FENPYRAZAMINE (298)

The first draft was prepared by Dr Y Yamada, Ministry of Agriculture, Forestry and Fisheries, Tokyo Japan

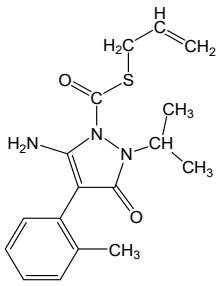
EXPLANATION

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 inhibit germ tube and mycelium elongation. It is not categorized as systemic but some translocation is observed in plants.

Fenpyrazamine has been registered in Europe, Canada, USA and many other countries for use on various crops including stone fruits, berries and other small fruits, fruiting vegetables (cucurbits and those other than cucurbits), lettuce, ginseng and tree nut for which supervised trial data were submitted to this Meeting.

No specification has been established by the Joint FAO/WHO Meeting on Pesticide Specifications.

Fenpyrazamine was listed in 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 Meeting received information on identity, chemical and physical properties, metabolism and environmental fate, residue analysis, use pattern, supervised trials on a number of crops and processing studies.

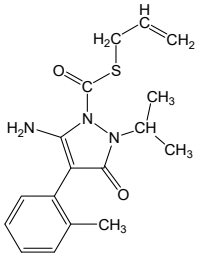


Fenpyrazamine

IDENTITY

ISO common name:	Fenpyrazamine
Chemical name	
IUPAC:	S-allyl 5-amino-2,3-dihydro-2-isopropyl-3-oxo-4-(o-tolyl)pyrazole-1-carbothioate
CAS:	S-2-propen-1-yl 5-amino-2,3-dihydro-2-(1-methylethyl)-4-(2-methylphenyl)-3-oxo-1H-pyrazole-1-carbothioate
CAS Registry No.:	473798-59-3
CIPAC No.:	832

Structural formula:



Molecular formula: C17H21N3O2S

Molecular weight: 331.43

PHYSICAL AND CHEMICAL PROPERTIES

Pure fenpyrazamine

The purity of fenpyrazamine used for the following was 99.3%, except that for hydrolysis in water and photochemical stability in water radiochemical purity was 99.4%.

Property	Results	Reference
Appearance	White with Munsell colour reference of N9.5/90% at 21.7 °C Solid with slight odour at 25 °C	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
Relative density	1.26 g/mL at 20 ºC	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
Vapour pressure	By gas saturation method: $<10^{-5}$ Pa at 25 °C (too low to be determined experimentally). By MPBPWin calculation: 2.89 x 10^{-8} Pa at 25 °C	DiFrancesco, D., 2006 (QNP-0004)
Melting point	116.4 °C (389.6 K)	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
Boiling point	239.8 °C (513.0 K) at 745 mmHg	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
Thermal stability	No decomposition was observed below 150 °C	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
Solubility in water	At neutral pH at 20 °C: 20.4 mg/L The effect of pH on water solubility was not determined as fenpyrazamine does not dissociate under acidic or basic conditions. No dissociation was observed in the approximate pH range of 1-13.	Lentz, N.R., 2005a (QNP-0003) Beckwith, R.C. & DiFrancesco, D., 2005 (QNP-0001)
Solubility in organic solvents	At 20 ± 0.5 ºC: n-hexane, 0.9 g/L n-octanol, 84 g/L (99 g/kg) toluene, 113 g/L (126 g/kg) acetone, > 250 g/L (> 250 g/kg) methanol, > 250 g/L (> 250 g/kg) dichloromethane, > 250 g/L (>250 g/kg) ethyl acetate, > 250 g/L (> 250 g/kg)	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
Octanol/water partition coefficient (Log Pow)	3307.32 (log Pow = 3.52) at 25±1 ºC	Lentz, N.R., 2005b (QNP-0002)
Hydrolysis in sterile buffer in the dark	pH 4: Stable (at 50 ºC for 5 days). pH 7: DT_{50} at 20 and 25 ºC extrapolated from the hydrolysis at 50, 60 and 70 ºC is >1 year. pH 9: DT_{50} at 25 ºC is 11 days (measured) and at 20 ºC extrapolated from the hydrolysis at 25, 40 and 50ºC is 24 days.	Lewis, C., 2007 (QNM-0017)

Property	Results	Reference
	Only two hydrolysis products detected at pH 7 and 9 at or above 10% of the applied radioactivity were: S-2188-DC (maximum of 89%); and S-2188-OH (maximum 18%).	
Photolysis in sterile water and buffer under artificial light	DT_{50} of fenpyrazamine in sterile water at pH 7 at 25 ºC (equivalent to the UK and US summer light) Pyrazolyl labelled: 1.7 days Phenyl labelled: 1.6 days Photodegradation products of fenpyrazamine: S-2188-DC (maximum 64%); and MCNI (maximum 18%)	Lewis, C.J. & Troth, K., 2007d (QNM-0029)

Technical grade

The purity of fenpyrazamine used for physical state, colour, odour, solubility in organic solvents was 94.7% except for stability. The minimum purity based on a pilot plant study was 94.0%.

Property	Results	Reference
Appearance	Very pale yellow with Munsell reference of 10Y 9/2 at 20.7 °C Solid with odour characteristic of garlic at 25 °C	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
Relative density	1.25 g/mL at 20 ºC	
Solubility in organic solvents	At 20 °C: n-hexane: 0.8 g/L n-octanol: 99 g/L (105 g/kg) toluene: 129 g/L (132 g/kg) acetone: > 250 g/L (> 250 g/kg) methanol: > 250 g/L (> 250 g/kg) dichloromethane: > 250 g/L (>250 g/kg) ethyl acetate: > 250 g/L (> 250 g/kg)	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
Flammability	Negative	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
Surface tension	66.9 mN/m at 20 ºC	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
Stability	At room temperature for 12 months (95.6-98.8% purity): No change of appearance and weight At ambient and at 54 ºC for 14 days (95.1% purity): Stable	Tani, N., 2009a (QNP-0011) Tani, N., 2009b (QNP-0010)

Formulations

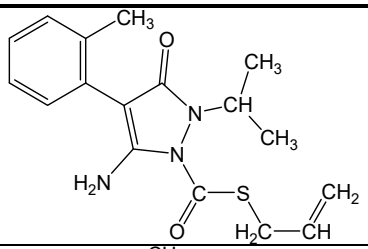
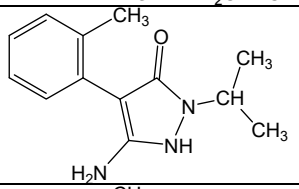
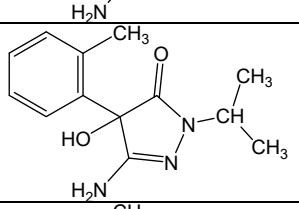
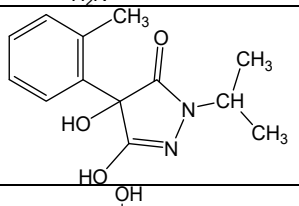
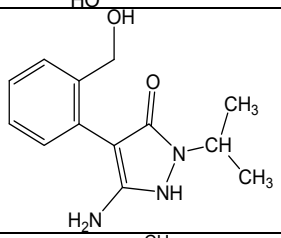
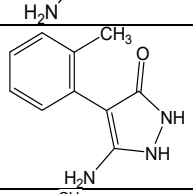
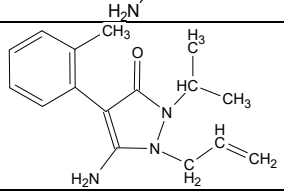
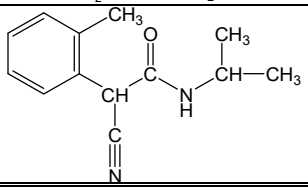
Fenpyrazamine is formulated as:

- Water dispersible granule (WG) formulation containing 500 g ai/kg; and
- Suspension concentration (=flowable concentrate) (SC) formulation containing 479 g ai/L.

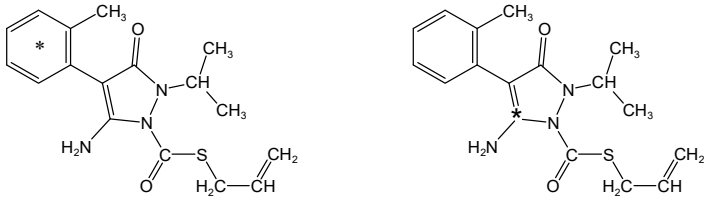
METABOLISM AND ENVIRONMENTAL FATE

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

Table 1 Structure of compounds appearing in metabolism and environmental fate studies

Name or Code IUPAC name (MW)	Structure	Found in:
Fenpyrazamine (S-2188) S-allyl 5-amino-2,3-dihydro-2-isopropyl-3-oxo-4-(o-tolyl)pyrazole-1-carbothioate (331.4)		Grape, Lettuce, Rapeseed Rotational crops (wheat, lettuce, carrot) Goat (liver, fat) Hen (liver, fat, egg) Rat
S-2188-DC 5-amino-1,2-dihydro-2-isopropyl-4-(o-tolyl)pyrazol-3-one (231.3)		Grape, Lettuce, Rapeseed Rotational crop (wheat) Goat (muscle, liver, kidney, fat, milk) Hen (muscle, liver, egg)
S-2188-OH 5-amino-2,4-dihydro-4-hydroxy-2-isopropyl-4-(o-tolyl)pyrazol-3-one (247.3)		Grape, Lettuce, Rapeseed Rotational crops (wheat, lettuce, carrot)
S-2188-$(OH)_2$ 2,4-dihydro-4,5-dihydroxy-2-isopropyl-4-(o-tolyl)pyrazol-3-one (248.3)		Rotational crops (wheat, lettuce, carrot)
S-2188-CH_2OH-DC 5-amino-1,2-dihydro-4-(2-hydroxymethylphenyl)-2-isopropyl-pyrazol-3-one (247.3)		Goat (muscle, liver, kidney)
MPPZ 5-amino-1,2-dihydro-4-(o-tolyl) pyrazol-3-one (189.2)		Hen (muscle, liver, egg)
S-2188-DCT 3-allyl-5-amino-2,3-dihydro-2-isopropyl-3-oxo-4-(o-tolyl)pyrazole-1-carbothioate (271.4)		Photolysis
MCNI (216.3)		Photolysis

The Meeting received information on plant and animal metabolism of fenpyrazamine, its environmental fate in soil and residues in rotational crops. The fate and behaviour of fenpyrazamine in plants, animals and soil were investigated using the radiolabelled fenpyrazamine with ^{14}C in the phenyl ring or at position 5 of the pyrazole ring as shown in Figure 1.



[Phenyl-UL-^{14}C] Fenpyrazamine [Pyrazolyl-5-^{14}C] Fenpyrazamine

Figure 1 Radiolabelled test materials used in the metabolism and environmental fate studies

Plant metabolism

The Meeting received information on metabolism of radiolabelled fenpyrazamine labelled with ^{14}C in the phenyl ring or at position 5 of the pyrazole ring in various plants in support of intended uses: grape vine (representing fruits), lettuce (leafy vegetables), and rapeseed (oilseeds). In the following texts, TRR is expressed in mg-fenpyrazamine equivalents/kg.

Grapes (Lewis C.J., Cooke C., 2006; QNM-0013)

The metabolism of fenpyrazamine in grapevine (variety Phoenix) grown on sandy loam topsoil and sand (5:2, v/v) in a container (capacity of approximately 70 L) was studied in a green house. Spray tents were placed around the vines before spraying, left for about 30 minutes after spraying, and then removed.

Both the [phenyl-^{14}C]- and [pyrazolyl-5-^{14}C]-fenpyrazamine were formulated as a WG formulation and applied twice using a hand pump sprayer over the grapevines at the stage of ripening of berries with an interval of 14 days between these applications. The application rates used were approximately 0.75 kg ai/ha (hereafter described as 1×), 1.5 times higher than the intended use in Europe of 0.6 kg ai/ha; and 3.75 kg ai/ha (5×).

Mature fruits and foliage of grape were harvested 14 and 21 days after the second application at BBCH 89. Only for 1× rate, the first harvest was made 14 days after the last application, collecting the foliage and about half of the fruits. The second harvest was made 21 days after the last application (both rates and control) collecting the foliage and the remaining fruits.

The samples of fruits and foliage from the 1× rate treatment were washed twice with acetonitrile before homogenization to powder in dry ice using a blender and kept frozen. The washes were combined for each crop fraction/treatment group for quantifying radioactivity. The samples from the 5× rate treatment were washed with acetonitrile and then frozen without homogenization or identification of metabolites.

The homogenized fruit and foliage samples were extracted 3 times by maceration with pre-chilled acetone/water (4:1, v/v). An aliquot of each acetone/water extract was concentrated by evaporation and reconstituted in acetone/water (4:1, v/v) for chromatography. The acetone/water extracts of the 14-day fruit and foliage samples were cleaned up by solid phase extraction using C18 SPE column to remove interference in chromatography.

The radioactivity in the surface washes and acetone/water extracts was determined by liquid scintillation counting (LSC). The amount remaining in the post-extraction solids was determined by combustion analysis. Analysis and identification of residues in the surface washes and concentrated acetone/water extracts was conducted by co-chromatography with reference standards using radio-HPLC and TLC. All the analysis was completed within three months after harvest.

The total radioactive residues (TRR) in the fruits and foliage collected 14 and 21 days after the last application of radioactive fenpyrazamine are shown in Table 2**Error! Reference source not found.**. The TRR in fruits collected 14 and 21 days after the last application of radioactive

fenpyrazamine at the 1× rate were 22 and 44 mg eq/kg, respectively, for the phenyl label, and 16 and 26 mg eq/kg, respectively, for the pyrazolyl label. The TRR in foliage collected 14 and 21 days after the last application of radioactive fenpyrazamine at the 1× rate were 246 and 321 mg eq/kg, respectively, for the phenyl label and 104 and 230 mg/kg, respectively, for the pyrazolyl label. The fruit and foliage samples in the control group collected 21 days after the last application contained 0.016 and 0.47 mg/kg, respectively.

Table 2 Total radioactive residues in the fruit and foliage samples from grapevines treated with radioactive fenpyrazamine at 0.75 kg ai/ha.

Sample	Sample collection (DALA)[a]	TRR (mg fenpyrazamine equivalents/kg)	
		[phenyl-^{14}C]-fenpyrazamine	[pyrazolyl-5-^{14}C]-fenpyrazamine
Fruit	14	21.6	15.7
Fruit	21	44.3	26.2
Foliage	14	245.7	103.6
Foliage	21	320.9	230.0

[a]: days after the last application

The distribution of radioactivity in the acetonitrile surface wash, acetone/water extracts and in the post extraction solid of each sample is shown in Table 3.

The distribution of radioactivity in fruit fractions was similar regardless of the position of ^{14}C or and the time between the last application and the harvest. Most of the radioactivity (94–96% TRR, 14.7–41.6 mg eq/kg) in fruit was recovered in the surface wash. While the acetone/water mixture extracted 3.4–5.3% TRR (0.79–2.23 mg eq/kg), 0.8–1.4% TRR (0.21–0.50 mg eq/kg) remained unextracted.

