutmeat from trials in the USA matching the GAP in the USA were: < 0.1 mg/kg (7). In the 12 trials using exaggerated rates (2× or 3× GAP rate), residues in nut, hull or nutmeat were all below the LOQ (0.05 or 0.1 mg/kg) (10).

The Meeting estimated a maximum residue level and STMR of 0.05* and 0 mg/kg, respectively for peanut.

The Meeting also estimated a median residue of 0 mg/kg for peanut hulls.

Rape seed

Three trials were conducted on imidazolinone-tolerant rape seed in Australia in 1997 and 1998.

The registered use on imidazolinone-tolerant rape seed in Australia allows one application at a maximum application rate of 0.0288 kg ai/ha at the 2–6 leaf stage of the crop. No PHI is required.

Residues of imazapic in rape seed from trials within

25% of GAP in Australia were: < 0.05 mg/kg (3). Residues from trials using exaggerated application rates (up to 2.4× GAP rate) were all < 0.05 mg/kg (2).

The Meeting estimated a maximum residue level and STMR of 0.05* and 0 mg/kg, respectively for rape seed.

Animal feed

Peanut fodder

Six trials involving peanut fodder were conducted in the USA in 1995. However, according to the GAP in the USA, grazing and feeding of treated peanut hay to livestock is not allowed. Therefore, there is no need to evaluate residues in peanut hay.

Wheat straw and fodder, dry, and forage

Five trials were conducted in Australia for wheat forage (four for straw).

According to GAP in Australia, grazing and cutting for stock feed are not allowed for 4 weeks after the application.

Residues of imazapic in wheat forage from trials matching GAP in Australia 4 weeks after application were: < 0.05 (3) mg/kg. Residues in forage from trials with exaggerated rates (up to 3.5× GAP rate; five trials) were < 0.05 mg/kg and residues in forage collected 15–19 days after application at any application rate were also < 0.05 mg/kg.

A median residue and highest residue were estimated for wheat forage at 0.05 and 0.05 mg/kg, respectively.

Residues of imazapic in wheat straw from trials matching GAP in Australia were: < 0.05 mg/kg (2). Residues in wheat straw from trials with exaggerated rates (up to 3.5× GAP rate) were all < 0.05 mg/kg (4).

The Meeting estimated a maximum residue level of 0.05* mg/kg for wheat straw and fodder, dry.

A median residue and highest residue for wheat straw and fodder, dry were 0 mg/kg.

Hay or fodder (dry) of grasses

A total of 10 trials were conducted on grasses in the USA.

The registered use on grasses in the USA allows one application at a maximum rate of 0.20 kg ai/ha after grass is full (100%) green-up for spring and summer application, or grass is dormant before green-up or full green-up for winter application. According to GAP cutting treated area for hay is not allowed within 7 days after treatment. There is no restriction on grazing on the label.

Residues of imazapic in forage from trials matching GAP in the USA were: 7.3, 7.5, 8.8, 10, 12, 13, 15, 15, 22 and 24 mg/kg.

The Meeting estimated a median residue and highest residue at 12.5 and 24 mg/kg respectively for grass forage on an "as received" basis.

Residues of imazapic in hay (> 7 DALA) from trials matching GAP in the USA were: < 0.50 (8), 0.91 and 2.3 mg/kg.

The Meeting estimated a maximum residue level, median residue and highest residue at 3, 0.5 and 2.3 mg/kg for hay or fodder (dry) of grasses, respectively.

Rape seed forage

Five trials were conducted in Australia for rape seed forage.

The approved uses in Australia do not allow grazing or cutting for stock feed for 6 weeks after application. Residues in rape seed forage 42 days after application at application rates within ±25% of the GAP rate were: < 0.05 (4) mg/kg.

The Meeting estimated a median residue and highest residue of 0.05 mg/kg.

Fate of residues during processing

High temperature hydrolysis

The hydrolysis of [^{14}C]-imazapic was investigated in sterile buffered aqueous solution.

After incubation at 90 °C (pH 4) for 20 minutes, 100 °C (pH 5) for 60 minutes or 120 °C (pH 6) for 20 minutes, 101–102% of the applied radioactivity remained. At the end of the incubation periods, the detectable radioactive component was unchanged imazapic only. Imazapic was stable under conditions representing pasteurization and baking/brewing/boiling and sterilization.

