5.13 FENPYRAZAMINE (298)

TOXICOLOGY

Fenpyrazamine is the International Organization for Standardization (ISO)–approved common name for allyl 5-amino-2,3-dihydro-2-isopropyl-3-oxo-4-(o-tolyl) pyrazole-1-carbothioate (International Union of Pure and Applied Chemistry name), with Chemical Abstracts Service number 473798-59-3. Fenpyrazamine is a fungicide whose mode of action is by inhibition of 3-ketoreductase in the ergosterol biosynthesis pathway.

Fenpyrazamine has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of Codex Committee on Pesticide Residues (CCPR).

All critical studies contained statements of compliance with good laboratory practice (GLP).

Biochemical aspects

In metabolism studies conducted in rats using fenpyrazamine labelled with ^{14}C at the 5-pyrazolyl position, the absorption of fenpyrazamine was rapid and extensive, with almost complete metabolism at low doses (3 mg/kw body weight [bw]). Approximately 4% was eliminated unmetabolized in the faeces in the high dose groups (300 mg/kg bw). There were no significant sex differences in pharmacokinetics. Maximum blood and plasma ^{14}C concentrations were observed at 1 and 6 hours (T_{max}) after administration in the low (3 mg/kg bw) and high dose (300 mg/kg bw) groups, respectively, and maximum concentration (C_{max}) values ranged between 1.5 and 2.0 μg/g at low doses and 45 and 68 μg/g at high doses. The half-lives were 2–3 hours for the low dose and 14–17 hours for the high dose. The area under the curve for the 300 mg/kg bw dose was 150- to 170-fold greater than that for the low dose (3 mg/kg bw), indicating some saturation in the elimination processes at the higher dose.

Less than 0.5% of the administered dose was retained in the tissues 12 hours following a single oral low dose. Peak radioactive residues were generally detected in the plasma, liver, kidneys and stomach within the first few hours after dosing. After repeated-dose administration, the radiolabel reached steady state from days 7 to 15 and then declined from the cessation of dosing until 97.2% had been excreted by day 25. The stomach (including contents) and the liver had the highest levels of radioactivity in the repeated-dose study.

Following administration of a single low and single high dose, the main route of elimination was urinary (80–87% administered dose). Faecal elimination of fenpyrazamine was minor (8–12% administered dose). Elimination was rapid, with about 90% of the radiolabel eliminated within 24 hours for the low-dose group and 48 hours for the high-dose group. There was no significant difference in route or rate of elimination between males and females. Elimination via expired air was negligible. With repeat dosing for 14 days, the excretion patterns of orally administered [^{14}C]fenpyrazamine were similar to those after single doses.

The principal routes of metabolism of fenpyrazamine involved hydrolysis to remove the allylsulfanylcarbonyl group to produce the main metabolites, 5-amino-1,2-dihydro-2-isopropyl-4-(*o*-tolyl)pyrazol-3-one (S-2188-DC), followed by dealkylation of S-2188-DC to 5-amino-1,2-dihydro-4-(*o*-tolyl)pyrazol-3-one (MPPZ) and hydroxylation to S-2188-CH_2OH-DC and S-2188-OH. MPPZ was then conjugated with sulfate and with glucuronide prior to elimination. The metabolite profile was qualitatively similar in males and females and at both dose levels as well as after single and repeat 14-day dosing.

Toxicological data

In rats, fenpyrazamine had an oral median lethal dose (LD_{50}) greater than 2000 mg/kg bw, a dermal LD_{50} greater than 2000 mg/kg and an inhalation median lethal concentration (LC_{50}) greater than

4.84 mg/L. Fenpyrazamine was non-irritating to the skin and eyes of rabbits. It did not induce dermal sensitization in guinea-pigs.

The main toxic effects of fenpyrazamine in short- and long-term toxicity studies in mice, rats and dogs were decreased body weight and body weight gain; additionally, liver effects were seen in the mouse and liver and thyroid effects in the rat.

In a 13-week oral toxicity study in which mice received dietary concentrations of fenpyrazamine of 0, 200, 2000, 4000 or 6000 parts per million (ppm; equal to 0, 28, 296, 640 and 1023 mg/kg bw per day in males and 0, 33, 363, 720 and 1098 mg/kg bw per day in females, respectively), the no-observed-adverse-effect-level (NOAEL) was 2000 ppm (equal to 296 mg/kg bw per day), based on decreased body weight and haematocrit at 4000 ppm (equal to 640 mg/kg bw per day).

In a 13-week oral toxicity study in which rats received dietary concentrations of fenpyrazamine of 0, 300, 600, 1000 or 3000 ppm (equal to 0, 19, 38, 64 and 196 mg/kg bw per day in males and 0, 21, 42, 69 and 207 mg/kg bw per day in females, respectively), the NOAEL was 1000 ppm (equal to 64 mg/kg bw per day) based on decreased body weight and body weight gain in males and females and increased thyroid follicular cell hypertrophy in males at the lowest-observed-adverse-effect level (LOAEL) of 3000 ppm (equal to 196 mg/kg bw per day).

In a 90-day oral capsule toxicity study, dogs received fenpyrazamine at doses of 0, 25, 50 or 150 mg/kg bw per day. The NOAEL was 25 mg/kg bw per day based on body weight loss in one male at the LOAEL of 50 mg/kg bw per day.

In a 1-year oral capsule toxicity study, dogs received fenpyrazamine at doses of 0, 5, 25 or 100 mg/kg bw per day. The NOAEL was 25 mg/kg bw per day based on increased mean corpuscular volume and alkaline phosphatase activity in males and increased platelets in females at the LOAEL of 100 mg/kg bw per day.

The Meeting concluded that the overall NOAEL for oral toxicity in dogs was 25 mg/kg bw per day.

In a 78-week dietary toxicity study in which mice received dietary concentrations of fenpyrazamine of 0, 100, 1500 or 3000 ppm in males (equal to 0, 11, 176 and 349 mg/kg bw per day) and 0, 100, 2000 or 4000 ppm in females (equal to 0, 14, 283 and 552 mg/kg bw per day), the NOAEL was 1500 ppm (equal to 176 mg/kg bw per day) based on decreased body weight and red blood cell counts and increased mean corpuscular volume and mean cell haemoglobin. The LOAEL was 3000 ppm (equal to 349 mg/kg bw per day). Findings suggestive of hepatocarcinogenicity were observed at the mid and high dose in male mice.

In a 2-year dietary toxicity study in which rats received dietary concentrations of fenpyrazamine of 0, 100, 300, 1200 or 2400 ppm (equal to 0, 4.3, 12.7, 52 and 107 mg/kg bw per day in males and 0, 5.3, 15.6, 64 and 130 mg/kg bw per day in females, respectively), the NOAEL was 1200 ppm (equal to 52 mg/kg bw per day) based on decreased body weights compared to controls. The LOAEL was 2400 ppm (equal to 107 mg/kg bw per day). Findings suggestive of thyroid carcinogenicity were observed at the high dose in male rats.

In an oral study investigating the time-course of changes in the liver and thyroid in which male rats received fenpyrazamine at dietary concentrations of 0 or 2400 ppm for 3, 7 or 14 days (equal to 0 and 216.5 in the 3-day group, 0 and 222.6 in the 7-day group and 0 and 216.6 mg/kg bw per day in the 14-day group, respectively), there were increases in cell proliferation seen following the 3-day treatment that was not seen following the 7- and 14-day treatments. However, the protein content in the rat liver S9 fraction was increased following the 7-day treatment only and 7-pentoxyresorufin *O*-dependentylase and thyroxine- UDP-glucuronosyltransferase (T_4-UGT) activities were increased in all treatment groups. Thyroid weights were increased in the 7- and 14-day treatment groups and follicular cell hypertrophy was increased in all treatment groups. Thyroid-stimulating hormone levels increased and triiodothyronine and T_4 levels decreased with treatment time. Changes were considered consistent with phenobarbital-like mode of action.