The distribution of radioactivity in foliage fractions was similar between the two different ^{14}C-labelled fenpyrazamine, but the samples harvested at the later date (21-day harvest), higher radioactivity was found in surface wash but at the lower percentage of TRR. At the 14-day harvest, 94–95% TRR (97.2–234.2 mg eq/kg) was recovered in the surface wash, 3.2–4.0% TRR (4.1–7.9 mg eq/kg) was extracted with acetone/water and 1.5–2.2% TRR (2.2–3.6 mg eq/kg) remained unextracted. At the 21-day harvest, 89–93% TRR (205–298 mg eq/kg) was recovered in the surface wash, 5.7–9.0% TRR (18.3–20.8 mg eq/kg) was extracted with acetone/water and 1.6–2.0% TRR (4.6–5.1 mg eq/kg) remained unextracted.

Table 3 Radioactive residues in fractions of the fruit and foliage samples from grapevines treated with radioactive fenpyrazamine at 0.75 kg ai/ha.

Fraction	[phenyl-^{14}C]-fenpyrazamine				[pyrazolyl-5-^{14}C]-fenpyrazamine			
	14 DALA		21 DALA		14 DALA		21 DALA	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Fruit								
Surface wash	20.3	93.7	41.6	93.8	14.7	93.6	25.1	95.8
Acetone/water extract	1.15	5.3	2.23	5.0	0.79	5.0	0.89	3.4
PESa	0.21	1.0	0.50	1.1	0.22	1.4	0.21	0.8
Total	21.6	100	44.3	100	15.7	100	26.2	100
Foliage								
Surface wash	234.2	95.3	297.6	92.7	97.2	93.9	204.5	88.9
Acetone/water extract	7.89	3.2	18.3	5.7	4.13	4.0	20.8	9.0
PES[a]	3.58	1.5	5.09	1.6	2.24	2.2	4.64	2.0
Total	245.7	100	320.9	100	103.6	100	230.0	100

[a], post-extraction solid

Identification of radioactive metabolites was attempted by using HPLC and TLC co-chromatography with S-2188-DC, S-2188-OH, S-2188-DTC, MCNI and MPPZ.

The distribution and identification of residues in grape berries and foliage from grape vines treated with 0.75 kg ai/ha are given in Table 4.

The predominant radioactive residue was the parent fenpyrazamine, accounting for 88-95% TRR (14–42 mg eq/kg) in grape berries; and 81–92% TRR (96–260 mg eq/kg) in foliage. As the distribution of radioactivity, most of the fenpyrazamine was present in the surface wash of berries and foliage. Similar or higher concentrations of fenpyrazamine were found in the samples obtained 21 days after the last application than those obtained 14 days after the last application.

Significantly lower concentrations of S-2188-DC and S-2188-OH and some other minor metabolites than those of fenpyrazamine were also found. The metabolite S-2188-DC was found in all the surface washes and acetone/water extracts and accounted for 1–4.9% TRR (0.22–1.17 mg eq/kg) in grape berries and 2.8–8.0% TRR (2.79–26 mg eq/kg) in foliage, most of which was also found in the surface wash. S-2188-OH accounted for 0.2–0.6% TRR (0.05–0.18 mg eq/kg) in grape berries and 0.2–0.3% TRR (0.26–0.79 mg eq/kg) in foliage. S-2188-OH was not detected (LOD equivalent to 0.012% TRR) in the surface wash samples, except in one foliage sample collected 21 days after the last application of pyrazolyl label (0.3% TRR and 0.79 mg eq/kg). No other radioactive compounds were identified.

Up to four unidentified radioactive components were found in fruit samples. The maximum sum of these components from samples was 2.1% TRR corresponding to 0.56 mg eq/kg. However, all of the individual components were at or below 1.6% TRR (0.43 mg eq/kg). These components in the acetone/water extracts of fruit samples were more polar than fenpyrazamine and common to the both labels. One unidentified component found in the surface wash of the fruit sample collected 21 days after the treatment with [pyrazolyl-^{14}C]-fenpyrazamine was more polar than fenpyrazamine, but less polar than the identified metabolites S-2188-DC and S-2188-OH.

Up to seven unidentified radioactive components were found in the foliage acetone/water extracts, accounting for a maximum of 1.4% TRR (1.7 mg/kg), with no individual component exceeding 0.3% TRR (0.72 mg/kg). This situation is common to the both radiolabels. No unidentified components were found in the foliage surface rinses. All the unidentified components were more polar than fenpyrazamine and commonality.

Table 4 Distribution and identification of components in fruit and foliage samples from grapevines treated with radioactive fenpyrazamine at 0.75 kg ai/ha.

Component	14 DALA						21 DALA					
	Surface wash		Acetone/water extract		Total		Surface wash		Acetone/water extract		Total	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Fruit: treatment with [phenyl-^{14}C]-fenpyrazamine												
Fenpyrazamine	20.1	92.8	0.45	2.1	20.5	94.9	40.6	91.6	0.97	2.2	41.5	93.7
S-2188-DC	0.11	0.5	0.11	0.5	0.22	1.0	0.88	2.0	0.23	0.5	1.11	2.5
S-2188-OH	ND	-	0.14	0.6	0.14	0.6	ND	-	0.18	0.4	0.18	0.4
Unknowns (1)	ND	-	0.04	0.2	0.04	0.2	ND	-	0.33	0.8	0.33	0.8
Unseparated regions (3) [a)]	ND	-	0.39	1.8	0.39	1.8	ND	-	0.40	0.9	0.40	0.9
Unresolved background	0.09	0.4	0.03	0.1	0.12	0.6	0.12	0.3	0.10	0.2	0.22	0.5
PES	-	-	-	-	0.21	1.0	-	-	-	-	0.50	1.1
Total	**20.3**	**93.7**	**1.15**	**5.3**	**21.6**	**100**	**41.5**	**93.8**	**2.23**	**5.0**	**44.3**	**100**
Fruit: treatment with [pyrazolyl-5-^{14}C]-fenpyrazamine												
Fenpyrazamine	13.5	86.1	0.33	2.1	13.8	88.2	23.5	89.4	0.36	1.4	23.8	90.7
S-2188-DC	0.69	4.4	0.09	0.6	0.77	4.9	1.1	4.2	0.07	0.3	1.17	4.4
S-2188-OH	ND	-	0.05	0.3	0.05	0.3	ND	-	0.06	0.2	0.06	0.2
Unknowns (3)	ND	-	0.11	0.7	0.11	0.7	0.43	1.6	0.13	0.5	0.56	2.1
Unseparated regions (3)	0.46	3.0	0.21	1.3	0.68	4.3	ND	-	0.25	1.0	0.25	1.0
Unresolved background	0.02	0.1	0.005	-	0.03	0.2	0.17	0.6	0.01	0.1	0.18	0.7
PES	-	-	-	-	0.22	1.4	-	-	-	-	0.21	0.8
Total	**14.7**	**93.6**	**0.79**	**5.0**	**15.7**	**100**	**25.1**	**95.8**	**0.89**	**3.4**	**26.2**	**100**
Foliage: treatment with [phenyl-^{14}C]-fenpyrazamine												
Fenpyrazamine	221.9	90.3	2.40	1.0	224.3	91.3	249.2	77.6	10.8	3.4	260.0	81.0

Component	14 DALA						21 DALA					
	Surface wash		Acetone/water extract		Total		Surface wash		Acetone/water extract		Total	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
S-2188-DC	11.2	4.5	0.72	0.3	11.9	4.8	23.9	7.4	1.80	0.6	25.7	8.0
S-2188-OH	ND	-	0.62	0.3	0.62	0.3	ND	-	0.53	0.2	0.53	0.2
Unknowns (6 or 1)[b)]	ND	-	1.71	0.7	1.71	0.7	ND	-	0.24	0.1	0.24	0.1
Unseparated regions (3 or 2)[c)]	ND	-	2.35	1.0	2.35	1.0	12.5	3.9	4.84	1.5	17.4	5.4
Unresolved background	1.18	0.5	0.10	0.0	1.28	0.5	12.0	3.7	0.07	0.0	12.1	3.8
PES	-	-	-	-	3.58	1.5	-	-	-	-	5.09	1.6
Total	**234.2**	**95.3**	**7.89**	**3.2**	**245.7**	**100**	**297.6**	**92.7**	**18.3**	**5.7**	**320.9**	**100**
Foliage: treatment with [pyrazolyl-5-^{14}C]-fenpyrazamine												
Fenpyrazamine	94.8	91.6	0.62	0.6	95.5	92.2	178.4	77.6	17.5	7.6	195.9	85.1
S-2188-DC	2.32	2.2	0.46	0.5	2.79	2.7	15.2	6.6	0.81	0.4	16.0	7.0
S-2188-OH	ND	-	0.26	0.3	0.26	0.3	0.79	0.3	ND	-	0.79	0.3
Unknowns (7 or 1)[d)]	ND	-	1.46	1.4	1.46	1.4	ND	-	0.31	0.1	0.31	0.1
Unseparated regions (2 or 1-2)[e)]	ND	-	1.24	1.2	1.24	1.2	3.71	1.6	2.18	0.9	5.88	2.6
Unresolved background	0.08	0.1	0.09	0.1	0.17	0.2	6.40	2.8	0.01	0.0	6.41	2.8
PES	-	-	-	-	2.24	2.2	-	-	-	-	4.64	2.0
Total	**97.2**	**93.9**	**4.13**	**4.0**	**103.6**	**100**	**204.5**	**88.9**	**20.8**	**9.0**	**230.0**	**100**

ND: not detected

[a] radioactivity which was not separated in the HPLC chromatograms as peaks

[b] 6 peaks in the 14-day sample and 1 peak in the 21-day sample

[c] 3 peaks in the 14-day sample and 2 peaks in the 21-day sample

[d] 2 peaks in the 14-ay sample and 1-2 peak(s) in the 21-day sample

Lettuce (Lewis C.J., Cooke C., 2007; QNM-0014)

Lettuce (variety Saladin) plants were transplanted into four containers (approximately 35 L capacity, 2 plants per container) of sandy loam topsoil. The containers were kept in plastic trays in a greenhouse. One container was used per treatment group. The plants were grown to full maturity. Glasshouses were heated to provide suitable growing conditions for the lettuces (13–23 °C day/8–16 °C night). Supplemental lighting was provided and plants were watered as necessary. The test material was formulated as a water dispersible granule (WG) formulation and applied three times as a spray, with an interval of 14 days between applications. At each application, spray tents were placed over each container and the formulation was applied using a hand pump sprayer as evenly as possible over the lettuce plants. The surrounding soil was not covered. After spraying, the spray tents were left for about 10 minutes to allow the spray to settle and were then removed. Based on a container surface area of about 0.2 m², the 1× application rate was equivalent to approximately 0.85 kg ai/ha, with a spray volume of 600 L/ha. The 5× application rate was equivalent to approximately 4.3 kg ai/ha, with a spray volume of 600 L/ha.

Mature lettuces were harvested 14 days after the final application. Obviously withered leaves were removed and discarded and the remaining lettuces were separated into individual leaves. Samples were pooled to give a single composite sample for each radiolabel.

Lettuce leaves from the 5× rate were washed with acetonitrile and then frozen without being homogenized and no metabolite identification was performed as sufficient radioactivity was obtained for metabolite identification at the 1× rate.

The lettuce leaves were washed twice with acetonitrile (*ca.* 1000 mL) to provide two surface wash solutions for each treatment group before homogenization. Duplicate aliquots (*ca.* 200 mL) of each surface wash were removed for quantification by LSC prior to the placing samples in the freezer (<-10 °C). A representative portion of each sample (except 5× pyrazolyl-label treated samples) was homogenized to a powder in dry ice using a blender. Afterwards, the samples were stored frozen with dry ice evaporating

A single weighed sub-sample (*ca.* 50 g) of each homogenate was extracted 3 times by maceration with pre-chilled acetone/water (4:1 v/v, 150 mL). The acetone/water extracts were pooled prior to duplicate aliquots being removed for LSC.

A weighed sub-sample (*ca.* 150 g) of each acetone/water extract from 1× group treated either by phenyl-^{14}C-fenpyrazamine or pyrazolyl-5-^{14}C-fenpyrazamine was concentrated by rotary evaporation and reconstituted with acetonitrile, water and methanol (1:1:1), or with water and methanol (1:1). The reconstituted extracts were clarified by centrifugation at 2460 g and the supernatant was transferred to a separate vial. Methanol was added to the pellet and the vial was centrifuged. The pellet wash was pooled with the original supernatant prior to quantification and chromatographic analysis.

The radioactivity in the surface washes and acetone/water extracts was determined by LSC. Post extraction solids were air-dried, ground and the radioactivity quantified by combustion. Identification of radioactive components in the surface washes and concentrated acetone/water extracts was conducted using HPLC and TLC using co-chromatography with reference standards. All the analyses were completed within three months after harvest.

The TRR in each sample was calculated from the sum of the radioactivity in the extracts and in PES and is shown in Table 5.

The TRR values in lettuces harvested 14 days after the final application of radioactive fenpyrazamine at approximately 0.85 kg ai/ha were similar for the both radiolabelled fenpyrazamine, amounting to 12.1 mg eq/kg for the phenyl label and 11.3 mg eq/kg for the pyrazolyl label.

Control lettuce samples were found to contain only a trace amount of radioactivity (0.002 mg eq/kg), all of which was recovered in the surface rinse.

Table 5 Total radioactive residues in leaf samples from lettuce plants treated with radioactive fenpyrazamine at 0.85 kg ai/ha.

Sample	Sample collection (DALA)[a]	TRR (mg fenpyrazamine equivalents/kg)	
		[phenyl-^{14}C]-fenpyrazamine	[pyrazolyl-5-^{14}C]-fenpyrazamine
Leaf	14	12.1	11.3

[a] days after the last application

The distribution of radioactive residues in the acetonitrile surface washes, acetone/water (4:1, v/v) extract and post-extraction solids is shown in Table 6.

The distribution of radioactivity in lettuce samples was similar between the two radiolabels. After treatment with [phenyl-^{14}C] fenpyrazamine, 84% of the TRR was recovered in the surface wash, 14% TRR was subsequently extracted with acetone/water (4:1) and 2.4% TRR remained unextracted. After treatment with [pyrazolyl-^{14}C] fenpyrazamine, 88% of the TRR was recovered in the surface wash, 10% TRR was subsequently extracted with acetone/water and 1.6% TRR remained unextracted.

Table 6 Radioactive residues in fractions of leaf samples collected from lettuce plants 14 days after the last treatment with radioactive fenpyrazamine at 0.85 kg ai/ha.

