5.22 IMAZAPIC (266)

TOXICOLOGY

Imazapic is the ISO-approved name for (*RS*)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid (IUPAC), with CAS No. 104098-48-8. It is a new herbicide that belongs to the imidazolinone family. The proposed mode of action is specific to plants and involves the disruption of protein synthesis via the inhibition of acetohydroxyacid synthase, an enzyme not found in mammalian tissues.

Imazapic has not been evaluated previously by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with GLP.

Biochemical aspects

Radiolabelled imazapic administered by oral gavage is rapidly and extensively absorbed, minimally metabolized and excreted primarily in the urine after single low (10 mg/kg bw) or high doses (1000 mg/kg bw) or repeated low doses (10 mg/kg bw per day) over 14 days to rats. Biliary excretion was minimal. The majority of radioactivity was excreted as the parent compound within the first 6 hours post-dosing. Less than 2% of the administered dose was detected in the carcass, with trace amounts detected in blood, kidneys and liver of the high-dose group at 168 hours post-dosing; however, radiolabelled test substance was not detected in any other organs. There was no evidence of accumulation. There were no notable differences in absorption or excretion between the sexes. Imazapic and its metabolites were not excreted in expired air. Parent compound accounted for more than 94% of the administered dose in the urine and 2.3% of the administered dose in the faeces. The metabolites produced from oxidation, reduction and hydrolysis, including CL 263,284 (M715H001 or 5-hydroxy methyl metabolite), CL 280,442 (no common name assigned) and several other unidentified metabolites, accounted for a total of approximately 6% of the administered dose in the urine and faeces.

Toxicological data

Imazapic is of low acute oral toxicity in the rat (LD_{50} > 5000 mg/kg bw) and low acute dermal toxicity in the rabbit (LD_{50} > 2000 mg/kg bw). Imazapic appears to be of low acute inhalation toxicity, but characterization by this route was limited due to the high median particle sizes. In the rabbit, imazapic was non-irritating to the skin and mildly irritating to the eyes. Imazapic was not a dermal sensitizer in guinea-pigs.

Overall, imazapic showed low mammalian toxicity on repeated administration. Most of the rodent studies found no adverse effects, including target organ toxicity, up to the limit dose. The dog and, to a lesser extent, the rabbit were more sensitive than the rat or mouse to imazapic-induced toxicity. The target tissues in the dog were skeletal muscle and, at higher doses, bone marrow.

In a 13-week oral toxicity study in the rat, the NOAEL was 20 000 ppm (equal to 1522 mg/kg bw per day), the highest dose tested.

In a 1-year oral toxicity study in the dog, animals were exposed to 0, 5000, 20 000 or 40 000 ppm (equal to 0, 137, 501 and 1141 mg/kg bw per day for males and 0, 180, 534 and 1092 mg/kg bw per day for females, respectively). There was an increase in lymphocyte and macrophage infiltration in the diaphragm, abdominal and thigh skeletal muscles and an increase in degeneration or necrosis of the abdominal and thigh muscles at doses greater than or equal to 5000 ppm (equal to 137 mg/kg bw per day), which was therefore the LOAEL for this study. The effects observed at the LOAEL were of minimal severity. Grade 1 lesions were seen primarily in one site per animal at the lowest dose tested, in multiple sites per animal at the middle dose and in

multiple sites at increased grades at the high dose. However, in the absence of any information on the mode of toxicological action for the effects of imazapic on the muscles of dogs, the effects were considered adverse and potentially relevant to humans.

The chronic toxicity and carcinogenicity of imazapic have been investigated in mice and rats. In the mouse study, there was no evidence of toxicity up to 7000 ppm (equal to 1134 mg/kg bw per day), the highest dose tested. In the rat study, there was no evidence of toxicity up to 20 000 ppm (equal to 1029 mg/kg bw per day), the highest dose tested.

The Meeting concluded that imazapic is not carcinogenic in mice or rats.

Imazapic has been tested in an adequate range of genotoxicity studies in vitro and in vivo. There was no evidence of genotoxicity.

The Meeting concluded that imazapic is unlikely to be genotoxic.

Based on the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that imazapic is unlikely to pose a carcinogenic risk to humans.

A multigeneration reproductive toxicity study was performed with imazapic in rats. The NOAEL for reproductive, parental and offspring toxicity was 20 000 ppm (equal to 1205 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, the NOAEL for maternal and embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, pregnant females were dosed at 0, 175, 350, 500 or 700 mg/kg bw per day; however, the highest dose was not evaluated for embryo and fetal toxicity due to excessive maternal mortality. The NOAEL for maternal toxicity was 350 mg/kg bw per day, based on a loss of body weight and a decrease in body weight gain compared with controls and a decrease in feed consumption at 500 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 500 mg/kg bw per day, the highest dose at which fetuses were evaluated.

The Meeting concluded that imazapic is not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats, animals were given imazapic as a single oral gavage dose at 0, 200, 600 or 2000 mg/kg bw per day. The NOAEL was 600 mg/kg bw per day, based on increased salivation in males and females at 2000 mg/kg bw per day.

In a 13-week dietary neurotoxicity study in rats, the NOAEL was 927 mg/kg bw per day, the highest dose tested.