Processing

The Meeting received information on processing of soya beans.

A processing factor was calculated for the processing of soya beans to oil to be 0.14.

Processing factors for meal and hulls which can be fed to animals were also calculated but not reported here as no maximum residue level was recommended for soya beans, dry.

Residues in animal commodities

Estimation of dietary burdens

The maximum and mean dietary burdens were calculated using the highest residues or median residues of imazapic estimated at the current Meeting on a basis of the OECD Animal Feeding Table.

Summary of livestock dietary burdens (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	max	mean	max	Mean	max	mean	Max	mean
Beef cattle	0.401	0.094	48.0	25.0	96.0	50.0	5.72	2.71
Dairy cattle	43.2	22.5	57.6	30.0	96.0 [a]	50.0 [b]	11.2	5.34
Broilers	0.009	0.009	0.008	0.008	0	0	0.008	0.008
Layers	0.009	0.009	9.63 [c]	5.03 [d]	0	0	0.009	0.009

[a] Suitable for estimating maximum residue levels for milk, meat, fat and edible offal of cattle.

[b] Suitable for estimating STMRs for meat, fat and edible offal of cattle.

[c] Suitable for estimating maximum residue levels for meat, fat and edible offal of poultry and eggs.

[d] Suitable for estimating STMRs for meat, fat and edible offal of poultry and eggs.

Residues in milk and cattle tissues

Four groups of lactating Holstein cows, each group containing three animals, were orally dosed with imazapic in gelatine capsules using a balling gun for 28 days following afternoon milking at levels equivalent to 0, 67, 223, and 676 ppm in the feed. Milk samples were collected twice daily during the dosing period with afternoon and morning milk pooled. Within 24 hours after the last dose, the cows were sacrificed. After sacrifice, loin muscle, omental fat, liver and kidney were collected and analysed.

In the lowest dose group (67 ppm), residues of imazapic in milk reached a plateau after Day 1 and averaged 0.025 mg/kg. In milk fat and kidney, average residues were 0.013 and 0.384 mg/kg, respectively. In muscle, liver and omental fat, average residues were < 0.050 mg/kg.

In the middle dose group (223 ppm), residues of imazapic in milk averaged 0.077 mg/kg. In milk fat and omental fat, average residues were 0.037 and 0.051 mg/kg, respectively. In loin muscle, kidney and liver, average residues were 0.054, 1.57 and 0.082 mg/kg, respectively.

In the highest dose group (676 ppm), residues of imazapic in milk averaged 0.274 mg/kg. In milk fat and omental fat, average residues were 0.127 and 0.051 mg/kg, respectively. In muscle, kidney and liver, average residues were 0.079, 2.71 and 0.192 mg/kg, respectively.

Levels of CL263284 were below the respective LOQ in all matrices and at all dosing levels.

The maximum and mean dietary burdens in cattle were 96 and 50 ppm of dry matter diet respectively for estimating a maximum residue level and STMR for milk and edible tissues. The maximum residue levels, STMRs and HRs for relevant commodities of animal origin were estimated using the residue levels in tissues and milk at 67 and 223 ppm feeding group.

	Feed level (ppm) for milk residues	Imazapic (mg/kg) in milk	Feed level (ppm) for tissue residues	Imazapic (mg/kg) in			
				Muscle	Liver	Kidney	Fat
Maximum residue level beef or dairy cattle							
Feeding study [a]	67 223	0.025 0.274	67 223	< 0.05 0.063	< 0.05 0.126	0.465 2.20	< 0.05 0.054
Dietary burden and highest residue	96	0.071	96	0.052	0.064	0.788	0.051
STMR beef or dairy cattle							
Feeding study [b]	67	0.025	67	< 0.05	< 0.05	0.384	< 0.05
Dietary burden and mean residue	50	0.019	50	< 0.05	< 0.05	0.287	< 0.05

[a] highest residues for tissues and mean residue for milk

[b] mean residues for tissues and mean residue for milk

The Meeting estimated STMRs of 0.019, 0.05, 0.05, 0.287 and 0.05 mg/kg for milk, meat, liver, kidney and fat, respectively.

Based on the above, the Meeting estimated maximum residue levels of 0.1, 0.1, 1 and 0.1 mg/kg respectively for milks, meat (from mammals other than marine mammals), edible offal (mammalian) and Mammalian fats (other than milk fat).