Fraction	[phenyl-^{14}C]-fenpyrazamine		[pyrazolyl-5-^{14}C]-fenpyrazamine	
	mg/kg	% TRR	mg/kg	% TRR
Surface wash	10.2	83.8	10.0	88.1
Acetone/water extract	1.68	13.8	1.16	10.3
PES[a]	0.29	2.4	0.18	1.6

Fraction	[phenyl-^{14}C]-fenpyrazamine		[pyrazolyl-5-^{14}C]-fenpyrazamine	
	mg/kg	% TRR	mg/kg	% TRR
Total	12.1	100	11.3	100

[a] post-extraction solid

The analysis of lettuce leaf surface wash and acetone/water extracts by HPLC and TLC co-chromatography with fenpyrazamine and S-2188-DC, S-2188-OH, S-2188-DTC and MCNI revealed that the predominant radioactive residue was the parent, fenpyrazamine, at 81–82% TRR (9.1–10 mg eq/kg) regardless of the position of radiolabel. Most of fenpyrazamine was recovered in acetonitrile surface wash (71–72% TRR, 8.2–8.6 mg eq/kg). S-2188-DC was found at lower concentrations 1.1–1.2 mg eq/kg accounting for 8.7–11% TRR, the majority of which was present in surface wash (7.9–11% TRR, 0.95–1.2 mg eq/kg). S-2188-OH found at 0.2–0.3% TRR (0.02–0.04 mg eq/kg). It was below the limit of detection of HPLC in surface wash samples but was detected by TLC.

An unidentified component comprising 0.1% TRR was found in the acetone/water extract of the lettuce leaf sample from [pyrazolyl-5-^{14}C]-fenpyrazamine treatment. The HPLC retention time of the unknown indicated that it was of similar polarity to the identified metabolites S-2188-DC and S-2188-OH.

Table 7 Distribution and identification of components in leaf samples collected from lettuce plants 14 days after the last treatment with radioactive fenpyrazamine at 0.85 kg ai/ha.

Component	[phenyl-14C]-fenpyrazamine						[pyrazolyl-5-14C]-fenpyrazamine					
	Surface wash		Acetone/water extract		Total		Surface wash		Acetone/water extract		Total	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Fenpyrazamine	8.63	71.1	1.33	11.0	9.96	82.1	8.19	72.1	0.96	8.5	9.14	80.6
S-2188-DC	0.95	7.9	0.10	0.8	1.05	8.7	1.20	10.5	0.04	0.4	1.24	10.9
S-2188-OH	ND	-	0.04	0.3	0.04	0.3	ND	-	0.02	0.2	0.02	0.2
Unknowns (1)	ND	-	ND	-	ND	-	ND	-	0.01	0.1	0.01	0.1
Unseparated regions (3) [a]	0.53	4.4	0.20	1.6	0.73	6.0	0.53	4.7	0.12	1.0	0.65	5.7
Unresolved background	0.05	0.4	0.01	0.1	0.06	0.5	0.09	0.8	0.01	0.1	0.10	0.9
PES	-	-	-	-	0.29	2.4	-	-	-	-	0.18	1.6
Total	10.2	83.8	1.68	13.8	12.1	100	10.0	88.1	1.16	10.3	11.3	100

[a] radioactivity which was not separated in the HPLC chromatograms as peaks

Rapeseed (Lewis C.J., Troth K., 2007; QNM-0019)

The metabolism of fenpyrazamine was studied in rapeseed (variety Coban Spring) transplanted into six containers (approximately 50 L capacity) of sandy loam topsoil. The containers were kept inside plastic trays in a greenhouse. One container was used per treatment group. The control container remained untreated.

The test material was formulated as a water dispersible granule (WG) formulation and applied twice as a spray with an interval of approximately 2 months between applications. The first application was made when the flower buds were present but still enclosed by leaves (BBCH 50) and the second was made at the end of flowering (BBCH 69), 45 days before the mature crop was harvested. Based on the surface area of one container of 0.2 m², the 1× application rate was equivalent to approximately 0.6 kg ai/ha and the 5× application rate was equivalent to approximately 3 kg ai/ha. The spray volumes were approximately 600 L/ha and 1200 L/ha for the first and second applications respectively. A higher spray volume application rate was used for the second application, for even and thorough application to significantly larger plants.

Immature whole plants (forage) were harvested 46 days after the first application (BBCH 67–69, approximately 8 weeks before the mature harvest) by cutting the plants just above ground levels. The plants were then washed twice with acetonitrile (2× 500 mL). The two wash solutions were pooled for each sample prior to concentration. After washing, the remaining plants were placed in the freezer (-10 °C).

Mature plants were harvested 45 days after the second application by cutting just above ground level. The harvested plants were dried in the greenhouse for 3–4 days before separation into 'heads' and 'remainder'. The crop fractions from the 1× rate were washed twice with acetonitrile to obtain surface wash fractions. The seeds were then separated from the pods, and the pod and 'remainder' samples were pooled to form a 'stalk' fraction. Samples of forage, stalk and seed were homogenized to a powder in dry ice. Samples from the 5× rate were washed with acetonitrile and then frozen without further processing but not further investigated as sufficient radioactivity for identification was obtained after the 1× treatment.

Homogenized forage, stalk and seed samples were extracted by maceration with pre-chilled acetone/water (4:1, v/v). The remaining sample was extracted sequentially with water, 0.1M hydrochloric acid, and then 0.1M sodium hydroxide solution.

Sub-samples of the forage and stalk acetone/water extracts were concentrated by rotary evaporation. Forage extracts were reconstituted in acetonitrile/water/methanol (5:1:7) or in water/methanol (2:13), and stalk samples were reconstituted in methanol for quantification and chromatographic analysis.

Sub-samples of the acetone/water extract of the seeds were subjected to partitioning with hexane to remove the oils, and the resulting aqueous fractions were concentrated and reconstituted in methanol for chromatographic analysis. The water extracts of the seeds were partitioned with hexane, and the concentrated hexane fraction was cleaned up by solid phase extraction (SPE) using a silica cartridge prior to chromatographic analysis. Residues remaining after the series of extraction were separated into protein, starch and lignin fractions. Protein was extracted using 5% lauryl sulphate at 50 °C for 16 hours. Starch was extracted with sulphuric acid under reflux conditions. The remaining radioactivity was classified as lignin.

The radioactivity in the surface washes and extracts was determined by LSC. The post-extraction solids were air-dried, ground and their radioactivity was quantified by combustion. Analysis and identification of residues in the concentrated surface washes, acetone/water and water extracts was conducted by HPLC and TLC co-chromatography with reference standards. All the samples were analysed within three months of harvest.

The TRR in each sample was calculated from the sum of the radioactivity in the extracts (acetonitrile/water, water, 0.1 M HCl and 0.1 M NaOH) and that remaining unextracted, and shown in Table 8.

The TRR in immature forage collected 46 days after the first application of fenpyrazamine at 0.6 kg ai/ha were 2.0 mg eq/kg for the phenyl-labelled fenpyrazamine and 1.3 mg eq/kg for the pyrazolyl-labelled fenpyrazamine. The TRR in samples collected 45 days after the second application of fenpyrazamine at 0.6 kg ai/ha were: in mature stalk 2.5 mg/kg for the phenyl label and 2.9 mg/kg for the pyrazolyl label; and in mature seed 0.023 mg/kg for the phenyl label and 0.046 mg/kg for the pyrazolyl label. The TRR in seeds were less than 1/50 of those in stalk.

Table 8 Total radioactive residues in portions from rapeseed plants treated with radioactive fenpyrazamine at 0.6 kg ai/ha.

Sample	Number of applications	Sample collection (DALA)[a]	TRR (mg fenpyrazamine equivalents/kg)	
			[phenyl-^{14}C]-fenpyrazamine	[pyrazolyl-5-^{14}C]-fenpyrazamine
Forage	1	46 (immature)	1.99	1.31
Stalk	2	45 (mature)	2.50	2.87
Seed	2	45 (mature)	0.023	0.046

[a] days after the last application

The distribution of radioactivity was studies for all rapeseed samples from the plants treated with the two different radiolabelled fenpyrazamine at 0.6 kg ai/ha. The difference of the position of radiolabel does not affect the radioactivity in the fractions of samples.

In the forage samples collected 46 days after the first application, most of the radioactivity was recovered in the surface wash (74–79% TRR). A further 12.3–16.3% TRR was extracted with acetone/water, 0.8–1.1% TRR with water, 0.4–0.5% TRR with 0.1M hydrochloric acid and 2.1% TRR with 0.1M sodium hydroxide solution. 5.3–6.5% of the TRR remained unextracted.

In the stalk samples collected 45 days after the second application, also the majority of the radioactivity was found in the surface wash (88–91% TRR). A further 5.7–7.3% TRR was extracted with acetone/water, 0.6–0.9% TRR with water, 0.2–0.5% TRR with 0.1M hydrochloric acid and 0.3–0.6% TRR with 0.1M sodium hydroxide solution. 2.4–3.0% of the TRR remained unextracted.

In the seed samples collected at the same time as the stalk samples, about the half of the radioactivity was extracted with acetone/water (19.6–38.2% TRR) and water (13.3–24.6% TRR). A further 12.7–13.7% TRR was extracted with 0.1M hydrochloric acid and 3.8–4.7% TRR was extracted with 0.1M sodium hydroxide. The remaining radioactivity in the seed samples (31.2–38.3% TRR) was found to have been incorporated into protein (6.8–10.1% TRR, 0.002–0.003 mg/kg), starch (3.6–5.6% TRR, 0.001-0.003 mg/kg) and lignin (17.4–25.9% TRR, 0.004–0.012 mg/kg) at very low levels.

Table 9 Radioactive residues in fractions of each sample from rapeseed plants treated with radioactive fenpyrazamine at 0.6 kg ai/ha.

Fraction	[phenyl-^{14}C]-fenpyrazamine		[pyrazolyl-5-^{14}C]-fenpyrazamine	
	mg/kg	% TRR	mg/kg	% TRR
Forage collected 46 days after the first application				
Surface wash	1.47	73.9	1.03	78.7
Extract sequentially with:				
Acetone/water	0.32	16.3	0.16	12.3
Water	0.02	0.8	0.02	1.1
0.1M HCl	0.01	0.5	0.01	0.4
0.1M NaOH	0.04	2.1	0.03	2.1
PES	0.13	6.5	0.07	5.3
Total	1.99	100	1.31	100
Stalk collected 45 days after the second application				
Surface wash	2.26	90.7	2.52	87.7
Extract sequentially with:				
Acetone/water	0.14	5.7	0.21	7.3
Water	0.02	0.6	0.03	0.9
0.1M HCl	0.01	0.2	0.02	0.5
0.1M NaOH	0.01	0.3	0.02	0.6
PES	0.06	2.4	0.09	3.0
Total	2.50	100	2.87	100
Seed collected 45 days after the second application				
Acetone/water extract	0.009	38.2	0.009	19.6
Aqueous/acetone	0.009	37.3	0.008	18.0
Hexane	< 0.001	0.9	0.001	1.6
Water extract	0.003	13.3	0.011	24.6
Aqueous acetonitrile	0.001	5.7	0.001	2.9
Hexane	0.001	5.8	0.006	14.2
Hexane	-	-	0.001	2.6
Ethyl acetate	-	-	0.001	3.0
Acetonitrile	-	-	0.004	8.5
Solid residue	< 0.001	1.8	0.003	7.5
0.1M HCl extract	0.003	12.7	0.006	13.7
0.1M NaOH extract	0.001	4.7	0.002	3.8
PES	0.007	31.2	0.018	38.3
Protein fraction	0.002	10.1	0.003	6.8
Starch fraction	0.001	3.6	0.003	5.6
Lignin fraction	0.004	17.4	0.012	25.9

Fraction	[phenyl-^{14}C]-fenpyrazamine		[pyrazolyl-5-^{14}C]-fenpyrazamine	
	mg/kg	% TRR	mg/kg	% TRR
Total	0.023	100	0.046	100

Radioactive residues were identified by HPLC and TLC co-chromatography using the reference standards of S-2188-DC, S-2188-OH, S-2188-DTC and MCNI (Table 10).

The most predominant radioactivity in the surface wash and acetone/water extract of immature foliage was parent fenpyrazamine (56–62% TRR in surface wash and 4.9% TRR in acetone/water extracts totalling 61–67% TRR, 0.88–1.2 mg eq/kg), with low levels of S-2188-DC (6.6–7.6% TRR in surface wash and 1.1–1.7% TRR in acetone/water extracts totalling 7.8–9.3% TRR, 0.10–0.19 mg eq/kg). The acetone/water extract of the sample from the pyrazolyl label fenpyrazamine also contained very low levels S-2188-OH (0.5% TRR, 0.006 mg eq/kg).

Also in mature stalk after the second application, the most predominant radioactivity was parent fenpyrazamine (48–58% TRR in surface wash and 1.0–1.3% TRR in acetone/water extracts, totalling 50–60% TRR, 1.4–1.5 mg eq/kg), with lower levels of S-2188-DC (8.7–10% TRR in surface wash and 0.5–0.6% TRR in acetone/water extracts totalling 9.3–11% TRR, 0.27 mg eq/kg) and S-2188-OH (1.8–3.4% TRR in surface wash and ND–0.9% in acetone/water extracts totalling 1.8–3.4% TRR, 0.05–0.12 mg eq/kg).

The acetone/water extracts of the seed samples were analysed after removal of oil with hexane. The main component of the acetone/water extracts was parent fenpyrazamine (9.6–22% TRR, 0.004–0.005 mg eq/kg), with lower levels of S-2188-DC (1.9–3.7% TRR, 0.001 mg eq/kg) and S-2188-OH (1.6–4.0% TRR, 0.001 mg eq/kg). In the water extract (acetonitrile eluate from SPE of hexane phase of water extract) from the pyrazolyl labelled fenpyrazamine treatment, parent fenpyrazamine was the only identified component. The PES fraction contained protein, starch and lignin components containing radioactivity.

Up to 2 unidentified metabolites were found in immature forage, comprising a maximum of 3.4% TRR (0.068 mg/kg), with no individual component exceeding 2.9% TRR (0.06 mg/kg). The unidentified metabolites in forage were found in the acetone/water extracts and the HPLC retention times indicated that they were less polar than the metabolite S-2188-DC. Up to 5 unidentified metabolites were found in stalk, comprising a maximum of 3.0% TRR (0.09 mg/kg), with no individual component exceeding 0.7% TRR (0.018 mg/kg). One unidentified metabolite in the haulm comprised a polar fraction; all other unknowns were less polar than the metabolite S-2188-DC.

Table 10 Distribution and identification of components in the extracts of samples collected from rapeseed plants treated with radioactive fenpyrazamine at 0.6 kg ai/ha.