In a related study, the role of constitutive androstane receptor (CAR) was investigated in fenpyrazamine-treated rat hepatocytes. Increased activity of CYP2B1, UGT1A and UGT2B1 messenger ribonucleic acid (mRNA) expression were observed in fenpyrazamine-treated hepatocytes. Following knockdown of CAR using small interfering ribonucleic acid, the induction of mRNA levels for CYP2B1, UGT1A and UGT2B1 was significantly reduced consistent with CAR-mediated induction of the hepatocytes.

The pattern of effects in the liver and thyroid is consistent with a CAR-mediated mode of action. Tumours in rodents induced by this mode of action are not relevant to humans.

Fenpyrazamine has been tested in an adequate range of genotoxicity studies, both in vitro and in vivo. No evidence of genotoxicity was found.

In view of the lack of genotoxicity and lack of human-relevant carcinogenicity in mice and rats, the Meeting concluded that fenpyrazamine is unlikely to pose a carcinogenic risk to humans.

In a two-generation dietary reproductive toxicity study in which rats received concentrations of fenpyrazamine at 0, 400, 1000 or 3000 ppm (equal to 0, 27, 69 and 213 mg/kg bw per day in males and 0, 32, 80, 237 mg/kg bw per day in females, respectively), the parental NOAEL was 400 ppm (equal to 27 mg/kg bw per day) based on increased liver and thyroid weights in both generations, decreased body weights in F_1 males and enlarged thyroids in F_1 females at the LOAEL of 1000 ppm (equal to 69 mg/kg bw per day). The NOAEL for reproductive toxicity was 1000 ppm (equal to 80 mg/kg bw per day) based on decreased birth weight, increased postimplantation loss and decreased implantations and litter size at the LOAEL of 3000 ppm (equal to 237 mg/kg bw per day). The NOAEL for offspring toxicity was 400 ppm (equal to 32 mg/kg bw per day) based on decreased pup weight in the $F_1$and F_2 pups at the LOAEL of 1000 ppm (equal to 80 mg/kg bw per day).

In a developmental toxicity study in which rats received fenpyrazamine by gavage at doses of 0, 30, 125 or 500 mg/kg per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day based on decreased body weight and body weight gain at the maternal LOAEL of 125 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 125 mg/kg based on decreased fetal weight and increased visceral and skeletal variations and delayed ossification at the LOAEL for embryo/fetal toxicity of 500 mg/kg bw per day. There was no evidence of teratogenicity.

In a developmental toxicity study in which rabbits received fenpyrazamine by gavage at doses of 0, 30, 50 or 90 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day based on increased late abortions accompanied by decreased food intake and increased gross pathological findings in the aborting animals at the maternal LOAEL of 50 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 30 mg/kg bw per day based on abortion at 50 mg/kg bw per day.

The Meeting concluded that fenpyrazamine is not teratogenic.

In an acute neurotoxicity study in which rats received fenpyrazamine by gavage at doses of 0, 80, 400 or 2000 mg/kg bw, the NOAEL was 80 mg/kg bw based on decreased locomotor activity as a result of general toxicity at the LOAEL of 400 mg/kg bw. There was no evidence of specific neurotoxicity.

In a 90-day neurotoxicity study in which rats received fenpyrazamine in the diet at concentrations of 0, 500, 1200 or 3000 ppm (equal to 0, 37, 88 and 224 mg/kg bw per day in males and 0, 42, 100 and 248 mg/kg bw per day in females, respectively), the NOAEL was 1200 ppm (equal to 88 mg/kg bw per day) based on decreased body weight and body weight gain. The LOAEL was 3000 ppm (equal to 224 mg/kg bw per day). There was no evidence of specific neurotoxicity.

The Meeting concluded that fenpyrazamine is not neurotoxic.

In a four-week immunotoxicity study in which female mice received fenpyrazamine in the diet at concentrations of 0, 500, 1500 or 4000 ppm (equal to 0, 36, 123 and 392 mg/kg bw per day), the NOAEL was 1500 ppm (equal to 123 mg/kg bw per day) based on decreased body weight and

body weight gain and increased liver and spleen weights. The LOAEL was 4000 ppm (equal to 392 mg/kg bw per day).

The Meeting concluded that fenpyrazamine is not immunotoxic.

Toxicological data on metabolites and/or degradates

The acute oral LD_{50} of S-2188-DC, a plant and rat metabolite, was greater than 500 mg/kg bw. It was negative in the Ames test. As S-2188-DC was a major rat metabolite, the toxicity of the metabolite would be covered by that of the parent.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted. No information on accidental or intentional poisoning in humans was available.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.3 mg/kg bw on the basis of the overall NOAEL of 25 mg/kg bw per day from the dog studies for body weight loss at 50 mg/kg bw per day. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 0.8 mg/kg bw on the basis of the NOAEL of 80 mg/kg bw in the acute neurotoxicity study for reduced locomotor activity at 400 mg/kg bw. A safety factor of 100 was applied.

The reference doses also cover the metabolite, S-2188-DC.

A toxicological monograph was prepared.

Levels relevant to risk assessment of fenpyrazamine

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	1 500 ppm, equal to 176 mg/kg bw per day	3 000 ppm, equal to 349 mg/kg bw per day
		Carcinogenicity	100 ppm, equal to 11 mg/kg bw per day	1 500 ppm, equal to 176 mg/kg bw per day[b]
	Immunotoxicity[a]	Immunotoxicity	4 000 ppm, equal to 392 mg/kg bw per day[c]	–
Rat	Acute neurotoxicity study[d]	Toxicity	80 mg/kg bw	400 mg/kg bw
		Neurotoxicity	400 mg/kg bw[e]	–
	Subchronic neurotoxicity study[a]	Toxicity	1 200 ppm, equal to 88 mg/kg bw per day	3 000 ppm, equal to 224 mg/kg bw per day
		Neurotoxicity	3 000 ppm, equal to 224 mg/kg bw per day[c]	–
	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	1 200 ppm, equal to 52 mg/kg bw per day	2 400 ppm, equal to 107 mg/kg bw per day[b]

Species	Study	Effect	NOAEL	LOAEL
		Carcinogenicity	1 200 ppm, equal to 52 mg/kg bw per day	2 400 ppm, equal to 107 mg/kg bw per day[b]
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1 000 ppm, equal to 69 mg/kg bw per day[c]	3 000 ppm, equal to 237 mg/kg bw per day
		Parental toxicity	400 ppm, equal to 27 mg/kg bw per day	1 000 ppm, equal to 80 mg/kg bw per day
		Offspring toxicity	400 ppm, equal to 32 mg/kg bw per day	1 000 ppm, equal to 80 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	30 mg/kg bw per day	125 mg/kg bw per day
		Embryo/fetal toxicity	125 mg/kg bw per day	500 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	30 mg/kg bw per day	50 mg/kg bw per day
		Embryo/fetal toxicity	30 mg/kg bw per day	50 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[e,f]	Toxicity	25 mg/kg bw per day	50 mg/kg bw per day

[a] Dietary administration.

[b] Not applicable to human toxicity.

[c] Highest dose tested.

[d] Gavage administration.

[e] Two or more studies combined.