Residues in eggs and poultry tissues

No feeding study on laying hens was conducted as expected dietary burdens for hen were low and the metabolism studies showed extremely low residues remaining in hen tissues.

In the metabolism study, the TRR in all edible tissues and eggs from hens fed orally radiolabelled imazapic at doses equivalent to 2.1 and 10.9 ppm in feed for 7 days were below the LOQ of 0.01 mg/kg.

At the maximum calculated dietary burden of 9.63 ppm in feed, the residues in eggs and edible tissues were estimated to be < 0.01 mg/kg. The Meeting therefore estimated a maximum residue level, STMR and HR at 0.01*, 0 and 0 mg/kg respectively applicable to poultry meat, poultry offal, poultry fat and eggs.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Definition of the residue for plant and animal commodities (for compliance with the MRL and for estimation of dietary intake): *Imazapic.*

Residue is not fat-soluble.

Commodity		Recommended MRL, mg/kg		STMR/STMR-P mg/kg
CCN	Name	New	Previous	
MO 0105	Edible offal (mammalian)	1	-	Liver, 0.05 Kidney, 0.287
PE 0112	Eggs	0.01*	-	0
AS 0162	Hay or fodder (dry) of grasses	3		(see table below)
GC 0645	Maize	0.01*	-	0
MF 0100	Mammalian fats (except milk fats)	0.1		0.05
MM 0095	Meat (from mammals other than marine mammals)	0.1		0.05
ML 0106	Milks	0.1		0.019
SO 0697	Peanut	0.05*	-	0
PF 0111	Poultry fats	0.01*	-	0
PM 0110	Poultry meat	0.01*	-	0
PO 0111	Poultry, edible offal of	0.01*	-	0
SO 0495	Rape seed	0.05*	-	0
GC 0649	Rice	0.05*	-	0
GS 0659	Sugar cane	0.01*	-	0
GC 0654	Wheat	0.05*	-	0
AS 0654	Wheat straw and fodder, dry	0.05*		(see table below)

For calculating animal dietary burdens.

Commodity		Median residue mg/kg	Highest mg/kg
CCN	Name		
	Grass forage	12.5	24
	Hay or fodder (dry) f grasses	0.5	2.3
	Peanut hull	0	-
	Rape seed forage	0.05	0.05
	Wheat forage	0.05	0.05
	Wheat straw and fodder, dry	0	0

Expressed on an “as received” basis.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of imazapic were calculated for the 13 GEMS/Food cluster diets using STMRs estimated by the current Meeting (Annex 3 of the 2013 JMPR Report). The ADI is 0–0.7 mg/kg bw and the calculated IEDIs were 0% of the maximum ADI. The Meeting concluded that the long-term intake of residues of imazapic resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term intake

The 2013 JMPR decided that an ARfD is unnecessary. The Meeting therefore concluded that the short-term intake of residues of imazapic is unlikely to present a public health concern.