Component	**[phenyl-^{14}C]-fenpyrazamine**						**[pyrazolyl-5-^{14}C]-fenpyrazamine**					
	Surface wash		Acetone/water extract		Total		Surface wash		Acetone/water extract		Total	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Forage collected 46 days after the first application												
Fenpyrazamine	1.12	56.2	0.10	4.9	1.22	61.1	0.81	62.2	0.07	4.9	0.88	67.2
S-2188-DC	0.15	7.6	0.03	1.7	0.19	9.3	0.09	6.6	0.02	1.1	0.10	7.8
S-2188-OH	ND	ND	ND	ND	ND	ND	ND	ND	0.006	0.5	0.006	0.5
Unknowns (2)	ND	ND	0.07	3.4	0.07	3.4	ND	ND	0.02	1.3	0.02	1.3
Unseparated regions (1-2) [a]	0.18	9.0	0.12	6.2	0.30	15.3	0.12	9.1	0.06	4.4	0.18	13.5
Unresolved background	0.02	1.1	0.002	0.1	0.02	1.2	0.01	0.7	0.001	0.1	0.01	0.8
PES	—	—	—	—	—	—	—	—	—	—	—	—
Total	1.47	73.9	0.32	16.3	1.80	90.2	1.03	78.7	0.16	12.3	1.19	91.0
Stalk collected 45 days after the second application												
Fenpyrazamine	1.46	58.4	0.03	1.0	1.48	59.5	1.39	48.2	0.04	1.3	1.42	49.5
S-2188-DC	0.26	10.3	0.01	0.5	0.27	10.8	0.25	8.7	0.02	0.6	0.27	9.3
S-2188-OH	0.05	1.8	ND	ND	0.05	1.8	0.10	3.4	0.03	0.9	0.12	4.3

Component	[phenyl-^{14}C]-fenpyrazamine						[pyrazolyl-5-^{14}C]-fenpyrazamine					
	Surface wash		Acetone/water extract		Total		Surface wash		Acetone/water extract		Total	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Unknowns (2-5 or 2-4)	0.02	0.6	0.05	2.1	0.07	2.7	0.03	1.0	0.06	1.9	0.09	3.0
Undifferentiated regions (2-3 or 2-4)	0.46	18.6	0.05	2.0	0.51	20.5	0.73	25.4	0.07	2.5	0.80	28.0
Unresolved background	0.03	1.0	0.001	< 0.1	0.03	1.1	0.03	0.9	0.002	< 0.1	0.03	0.9
PES	—	—	—	—	—	—	—	—	—	—	—	—
Total	2.26	90.7	0.14	5.7	2.41	96.4	2.52	87.6	0.21	7.3	2.73	94.9

Component	[phenyl-^{14}C]-fenpyrazamine				[pyrazolyl-5-^{14}C]-fenpyrazamine					
	Acetone/water Extract [b)]		Total		Acetone/water extract [b)]		Water extract [c)]		Total	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Seed collected 45 days after the second application										
Fenpyrazamine	0.005	21.8	0.005	21.8	0.004	9.6	0.003	6.6	0.007	16.2
S-2188-DC	0.001	3.7	0.001	3.7	0.001	1.9	ND	ND	0.001	1.9
S-2188-OH	0.001	4.0	0.001	4.0	0.001	1.6	ND	ND	0.001	1.6
Unknowns	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Undifferentiated regions (3 or 3-9)	0.001	7.1	0.001	7.1	0.001	4.5	< 0.001	1.7	0.001	6.2
Unresolved background	< 0.001	0.8	< 0.001	0.8	< 0.001	0.4	< 0.001	0.1	< 0.001	0.5
PES	—	—	—	—	—	—	—	—	—	—
Total	0.009	37.3	0.009	37.3	0.008	18.0	0.004	8.5	0.012	26.5

ND, not detected

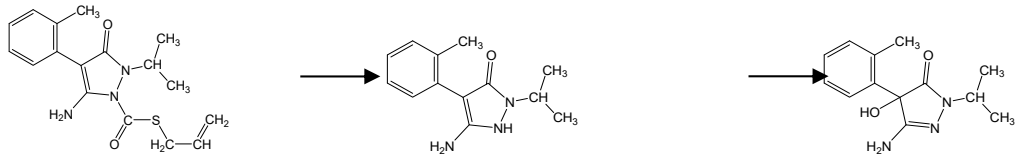
[a] radioactivity which was not separated in the HPLC chromatograms as peaks

[b] Remaining in the extract after removal of oils by partitioning with hexane

[c] Acetonitrile eluate from SPE of hexane phase of water extract

Summary of plant metabolism of fenpyrazamine

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. Proposed metabolic pathway of fenpyrazamine is shown in Figure 1.



Fenpyrazamine S-2188-DC S-2188-OH

Figure 2 Proposed metabolic pathway of fenpyrazamine in plants

Residues in succeeding or rotational crops

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

Confined rotational crop studies (Jalal M.A.F., 2009; QNM-0039)

A total of 6 plots (4 treated, 2 untreated), each consisting of a plastic sheet-lined box (90 cm wide × 150 cm long × 38 cm deep with a total plot area of 1.39 m^2) were used for the study. [Pyrazolyl-5-^{14}C]-fenpyrazamine was dissolved in acetonitrile/water (3:1, v/v) and applied by spray at a rate of 2.83 kg ai/ha to bare sandy loam soil at pH 6.9. The control plots were treated with a solvent blank spray. The plots remained fallow until crops were sown at the designated plant back times (30, 120 and 365 days), with the exception of the 30 DAT plots which were replanted at 365 DAT.

Cereal (wheat), leafy vegetable (lettuce) and root vegetable (carrot) were sown 30, 120 and 365 days after treatment (plant back interval: PBI). At each planting time, wheat was planted in one plot, and lettuce and carrots were planted in separate sections of another plot. Plots were maintained outside and the crops grown and harvested according to normal agricultural practices.

Soil samples were collected before and after the application.

Wheat forage (immature) was collected when plants were at the 15–20 cm stage to stem elongation (jointing) stage. Wheat hay was collected when plants were at the early flower (boot) to soft dough stage of grain development and air dried in a greenhouse. Wheat straw and grain samples were harvested at maturity. In all cases, the aerial parts of the plant were cut near the soil surface. Mature wheat was separated into wheat grain and straw (including chaff). Samples were stored frozen between sampling and analysis.

Immature and mature lettuce heads were harvested by cutting above ground level. Carrots were harvested at maturity and were separated into leaves and roots. Immature carrot tops and roots were also collected from the 120 PBI and 365 PBI plots. The carrot roots were gently washed to remove adhering soil.

The total radioactive residues (TRR) in soil and plant samples were determined by combustion/liquid scintillation counting (LSC).

Crop samples were homogenized and extracted 3 times with acetonitrile. The remaining solids were then extracted twice with water and rinsed with acetonitrile. The acetonitrile extracts and rinse were combined. The radioactivity in the acetonitrile extracts and water extracts was determined by LSC. The radioactivity remaining in the post-extraction solids (PES) was quantified by combustion.

The acetonitrile extracts were evaporated to dryness and reconstituted in 50% acetonitrile for identification of radioactive components by HPLC and 2D-TLC analysis. Similarly, the water extracts were dried and reconstituted in a small volume of water for chromatographic analysis. The polar residues extracted from selected crop matrices were characterized by enzymatic hydrolysis with protease and glucosidase and 2M hydrochloric acid hydrolysis.

Separately, representative PES samples containing significant unextracted radioactive residues were further characterized by sequential ultrasonic and hydrolytic treatments (cellulase and protease digestion, 2M HCl reflux at 102–104 °C for 4 hr, and then 2M NaOH reflux at around 100 °C for 4 hr).

Representative freezer-stored samples were re-extracted approximately 2 years after the initial extraction and analysed. The distribution of radioactive residues in the extracts and post extraction solids (PES) was shown to be similar in the initial and final extractions, confirming the storage stability of the residues.

The total TRR in each sample, determined by combustion, is presented in Table 11**Error! Reference source not found.**. No radioactivity was detected in the control crops.

In general residue levels declined over the sowing intervals, and were lowest in the 365 PBI samples.

Among wheat samples, the TRR levels in wheat forage, hay and straw samples were generally higher in the 30 PBI samples (1.33–3.72 mg eq/kg) compared to the 120 PBI samples (0.44–1.33 mg eq/kg), and were lowest in the 365 PBI samples (0.06–0.14 mg eq/kg). The TRR were lower in the wheat forage than in hay and straw at 30 and 120 PBI. The TRR in grain was also much lower at 0.05 mg eq/kg in the 365 PBI samples, compared to 0.11 and 0.19 mg/kg in the 30 and 120 PBI samples.

Among lettuce samples, the TRR was higher in the 30 PBI immature lettuce (0.69 mg eq/kg) compared to the immature samples of 30 and 120 PBI and the mature samples of all the plant back intervals (0.23–0.27 mg eq/kg). The TRR was significantly lower in the both immature and mature samples of 365 PBI (0.05–0.06 mg eq/kg).

Among carrot samples, the TRR levels were higher in the 120 and 365 PBI immature roots (1.42 and 0.24 mg/kg) compared to the immature tops (0.47 and 0.17 mg eq/kg). Similarly, TRR levels were higher in the 30 PBI mature roots (0.85 mg eq/kg) compared to the 30 PBI mature tops (0.44 mg eq/kg). However, TRR levels were lower in mature roots (0.28 and 0.03 mg eq/kg) than in mature tops (0.51 and 0.08 mg eq/kg) of 120 and 365 PBI.

Table 11 Total radioactive residues (by combustion) in confined rotational crops planted at 30, 120 and 365 days after the treatment of soil with [pyrazolyl-5-^{14}C]-fenpyrazamine at 2.83 kg ai/ha.

Crop	Maturity and/or Part	Total Radioactive Residue (mg/kg)		
		30 PBI	120 PBI	365 PBI
Wheat	Forage	1.33	0.44	0.08
	Hay	1.50	1.20	0.14
	Straw	3.72	1.33	0.06
	Grain	0.11	0.19	0.05
Lettuce	Immature	0.69	0.27	0.06
	Mature	0.26	0.23	0.05
Carrot	Immature tops	-	0.47	0.17
	Immature roots	-	1.42	0.24
	Mature tops	0.44	0.51	0.08
	Mature roots	0.85	0.28	0.03

Crop samples were extracted to determine the nature and distribution of the residue. The extractable residues amounted to 42–58% TRR in the 30 and 120 PBI wheat samples, and 70–77% TRR in the 365 PBI wheat samples. Approximately 23–58% TRR was found in the wheat PES. The amounts of extracted radioactive residues were higher in the lettuce and carrot samples at all plant back intervals (65–89% TRR) with approximately 11–35% TRR remaining in the PES.

The radioactive components were better resolved by 2D-TLC than by HPLC, and the results of the identification and characterization in Table 12 to 14 were from the 2D-TLC analyses.

Parent fenpyrazamine was present in the 30 PBI wheat forage at 0.088 mg/kg (6.7% TRR). Fenpyrazamine was present in trace amounts (0.003–0.008 mg/kg, 0.2–0.4% TRR) in the 30 PBI wheat hay and straw and 120 PBI hay samples. Fenpyrazamine was not detected in wheat grain from any plant back intervals.

Low levels of fenpyrazamine were found in 30 and 120 PBI mature and immature lettuce samples, but fenpyrazamine was not detected in the 365 PBI lettuce samples.

Fenpyrazamine was the major residue found in the 30 and 120 PBI carrot samples (0.16–0.77 mg/kg, 30–68% TRR), but generally at lower concentrations in the carrot tops compared to the roots. Fenpyrazamine was not detected in the 365 PBI mature carrot samples, and was present at a significantly reduced level in the 365 PBI immature carrot roots (0.02 mg/kg, 6% TRR).

The metabolites, S-2188-OH and S-2188-$(OH)_2$, were found in low amounts in all three crops at various planting intervals.

S-2188-OH was found in 30 PBI wheat forage at 0.13 mg/kg (9.9% TRR). It was also found in the 30 and 120 PBI wheat hay and straw samples at a significantly lower level. S-2188-OH was not detected in wheat grain.

In lettuce, S-2188-OH comprised 19% TRR (0.127 mg/kg) in 30 PBI immature lettuce, but decreased to 7.5% TRR (0.021 mg/kg) in the 30 PBI mature lettuce sample. Trace levels (< 0.01 mg/kg) of S-2188-OH were found in lettuce samples from later plant back intervals.

S-2188-OH was not detected or present only at trace levels (< 0.01 mg/kg) in the carrot samples.

S-2188-$(OH)_2$ was found at levels of 0.010–0.18 mg/kg (0.7–5.1% TRR) in 30 and 120 PBI wheat forage, hay and straw, and at 0.006 mg/kg (8.5% TRR) in the 365 PBI wheat forage. S-2188-$(OH)_2$ was not detected in wheat grain.

S-2188-$(OH)_2$ was found in all lettuce samples at levels from 0.002 mg/kg to 0.038 mg/kg (4.1–9.1% TRR), with the lowest levels in the 365 PBI samples.

S-2188-$(OH)_2$ was present in 120 PBI immature and mature carrot tops at levels of 0.051–0.056 mg/kg (10.5–10.8% TRR). S-2188-$(OH)_2$ was found at lower levels (0.001–0.014 mg/kg, 0.9–10.2% TRR) in the 30 PBI mature carrot top, 120 PBI immature carrot roots and all 365 PBI carrot samples. S-2188-$(OH)_2$ was not detected in the 30 PBI and 120 PBI mature carrot root samples.

Fenpyrazamine residues were also recovered from the extracted polar fraction but could not be released by enzyme or acid hydrolysis. Analysis of the unextracted or released residues in wheat, lettuce and carrot indicated that radioactive residues may be degraded further and became associated with starch, protein, lignin, cellulose and other natural plant constituents.

Table 12 Distribution and identification of radioactive residues in wheat samples of 30, 120 and 365 PBI (treatment with [pyrazolyl-5-^{14}C]-fenpyrazamine at 2.83 kg ai/ha)

Component	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
30 PBI								
Fenpyrazamine	6.7	0.09	0.4	0.007	0.2	0.008	ND	ND
S-2188-DC	1.0	0.1	ND	ND	ND	ND	ND	ND
S-2188-OH	9.9	0.13	0.5	0.007	1.1	0.04	ND	ND
S-2188-$(OH)_2$	4.8	0.06	0.7	0.010	5.1	0.18	ND	ND
Polar unknowns (1-2)	24.0	0.32	39.7	0.63	31.1	1.11	58.2	0.05
Unknowns (4-7)	6.4	0.10	1.9	0.03	4.1	0.15	ND	ND
Total extracted	52.7	0.69	43.1	0.68	41.7	1.49	58.2	0.05
Cellulase digest of PES	8.8	0.12	3.4	0.05	5.9	0.21	NA	NA
Protease digest of PES	7.7	0.10	8.6	0.14	5.7	0.20	NA	NA
Acid-hydrolysed starch fraction of PES	6.7	0.09	7.1	0.11	5.7	0.21	NA	NA
Base-hydrolysed starch fraction of PES	3.3	0.04	4.8	0.08	4.3	0.15	NA	NA
Base-hydrolysed lignin fraction of PES	13.6	0.18	21.3	0.34	22.1	0.79	NA	NA
Unhydrolysed cellulose fraction of PES	6.4	0.09	9.4	0.15	10.6	0.38	NA	NA
Remaining in PES	0.8	0.01	2.3	0.04	4.1	0.15	NA	NA
Total in PES	47.3	0.62	56.9	0.90	58.3	2.09	41.8	0.04
Total radioactive residue	100	1.31	100	1.58	100	3.58	100	0.09
120 PBI								
Fenpyrazamine	ND	ND	0.2	0.003	ND	ND	ND	ND
S-2188-OH	1.2	0.006	0.7	0.008	2.0	0.03	ND	ND
S-2188-$(OH)_2$	3.9	0.02	0.8	0.01	3.7	0.05	ND	ND
Polar unknowns (1-2)	48.3	0.22	52.0	0.65	37.5	0.53	51.1	0.09
Unknowns (2-4)	4.3	0.02	4.1	0.05	2.1	0.03	ND	ND
Total extracted	57.7	0.26	57.8	0.72	45.3	0.64	51.1	0.09
Cellulase digest of PES	NA	NA	NA	NA	NA	NA	13.5	0.02
Protease digest of PES	NA	NA	NA	NA	NA	NA	11.5	0.02
Acid-hydrolysed starch fraction of PES	NA	NA	NA	NA	NA	NA	11.9	0.02
Base-hydrolysed starch fraction of PES	NA	NA	NA	NA	NA	NA	2.6	0.004
Base-hydrolysed lignin fraction of PES	NA	NA	NA	NA	NA	NA	3.0	0.005