[f] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–0.3 mg/kg bw

Estimate of acute reference dose (ARfD)

0.8 mg/kg bw

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 fenpyrazamine

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and extensive with some saturation of excretion at high doses
Dermal absorption	No data
Distribution	Widely distributed; higher concentrations in plasma, liver, kidneys and stomach

Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Primarily via the urine; about 90% of radiolabel eliminated within 24/48 h in low and high doses, respectively
Metabolism in animals	Extensively metabolized
Toxicologically significant compounds in animals and plants	Parent, S-2188-DC
Acute toxicity	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kw bw
Rat, LC_{50}, inhalation	> 4.84 mg/kg bw
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Non-irritating
Guinea-pig, dermal sensitization	Non-sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Body weight loss
Lowest relevant oral NOAEL	25 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight
Lowest relevant NOAEL	52 mg/kg bw per day (rat)
Carcinogenicity	Evidence suggestive of carcinogenicity in male mice and rats; however, not relevant to humans[a]
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Target/critical effect	Decreased birth weight (rat).
Lowest relevant parental NOAEL	27 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	80 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	32 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Abortion (rabbit)
Lowest relevant maternal NOAEL	30 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	30 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	80 mg/kg bw (rat)
Subchronic neurotoxicity NOAEL	88 mg/kg bw per day(rat)
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	123 mg/kg bw per day (mouse)

Studies on toxicologically relevant metabolites	
S-2188-DC	LD_{50} > 500 mg/kg bw No evidence of genotoxicity
Human data	
	No evidence of adverse effects from reports on health status of factory workers

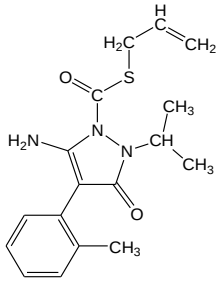
[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.3 mg/kg bw	Dog toxicity studies	100
ARfD	0.8 mg/kg bw	Acute neurotoxicity	100

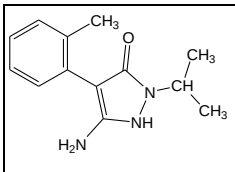
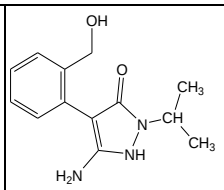
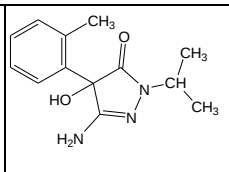
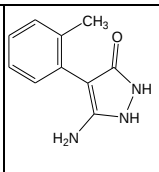
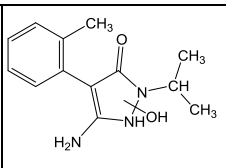
RESIDUE AND ANALYTICAL ASPECTS

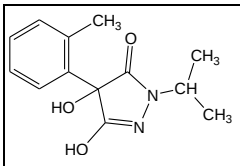
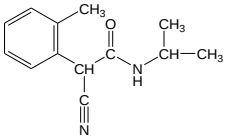
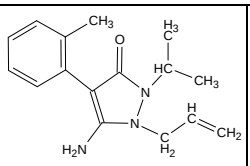
Fenpyrazamine, *S*-allyl 5-amino-2,3-dihydro-2-isopropyl-3-oxo-4-(o-tolyl)pyrazole-1-carbothioate (IUPAC name), is a member of phenylpyrazole fungicide. It can be used for the control of *Botrytis cinerea* (Grey mould) and *Monilia* species (fruit rot and brown rot). Its mode of fungicidal action is still unclear but it is thought to inhibit germ tube and mycelium elongation. It is not categorized as systemic but some translocation is observed in plants.



Fenpyrazamine was listed on the Codex Priority List by the 47th Session of CCPR in 2015 for toxicological and residue evaluation by the current Meeting as a new compound.

The following abbreviated names were used for the metabolites referred to in this Appraisal.

				
S-2188-DC	S-2188-OH	S-2188-CH_2OH-DC	MPPZ	OH-S-2188-DC

				
S-2188-$(OH)_2$	MCNI	S-2188-DTC		

Plant metabolism

The Meeting received information on the fate of fenpyrazamine in grapevine, lettuce and rape seed. For the studies, fenpyrazamine labelled with ^{14}C at phenyl ring ([U-phenyl-^{14}C]-fenpyrazamine) or at position 5 of the pyrazole ring ([pyrazolyl-5-^{14}C]-fenpyrazamine) were used. In metabolism studies, total radioactive residues (TRR) are expressed in mg fenpyrazamine equivalents/kg unless otherwise stated.

When ^{14}C-fenpyrazamine was applied twice as a spray to grapevine in a container and grown in a greenhouse at the ripening stage with an interval of 14 days at a rate of 0.75 kg ai/ha, the TRR in the mature fruit and foliage collected 14 days after the last treatment (DALA) were 22 and 246 mg eq/kg respectively for phenyl-labelled fenpyrazamine and 16 and 104 mg eq/kg, respectively, for pyrazolyl-labelled fenpyrazamine. The TRR in the mature fruit and foliage collected 21 DALA were 44 and 320 mg eq/kg, respectively for phenyl-labelled fenpyrazamine, and 26 and 230 mg eq/kg, respectively for pyrazolyl-labelled fenpyrazamine.

The distribution of radioactivity in fruits and foliage was similar between the two fenpyrazamine radiolabels. Most of the TRR (89–96% TRR), regardless of the DALA or label position, was recovered in the acetonitrile surface wash fractions of fruit and foliage. After washing with acetonitrile and extraction with acetonitrile/water (4:1, v/v), 0.8–1.4% TRR (0.21–0.50 mg eq/kg) and 1.5–2.2% TRR (2.2–5.1 mg eq/kg) remained unextracted, respectively from fruit and foliage.

In the extracted radioactivity, regardless of the DALA, the majority was the parent fenpyrazamine: 88–95% TRR (14–42 mg/kg) in fruit; and 81–92% TRR (96–260 mg/kg) in foliage. Metabolite S-2188-DC was found at 1.0–4.9% TRR (0.22–1.2 mg/kg) in fruit; and 2.7–8.0% TRR (2.8–26 mg/kg) in foliage. No other individual metabolites exceeded 10% TRR and 0.01 mg eq/kg.

When ^{14}C-fenpyrazamine was applied as a spray to lettuce in a container (grown in a greenhouse) at a rate of 0.85 kg ai/ha, the TRRs in lettuce collected at maturity (14 days after treatment) were 12 mg eq/kg for phenyl-labelled fenpyrazamine and 11 mg eq/kg for pyrazolyl-labelled fenpyrazamine.

For the both fenpyrazamine radiolabels, acetonitrile surface wash removed 84–88% TRR. After washing the surface with acetonitrile and subsequent extraction with acetonitrile/water (4:1), 1.6–2.4% TRR (0.18–0.29 mg eq/kg) remained unextracted.

In the surface wash and the extract, most radioactivity was attributed to the parent fenpyrazamine: 81–82% TRR (9.1–10 mg eq/kg). S-2188-DC accounted for 8.7–11% TRR (1.1–1.2 mg eq/kg). No other individual metabolites exceeded 10% TRR and 0.01 mg eq/kg.

Two spray applications of ^{14}C-fenpyrazamine at a rate of 0.60 kg ai/ha were applied to rapeseed plants in containers and grown in a greenhouse (BBCH 50 and 69) . The TRRs in the seed and stalk collected at maturity (45 DALA) were 0.023–0.046 mg eq/kg, and 2.5–2.9 mg eq/kg respectively. Immature forage collected 46 days after the first application contained a TRR of 1.3–2.0 mg eq/kg.

Acetonitrile surface wash of mature stalk and immature forage removed 88–91% TRR and 74–79% TRR. For these plant parts, in addition to the extraction with acetone/water, sequential extraction with water, 0.1M HCl and then 0.1M NaOH was attempted. Surface wash and these

extractions recovered a total of 97–98% and 94–95% TRR, respectively from mature stalk and immature forage.

However, the surface wash recovered only 20–38% TRR from mature seed. After subsequent extractions, 31–38% TRR still remained unextracted although the concentrations were low at 0.007–0.018 mg eq/kg. The unextracted radioactivity was treated with lauryl sulphate at 50°C for 16 hours and then sulphuric acid reflux to separate protein and starch and the remainder was classified as lignin. They accounted for 6.8–10%, 3.6–5.6%, and 17–26% TRR in seed, respectively.

In the extracts of mature stalk and immature forage, the predominant identified radioactive component was, as in other plants tested, the parent fenpyrazamine. It accounted for 50–60% TRR (1.4–1.5 mg eq/kg) in the stalk and 61–67% TRR (0.88–1.2 mg eq/kg) in the forage. In these plant parts, S-2188-DC was found at 9.3–11% TRR (0.27 mg eq/kg) in the stalk and 7.8–9.3% TRR (0.10–0.19 mg eq/kg) in the forage.