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IA-740-001	Kleiner A.	1993 a	CL 263,222 (2AS): Residues of CL 263,222 and CL 263,284 in peanuts (POST; GA, 1992) American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-002	Kleiner A.	1993 b	CL 263,222 (2AS): Residues of CL 263,222 and CL 263,284 in peanuts (POST; FL, 1992) American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-003	Kleiner A.	1993 c	CL 263,222 (2AS): Residues of CL 263,222 and CL 263,284 in peanuts (POST; AL, 1992) American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-004	Kleiner A.	1993 d	CL 263,222 (2AS): Residues of CL 263,222 and CL 263,284 in peanuts (POST; OK, 1992) American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-005	Kleiner A.	1993 e	CL 263,222 (2AS): Residues of CL 263,222 and CL 263,284 in peanuts (POST; NC, 1992) American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-006	Kleiner A.	1993 f	CL 263,222 (2AS): Residues of CL 263,222 and CL 263,284 in peanuts (POST; TX, 1992) American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-009	Kleiner A., Haynes D.	1994 a	CL 263,222 (2AS): Residues of CL 263,222, CL 263,284, and CL 189,215 in peanuts (POST; OK, 1993), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-010	Kleiner A.	1994 b	CL 263,222 (2AS): Residues of CL 263,222, CL 263,284 and CL 189,215 in peanuts (POST; NC, 1993), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-011	Kleiner A.	1994 c	CL 263,222 (2AS): Residues of CL 263,222, CL 263,284 and CL 189,215 in peanuts (POST; AL, 1993), American Cyanamid Co., Princeton NJ, USA, Unpublished
2012/1010149	Kroehl T.	2012 a	Physical properties of Imazapic technical grade active ingredient (TC/TGAI) manufactured at Hannibal site, BASF SE, Limburgerhof, Germany Fed.Rep., Unpublished
2005/1040166	Leite R.	2005 a	Estudo de validacao do POP-PA.0249 para determinacao de residuos de Imazapyr, Imazapic, Imazamox, Imazethapyr e Imazaquin em arroz e soja, BASF SA, Resende, Brazil, Unpublished
2005/3001521	Leite R.	2005 b	Estudo de residuo de Imazapic em amendoim (grao) apos tratamento com Plateau (BAS 715 01 H) no Brasil, BASF SA, Resende, Brazil, Unpublished
2010/1090461	Leite R.	2008 a	SOP-PA.0288_E - Determination of Imidazolinones residues and its metabolites in several matrices and processed fractions by LC/MS/MS, BASF SA, Guaratingueta, Brazil, Unpublished
2009/1094340	Leite R.	2009 a	Study of Imazapic (BAS 715 H) residues in sugar cane after application of BAS 715 01 H under field conditions in Brazil for import tolerance, BASF SA, Guaratingueta, Brazil Unpublished
2011/3005983	Leite R.	2011 a	Amendment 01: Study of residues of Imazapyr and Imazapic in soya bean cultivance (grains) and processed fractions (meal, oil and flaked soya bean) after treatment with BAS 714 01 H, under field conditions in Brazil, BASF SA, Guaratingueta, Brazil, Unpublished
2008/1019445	Leite R., Souza C.	2009 a	Validation study of the SOP-PA.0288 for determination of Imazapic and its metabolites residues in sugarcane and processed fractions, BASF SA, Guaratingueta, Brazil, Unpublished
2010/1057141	Leite R., Marinho E.	2011 a	Estudo de investigacao da estabilidade de Imidazolinonas e seus metabolitos em solucoes padrao de trabalho sob circunstancias rotineira de armazenamento BASF SA, Guaratingueta, Brazil, Unpublished
IA-740-013	Leonard R.	1997 a	CL 263,222 crop residue study: CL 263,222, CL 263,284 and CL 189,215 residues in peanut commodities after a post emergence application with CADRE 2AS herbicide at 0.063 lb a.e./A (NC, 1995), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-014	Leonard R.	1998 a	CL 263,222 crop residue study: CL 263,222, CL 263,284 and CL 189,215 residues in peanut hay after a post emergence application with Cadre 2AS herbicide at 0.063 lb ae/A (GA, 1995), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-015	Leonard R.	1998	CL 263,222 crop residue study: CL 263,222, CL 263,284 and CL 189,215