Component	Forage		Hay		Straw		Grain	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Unhydrolysed cellulose fraction of PES	NA	NA	NA	NA	NA	NA	1.5	0.002
Remaining in PES	NA	NA	NA	NA	NA	NA	4.9	0.008
Total in PES	42.3	0.19	42.2	0.52	54.7	0.77	48.9	0.08
Total radioactive residue	100	0.45	100	1.24	100	1.41	100	0.17
365 PBI								
S-2188-$(OH)_2$	8.5	0.006	ND	ND	ND	ND	ND	ND
Polar unknowns (1-2)	53.2	0.04	72.4	0.10	70.3	0.04	73.8	0.04
Unknowns (1-2)	9.9	0.007	4.8	0.007	ND	ND	ND	ND
Total extracted	71.7	0.05	77.2	0.11	70.3	0.04	73.8	0.04
Total in PES	28.3	0.02	22.8	0.03	29.7	0.02	26.2	0.01
Total radioactive residue	100	0.07	100	0.14	100	0.06	100	0.05

NA = not analysed; ND = not detected

Table 13 Distribution and identification of radioactive residues in lettuce samples of 30, 120 and 365 PBI (treatment with [pyrazolyl-5-^{14}C]-fenpyrazamine at 2.83 kg ai/ha)

Component	Immature Lettuce		Mature Lettuce	
	% TRR	mg/kg	% TRR	mg/kg
30 PBI				
Fenpyrazamine	13.0	0.09	2.9	0.008
S-2188-OH	19.0	0.13	7.5	0.02
S-2188-$(OH)_2$	5.7	0.04	4.1	0.01
Polar unknowns (3)	33.7	0.23	47.0	0.13
Unknowns (3-4)	6.7	0.05	3.5	0.01
Total extracted	78.2	0.52	65.0	0.18
Cellulase digest of PES	1.4	0.01	1.2	0.003
Protease digest of PES	5.1	0.03	5.2	0.02
Acid-hydrolysed starch fraction of PES	4.0	0.03	8.4	0.02
Base-hydrolysed starch fraction of PES	1.4	0.009	3.1	0.009
Base-hydrolysed lignin fraction of PES	3.7	0.02	7.7	0.02
Unhydrolysed cellulose fraction of PES	5.7	0.04	8.6	0.02
Remaining in PES	0.5	0.004	0.8	0.002
Total in PES	21.8	0.15	35.0	0.10
Total radioactive residue	100	0.67	100	0.28
120 PBI				
Fenpyrazamine	5.2	0.01	5.8	0.01
S-2188-OH	2.1	0.006	3.8	0.009
S-2188-$(OH)_2$	5.6	0.02	6.9	0.02
Polar unknowns (1-2)	53.6	0.14	46.4	0.12
Unknowns (2-3)*	13.3	0.04	11.8	0.03
Total extracted	79.8	0.21	74.8	0.19
Total in PES	20.2	0.05	25.2	0.06
Total radioactive residue	100	0.27	100	0.25
365 PBI				
Fenpyrazamine	ND	ND	ND	ND
S-2188-OH	0.3	< 0.001	4.0	0.002
S-2188-$(OH)_2$	9.1	0.005	4.5	0.002
Polar unknowns (2-3)	54.9	0.03	47.4	0.02
Unknowns (2-6)	15.7	0.008	24.1	0.01
Total extracted	80.1	0.04	80.1	0.04
Total in PES	19.9	0.01	19.9	0.009
Total radioactive residue	100	0.05	100	0.05

NA = not analysed; ND = not detected

* = any of the components exceeded both 10%TRR and 0.01 mg/kg

Table 14 Distribution and identification of radioactive residues in carrot samples of 30, 120 and 365 PBI (treatment with [pyrazolyl-5-^{14}C]-fenpyrazamine at 2.83 kg ai/ha)

Component	Immature Tops		Immature Roots		Mature Tops		Mature Roots	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
30 PBI								
Fenpyrazamine					46.4	0.20	67.9	0.57
S-2188-OH					1.2	0.005	0.3	0.002
S-2188-$(OH)_2$					2.3	0.01	ND	ND
Polar unknowns (2)					21.2	0.09	9.3	0.08
Unknowns					ND	ND	ND	ND
Total extracted					71.2	0.31	77.5	0.65
Cellulase digest of PES					1.1	0.005	0.6	0.005
Protease digest of PES					2.2	0.01	1.6	0.01
Acid-hydrolysed starch fraction of PES					4.9	0.02	2.6	0.02
Base-hydrolysed starch fraction of PES					2.5	0.01	3.2	0.03
Base-hydrolysed lignin fraction of PES					8.3	0.04	6.0	0.05
Unhydrolysed cellulose fraction of PES					9.5	0.04	8.4	0.07
Remaining in PES					0.3	0.001	0.3	0.002
Total in PES					28.8	0.13	22.5	0.19
Total radioactive residue					100	0.44	100	0.84
120 PBI								
Fenpyrazamine	32.8	0.16	51.3	0.77	30.2	0.16	58.7	0.17
S-2188-OH	1.6	0.008	ND	ND	1.1	0.006	ND	ND
S-2188-$(OH)_2$	10.5	0.05	0.9	0.01	10.8	0.06	ND	ND
Polar unknowns (1-2)	26.7	0.13	14.0	0.21	37.4	0.20	22.8	0.06
Unknowns (1-2)	4.3	0.02	0.4	0.007	2.9	0.02	ND	ND
Total extracted	75.8	0.37	66.6	1.00	82.4	0.43	81.5	0.23
Cellulase digest of PES	0.7	0.003	1.1	0.02	NA	NA	NA	NA
Protease digest of PES	1.7	0.008	2.5	0.04	NA	NA	NA	NA
Acid-hydrolysed starch fraction of PES	9.8	0.05	2.4	0.04	NA	NA	NA	NA
Base-hydrolysed starch fraction of PES	2.1	0.01	6.1	0.09	NA	NA	NA	NA
Base-hydrolysed lignin fraction of PES	5.1	0.03	10.4	0.16	NA	NA	NA	NA
Unhydrolysed cellulose fraction of PES	4.3	0.02	10.1	0.15	NA	NA	NA	NA
Remaining in PES	0.4	0.002	0.8	0.01	NA	NA	NA	NA
Total in PES	24.2	0.12	33.4	0.50	17.6	0.09	18.5	0.05
Total radioactive residue	100	0.48	100	1.50	100	0.52	100	0.28
365 PBI								
Fenpyrazamine	ND	ND	6.1	0.02	ND	ND	ND	ND
S-2188-OH	1.3	0.002	ND	ND	ND	ND	1.4	< 0.001
S-2188-$(OH)_2$	10.2	0.01	2.6	0.006	7.7	0.006	2.9	0.001
Polar unknowns (1-2)	36.3	0.05	52.6	0.13	71.9	0.05	85.1	0.03
Unknowns (1-6)	23.6	0.03	3.7	0.009	ND	ND	ND	ND
Total extracted	71.4	0.10	64.9	0.16	79.6	0.06	89.4	0.03
Total in PES	28.6	0.04	35.1	0.08	20.4	0.02	10.6	0.003
Total radioactive residue	100	0.14	100	0.24	100	0.07	100	0.03

NA = not analysed; ND = not detected

The extraction efficiency of fenpyrazamine was verified using fortified control samples. The extraction efficiency of fenpyrazamine from various samples matrices using acetonitrile was in the range 96–107%.

The confined rotational crops used in this study were found to contain parent fenpyrazamine as well as the metabolites S-2188-OH and S-2188-$(OH)_2$. The metabolite S-2188-OH was probably taken up directly from the soil, and may also have been formed from parent or S-2188-DC in the plants. The first step in the metabolism of fenpyrazamine in plants involves the loss of the allyl-carbothioate side chain to form S-2188-DC. S-2188-DC did not accumulate more than quantifiable levels in any of the crops, and was probably rapidly converted into its 4-hydroxylation product, S-2188-OH. The metabolite S-2188-$(OH)_2$ was probably formed by enzyme-mediated deamination of S-2188-OH.

Field Rotational Crop Study (Grolleau, G., 2009; QNR-0058)

A field rotational crop study was conducted in Italy (trial IT01) and Spain (trial SP01). Each trial consisted of two plots, one untreated and one treated plot. After harvest of the preceding crop, each plot was divided into 12 sub-plots. The 1-month rotational tomato crop was damaged by frost in October 2007 in the trial in Italy (Lombardy), and therefore this part of the study was repeated in June 2008 in Southern Italy (Puglia). To avoid the risk of frost damage, the second rotational interval for tomato was postponed to 8 months. The open-field tomato preceding crop was treated three times in July 2007 (June-July 2008 for the repeated trial) with fenpyrazamine 50WG at a rate of 0.6 kg ai/ha, with a 6 to 8-day spray interval, and the tomatoes were harvested 3 days after the final application. The plants were incorporated into the soil by ploughing in accordance with local practices and the seed bed was prepared.

Succeeding crops (carrot, lettuce, tomato and barley) were sown or transplanted into the plots 1 month and 4 months (or 8 months for tomatoes), or 12 months after the last application to open field tomato preceding crop.

The planted succeeding crops were harvested at their commercial harvest dates and the following samples were collected: tomato fruits, carrot roots and leaves, lettuce heads and barley grain and straw.

Preceding open-field tomatoes were analysed for fenpyrazamine. Rotational crop samples were analysed for fenpyrazamine and S-2188-OH. Fenpyrazamine was analysed using method SUM-0731V and S-2188-OH using method SUM-0706V. These methods are described in the section on analytical methods. The limit of quantitation (LOQ) for both of these compounds was 0.01 mg/kg.

Residues of fenpyrazamine and S-2188-OH in the primary and succeeding crops are given in Table 15. No detectable residues of fenpyrazamine or S-2188-OH were found in any of the control samples.

Table 15 Residues arising in the field rotational crop studies conducted in Italy and Spain

Plant back interval	Crop / part	Days after the last application to tomato	Fenpyrazamine (mg/kg)	S-2188-OH (mg/kg)
Trial IT01 in Italy				
Primary Crop	Tomato	3	0.20, 0.08[a)]	—
1 month	Lettuce	85	< 0.01	< 0.01
	Tomato [a]	108	< 0.01	< 0.01
	Barley grain	337	< 0.01	< 0.01
	Barley straw	337	< 0.01	< 0.01
	Carrot roots	307	< 0.01	< 0.01
	Carrot leaves	307	< 0.01	< 0.01
4 months	Lettuce	254	< 0.01	< 0.01
	Barley grain	337	< 0.01	< 0.01
	Barley straw	337	< 0.01	< 0.01
	Carrot roots	366	< 0.01	< 0.01
	Carrot leaves	366	< 0.01	< 0.01
8 months	Tomato	380	< 0.01	< 0.01
12 months	Lettuce	425	< 0.01	< 0.01
	Tomato	482	< 0.01	< 0.01
	Carrot roots	715	< 0.01	< 0.01
	Carrot leaves	715	< 0.01	< 0.01
	Barley grain	715	< 0.01	< 0.01
	Barley straw	715	< 0.01	< 0.01
Trial SP01 in Spain				
Primary Crop	Tomato	3	0.06	—
1 month	Lettuce	80	< 0.01	< 0.01
	Tomato	122	< 0.01	< 0.01
	Barley grain	229	< 0.01	< 0.01
	Barley straw	229	< 0.01	< 0.01
	Carrot roots	138	< 0.01	< 0.01
	Carrot leaves	138	< 0.01	< 0.01

Plant back interval	Crop / part	Days after the last application to tomato	Fenpyrazamine (mg/kg)	S-2188-OH (mg/kg)
4 months	Lettuce	252	< 0.01	< 0.01
	Barley grain	290	< 0.01	< 0.01
	Barley straw	290	< 0.01	< 0.01
	Carrot roots	300	< 0.01	< 0.01
	Carrot leaves	300	< 0.01	< 0.01
8 months	Tomato	363	< 0.01	< 0.01
12 months	Lettuce	444	< 0.01	< 0.01
	Tomato	472	< 0.01	< 0.01
	Carrot roots	525	< 0.01	< 0.01
	Carrot leaves	525	< 0.01	< 0.01
	Barley grain	683	< 0.01	< 0.01
	Barley straw	683	< 0.01	< 0.01

[a] Repeated trial for tomato 1-month plant back interval conducted in Puglia, Italy, next year, due to crop damage by unexpected frost

Residues of fenpyrazamine in preceding open-field tomatoes were 0.06–0.20 mg/kg. No residues of fenpyrazamine or S-2188-OH were found above the LOQ (< 0.01 mg/kg) in any of the succeeding crops (lettuce, tomato, barley, carrot) planted in rotation 1, 4 (or 8), and 12 months after the final application to open-field tomato.

Animal metabolism

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

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

Rat

The principal routes of metabolism of fenpyrazamine in the rat involved an hydrolysis to remove the allylsulfanylcarbonyl group to produce the main metabolites, 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 both dose levels, as well as after single and repeat 14-day dosing.

Lactating goats

The metabolism of fenpyrazamine in the lactating domestic goat (variety not reported) was studied using [pyrazolyl-5-^{14}C]-fenpyrazamine administered orally in gelatin capsules (by ball gun), once daily after the morning milking for five consecutive days (Panthani A.M., Herczog K.J.S., and Savides M.C., 2007; QNM-0024). One goat received an average daily dose of 0.36 mg/kg bw equivalent to 7.2 ppm in the diet. Another goat served as a control and received placebo capsules daily for five days.

Urine and faeces samples were collected daily after the morning milking but before the dosing. Milk samples were collected twice daily (1600–1700 g daily), and the afternoon milk and the morning milk of the following day were combined after radioactivity analysis to give one sample per day. The animals were sacrificed 8 hours after the final dose, and the following samples were collected: blood, bile (from the gall bladder), kidneys, heart, liver, muscle (composite of loin, front and rear leg muscle), fat (composite of omental and perirenal fat) and the gastrointestinal tract (GIT) and its contents.

Urine, faeces, milk and tissue samples were stored frozen, and blood samples were refrigerated at 4 °C prior to analysis. Heart, liver, kidney, muscle, fat, GI tract, GI tract contents and

faeces samples were homogenized with dry ice. All samples were analysed for radioactivity by liquid scintillation counting (LSC), either directly or following combustion.

Milk samples were extracted with acetonitrile. The precipitated milk solids were separated by centrifugation and extracted with acetonitrile/water (80:20; v/v). After centrifugation, the extracts were combined and partitioned with hexane. The aqueous fraction was concentrated by rotary evaporation and analysed by HPLC. The polar HPLC fraction was isolated and analysed by HPLC using a Rezex RCM Monosaccharide column. A ^{14}C-lactose standard was also analysed using this HPLC method.