However, in the seed, fenpyrazamine accounted for only 16–22% TRR (0.005–0.007 mg eq/kg) but still was the most abundant radioactive component. Metabolite S-2188-DC was found at around 1.9–3.7% TRR (0.001 mg eq/kg). No other individual radioactive components exceeded 10% TRR and 0.01 mg eq/kg.

In all of the metabolism studies, another metabolite S-2188-OH was detected at <5% TRR.

The metabolism of fenpyrazamine was studied on grape, lettuce and rapeseed with ^{14}C in the phenyl ring or on position 5 of pyrazolyl ring. The metabolic profiles were qualitatively similar among these crops with the two radiolabelled fenpyrazamine with only fenpyrazamine, S-2188-DC and S-2188-OH as identified radioactive components. The metabolism of fenpyrazamine proceeds with cleavage of the carbamate linkage on the pyrazolyl ring producing S-2188-DC. Subsequent hydroxylation at position 4 of pyrazolyl ring forms S-2188-OH. These metabolites also occur in rat metabolism.

These plant metabolism studies were conducted in a greenhouse condition while fenpyrazamine is known to be susceptible to photolysis in sterile water at pH 7 (25 ºC) and degrades to S-2188-DC (maximum of 64% on day 7) and MCNI (maximum of 18% at the end of the test period of 30 days). In the surface wash of the plants studied, S-2188-DC was detected as a minor radioactive component and MCNI was not detected, indicating that photolysis on the surface of plants may not be significant. However, the potential for higher levels of S-2188-DC or MCNI could not be excluded had the metabolism studies been conducted outdoors.

Residues in Succeeding or Rotational Crops

The Meeting received information on confined and field rotational crop studies.

Confined rotational crop study

A confined rotational crop study was conducted using wheat, lettuce and carrot. [Pyrazolyl-5-^{14}C]-fenpyrazamine was applied to bare sandy loam soil (pH 6.9) at an actual rate of 2.83 kg ai/ha.

In general, radioactive residue levels declined over the plant back interval (PBI) and were the lowest in the 365-day PBI. For example, at the 365-day PBI, TRR values were at or below 0.06 mg eq/kg in wheat grain, immature and mature lettuce, and mature carrot root, compared to 0.11–0.85 mg eq/kg at the 30-day PBI.

A wider variety of radioactive metabolites were identified in mature parts of rotated crops than in plant metabolism studies, but at very low concentrations and contribution to TRR, mostly around or below 10% of TRR, except fenpyrazamine in mature and immature carrot samples at the 30-day PBI and the 120-day PBI. Fenpyrazamine was not detected at the 365-day PBI, except in immature carrot root (6.1% TRR and 0.02 mg eq/kg).

S-2188-DC and S-2188-$(OH)_2$ were found in succeeding crops at low levels: at or below 10% TRR and less than 0.06 mg eq/kg. Hydrolytic treatments of unextracted radioactivity in the three

crops indicated that radioactive residues may be further degraded to polar fractions and became incorporated or associated with starch, protein, lignin, cellulose and other natural constituents.

The rotational crops were found to contain fenpyrazamine and metabolites S-2188-OH and S-2188-$(OH)_2$. Metabolite S-2188-DC was found only in wheat forage and hayat the 30-day PBI. S-2188-OH may be taken up from the soil or produced in the plants from S-2188-DC. S-2188-$(OH)_2$ was likely produced by deamination of S-2188-OH.

Field rotational crop study

A field rotational crop study was conducted using carrot, lettuce, tomato and barley as succeeding crops. The preceding crop was tomato grown outdoor which was treated three times with a fenpyrazamine WG formulation at a rate of 0.6 kg ai/ha (total rate of 1.8 kg ai/ha, equivalent to the maximum seasonal GAP rate in Austria and many other countries in the EU). After harvesting the tomato crop 3 days after the last application, the remaining plant parts were incorporated into the soil. Succeeding crops were sown or transplanted into the plots 1 month, 4 months (8 months for tomato), or 12 months after the last application to the preceding tomato crop, and harvested at their commercial maturity.

Residues of fenpyrazamine in the preceding tomato crop were 0.06–0.20 mg eq/kg. No residues of fenpyrazamine or S-2188-OH above the LOQ of 0.01 mg/kg were found in any of the succeeding crop samples planted with 1, 4 (or 8) and 12 months after the last application to the preceding tomato crop.

Animal metabolism

The Meeting received information on the results of studies on a lactating goat and laying hens which were fed ^{14}C-labelled fenpyrazamine.

Rat

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

Lactating goat

[Pyrazolyl-5-^{14}C]-fenpyrazamine was orally dosed once daily in gelatin capsules to lactating goat at an average daily dose of 7.2 ppm in the diet, equivalent to 0.36 mg/kg body weight, for five consecutive days.

The great majority (84%) of the administered dose was excreted in the urine (58%), feces (24%) and cage wash (2.4%). An additional 7.0% of the administered dose was recovered in the gastrointestinal tract and contents. Bile contained 0.17% of the administered dose at 4.4 mg eq/kg.

The total radioactivity in muscle, liver, kidney, heart and fat, taken 8 hours after the final dose, accounted for about 1.0% of the administered dose but all below 0.3 mg eq/kg. The total radioactivity excreted in milk was 0.15% of the administered dose. The TRR of daily collected milk samples (day 1–4) were 0.01–0.02 mg eq/kg reaching a plateau on day 2 and remained at 0.017–0.019 mg eq/kg.

For milk, acetonitrile and then acetonitrile/water (4:1) extracted 96% TRR. Sequential extraction with acetonitrile, acetonitrile/water (4:1) and then acetonitrile/water (1:1) extracted 91–100% of TRR in muscle, heart, kidney and fat with up to 8.6% TRR (0.004 mg eq/kg) unextracted. However, from liver, only 63% TRR was extracted with 37% TRR unextracted, from which 28% TRR was released as protein.

Unchanged fenpyrazamine was detected only in the liver (14% TRR) and fat (18% TRR). S-2188-DC was found at 8.6% TRR in day 4 milk; 25% TRR in muscle; 17% TRR for free and conjugated in liver; 41% TRR for free and released in kidney; and 26% TRR in fat. S-2188-CH_2OH-DC was found at 29% TRR in muscle; 20% TRR for free and conjugated in liver; and 26% TRR for

free and conjugated in kidney. S-2188-DC and S-2188-CH_2OH-DC were the major metabolites found in goat samples.

Laying hens

[Pyrazolyl-5-^{14}C]-fenpyrazamine was orally administered in gelatin capsules to laying hens once daily at an average daily dose of 9.4 ppm in the feed, equivalent to 0.70 mg/kg bw, for 7 consecutive days.

Approximately 95% of the administered dose was recovered from excreta (89%), cage wash (2.3%), and GI tract and its contents (3.3%).

The sum of TRR in muscle, fat and liver, taken 8–9 hours after the final dose, accounted for 0.10% of the administered dose. The highest residue concentration was found in the liver at 0.18 mg eq/kg. Residues in muscle and fat amounted to 0.02 mg eq/kg. The TRR in eggs (white and yolk) accounted for 0.06% of the administered dose.

Radioactive residues in egg yolk increased from 0.003 mg eq/kg on day 2 to 0.047 mg eq/kg on day 6. Radioactive residues in egg white reached a plateau after 2 days and remained constant at around 0.017–0.022 mg eq/kg.

Sequential extraction by acetonitrile, acetonitrile/water (4:1) and then acetonitrile/water (1:1) released 96% TRR in egg white, 84% TRR in muscle, and 66% TRR in liver. Acetonitrile and acetonitrile/water (4:1) extracted a total of 70% TRR in egg yolk. Hexane extracted a total of 84% TRR in fat.