Doc. ID	Author(s)	Year	Title, Source, published or not
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IA-740-016	Leonard R.	1998 c	CL 263,222 crop residue study: CL 263,222, CL 263,284 and CL 189,215 residues in peanut commodities after a post emergence application with Cadre 2AS herbicide at 0.063 lb a.e./A (NC, 1995), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-017	Leonard R.	1998 d	CL 263,222 crop residue study: CL 263,222, CL 263,284 and CL 189,215 residues in peanut commodities after a post emergence application with Cadre 2AS herbicide at 0.063 lb ae/A (TX, 1995), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-740-018	Leonard R.	1998 e	CL 263,222 crop residue study: CL 263,222, CL 263,284 and CL 189,215 residues in peanut commodities after a post emergence application with Cadre 2AS herbicide at 0.063 lb ae/A (FL, 1995), American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-731-008	Leonard R.C.	1998 f	CL 263,222 (Imazapic): Residues of CL 263,222, CL 263,284 and CL 189,215 in established common bermuda grass after postemergence treatment with Plateau 2AS herbicide in Mississippi, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-731-010	Leonard R.C.	1998 g	CL 263,222 (Imazapic): Residues of CL 263,222, CL 263,284 and CL 189,215 in established common Bermuda grass after postemergence treatment with Plateau 2AS herbicide in Georgia, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-705-002	Leonard R.C.	1999 a	CL 263222 (Imazapic): Determination of CL 263222 and CL 263284 residues in milk, milk fat and edible tissues from dairy cattle after oral administration of CL 263222 for 28 consecutive days, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-620-003	Madsen S.	1993 a	Aerobic soil metabolism of 14C-CL 263,222 ABC - Analytical Bio-Chemistry Laboratories Inc., Columbia MO, USA, Unpublished
IA-630-001	Madsen S.	1993 b	Anaerobic aquatic metabolism of 14C-CL 263,222 ABC - Analytical Bio-Chemistry Laboratories Inc., Columbia MO, USA, Unpublished
IA-306-001	Mangels G.	1986 a	AC 263,222: Determination of the vapor pressure ABC - Analytical Bio-Chemistry Laboratories Inc., Columbia MO, USA, Unpublished
IA-311-001	Mangels G.	1986 b	AC 263,222: The determination of the water solubility American Cyanamid Co., Princeton NJ, USA, (Studies were conducted prior to the implementation of GLP but are scientifically valid), Unpublished
IA-322-003	Mangels G.	1986 c	AC 263,222: The determination of the dissociation constants American Cyanamid Co., Princeton NJ, USA, (Studies were conducted prior to the implementation of GLP but are scientifically valid), Unpublished
IA-640-001	Mangels G.	1988 a	AC 263,222: Metabolism in sugarcane American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-322-004	Mangels G.	1992 a	AC 263,222: Hydrolysis American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-322-002	Melcer M.	1993 a	AC 263,222: Determination of the dissociation constants American Cyanamid Co., Princeton NJ, USA, Unpublished
2008/7012758	Mickelson K.R., Jordan J.M.	2008 a	Study of BAS 715 01 H residues in sugarcane and processed fractions of sugarcane after application of Cadre 70 DG under field conditions in Costa Rica and Guatemala for import tolerance, IAR - International Agricultural Research Inc., Apex NC, USA, Unpublished
2008/7012758	Mickelson K.R., Jordan J.M.	2008 a	Study of BAS 715 01 H residues in sugarcane and processed fractions of sugarcane after application of Cadre 70 DG under field conditions in Costa Rica and Guatemala for import tolerance, IAR - International Agricultural Research Inc., Apex NC, USA, Unpublished
IA-244-012	Minoura M.	1995 a	CL 263,222: Validation of method M-2253.01 for the determination of CL 263,222 and CL 263,284 residues in wheat stubble, Cyanamid Japan Ltd., Tahara Aichi 441-34, Japan Unpublished
IA-244-011	Minoura M.	1995 b	CL 263,222: Validation of method M-2253.01 for the determination of CL 263,222 and CL 263,284 residues in chick-pea grain, Cyanamid Japan Ltd., Tahara Aichi 441-34, Japan Unpublished
IA-244-010	Minoura M.	1995 c	CL 263,222: Validation of method M-2253.01 for the determination of CL 263,222 and CL 263,284 residues in barley grain, Cyanamid Japan Ltd., Tahara