Tissue and faeces samples were sequentially extracted with acetonitrile, acetonitrile/water (80:20; v/v) and acetonitrile/water (50:50; v/v). Extracts containing detectable levels of radioactivity were pooled, concentrated and analysed by HPLC. Urine samples were analysed directly by HPLC.

Extracts, fractions or isolates containing conjugated metabolites were subjected to enzymatic hydrolysis with sulfatase (*Helix pomatia*) and/or 1.0M HCl hydrolysis at 80 °C to release the free metabolites.

A sub-sample of the PES from liver was sequentially hydrolysed with 1.0M HCl at 80 °C, 6.0M HCl at 80 °C and 0.1M NaOH at 40 °C. A sub-sample of the PES from liver was also subjected to protease hydrolysis.

Identification of radioactive residues in the extracts was conducted by radio-HPLC and TLC using co-chromatography with reference standards. Isolated metabolites were analysed by LC/MS and LC-MS/MS in negative and/or positive ion mode.

Liver, muscle and milk samples were re-extracted and analysed after storage in the freezer for approximately 9 months to verify the stability of fenpyrazamine and metabolites in the matrices during storage. Similar metabolite profiles were obtained from the initial and final analyses of these samples, demonstrating that fenpyrazamine and its metabolites were stable in these matrices under freezer storage conditions.

Approximately 92% of the administered radioactivity was recovered from the goat. The great majority (84%) of the administered dose was excreted in the urine, faeces and cage wash. Additional 7.0% of the administered dose was recovered in the gastrointestinal tract and contents.

The TRR levels in milk, tissues, blood, bile, GI tract and its contents, urine, faeces and cage washes are shown in Table 16. While the plant metabolism studies indicated that fenpyrazamine residues are expected to be not higher than 0.1 mg/kg, which is much lower than the dose level used in the study, the TRRs in milk, muscle, liver, kidney, heart and fat were all lower than 0.3 mg/kg. The TRR in these tissues, blood and bile accounted for a total of 1.0% of the administered dose, and the TRR in milk accounted for 0.15% of the administered dose.

The highest residue concentrations were found in the liver and kidney at 0.26 mg/kg and 0.16 mg/kg, respectively. Residues in muscle and fat amounted to 0.01 mg/kg.

Table 16 Total radioactive residues in milk, tissues and excreta following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine to lactating goat at 7.2 ppm in the diet

Tissue/excreta	Total Radioactive Residues (TRR)	
	mg eq/kg	% of administered dose
Milk	0.01-0.02[1)]	0.15
Muscle	0.01	0.30
Liver	0.26	0.30
Kidney	0.16	0.03
Heart	0.02	0.01
Fat	0.01	0.01
Blood	0.05	0.18
Bile	4.38	0.17
GI tract	0.18	0.83
GI tract contents	0.75	6.20
Urine	-	58.04

Tissue/excreta	Total Radioactive Residues (TRR)	
	mg eq/kg	% of administered dose
Faeces	-	23.61
Cage washes	-	2.40
Total Recovered	**-**	**92.23**

[a] Residue values for Day 1-4. Day 5 residues are not comparable due to the reduced collection period

The radioactivity in milk reached a plateau on day 2 at 0.017–0.019 mg eq/kg (total, morning as well as afternoon milk). The afternoon milk contained 2–3 times higher radioactivity as radiolabelled fenpyrazamine than the morning milk each day. The TRR in milk samples during the study period are given in Table 17. The milk from before the first administration contained no radioactivity above the limit of detection.

Table 17 Total radioactive residues in milk during the study period following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine to lactating goat at 7.2 ppm in the diet

Day	Total Radioactive Residues (TRR)	
	mg eq/kg	% of administered dose
1[a)] (day 1 afternoon + day2 morning)	0.011	0.02
2 (day 2 afternoon + day 3 morning)	0.017	0.03
3 (day 3 afternoon + day 4 morning)	0.018	0.04
4 (day 4 afternoon + day 5 morning)	0.019	0.04
5 (day 5 afternoon) [b)]	0.032	0.02

[a] Fenpyrazamine was administered after the morning milking each day.

[b] The milk from Day 5 was collected just before sacrifice (*ca.* 8 hours after last dose) and not comparable to the milk from the previous days. However, the level shown is comparable to the afternoon milk of the previous days.

The radioactivity extracted from muscle, liver, kidney, heart and fat samples accounted for 91.4%, 63%, 97%, 94% and 100% of the respective TRR. The radioactivity extracted from milk accounted for 96% TRR, all of which remained in the aqueous fraction when partitioned with hexane. Close to 40% TRR remained in the PES of liver while <10% TRR remained in the PES of other tissues or milk. The distribution of radioactive residues in the extracts and post-extraction solids (unextracted material) are shown in Table 18 for day 4 milk and tissues.

The radioactivity extracted from faeces accounted for 83% TRR. Urine samples were analysed directly, without extraction.

Table 18 Distribution of radioactive residues in fractions of day 4 milk and tissues of lactating goat following administration of [pyrazolyl-5-14C]-fenpyrazamine at 7.2 ppm in the diet

Fraction	Day 4 Milk		Muscle		Heart	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
Acetonitrile and acetonitrile/water extracts	-	-	0.01	91.4	0.02	93.5
Aqueous fraction	0.020	95.6	-	-	-	-
Hexane fraction	0.000	0.0	-	-	-	-
PES	0.001	4.4	0.001	8.6	0.001	6.5
Total	0.021	100	0.01	100	0.02	100

Fraction	Liver		Kidney		Fat	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
Acetonitrile and acetonitrile/water extracts	0.15	62.7	0.16	97.4	0.01	100
PES	0.09	37.3	0.004	2.6	<LOD [a]	<LOD
Total	0.24	100	0.16	100	0.01	100

[a] LOD for PES is 0.0013 mg/kg.

Radioactive components were identified by HPLC and TLC co-chromatography with S-2188-DC, S-2188-OH, S-2188-CH_2OH-DC, MCNI and MPPZ. Unknown components were characterized by LC-MS/MS.

Unchanged fenpyrazamine was found in the liver, fat and faeces. S-2188-DC and S-2188-CH_2OH-DC were the major metabolites found in the goat samples. Conjugated metabolites were characterized from liver, kidney and excreta extracts and hydrolysed with enzyme. However, the exact molecular structures were not determined. The results of characterization and identification are shown in Table 19.

In aqueous fraction of the acetonitrile and acetonitrile/water extracts of day 4 milk, fenpyrazamine was not detected. The metabolite S-2188-DC was detected at 8.6% TRR (0.002 mg/kg). The polar component fraction accounted for 50% TRR (0.010 mg/kg) and was characterized as radioactivity incorporated into lactose. A number of minor metabolites were found in the milk extracts but each represented less than 10% TRR and < 0.01 mg/kg.

In the muscle extracts, S-2188-DC and S-2188-CH_2OH-DC were identified at 25% TRR (0.003 mg/kg) and 29% TRR (0.003 mg/kg), respectively. All other radioactive components were present at 0.001-0.002 mg/kg. In the heart extract, S-2188-DC (0.004 mg/kg, 23.1% TRR) was the major residue found. All other components of the residue were present at 0.001-0.003 mg/kg.

In the liver extracts, fenpyrazamine (0.033 mg/kg, 13.7% TRR) and the metabolites S-2188-DC (0.041 mg/kg, 17.1% TRR) and S-2188-CH_2OH-DC (0.039 mg/kg, 16.2% TRR) were identified. The unhydrolysed glucuronic acid conjugate of S-2188-CH_2OH-DC was identified by HPLC comparison with the equivalent peak identified in urine and faeces. A minor metabolite was characterized by LC/MS/MS as dihydroxylated fenpyrazamine (0.011 mg/kg, 4.8% TRR). A number of minor metabolites were detected in liver, each representing less than 5% TRR. The unextracted radioactive residue accounted for 37% TRR (0.089 mg/kg). From the unextracted residue, protease hydrolysis released 28.2% TRR and was shown by HPLC to consist of multiple radiolabelled components, indicating that the unextracted radioactivity was either bound to or incorporated into protein.

In the kidney extracts, S-2188-DC (sum of free and conjugated)(0.067 mg/kg, 41.0% TRR) and S-2188-CH_2OH-DC (free and conjugated)(0.043 mg/kg, 26.3% TRR) were the major metabolites identified. The unhydrolysed glucuronic acid conjugate of S-2188-CH_2OH-DC was also identified. A number of minor metabolites were detected in kidney, each representing less than 7% TRR (0.01 mg/kg).

In the fat extracts, parent fenpyrazamine (0.002 mg/kg, 17.5% TRR) and S-2188-DC (0.003 mg/kg, 25.9% TRR) were identified. The polar fraction accounted for 37.5% TRR (0.004 mg/kg).

Table 19 Characterization and identification of radioactive residues in day 4 milk and tissues of lactating goat following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine at 7.2 ppm in the diet

Component	Day 4 milk	
	mg/kg	% TRR
Aqueous fraction of Acetonitrile and acetonitrile/water extracts	0.020	95.6
S-2188-DC	0.002	8.6
Unidentified peaks		
49.60 min	< 0.001	1.7
49.07 min	0.001	3.2
46.60 min	0.001	2.8
46.20 min	0.001	4.7
45.87 min	0.001	5.0
44.53 min	< 0.001	1.9
40.07 min	< 0.001	2.4
23.53 min	0.001	7.1
22.27 min	0.002	8.5
Polar 4-6 min	0.010	49.7

Component	Muscle	
	mg/kg	% TRR
Acetonitrile and acetonitrile/water extracts	0.010	91.4
S-2188-DC	0.003	24.9
S-2188-CH_2OH-DC	0.003	29.2
Unidentified peaks		
46.13 min	0.001	7.1
22.07 min	0.001	6.4
19.60 min	0.001	9.0
Polar 4-6 min	0.002	14.8
PES	0.001	8.6
Total	**0.011**	**100**

Component	Day 4 milk	
	mg/kg	% TRR
PES	0.001	4.4
Total	**0.021**	**100**

Component	Muscle	
	mg/kg	% TRR

Component	Liver	
	mg/kg	% TRR
Acetonitrile and acetonitrile/water extracts	0.15	62.7
Fenpyrazamine	0.03	13.7
S-2188-DC free and conjugated [a)]	0.04	17.1
S-2188-CH_2OH-DC free and conjugated [a)]	0.04	16.2
S-2188-CH_2OH-DC conjugated [b)]	0.009	3.7
45-50 min region		
Unknown L1	0.005	1.9
Unknown L2 [c)]	0.01	4.8
17-25 min region		
22.93 min unknown	0.005	2.2
19.4 min unknown [b)]	0.005	2.3
18.5 min unknown [b)]	0.002	0.9
PES	0.09	37.3
Total	**0.24**	**100**

[a)] Sum of the free metabolite plus the conjugated metabolite released after enzyme hydrolysis of the 17-20 min region

[b)] Released metabolites after enzyme hydrolysis of the 17-20 min region

[c)] Tentatively identified as dihydroxylated S02188-CH_2OH (MW = 365 by LC-MS)

Component	Kidney	
	mg/kg	% TRR
Acetonitrile and acetonitrile/water extracts	0.16	97.4
S-2188-DC (free)	0.03	19.5
45-50 min Region 2 [d)]	0.02	13.8
Unknown K1 (matches urine unknown U1) [d)]	0.01	6.7
Unknown K2 [e)]	0.002	1.0
Unknown K3 [e)]	0.004	2.2
Unknown K4 [e)]	0.006	3.9
41.27 min unknown	0.008	4.9
40.60 min unknown	0.008	4.7
19-25 min Region 1 [f)]	0.09	54.6
S-2188-DC [g)]	0.04	21.5
S-2188-CH_2OH-DC [g)]	0.02	12.7
S-2188-CH_2OH-DC conjugate [g) h)]	0.02	13.6
Other unknowns formed during acid hydrolysis		
Unknown K5 [g)]	0.005	3.3
Unknown K6 [g)]	0.006	3.5
PES	0.004	2.6
Total	**0.16**	**100**

[d)] Region 2 was isolated and re-analysed using HPLC method 2 showing a broad region of radioactivity from 46-48 minutes and a major peak at 48.17 minutes

[e)] The 46-48 min region was re-analysed using HPLC method 4 and showed 3 peaks

[f)] Region 1 was isolated and hydrolysed with 1N HCl

[g)] Released metabolites after acid hydrolysis

[h)] Unhydrolysed S-2188-CH_2OH-DC conjugate

Component	Fat	
	mg/kg	% TRR
Aqueous fraction of Acetonitrile and acetonitrile/water extracts	0.010	100
Fenpyrazamine	0.002	17.5
S-2188-DC	0.003	25.9
Unidentified peaks		
44.33 min	0.001	6.8
22.20 min	0.001	6.0
19.47 min	0.001	6.4
Polar 4-6 min	0.004	37.5
PES	<LOD	<LOD
Total	**0.010**	**100**

LOD = 0.0013 mg/kg

The extracts of Day 4 faeces were extracted for metabolite identification. Fenpyrazamine was the major radioactive residue found, accounting for 39.0% TRR. HPLC Region 1 of the faeces extract was isolated and subjected to acid hydrolysis; releasing S-2188-DC (6.4% TRR), S-2188-CH_2OH-DC (13.5% TRR) and unhydrolysed S-2188-CH_2OH-DC conjugate (0.5% TRR). All other components of the residue in faeces were present at less than 5% TRR.

Day 4 urine was analysed directly by HPLC for metabolite identification. The metabolites S-2188-DC (20.7% TRR) and S-2188-OH (10.4% TRR) were found in the urine. HPLC Region 1 was isolated and hydrolysed with 1M HCl, releasing S-2188-DC (30.0% TRR), S-2188-CH_2OH-DC (11.4% TRR) and unhydrolysed S-2188-CH_2OH-DC conjugate (7.7% TRR). Unknown U1 (4.4% TRR) corresponded to unknown K1 found in kidney. No other minor individual component was present in urine above 5% TRR.

Laying Hens

The metabolism of fenpyrazamine in the laying hen was studied using [pyrazolyl-5-^{14}C]-fenpyrazamine administered orally by hand in gelatin capsules, once daily for seven consecutive days (Panthani A.M., Herczog K.J.S., Savides M.C., 2007; QNM-0025). Ten hens received an average daily dose of 0.70 mg/kg bw equivalent to 9.4 ppm in the diet. Another five hens served as a control and received placebo capsules daily for seven days.

Excreta were collected daily, and eggs were collected twice daily (am and pm). Eggs from the pm collection were refrigerated and then added to the am eggs of the following day to give one sample per day, and were separated into yolks and whites. The egg shells were discarded. Eight to nine hours after the final dose, the animals were necropsied by CO_2 anaesthesia followed by exsanguination and the following tissues were collected: blood, muscle (thigh and breast muscle), fat (abdominal and skin fat), liver, undeveloped eggs and the gastrointestinal (GI) tract and its contents. Tissue, egg and excreta samples were stored frozen, and blood samples were refrigerated prior to analysis at approximately 4 °C. Egg yolk, egg white and muscle samples were extracted and analysed after storage in the freezer for approximately 4 months to verify the stability of fenpyrazamine and metabolites in the matrices during frozen storage. The metabolite profiles from the two analyses were similar indicating that fenpyrazamine and metabolites were stable under freezer storage conditions.