Unchanged fenpyrazamine was detected in the acetonitrile and acetonitrile/water extracts of egg yolk (2.6% TRR), egg white (3.5% TRR) and liver (2.1% TRR), and was a major residue in the acetonitrile fraction of hexane extract of fat (43% TRR). Fenpyrazamine was not detected in the acetonitrile and acetonitrile/water extracts of muscle.

S-2188-DC was detected in the acetonitrile and acetonitrile/water extracts of egg yolk (6.6% TRR), egg white (25% TRR), muscle (4.5% TRR) and liver (3.1% TRR after sulfatase treatment) but was not detected in the acetonitrile fraction of the hexane extract of fat.

The concentrations of unchanged fenpyrazamine and S-2188-DC were lower than 0.01 mg eq/kg.

MPPZ was identified from the extracts of egg yolk, egg white, muscle and liver through two-dimensional TLC co-chromatography. It was not quantified individually. It was estimated to be present at low levels as the fraction containing MPPZ accounted for 0.003–0.031 mg eq/kg, but may be predominant in egg yolk and white (16–34% TRR).

In the goat, the major radioactive residue was S-2188-DC followed by S-2188-CH_2OH-DC and fenpyrazamine. In the laying hen, the radioactive metabolites identified were S-2188-DC, fenpyrazamine and MPPZ. These compounds occur in the rat metabolism.

The metabolism of fenpyrazamine in animals proceeds via cleavage of the carbothioate side chain of the pyrazolyl ring to produce S-2188-DC. In the goat, S-2188-DC was hydroxylated to S-2188-CH_2OH-DC or S-2188-OH. In the laying hen, S-2188-DC was hydroxylated to produce OH-S-2188-DC or dealkylated to form MPPZ. These compounds are further metabolized by conjugation and incorporated into natural components.

Environmental fate

The Meeting received information on hydrolysis of fenpyrazamine in sterilized buffers.

Hydrolysis

Fenpyrazamine was stable in buffers at pH 4 and pH 7.

At pH 9 at 25 °C, after incubation for 17 days, an average of 33% of fenpyrazamine remained with S-2188-DC and S-2188-OH formed at amounts equivalent to 54% and 4.9% of the applied fenpyrazamine. The DT_{50} at pH 9 at 20 °C was calculated to be 24 days.

Photolysis in sterile buffer

The photolytic fate of radiolabelled fenpyrazamine was investigated in sterilized buffer at pH 7 at 25 ± 1 °C over a period of 30 days.

Fenpyrazamine degraded to 1 or 2% of the applied radioactivity after 30 days. S-2188-DC increased and reached a peak concentration (62–64% of the applied radioactivity) after 7 days then decreased to 7 to 10% of applied radioactivity after 30 days. MCNI gradually increased from day 5 to 16–18% of the applied radioactivity after 30 days. There were up to seven to ten other compounds, including S-2188-DTC (propene side chain at position 1 of pyrazolyl ring) and one characterized as dioxygenated S-2188-DC at less than 10% of the applied radioactivity. These photolytic degradates were not found in the dark samples.

DT_{50} values were calculated to be 1.6–1.7 days showing susceptibility of fenpyrazamine to photolysis.

Photolysis on soil

Photodegradation of fenpyrazamine was studied on soil. There was no significant difference in the degradation of fenpyrazamine between the irradiated sample and the control (dark) sample.

Methanol/water (5:1) extracted 96–97% of the applied radioactivity (AR) on day 0, gradually declining to 67–73% AR after 30 days. In the methanol/water (5:1) extracts of soil, fenpyrazamine decreased from 96% AR at the beginning to 67–70% after 30 days. S-2188-OH increased after 2 or 7 days through 30 days (<1% AR). S-2188-DC was detected only in the test with phenyl-labelled fenpyrazamine after 21 days (<1% AR). There were 3 unknown compounds but none exceeded 0.7% of the applied radioactivity. MCNI was not detected with either of the fenpyrazamine radiolabels.

DT_{50} values were calculated to be 74–80 days at 20 °C indicating that fenpyrazamine is moderately stable on the surface of soil.

Methods of analysis

Analytical methods for determination of residues of fenpyrazamine, S-2188-DC and/or S-2188-OH were developed for a wide range of matrices of plant origin. Descriptions and validation results of the analytical methods were provided to the Meeting to cover plants on which supervised trials were conducted.

In general, the methods employ extraction by homogenization with a mixture of acetone and water (4:1, v/v) with or without the presence of sodium ascorbate, clean-up with an SPE cartridge, and determination of analytes using LC-MS/MS. The analytical methods do not involve acid hydrolysis.

A number of methods for plant matrices were found suitable for analysis of fenpyrazamine, S-2188-DC with LOQ ranging from 0.01–0.02 mg/kg for these analytes. The mean recoveries and RSD values were within the acceptable range of 70–120% and below 20%, respectively. One method was found suitable for analysis of S-2188-OH with a LOQ of 0.01 mg/kg, mean recoveries 78–108% and RSD values below 20%.

One multi-residue method (LC-MS/MS) was found suitable for analysis of fenpyrazamine with a LOQ of 0.01 mg/kg for grapes, peppers, carrot, cereals (grain and straw) and rapeseed.

One analytical method was found suitable for determination of fenpyrazamine in matrices of animal origin (milk, eggs, tissues). The method employs extraction by homogenization with a mixture of acetonitrile and water (10:1 for milk, and 10:6 for eggs and tissues), addition of salts, clean-up with an SPE cartridge, and determination of fenpyrazamine using LC-MS/MS. The LOQ was 0.005 mg/kg in milk and 0.01 mg/kg in eggs and edible tissues. The mean recoveries and RSD values were within

the acceptable range of 70–120% and below 20%, respectively. The method was not validated for S-2188-DC in animal commodities.

Stability of residues in stored analytical samples

The stability of fenpyrazamine, S-2188-DC and S-2188-OH during frozen storage at -20 to -18 ºC was investigated in a range of plant matrices for which supervised residue trials were submitted. Each tested compound was spiked in matrices at 0.1 or 0.2 mg/kg.

All of the three compounds tested were found to be stable (>70% remaining) in the matrices tested, except strawberry, for up to about one year in the homogenized samples.

In strawberry, after 337 days of frozen storage, fenpyrazamine and S-2188-DC were below 70% of the initial concentrations except for S-2188-DC with 1M ascorbate. Fenpyrazamine with or without 1M ascorbate was stable in strawberry up to 114 days, and S-2188-DC was stable up to 114 days without 1M ascorbate.

No storage stability data were available for animal commodities.

Definition of the residue

In the plant metabolism studies conducted indoors on grapevine, lettuce and rapeseed, fenpyrazamine, S-2188-DC and S-2188-OH were identified. The predominant or most abundant residue was parent fenpyrazamine (around 90% TRR in grape berries and foliage; around 80% in lettuce, around 60% in rapeseed stalk and forage, and around 20% in the seed of rape). S-2188-DC was in some cases present at slightly higher than 10% TRR but in concentrations higher than 0.01 mg eq/kg. S-2188-OH was found at lower ratios of TRR than S-2188-DC.

Fenpyrazamine is known to be susceptible to photolysis to produce S-2188-DC and MCNI. In irradiated sterile buffer at pH 7, MCNI gradually increased from day 5. However, 20 days after incubation (comparable to the longest PHI) it was 1/5 to 1/4 of S-2188-DC. MCNI was not detected in the surface wash in the plant metabolism studies conducted in the greenhouse or on the surface of irradiated soil.

The confined and field rotational crop studies indicate that it was unlikely to find fenpyrazamine or its degradates in succeeding crops.

Suitable analytical methods are available for plant commodities to analyse these three compounds.

The Meeting considered that fenpyrazamine, which is the predominant residue, was a suitable marker for enforcement of MRLs.

In most supervised trials, S-2188-DC was detected at comparable levels or lower than fenpyrazamine at longer DALA. However, in the outdoor trials on lettuce, S-2188-DC was detected at a maximum of 3 times the concentration of fenpyrazamine.