In a 28-day dietary immunotoxicity study in mice, no effects on IgM response to sheep red blood cells or any other signs of immunotoxicity were observed at 5000 ppm (equal to 1364 mg/kg bw per day), the highest dose tested.

Human data

No specific information on the effects of impazapic on production plant workers or others was available. There are no reports of poisoning cases with imazapic.

The Meeting concluded that the existing database on imazapic was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.7 mg/kg bw on the basis of the LOAEL of 5000 ppm (equal to 137 mg/kg bw per day), the lowest dose tested, for effects on skeletal muscles in a 1-year study of

toxicity in dogs. A safety factor of 200 was applied, with an additional safety factor of 2 being used to account for the use of a LOAEL instead of a NOAEL; the effects observed at the LOAEL were of minimal severity. No adverse effects were observed in chronic studies in rats or mice, up to the limit dose.

The plant metabolite CL 189,215 is the glucoside conjugate of the 5-hydroxy methyl imazapic, which occurs as a minor metabolite in rats. The Meeting concluded that this plant metabolite would be covered by the ADI for imazapic.

The Meeting concluded that it was not necessary to establish an ARfD for imazapic in view of its low acute toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose. The effects observed in the acute neurotoxicity study occurred above 500 mg/kg bw.

A toxicological monograph was prepared.

Levels relevant to risk assessment of imazapic

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	7000 ppm, equal to 1134 mg/kg bw per day[b]	—
		Carcinogenicity	7000 ppm, equal to 1134 mg/kg bw per day[b]	—
Rat	Thirteen-week study of toxicity[a]	Toxicity	20 000 ppm, equal to 1522 mg/kg bw per day[b]	—
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 000 ppm, equal to 1029 mg/kg bw per day[b]	—
		Carcinogenicity	20 000 ppm, equal to 1029 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	20 000 ppm, equal to 1205 mg/kg bw per day[b]	—
		Parental toxicity	20 000 ppm, equal to 1205 mg/kg bw per day[b]	—
		Offspring toxicity	20 000 ppm, equal to 1205 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	350 mg/kg bw per day	500 mg/kg bw per day
		Embryo and fetal toxicity	500 mg/kg bw per day[b]	—
Dog	One-year study of toxicity[a]	Toxicity	—	5000 ppm, equal to 137 mg/kg bw per day[d]

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

[d] Lowest dose tested.

Estimate of acceptable daily intake

0–0.7 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to imazapic

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; extensive
Dermal absorption	No data
Distribution	Rapidly eliminated; highest residues in blood, bone, carcass and liver in males and females and fat and kidneys in females
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Largely complete within 48 h; primarily via urine (94.50–102%) with < 5% via faeces
Metabolism in animals	Mostly excreted unchanged
Toxicologically significant compounds in animals, plants and the environment	Imazapic, 5-hydroxy methyl metabolite
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	No reliable data
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Not sensitizing (Buehler method)
Short-term studies of toxicity	
Target/critical effect	Skeletal muscle
Lowest relevant oral NOAEL	< 137 mg/kg bw per day, the lowest dose tested (dog)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	No long-term effects up to limit dose
Lowest relevant oral NOAEL	1029 mg/kg bw per day, the highest dose tested (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic

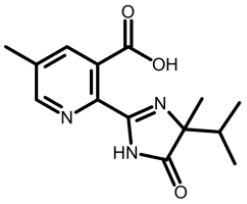
Reproductive toxicity	
Target/critical effect	No reproductive toxicity
Lowest relevant parental NOAEL	1205 mg/kg bw per day, the highest dose tested
Lowest relevant offspring NOAEL	1205 mg/kg bw per day, the highest dose tested
Lowest relevant reproductive NOAEL	1205 mg/kg bw per day, the highest dose tested
Developmental toxicity	
Target/critical effect	Body weight and feed consumption in dams
Lowest relevant maternal NOAEL	350 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	500 mg/kg bw per day, the highest dose tested (rabbit)
Neurotoxicity	
Acute and subchronic neurotoxicity NOAEL	Salivation: 600 mg/kg bw
Immunotoxicity	
	Not immunotoxic
Medical data	
	No studies submitted

Summary

	Value	Study	Safety factor
ADI	0–0.7 mg/kg bw	One-year oral toxicity study (dog)	200
ARfD	Unnecessary	—	—

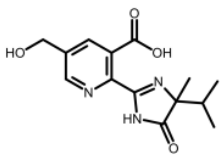
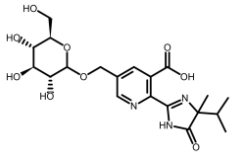
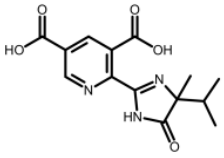
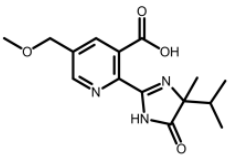
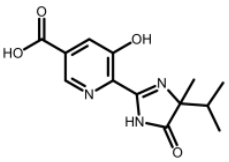
RESIDUE AND ANALYTICAL ASPECTS

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 $\log P_{ow}$ 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.

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). 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.