Muscle, fat, liver, GI tract and excreta samples were homogenized with dry ice. Egg whites and egg yolks were blended by hand to give homogenous samples. Undeveloped eggs were homogenized using a blender, without the use of dry ice. All samples were analysed for radioactivity by liquid scintillation counting (LSC), either directly or following solubilisation or combustion.

Egg yolk samples were extracted with acetonitrile, and then with acetonitrile/water (80:20 v/v) and acetonitrile/water (50:50; v/v). The combined extracts were concentrated and partitioned with hexane and the resulting aqueous fraction was analysed by HPLC. The egg yolk post-extraction solids were subjected to protease hydrolysis.

Fat samples were sequentially extracted with hexane, acetonitrile and acetonitrile/water (80:20; v/v). The combined hexane extracts were partitioned with acetonitrile and the resulting acetonitrile fraction was analysed by HPLC and detected by a radioactivity flow detector.

Egg white, liver, muscle and excreta samples were sequentially extracted with acetonitrile, acetonitrile/water (80:20; v/v) and acetonitrile/water (50:50; v/v). Extracts containing detectable levels of radioactivity were pooled and analysed by HPLC. The liver post-extraction solids were subjected to pronase hydrolysis and the solubilized fraction was analysed by HPLC.

Selected extracts, fractions or isolates containing conjugated metabolites were subjected to enzyme hydrolysis (sulfatase) and/or acid hydrolysis to release the free metabolites.

Analysis and identification of residues in the extracts was conducted by radio-HPLC and TLC using co-chromatography with reference standards. Isolated metabolites were analysed by LC/MS and LC/MS/MS in negative and/or positive ion mode.

Approximately 95% of the administered radioactivity (AR) was recovered with the majority of the total AR in excreta (88%). A 2.3% of AR was recovered in cage wash and 3.3% AR in the GI tract and its contents. At the higher dose level than that expected from the plant metabolism studies, the radioactive residues in egg, liver, fat and muscle were very low. The TRR in muscle, fat and liver accounted for 0.10% of the administered dose. The TRR in eggs (white and yolk) accounted for 0.06% of the 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/kg. The total radioactive residues in egg, tissues and blood are presented in Table 20.

Table 20 Total radioactive residues in eggs, tissues and excreta following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine to laying hens at 9.4 ppm in the diet

Tissue/egg/excreta	Total Radioactive Residues (TRR)	
	mg eq/kg	% of administered dose
Egg yolk	0.003-0.05[a)]	0.02
Egg white	0.02[a)]	0.04
Undeveloped eggs	0.05	0.04
Muscle	0.02	0.02
Fat	0.02	0.01
Liver	0.18	0.07
Blood	0.05	0.13
GI tract and its contents	1.79	3.31
Excreta	-	88.72
Cage wash	-	2.30
Total Recovered	-	**94.61**

[a] Residue values for Day 2-6 (day 1 residues were <LOD; day 7 value is not comparable due to the reduced collection period)

Radioactive residues in egg yolk increased from 0.003 mg/kg on day 2 to 0.047 mg/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/kg (Table 21). No residues above the limit of detection of 0.0013 mg/kg were observed in eggs before the first administration or in day 1 eggs (both egg white and egg yolk).

Table 21 Total radioactive residues in egg white and egg yolk during the study period following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine to laying hens at 9.4 ppm in the diet

Day	Egg White		Egg Yolk	
	mg eq/kg	% of dose	mg eq/kg	% of dose
1 (day 1 pm + day2 am)	<LOD	<LOD	<LOD	<LOD
2 (day 2 pm + day 3 am)	0.017	0.01	0.003	< 0.01
3 (day 3 pm + day 4 am)	0.020	0.01	0.013	< 0.01
4 (day 4 pm + day 5 am)	0.018	0.01	0.028	< 0.01
5 (day 5 pm + day 6 am)	0.019	0.01	0.035	0.01
6 (day 6 pm + day 7 am)	0.018	0.01	0.047	0.01
7 (day 7 pm) [a)]	0.022	<LOD	0.046	< 0.01

[a] The eggs from Day 7 were collected just before sacrifice (8-9 hours after last dose), and due to the reduced collection period the mg/kg residue values are not comparable to the other days.

The radioactivity extracted with acetonitrile and acetonitrile/water from egg white, egg yolk, muscle and liver accounted for 96%, 70%, 84% and 66% of TRR, respectively. Most of the radioactivity in the acetonitrile extract and acetonitrile/water extracts of egg yolk was not partitioned in hexane but remained in the aqueous layer. Approximately 95% of the radioactivity in the fat was extracted with hexane (84% TRR), acetonitrile and acetonitrile/water (11% TRR). Most of the radioactivity extracted by hexane from fat was partitioned into acetonitrile fraction (71% TRR). The distribution of radioactive residues in the extracts and post-extraction solids (PES) of egg, tissues and excreta are shown in Table 22.

Table 22 Distribution of radioactive residues in fractions of day 6 eggs and tissues of laying hens following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine at 9.4 ppm in the diet

Fraction	Egg Yolk		Egg White		Muscle		Liver	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
Acetonitrile and acetonitrile/water extracts	-	-	0.017	96.4	0.014	83.9	0.115	66.4
Aqueous fraction	0.032	67.0	-	-	-	-	-	-
Hexane fraction	0.001	2.7	-	-	-	-	-	-

Fraction	Egg Yolk		Egg White		Muscle		Liver	
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR
PES	0.014	30.2	0.001	3.6	0.003	16.1	0.058	33.6
Total	0.048	100	0.017	100	0.017	100	0.173	100

Fraction	Fat	
	mg eq/kg	% TRR
Hexane extract	-	-
Acetonitrile fraction	0.014	70.9
Hexane fraction	0.002	13.0
Acetonitrile and acetonitrile/water extracts	0.002	11.3
PES	0.001	4.8
Total	0.019	100

Radioactive components were identified by HPLC and TLC co-chromatography using the following reference standards: S-2188-DC, S-2188-OH, S-2188-CH_2OH-DC, MCNI and MPPZ. Unknown metabolites were characterized by LC-MS/MS.

Fenpyrazamine, S-2188-DC and MPPZ were identified from hen samples. The major metabolite present in excreta did not correspond to any of the fenpyrazamine reference standards and was tentatively identified as a hydroxylated S-2188-DC. Conjugated metabolites regions were isolated from liver and excreta extracts and hydrolysed with enzyme. However, the exact molecular structures were not determined.

In the egg yolk extracts, fenpyrazamine and the metabolites S-2188-DC and MPPZ were identified. No individual component was present in the extracts at levels above 0.01 mg/kg fenpyrazamine equivalents. The polar fraction (0.008 mg/kg, 16% TRR) contained multiple components. The unextracted radioactivity in the post-extraction solids from egg yolk represented 30% TRR (0.014 mg/kg) and was characterized by protease hydrolysis. The hydrolysate contained 20% TRR and was shown by HPLC to consist of a number of minor radiolabelled components.

In the egg white extracts, fenpyrazamine and the metabolites S-2188-DC and MPPZ were identified, and no individual component was present at levels greater than 0.005 mg/kg. The polar fraction (0.004 mg/kg, 24.2% TRR) contained multiple components.

In the muscle extracts, fenpyrazamine was not found above the LOQ and S-2188-DC and MPPZ were present as minor residues. A number of minor unidentified components were present in the muscle extracts, each less than 0.002 mg/kg fenpyrazamine equivalents. The unextracted residue accounted for 16% TRR (0.003 mg/kg) but was not characterized further due to the low concentration of radioactivity.

In the liver extracts, fenpyrazamine (0.004 mg/kg, 2.1% TRR) and a metabolite tentatively identified as an aldehyde of fenpyrazamine (0.015 mg/kg, 8.8% TRR) were present in the liver extracts. HPLC Region 1 (0.072 mg/kg, 42% TRR) contained several radioactive components. Sulfatase hydrolysis of the isolated Region 1 gave a number of minor components, including S-2188-DC (0.005 mg/kg, 3.1% TRR) and MPPZ. The unextracted residue accounted for 34% TRR (0.058 mg/kg) and was characterized by pronase hydrolysis. The hydrolysate contained 26% TRR and was shown by HPLC to consist of multiple radiolabelled components.

In the acetonitrile fraction of hexane extract of fat, fenpyrazamine was the major residue, accounting for 43% TRR (0.008 mg/kg). No other component was present in the fat extract at levels above 0.005 mg/kg fenpyrazamine equivalents.

Table 23 Characterization and identification of radioactive residues in day 6 eggs and tissues of laying hens following administration of [pyrazolyl-5-^{14}C]-fenpyrazamine at 9.4 ppm in the diet

	mg/kg	% TRR
Component	Egg yolk	
Aqueous fraction of Acetonitrile and acetonitrile/water extracts	0.032	67

	mg/kg	% TRR
Fenpyrazamine	0.001	2.6
S-2188-DC	0.003	6.6
Unidentified peaks		
48.60 min	0.001	1.8
46.67 min	0.001	2.4
23.80 min (MPPZ)[a]	0.011	22.9
22.07 min	0.003	6.4
21.47 min	0.001	2.2
20.00 min	0.001	1.6
19.00 min	0.001	2
18.27 min	0.001	2.2
Polar 3-6 min	0.008	16.4
Hexane extract	0.001	2.7
PES	0.014	30.2
Total	**0.048**	**100**
Component	Egg white	
Acetonitrile and acetonitrile/water extracts	0.017	96.4
Fenpyrazamine	0.001	3.5
S-2188-DC	0.004	25.1
Unidentified peaks		
45.20 min	0	2.1
25.73 min	0.001	3.8
24.87 min	0.002	9.3
24.07 min (MPPZ)[b]	0.003	15.9
22.13 min	0.001	8.1
20.73 min	0.001	4.4
Polar 3-6 min	0.004	24.2
PES	0.001	3.6
Total	**0.017**	**100**
Component	Liver	
Acetonitrile and acetonitrile/water extracts	0.115	66.4
Fenpyrazamine	0.004	2.1
47.60 min [c]	0.015	8.8
46.80 min	0.009	5.0
45.00 min	0.002	1.4
44.60 min	0.002	1.3
Region 1 (17-35 min)	0.072	41.9
S-2188-DC [d]	0.005	3.1
23.80 min (MPPZ)[e]	0.032	18.3
23.10 min	0.004	2.5
21.90 min	0.005	2.8
21.20 min	0.008	4.4
21.00 min	0.003	1.8
20.10 min	0.002	1.1
19.80 min	0.007	4.3
19.40 min	0.006	3.6
Polar 3-6 min	0.010	5.9
PES	0.058	33.6
Total	**0.173**	**100**
Component	Muscle	
Acetonitrile and acetonitrile/water extracts	0.014	83.9
S-2188-DC	0.001	4.5
Unidentified peaks		
46.33 min	0	1.8
26.53 min	0	1.7
25.53 min	0.001	6.4
24.73 min	0.001	5.8
23.80 min (MPPZ)[g]	0.006	34.1
22.13 min	0.002	10.2
20.20 min	0.001	3.8

	mg/kg	% TRR
19.47 min	0	2.1
18.40 min	0.001	3.2
17.80 min	0	1.6
Polar 3-6 min	0.001	8.6
PES	0.003	16.1
Total	**0.017**	**100**
Component	Fat	
Acetonitrile fraction of hexane extract	0.014	70.9
Fenpyrazamine	0.008	42.6
Unidentified peaks		
59.40 min	0.001	5.5
56.53 min	0.003	14
52.87 min	0.001	4.8
48.40 min	0.001	4
Hexane fraction of hexane extract	0.002	13
Acetonitrile/water extract (following the hexane extraction)	0.002	11.3
Total extracted	0.018	95.2
PES	0.001	4.8
Total	**0.019**	**100**

Excreta samples from Day 6 were used for profiling and metabolite identification. More than 85% of the radioactivity in the excreta was extracted. Fenpyrazamine accounted for 13% TRR and the metabolite S-2188-DC accounted for 2.4% TRR. HPLC Region 1 contained both free and conjugated metabolites. Acid hydrolysis of Region 1 released a major metabolite characterized as hydroxylated S-2188-DC (OH-S-2188-DC) (30% TRR). This metabolite was not present at detectable levels in egg, muscle and tissues and therefore no attempts were made to confirm the position of hydroxylation. MPPZ was present in the hydrolysate as a major metabolite at less than 5.0% TRR. HPLC Region 2 contained 6 minor unknown metabolites, one of which was tentatively identified by LC/MS/MS as dihydroxylated S-2188-CH_2OH. All other components were present at less than 5% TRR, and no attempt was made to identify them.

Summary of animal metabolism of fenpyrazamine

In lactating goat, the major radioactive metabolite found was S-2188-DC followed by S-2188-CH_2OH-DC or fenpyrazamine. In laying hens, the radioactive metabolites identified were MPPZ, S-2188-DC, and fenpyrazamine. These compounds were also found in rats.

The metabolism of fenpyrazamine in lactating goat and laying hens proceeds via cleavage of the carbothioate side chain of the pyrazolyl ring to produce S-2188-DC.

In the goat, S-2188-DC is hydroxylated to S-2188-CH_2OH-DC and S-2188-OH. These compounds are metabolized by conjugation with glucuronic acid or further metabolized to polar metabolites and incorporated into lactose in milk and protein in liver.

In the hen, S-2188-DC is also hydroxylated to produce hydroxylated S-2188-DC (OH-S-2188-DC). Dealkylation of S-2188-DC results in the formation of MPPZ. Through Oxidation of the allylic carbons on the carbothioate side chain, dihydroxylated S-2188-CH_2OH and the aldehyde of fenpyrazamine are formed but present minor amounts. These metabolites are further metabolized by conjugation and to polar metabolites and incorporated into protein in egg yolk and liver. The metabolic pathway in lactating goat and laying hen is presented in Figure 3.