S-2188-DC is the initial metabolite in rats and therefore covered by the toxicological guidance values.

The Meeting considered that for dietary risk assessment, both fenpyrazamine and S-2188-DC were suitable markers.

In animal metabolism, orally administered parent fenpyrazamine was excreted efficiently and rapidly. Fenpyrazamine was extensively metabolized into various compounds including incorporation into natural components. In the goat, the major radioactive residue was S-2188-DC followed by S-2188-CH_2OH-DC and fenpyrazamine. In the goat, MPPZ was not detected. In the laying hen, the radioactive metabolites identified were S-2188-DC, fenpyrazamine and MPPZ. However, these compounds were present at low concentrations to a maximum of 0.04 mg eq/kg in goat liver (S-2188-DC). S-2188-DC (including its conjugates), S-2188-CH_2OH-DC, MPPZ (including its conjugates) and S-2188-OH were found in rats.

The animal dietary burden was calculated to be zero for poultry, and therefore it is not likely that residues would be detected in foods of poultry origin above the LOQ.

The Meeting considered that fenpyrazamine and S-2188-DC were suitable markers for enforcement of MRLs and for dietary risk assessment for animal commodities.

The $logP_{ow}$ of 3.5 indicates some fat solubility. The ratio of residue concentrations in egg yolk to those in egg white was around 2 to 1. The residues in muscle and those in fat were not different. Therefore the Meeting considered the fenpyrazamine residue not fat-soluble.

One analytical method was found suitable for determination of fenpyrazamine in animal commodities but this method was not validated for S-2188-DC.

Based on the above, the Meeting recommended the following residue definitions.

Definition of the residue (for compliance with MRLs) for plant commodities: *Fenpyrazamine*.

Definition of the residue (for dietary risk assessment) for plant commodities: *Sum of fenpyrazamine and 5-amino-1,2-dihydro-2-isopropyl-4-(o-tolyl)pyrazol-3-one (S-2188-DC), expressed as fenpyrazamine.*

Definition of the residue (for compliance with MRLs and for dietary risk assessment): *Sum of fenpyrazamine and 5-amino-1,2-dihydro-2-isopropyl-4-(o-tolyl)pyrazol-3-one (S-2188-DC), expressed as fenpyrazamine.*

The residue is not fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for fenpyrazamine on cherry, plum, apricot, peach, blackberry, raspberry, blueberry, grapes, strawberry, cucumber, peppers, tomato, lettuce, ginseng and almond.

The sum of residues of fenpyrazamine and S-2188-DC was calculated by conversion of the S-2188-DC residues into fenpyrazamine equivalents using the molecular weight ratio [331.4/231.3 = 1.433].

Stone fruits

Cherries

A total of 12 trials were conducted in Europe in 2011 and 2012. Fenpyrazamine was applied three times at a rate of 600 g ai/ha as a foliar spray with an interval of 7 days. The critical GAP in Austria for apricot, cherry, nectarine, peach and plum allows three applications at a rate of 600 g ai/ha with an interval of 7 days. PHI is 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=12): 0.33, 0.34, 0.36, 0.41, 0.54, 0.60, 0.61, 0.82, 1.0, 1.0, 1.8, and 1.9 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.45, 0.47, 0.48, 0.59, 0.61, 0.70, 0.78, 1.0, 1.2, 1.4, 1.9, 2.2 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg, STMR of 0.74 mg/kg and HR of 2.2 mg/kg for the subgroup of cherries.

Plums

A total of 16 trials were conducted in Europe in 2011 and 2012. Fenpyrazamine was applied three times at a rate of 600 g ai/ha as a foliar spray with an interval of 7 days. The critical GAP in Austria for apricot, cherry, nectarine, peach and plum allows three applications at a rate of 600 g ai/ha with an interval of 7 days. PHI is 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=16): 0.12, 0.18, 0.19, 0.23, 0.23, 0.30, 0.33, 0.36, 0.40, 0.40, 0.56, 0.67, 0.70, 0.84, 0.87, and 1.5 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.15, 0.21, 0.22, 0.26, 0.37, 0.43, 0.44, 0.44, 0.47, 0.49, 0.67, 0.77, 0.83, 0.93, 1.1 and 1.7 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg, STMR of 0.455 mg/kg and HR of 1.7 mg/kg for the subgroup of plums.

Apricots

A total of six trials were conducted in Europe in 2012. Fenpyrazamine was applied three times at a rate of 600 g ai/ha as a foliar spray with an interval of 7 days. The critical GAP in Austria for apricot, cherry, nectarine, peach and plum allows three applications at a rate of 600 g ai/ha with an interval of 7 days. PHI is 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=6): 0.43, 0.52, 0.89, 1.1, 1.6, and 3.0 mg/kg.

Peaches

A total of 12 trials were conducted in Europe in 2010 and 2011. Fenpyrazamine was applied three times at a rate of 600 g ai/ha as a foliar spray with an interval of 7 days. The critical GAP in Austria for apricot, cherry, nectarine, peach and plum allows three applications at a rate of 600 g ai/ha with an interval of 7 days. PHI is 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=12): 0.36, 0.44, 0.55, 0.61, 0.70, 0.76, 0.85, 0.94, 0.95, 1.1, 1.5, and 2.5 mg/kg.

Since the Codex subgroup of peaches includes both apricot and peach, and the critical GAP in Austria as well as GAP in Spain covers both apricot and peach, the Mann-Whitney U test was conducted on residue populations of apricot and peach trials. The populations were not significantly different. Therefore, the Meeting used the combined residues from apricot and peach trials. The combined residues were (n=18): 0.36, 0.43, 0.44, 0.52, 0.55, 0.61, 0.70, 0.76, 0.85, 0.89, 0.94, 0.95, 1.1, 1.1, 1.5, 1.6, 2.5, and 3.0 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.58, 0.61, 0.73, 0.77, 0.83, 0.88, 0.94, 0.95, 1.1, 1.1, 1.2, 1.3, 1.3, 1.6, 1.6, 1.9, 2.5, 3.8 mg/kg

The Meeting estimated a maximum residue level of 4 mg/kg, STMR of 1.1 mg/kg and HR of 3.8 mg/kg for the subgroup of peaches (including nectarines and apricots).

Berries and other small fruits

Cane berries

Four independent supervised residue trials were conducted on cane berries (two on blackberries and two on raspberries) in the USA in 2009. In each trial, fenpyrazamine was applied three times as a foliar spray at a rate of 560 g ai/ha with an interval of 7 days. The critical GAP for cane berry in the USA allows three applications at 556 g ai/ha with an interval of 7 days and. a PHI of 0 days. Residue data from blackberry and raspberry trials were combined for mutual support.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=4): 0.53, 1.6, 1.9 and 2.8 mg/kg.

Sum of residues in these trials were: 0.61, 1.9, 2.2 and 3.2 (mean of 3.29 and 3.16) mg/kg.

The Meeting estimated a maximum residue level of 5 mg/kg, STMR of 2.05 mg/kg and HR of 3.3 mg/kg, based on the highest individual sample concentration, for the subgroup of cane berries.

Blueberry (Bush berry)

Eight trials were conducted on blueberry in the USA in 2009. In each trial fenpyrazamine was applied three times as a foliar spray at a rate of 560 g ai/ha with an interval of seven days. The critical GAP for bush berry in the USA allows three applications at a rate of 560 g ai/ha with an interval of 7 days and a PHI of 0 days.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=8): 0.15, 0.35, 0.38, 0.74, 0.92, 1.0, 1.8, and 2.3 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.22, 0.49, 0.51, 0.85, 1.1, 1.3, 2.0 and 2.8 (mean of 2.91 and 2.67) mg/kg.

The Meeting estimated a maximum residue level of 4 mg/kg, STMR of 0.985 mg/kg and HR of 2.9 mg/kg, based on the highest individual sample concentration, for bush berries.

Grapes – Trials in Europe

Seventeen trials were conducted on grapevine in Europe (Austria, Germany, Italy, Spain) in 2006–2008. In each trial fenpyrazamine was applied once as a foliar spray at a rate of 600 g ai/ha.