Doc. ID	Author(s)	Year	Title, Source, published or not
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IA-244-008	Minoura M.	1995 e	CL 263,222: Validation of method M-2253.01 for the determination of CL 263,222 and CL 263,284 residues in chick-pea forage, Cyanamid Japan Ltd., Tahara Aichi 441-34, Japan Unpublished
IA-244-007	Minoura M.	1995 f	CL 263,222: Validation of method M-2253.01 for the determination of CL 263,222 and CL 263,284 residues in barley forage, Cyanamid Japan Ltd., Tahara Aichi 441-34, Japan Unpublished
IA-244-006	Minoura M.	1995 g	CL 263,222: Validation of method M-2253.01 for the determination of CL 263,222 and CL 263,284 residues in wheat forage, Cyanamid Japan Ltd., Tahara Aichi 441-34, Japan Unpublished
IA-315-003	Morelli D.	1993 a	AC 263,222: Determination of the n-Octanol/water partition coefficient American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-244-001	Nejad H.	1993 a	Validation of HPLC method M 2253.01 for the determination of CL 263,222 and CL 263,284 residues in peanut hull and peanut meat, American Cyanamid Co., Princeton NJ, USA Unpublished
IA-244-003	Nejad H.	1994 a	CL 263,222: Validation of capillary electrophoresis (CE) determinative and LC/MS confirmatory method for CL 263,222, CL 263,284 and CL 189,215 residues in peanut hull and peanut nutmeat, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-244-004	Nejad H.	1994 b	CL 263,222 (Herbicide): Extractability of total carbon-14 residues of CL 263,222 and related metabolites from peanut hulls, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-244-002	Nejad H.	1995 a	CL 263,222: Capillary electrophoresis (CE) determination and LC/MS confirmatory method for CL 263,222, CL 263,284, and CL 189,215 residues in peanut hull and peanut nutmeat American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-244-018	Nejad H.	1998 a	CL 263,222 (Imazameth): Independent laboratory validation of capillary electrophoresis (CE) method M 2599 for the determination of CL 263,222, CL 263,284 and CL 189,215 residues in peanut hay, Centre Analytical Laboratories Inc., State College PA, USA Unpublished
IA-244-020	Nejad H.	1999 a	CL 263,222 (Imazapic): Radiovalidation of method M 3114 for the determination of residues of CL 263,222 and metabolites CL 263,284 and CL 189,215 in grass American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-245-003	Nejad H.	1999 b	CL 263222 (Imazapic): Radiovalidation of methods M 3188 and M 3222 for the determination of CL 263222 and metabolite CL 263284 using goat milk and kidney, respectively, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-244-013	Nejad H., Cenni M.	1995 a	Independent laboratory validation of capillary electrophoresis (CE) method M 2463 for the determination of CL 263,222, CL 263,284 and CL 189,215 residues in wheat straw, wheat hay, wheat forage and wheat grain, Centre Analytical Laboratories Inc., State College PA, USA, Unpublished
IA-244-014	Nejad H., Cenni M.	1996 a	CL 263,222 (Imazameth): Extractability of total carbon-14 residues of CL 263,222 and related metabolites in barley straw, American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-240-002	Nejad H., Cenni M.	1997a	CL 263,222 (Imazameth): Independent laboratory validation of HPLC method M 2253.01 for the determination of CL 263,222 and metabolite CL 263,284 residues in peanut hull and peanut meat, American Cyanamid Co., Princeton NJ, USA, Unpublished
2012/7000340	Ostermann B., Chu J.	2012 a	High temperature hydrolysis of 14C-BAS 715 H (Imazapic) at 90°C, 100°C, and 120°C PTRL West Inc., Hercules CA, USA, Unpublished
2008/1097471	Resende G.	2008 a	Amendment report 02 RF-1088-06 - Study of residues of Imazapyr and Imazapic in soya bean cultivance (grains) after treatment with BAS 714 01 H, under field conditions in Brazil BASF SA, Guaratingueta, Brazil, Unpublished
2009/700008	Resende G.	2009 a	Study of residues of Imazapyr and Imazapic in soya bean cultivance (grains) and