Critical GAP in France allows one application at 600 g ai/ha with PHI of 7 days for table grapes and 14 days for wine grapes. Although all the trials were conducted on wine grapes, a PHI of 7 days was used for evaluation to cover table grapes.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=16): 0.06, 0.14, 0.15, 0.22, 0.23, 0.25, 0.37, 0.37, 0.45, 0.54, 0.62, 0.74, 0.77, 1.0, 1.2, and 1.2 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.07, 0.16, 0.17, 0.26, 0.28, 0.35, 0.44, 0.44, 0.54, 0.63, 0.73, 0.85, 0.92, 1.1, 1.6 and 1.8 mg/kg.

Grape – Trials in North America

Fourteen trials were conducted on grapevine in North America (13 × U.S.A., 1 × Canada) in 2006–2008. In each trial fenpyrazamine was applied three times as a foliar spray at a rate of 560 g ai/ha with a PHI of 3 days for table grapes and 14 days for wine grapes. The trials were conducted on wine grapes and table grapes and evaluated against the PHI for table grapes.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=14): 0.33, 0.53, 0.55, 0.71, 0.80, 0.88, 0.91, 0.93, 1.0, 1.1, 1.1, 1.1, 1.2, and 2.1 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.37, 0.62, 0.66, 0.95, 1.0, 1.1, 1.2, 1.3, 1.3, 1.3, 1.4, 1.4, 1.6 and 3.2 (mean of 2.90 and 3.42) mg/kg.

The Meeting decided to use the North American dataset which would lead to a higher maximum residue level.

The Meeting estimated a maximum residue level of 4 mg/kg, STMR of 1.25 mg/kg and HR of 3.4 mg/kg, based on the highest individual sample concentration, for grapes.

Strawberry - Trials in Europe

Eight trials were conducted on indoor strawberries and another eight trials were conducted on outdoor strawberries in Europe (France, Germany, Greece, Hungary, Italy, Poland and Spain) in 2010/11. In each trial fenpyrazamine was applied three times as a foliar spray at a rate of 600 g ai/ha.

Critical GAP in France for strawberry is applicable to both indoor and outdoor and allows three applications at 600 g ai/ha with an interval of 7 days with a PHI of 1 day.

Fenpyrazamine residues from indoor trials matching the above GAP were in rank order (n=7): 0.24, 0.28, 0.35, 0.45, 0.86, 0.92 and 1.4 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.28, 0.36, 0.47, 0.54, 1.0, 1.2 and 2.0 mg/kg

Fenpyrazamine residues from outdoor trials matching the above GAP were in rank order (n=7): 0.28, 0.30, 0.54, 0.64, 0.65, 1.3, 1.4 mg/kg.

Sum of residues in these trials were: 0.39, 0.41, 0.81, 0.84, 0.84, 1.4 and 1.7 mg/kg.

Strawberry - Trials in North America

Eight trials were conducted on outdoor strawberries in North America (USA, Canada) in 2006–2009. In each trial, fenpyrazamine was applied four times as a foliar spray at a rate of 560 g ai/ha.

Critical GAP in the USA allows four applications at a rate of 560 g ai/ha with an interval of 7–14 days and a PHI of 0 days.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=7): 0.39, 0.41, 0.54, 0.87, 0.88, 0.95, 1.3 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.50, 0.58, 0.59, 0.94, 0.99, 1.2 and 1.4 (mean of 1.49 and 1.37) mg/kg.

Noting that the three residue populations from indoor and outdoor trials in Europe and outdoor trials in the USA would lead to the same maximum residues level of 3 mg/kg, the Meeting used the highest mean value and highest residue as STMR and HR.

The Meeting estimated a maximum residue level of 3 mg/kg, STMR of 0.94 mg/kg and HR of 2.0 mg/kg for strawberry.

Fruiting vegetables, Cucurbits

Cucumber

Eight trials were conducted on indoor cucumber in Europe (Hungary, The Netherlands, Italy, Spain) in 2007 and 2008. In each trial, fenpyrazamine was applied three times as a foliar spray at a rate of 600 g ai/ha.

Critical GAP in France for Cucurbits edible peel (indoor) allows three applications with an interval of 10 days and a PHI of 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=8): 0.12, 0.14, 0.15, 0.16, 0.22, 0.25, 0.33, and 0.34 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.13, 0.16, 0.17, 0.23, 0.23, 0.26, 0.34 and 0.38 mg/kg.

The Meeting estimated a maximum residue level of 0.7 mg/kg, STMR of 0.23 mg/kg and HR of 0.38 mg/kg for cucumber.

Fruiting vegetables, other than Cucurbits

Tomato

Eight trials were conducted on indoor cherry tomato in Europe (Hungary, The Netherlands, Italy, Spain) in 2007 and 2008. In each trial, fenpyrazamine was applied three times as a foliar spray at a rate of 600 g ai/ha.

Critical GAP in France for tomato, pepper and eggplant (indoor) allows three applications with an interval of 10 days at a rate of 600 g ai/ha and a PHI of 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=8): 0.56, 0.65, 0.66, 0.71, 0.85, 1.4, 1.5, and 1.8 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.65, 0.67, 0.71, 0.74, 0.88, 1.4, 1.5 and 1.8 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg, STMR of 0.81 mg/kg and HR of 1.8 mg/kg for cherry tomato and tomato.

Peppers, sweet

Eight trials were conducted on indoor sweet pepper in Europe (Hungary, the Netherlands, Italy, Spain) in 2007 and 2008. In each trial, fenpyrazamine was applied three times as a foliar spray at a rate of 600 g ai/ha.

Critical GAP in France for tomato, pepper and eggplant (indoor) allows three applications with an interval of 10 days at a rate of 600 g ai/ha and a PHI of 1 day.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=8): 0.47, 0.58, 0.60, 0.69, 0.94, 1.2, 1.3, and 1.4 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.55, 0.59, 0.75, 0.80, 1.0, 1.4, 1.5 and 1.5 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg, STMR of 0.90 mg/kg and HR of 1.5 mg/kg for peppers, sweet.

Eggplant

As the GAP in France and in some other countries in the EU for tomato also covers eggplant, the Meeting agreed to extrapolate the maximum residue level, STMR and HR for tomato/cherry tomato to the subgroup of eggplants.

Leafy vegetables

Lettuce

Sixteen trials were conducted on lettuce in the USA (eight head lettuce and eight leaf lettuce) in 2009 and 2010. In each trial, fenpyrazamine was applied three times as a foliar spray at a nominal rate of 840 g ai/ha.

Critical GAP for both head and leaf lettuce in the USA allows three applications at a rate of 560 g ai/ha with an interval of 7–10 days and a PHI of 14 days.

As no trials exactly match the critical GAP with deviations in application rate, the Meeting decided to apply the proportionality concept to use the trial results. The decline studies indicate that at the time of the last application, residues from the previous application may not be negligible and therefore, the average application rate was used for scaling.

Based on residues from trials in the USA and the scaling factors (0.330–0.683), residues for estimating a maximum residue level were:

For head lettuce (n=8): < 0.01 (2), 0.013, 0.033, 0.070, 0.090, 0.22 and 0.60 mg/kg; and

For leaf lettuce (n=8): < 0.01 (2), 0.080, 0.094, 0.23, 0.29, 0.52 and 0.70 mg/kg.

The Mann–Whitney U test indicates that the data population from head lettuce trials and that from leaf lettuce trials were not significantly different. And therefore, the combined list was used to estimate a maximum residue level.

Combined residues are in rank order (n=16): < 0.01 (4), 0.013, 0.033, 0.070, 0.080, 0.090, 0.094, 0.22, 0.23, 0.29, 0.52, 0.60, and 0.70 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: < 0.020 (3), 0.020, 0.040, 0.090, 0.165, 0.19, 0.20, 0.32, 0.51, 0.76, 0.83, 0.88, 0.99 and 2.3 (highest individual residue from duplicate samples was 2.4) mg/kg

The Meeting estimated a maximum residue level of 1.5 mg/kg, STMR of 0.195 mg/kg and HR of 2.4 mg/kg, based on the highest individual sample concentration, for head lettuce and leaf lettuce.