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2010/1079212	Resende G.	2010 a	Amendment 01 - Final report 1273-07 - Study of residues of Imazapyr and Imazapic in soya bean cultivance (grains) after treatment with BAS 714 01 H under field conditions in Brazil for import tolerance, BASF SA, Guaratingueta, Brazil, Unpublished
2008/1097470	Resende G., Souza C.	2008 a	Amendment report 01 RF-1088-06 - Study of residues of Imazapyr and Imazapic in soya bean cultivance (grains) after treatment with BAS 714 01 H, under field conditions in Brazil BASF SA, Guaratingueta, Brazil, Unpublished
2010/1033332	Resende G., Takahashi J.	2010 a	Validation study of the SOP-PA.0288 for the determination of residues of Imazapyr, Imazapic and their metabolites CL 263,284 and CL 189,215 in soya bean and processed fractions of soya bean by LC/MS/MS, BASF SA, Guaratingueta, Brazil, Unpublished
2010/1010261	Resende G., Marinho E.	2010 a	Study of residues of Imazapyr and Imazapic in soya bean cultivance (grains) after treatment with BAS 714 01 H under field conditions in Brazil for import tolerance BASF SA, Guaratingueta, Brazil, Unpublished
2012/7000359	Robaugh D.A.	2012 a	Independent laboratory validation of analytical method number SOP-PA.0288: Determination of Imidazolinones residues and its metabolites in several matrices and processed fractions by LC/MS/MS Pyxant Labs Inc., Colorado Springs CO, USA, Unpublished
2012/7000359	Robaugh D.A.	2012 a	Independent laboratory validation of analytical method number SOP-PA.0288: Determination of Imidazolinones residues and its metabolites in several matrices and processed fractions by LC/MS/MS Pyxant Labs Inc., Colorado Springs CO, USA, Unpublished
IA-244-005	Safarpour M.	1994 a	Independent laboratory validation of capillary electrophoresis (CE) method M 2379 for the determination of CL 263,222, CL 263,284, and CL 189,215 residues in peanut hull and peanut nutmeat by Centre Analytical Laboratories, Inc. Centre Analytical Laboratories Inc., State College PA, USA, Unpublished
IA-440-006	Sharp D., Thalacker F.W.	1999 a	AC 263222 (Imazapic): Metabolic fate of 14C AC 263222 in lactating goats Covance Laboratories Inc., Madison WI, USA, Unpublished
IA-731-007	Steling C.	1998 a	CL 263,222: Determination of CL 263,222 (Imazapic) in corn samples from Brazil LAADL - Latin America Agricultural Development Laboratory, Rio de Janeiro, Brazil Unpublished
IA-790-011	Steling C.	1998 a	CL 263,222: Determination of CL 263,222 residues in sugar cane samples from Argentina LAADL - Latin America Agricultural Development Laboratory, Rio de Janeiro, Brazil Unpublished
IA-740-019	Steling C.	1998 b	CL 263,222: Determination of CL 263,222 (Imazapic) residues in peanut samples from Brazil, LAADL - Latin America Agricultural Development Laboratory, Rio de Janeiro, Brazil, Unpublished
2001/3001704	Steling C.	2001 a	Estudo de residuo de Imazapic (CL 263,222) em arroz (grao com casca) do Brasil (curva de degradacao), LAADL - Latin America Agricultural Development Laboratory, Rio de Janeiro, Brazil, Unpublished
2001/3001705	Steling C.	2001 b	Estudo de residuos de Imazapic (CL 263,222) em arroz (grao com casca) do Brasil LAADL - Latin America Agricultural Development Laboratory, Rio de Janeiro, Brazil Unpublished
2001/3001706	Steling C.	2001 c	Estudo de residuo de Imazapic (CL 263,222) em arroz (grao com casca) do Brasil LAADL - Latin America Agricultural Development Laboratory, Rio de Janeiro, Brazil Unpublished
IA-245-001	Sweeney R.A., Nejad H.	1998 a	CL 263222 (Imazapic): Independent laboratory validation of capillary electrophoresis (CE) determinative and LC/MS confirmatory method for CL 263222 and CL 263284 residues in milk, ABC - Analytical Bio-Chemistry Laboratories Inc., Columbia MO, USA, Unpublished
IA-245-002	Sweeney R.A.,	1998 b	CL 263222 (Imazapic): Independent laboratory validation of capillary electrophoresis (CE) determinative and LC/MS confirmatory method for CL

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IA-630-002	Ta C.	1994 a	AC 263,222: Photodegradation in water American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-620-001 US	Ta C.	1994 b	AC 263,222: Photodegradation on soil American Cyanamid Co., Princeton NJ, USA, Unpublished
IA-620-009	Ta C.T.	1997 a	Aerobic soil metabolism of AC 263222 and its metabolites CL 263284 and CL 312622 (non GLP), American Cyanamid Co., Ewing NJ, USA, Unpublished
IA-310-001	Teeter D.	1992 a	AC 263222: The determination of the solubility in deionized water, water at pH 5, 7 and 9 and various organic solvents, American Cyanamid Co., Princeton NJ, USA Unpublished
2000/1023962	Thomas G.	2000 a	Residues evaluation report - Midas herbicide National Registration Authority for Agricultural & Veterinary Chemicals, Kingston Australian Capital Territory 2604, Australia, No, not subject to GLP regulations Unpublished
2010/1204819	Vieira F.A.	2010 a	Determination of the surface tension in water of BAS 715 H BIOAGRI Laboratorios Ltda., Piracicaba, Brazil, Unpublished
IA-640-003	Wu S.-S.	1993 a	CL 263,222: Metabolism of carbon-14 labelled CL 263,222 in peanuts under field conditions American Cyanamid Co., Princeton NJ, USA, Unpublished