Root and tuber vegetables

Ginseng

Three trials were conducted on ginseng in the USA in 2008. In each trial, fenpyrazamine was applied four times as a foliar spray at a rate of 560 g ai/ha.

Critical GAP for ginseng in the USA allows 4 applications at a rate of 560 g ai/ha with an interval of 7–14 days and a. PHI of 2 days.

Fenpyrazamine residues from trials matching the above GAP were in rank order (n=3): 0.15, 0.17 and 0.32 mg/kg.

Sum of residues expressed as fenpyrazamine in these trials were: 0.18, 0.20 and 0.35 (mean of 0.38 and 0.32) mg/kg.

The Meeting estimated a maximum residue level of 0.7 mg/kg, STMR of 0.20 mg/kg and HR of 0.38 mg/kg, based on the highest individual sample concentration, for ginseng.

Tree nuts

Almond

Five trials were conducted on almond in the USA in 2008. In each trial, fenpyrazamine was applied three times as a foliar spray at a rate of 447 g ai/ha.

Critical GAP for almond in the USA allows three applications at a rate of 420 g ai/ha with a PHI of 21 days.

Fenpyrazamine residues in almond nut meat from trials matching the above GAP were (n=3): < 0.01 mg/kg.

In two trials, 2× rate applications were made and fenpyrazamine residues in nutmeat were < 0.01 mg/kg, and corresponding sum of residues were < 0.02 mg/kg. Based on the residue levels from 2x applications and it is quite unlikely that nutmeat is exposed to fungicide sprayed 21 days before the harvest, the Meeting estimated a maximum residue level of 0.01 * mg/kg and STMR of 0.02 mg/kg for almond.

Animal feed items

Almond hulls

It is not possible to evaluate the data on almond hulls as the storage period of samples were not covered by the storage stability study.

Fate of residues during processing

High temperature hydrolysis

The hydrolysis of [pyrazolyl-5-^{14}C]-fenpyrazamine was studied in sterile buffered aqueous solution under conditions simulating pasteurization, baking/brewing/boiling, and sterilization.

Fenpyrazamine was stable under the conditions representing pasteurization (pH 4) and baking/brewing/boiling (pH 5) with only less than 1% of fenpyrazamine transformed to S-2188-DC. Fenpyrazamine was slightly less stable under the condition representing sterilization (pH 6), with about 89% of fenpyrazamine remaining after 20 minutes of incubation at 120°C and 8.6% of fenpyrazamine being transformed to S-2188-DC. There were no other significant hydrolysis products.

Processing

The Meeting received information on processing of grape to wines, juice and dried grape. Processing factors of grape products from four studies are summarized below.

Processed Commodity	Fenpyrazamine		Fenpyrazamine + S-2188-DC		STMR/ STMR-P
	Individual processing factor	Best estimate	Individual Processing Factors	Best estimate	
Grape					1.25
Dried grape	1.1, 1.4, 4.7, 4.8	3.1	1.2, 1.2, 4.1, 4.5	2.7	3.38
Juice	0.2, 0.3, 0.3, 0.4, 2.0	0.3	0.3, 0.4, 0.4, 0.6, 1.9	0.4	0.5
White wine (at bottling)	1.1, 1.5, 2.4	1.5	1.3, 1.5, 2.8	1.5	1.88
White wine (after 6 months storage)	1.0, 1.2, 2.5	1.2	1.2, 1.3, 2.9	1.3	
Red wine (at bottling)	0.3, 0.4, 0.4,	0.4	0.6, 0.8, 1.1	0.8	1.12
Red wine (after 6 months storage)	0.3, 0.4, 0.5	0.4	0.8, 0.9, 1.1	0.9	
Wet pomace	1.6, 1.9, 2.3, 5.8,	2.1	1.6, 1.6, 2.2, 5.0	1.9	-

Using the best estimates of processing factors and the STMR of 1.25 mg/kg for grapes, the STMR-P values were calculated for processed commodities of grapes.

As the residues concentrate in dried grape, the Meeting estimated a maximum residue level of 12 mg/kg for dried grape. HR-P was calculated to be 9.2 mg/kg.

The mean residue for wet pomace was calculated to be 2.4 mg/kg (as received) for animal dietary burden.

Residues in animal products

Estimation of dietary burdens

The maximum and mean dietary burdens were calculated using the mean residues for grape wet pomace 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	0	0	0	3.17 [a]	3.17 [b]	0	0
Dairy cattle	0	0	0	0	3.17 [c]	3.17 [d]	0	0
Broilers	0	0	0	0	0	0	0	0
Layers	0	0	0	0	0	0	0	0

[a] Suitable for estimating maximum residue levels for 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 level for milk.

[d] Suitable for estimating STMR for milk

Residues in milk and cattle tissues

No feeding study was conducted on cattle. An animal metabolism study on a goat was conducted at a dose equivalent to 7.2 ppm in the diet. An analytical method was available for fenpyrazamine in animal commodities.

The sum of fenpyrazamine and S-2188-DC in the extracts of acetonitrile and acetonitrile/water (4:1) in the tissue after adjustment for molecular weight is calculated for each tissue as follows:

Milk or tissue	Fenpyrazamine+S-2188-DC mg/kg from the 7.2 ppm fenpyrazamine in the diet	Note
Day 4 milk	< 0.01	Reached a plateau on day 2
Muscle	< 0.01	Free S-2188-DC
Liver	0.087	Free fenpyrazamine and free and conjugated S-2188-DC. Calculated as free S-2188-DC May lead to over-estimation
Kidney	0.043	Free S-2188-DC
Fat	< 0.01	

The calculated maximum and mean dietary burdens for beef and dairy cattle were 3.17 ppm in diet. Using the residue levels from the goat metabolism study and the LOQ for fenpyrazamine, the Meeting estimated maximum residue levels of 0.01* mg/kg for milks and 0.02 * mg/kg for mammalian meat and fat. STMRs and HRs (except milk) of 0 mg/kg were estimated for milks and mammalian meat and fat.

Using the ratio of 3.17/7.2, the Meeting estimated a maximum residue level of 0.05 mg/kg (from 0.043×3.17/7.2=0.018 for kidney and 0.087×3.17/7.2=0.038 for liver) for edible offal (mammalian). STMRs and HRs were estimated at 0.038 mg/kg for liver and 0.018 mg/kg for kidney.

Residues in egg and poultry tissues

No feeding study was conducted on laying hens. However, since the dietary burden is zero, no residue was expected to occur in foods of poultry origin.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Definition of the residue for plant commodities (for enforcement of MRLs): Fenpyrazamine

Definition of the residue for plant commodities (for dietary risk assessment): *Sum of fenpyrazamine and 5-amino-1,2-dihydro-2-isopropyl-4-(o-tolyl)pyrazol-3-one (S-2188-DC), expressed as fenpyrazamine*

Definition of the residue for animal commodities (for enforcement of MRLs and for dietary risk assessment): *Sum of fenpyrazamine and 5-amino-1,2-dihydro-2-isopropyl-4-(o-tolyl)pyrazol-3-one (S-2188-DC), expressed as fenpyrazamine*

The residue is not fat-soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The current Meeting established an ADI of 0–0.3 mg/kg bw.

The International Estimated Dietary Intakes (IEDIs) of fenpyrazamine were calculated for the 17 GEMS/Food cluster diets using STMRs estimated by the current Meeting (Annex 3 to the 2017 Report). The calculated IEDIs were 0–2% of the maximum ADI (0.3 mg/kg bw). The Meeting concluded that the long-term dietary exposure to residues of fenpyrazamine resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term dietary exposure

The current Meeting established an ARfD of 0.8 mg/kg bw.