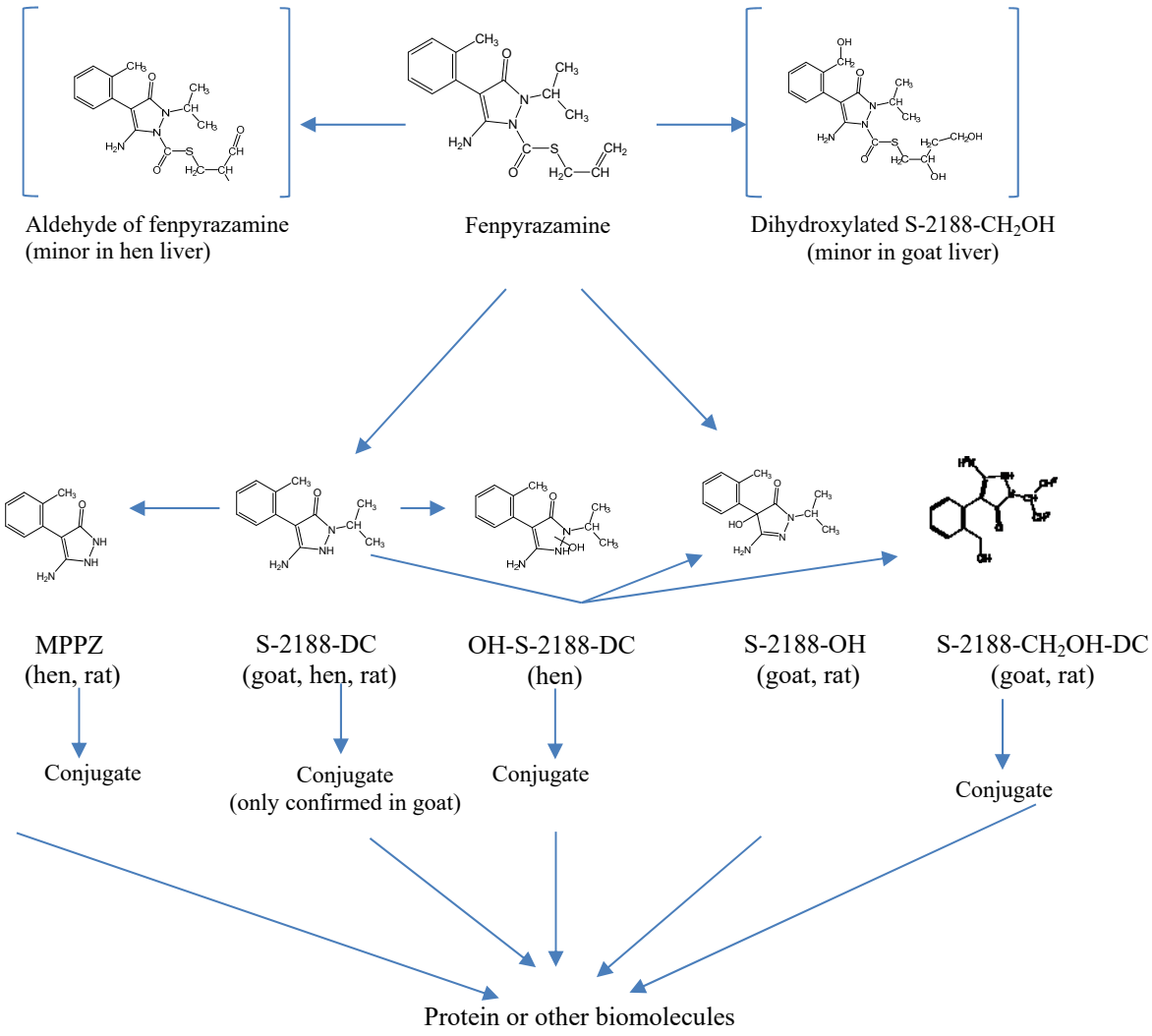


Figure 3 Proposed metabolic pathway for fenpyrazamine in animals

Environmental fate

Hydrolytic degradation

Aqueous buffers at pH 4, 7 and 9 were sterilized by autoclaving. Their oxygen content was reduced by sonication and nitrogen bubbling. [Phenyl-^{14}C]-fenpyrazamine or [Pyrazolyl-5-^{14}C]-fenpyrazamine (in acetonitrile, 21–22 µL), were applied to glass vials containing 3 mL of each buffer to the concentration of *ca.* 1 µg fenpyrazamine/Ml. Duplicate incubation units were analysed immediately after dosing and at various sampling intervals. Tests were conducted in 2 tiers: in tier 1 at pH 4 and 7 at 50 °C; and in tier 2 at pH 7 at 50, 60 and 70 °C and at pH 9 at 25, 40 and 50 °C (Lewis, C.J., 2007; QNM-0017).

Radioactivity present in the test solution at each sampling interval was determined by liquid scintillation counting (LSC). All samples were analysed for [^{14}C]-fenpyrazamine and hydrolysis products by high performance liquid chromatography (HPLC) and selected samples were also analysed by two-dimensional thin layer chromatography (2D-TLC) to identify fenpyrazamine and its hydrolysis products. Additional vials, treated with non-radiolabelled fenpyrazamine, were incubated

concurrently with the test samples to confirm the pH and sterility of samples at the end of each incubation period.

In Tier 1 test, fenpyrazamine was hydrolytically stable at pH 4 with > 94% of applied radioactivity was recovered as fenpyrazamine. At pH 7, slightly more than 10% hydrolysis occurred after 5 days. Tier 1 test was not conducted at pH 9 because fenpyrazamine is known to be unstable under alkaline conditions. Total recovered radioactivity at pH 4 and 7 at 50 °C were in a range of 95–98% of the applied radioactivity indicating that formation of $^{14}CO_2$ or any other volatile compounds was negligible. No distinct differences were observed between the two labelled test substances used in the test.

Table 24 Hydrolysis of radiolabelled fenpyrazamine at pH 4 and 7 at 50 °C (Tier 1)

Days	Label position	% of Applied radioactivity					
		Fenpyrazamine	S-2188-OH	S-2188-DC	Unknown	Unresolved background	Total
pH 4							
0	Phenyl	94.5	< 0.1	< 0.1	0.3	0.2	95.0
0	Pyrazolyl	96.3	< 0.1	< 0.1	0.2	0.3	96.8
0.1	Phenyl	95.0	< 0.1	< 0.1	0.4	0.2	95.7
0.1	Pyrazolyl	96.3	< 0.1	< 0.1	< 0.1	0.2	96.5
5	Phenyl	96.0	0.8	< 0.1	0.2	0.2	97.2
5	Pyrazolyl	95.9	0.4	< 0.1	< 0.1	0.1	96.4
pH 7							
0	Phenyl	95.3	< 0.1	< 0.1	0.3	0.3	95.9
0	Pyrazolyl	96.7	< 0.1	< 0.1	0.1	0.1	97.0
0.1	Phenyl	96.4	< 0.1	< 0.1	0.6	0.3	97.3
0.1	Pyrazolyl	95.5	< 0.1	< 0.1	0.1	0.2	95.7
5	Phenyl	85.6	10.6	0.3	0.5	0.2	97.2
5	Pyrazolyl	88.8	8.8	< 0.1	0.1	0.4	98.1

In all the Tier 2 tests, 97–100% of the applied radioactivity was recovered, indicating that formation of $^{14}CO_2$ or any other volatile compounds was negligible. No distinct differences were observed between the two labelled test substances used in the test. The hydrolysis at pH 7 (50 °C) and 9 (25 °C) in Tier 2 tests are presented in Table 25. Additional incubations were carried out at pH 7 at 60 and 70 °C and at pH 9 at 40 and 50 °C. While the results are not presented here, they were used to estimate DT_{50} and DT_{90} at each pH using the Arrenius plots.

After 50 days of incubation at pH 7 at 50 °C of fenpyrazamine, metabolite S-2188-DC was formed to a maximum of 59% of applied radioactivity (pyrazolyl label) and 49% (phenyl label); and S-2188-OH was found at a maximum of 10% of applied radioactivity (phenyl label) and 7.4% (pyrazolyl label). After 17 days of incubation at pH 9 at 25 °C of fenpyrazamine, metabolite S-2188-DC was formed at a maximum of 54% of applied radioactivity (both labels); and S-2188-OH was formed at a maximum of 4.7% of applied radioactivity (phenyl label) and 5.1% (pyrazolyl label).

Unidentified radioactivity was below 10% of applied radioactivity in all the tests.

Table 25 Hydrolysis of radiolabelled fenpyrazamine at pH 7 at 50 °C and 9 at 25 °C (lowest temperature tested at each pH) (Tier 2)

Days	Label position	% of Applied radioactivity				
		Fenpyrazamine	S-2188-DC	S-2188-OH	Others [a]	Total
pH 7, 50 °C						
0	Phenyl	96.4	< 0.1	< 0.1	0.6	97.0
0	Pyrazolyl	98.7	< 0.1	< 0.1	0.3	99.1
9	Phenyl	81.6	14.0	0.7	1.6	97.8
9	Pyrazolyl	82.2	14.9	0.2	0.6	97.9
17	Phenyl	69.0	25.3	1.4	3.4	99.1
17	Pyrazolyl	68.7	26.4	1.3	2.9	99.3
26	Phenyl	56.0	34.8	3.0	4.0	97.9
26	Pyrazolyl	55.9	37.1	2.8	3.4	99.3

Days	Label position	% of Applied radioactivity				
		Fenpyrazamine	S-2188-DC	S-2188-OH	Others [a]	Total
34	Phenyl	46.1	41.5	5.3	6.2	99.0
34	Pyrazolyl	48.6	43.1	4.4	4.3	100.4
42	Phenyl	41.5	45.2	6.6	5.9	99.2
42	Pyrazolyl	41.6	47.5	5.6	4.2	98.9
50	Phenyl	32.9	49.0	10.0	6.1	98.0
50	Pyrazolyl [b]	31.1	59.4	7.4	1.5	99.4
pH 9, 25 °C						
0	Phenyl	95.1	1.4	< 0.1	1.0	97.6
0	Pyrazolyl	96.3	1.3	< 0.1	0.4	98.1
2	Phenyl	85.0	10.9	0.8	1.4	98.2
2	Pyrazolyl	86.4	11.5	0.3	0.7	99.0
5	Phenyl	66.3	21.0	3.5	6.7	97.6
5	Pyrazolyl	68.7	22.5	2.6	3.9	97.7
8	Phenyl	57.4	34.5	2.8	4.7	99.4
8	Pyrazolyl	58.7	33.7	2.1	4.6	99.2
11	Phenyl	46.4	45.4	2.8	2.8	97.5
11	Pyrazolyl	47.8	47.4	1.6	2.0	98.8
14	Phenyl	39.2	47.6	4.7	5.9	97.3
14	Pyrazolyl	39.2	49.4	3.7	5.6	98.0
17	Phenyl	32.1	54.3	4.7	7.9	99.2
17	Pyrazolyl	34.7	54.0	5.1	5.8	99.5

[a] Sum of four and other unknowns and unresolved background.

[b] single sample only

Hydrolysis of fenpyrazamine in aqueous solution proceeds with the 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. The mean hydrolytic DT_{50} and DT_{90} values of two radiolabelled fenpyrazamine in sterile buffers at pH 7 and 9 calculated from the tests are presented in Table 26. Fenpyrazamine is stable at pH 4. It is also stable at an ambient temperature at pH 7.

Table 26 Calculated mean hydrolytic DT_{50} and DT_{90} values of radiolabelled fenpyrazamine in sterile buffers at pH 7 and 9 tested using the SFO kinetics

pH	Temperature (°C)	DT_{50} (days)	DT_{90} (days)
4	50	Stable	Stable
7	50	32.6	108.3
	20 [a]	2503	8314
9	25	11	36
	20 [a]	24	78

[a] extrapolated using Arrhenius plot and activation energy

Photolysis in aqueous solution (Lewis, C.J. & Troth, K., 2007d; QNM-0029)

The photolytic fate of radiolabelled fenpyrazamine (both pyrazolyl-5-labelled and phenyl labelled) was investigated in sterilized butter at pH 7 at 25±1 °C, over a period of 30 days. Phosphate buffer (0.01 M, pH 7) was dispensed into glass units and sterilized by autoclaving. Samples containing radiolabelled fenpyrazamine at ca 1.0 µg/mL were exposed to simulated sunlight for up to 30 days or were kept in the dark for a similar period. The lighting system filtered off light with a wavelength of < 290 nm and produced light with a similar spectrum to sunlight. The irradiation intensity was adjusted so that the light received per day was approximately equivalent to one day of UK/US summer sunlight (ca 25 W/m^2, 300-400 nm) with the resulting energy (2.2 MJ/m^2/day) about 3.3 times that of natural Japanese spring sunlight (0.672 MJ/m^2/day, 300–400 nm).

Units exposed to light were equipped with polyurethane foam bungs and attached to an ethanediol and two 2 M sodium hydroxide traps. Fenpyrazamine and degradation products were

detected and quantified by HPLC. Degradation products were confirmed by co-chromatography with reference compounds using two-dimensional TLC.

After 30 days, from irradiated and dark samples, 94–101% of the initial radioactivity was recovered, mostly in solutions.

Fenpyrazamine degraded to 1 or 2% of the applied radioactivity after 30 days. Carbon dioxide formed up to 10% from pyrazolyl-labelled fenpyrazamine but 1.5% from phenyl-labelled fenpyrazamine.

S-2188-DC was at its highest concentration (62–64% of the initial radioactivity) at 7 days then decreased to 7 to 10% of at 30 days. Another major degradation product was MCNI gradually increasing to 16–18% of the applied radioactivity after 30 days. There were up to seven and ten other compounds detected by HPLC after treatment with the pyrazolyl and phenyl labels, respectively, including S-2188-DTC (at its maximum at the end of the test) and one characterized as deoxygenated S-2188-DC. However, these were present at less than 10% of the applied radioactivity. (Table 27, Table 28 and Figure 4) These photolytic degradates were not found in the dark samples.

Table 27 Effect of irradiation on [pyrazolyl-5-^{14}C]-fenpyrazamine and its degradation products in pH 7 buffer (mean percent of applied radioactivity)

Days	Parent	DC	DTC	MCNI	Unk A	Polar Peak	Unk F	Unk G	Other Unk's	Undiff	Unres Bkg	Total
0	**96.8**	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.5	**97.4**
1	**63.4**	**29.7**	1.2	ND	ND	ND	ND	ND	1.8	**1.5**	0.6	**98.2**
2	**38.5**	**47.7**	2.1	0.4	2.1	ND	ND	ND	3.6	2	**1.4**	**97.8**
3	**29.5**	**55.3**	2.2	0.6	1.3	ND	ND	ND	3.4	3	1	**96.3**
7	**7.1**	**63.8**	4.2	2.6	3.8	ND	ND	0.7	8.4	7.3	0.4	**98.2**
20	2	**48.1**	**4.4**	9	2.8	4.2	ND	3.6	**7.1**	**11.2**	0.6	**92.9**
30	**1.1**	9.5	4.2	**17.7**	5	**13.9**	2.6	**4.5**	9.7	**18.1**	0.6	**86.8**

Table 28 Effect of irradiation on [phenyl-^{14}C]-fenpyrazamine and its degradation products in pH 7 buffer (mean percent of applied radioactivity)

Days	Parent	DC	DTC	MCNI	Unk A	Polar Peak	Unk F	Unk G	Other Unk's	Undiff	Unres Bkg	Total
0	**95.6**	ND	ND	ND	ND	ND	ND	ND	ND	**1**	**1.1**	**97.7**
1	**62.3**	**23.7**	**1.1**	0.3	2	ND	ND	ND	3.1	2.2	0.9	**95.6**
2	**40.8**	**36.4**	2.1	**1**	3	ND	ND	ND	2.7	9.5	**1.1**	**96.6**
3	**26.5**	**54.7**	3	**1**	3.8	ND	ND	ND	4.2	2.9	1.6	**97.7**
7	**4.4**	**61.7**	**4.8**	**4**	3.5	ND	ND	**1.1**	**8.7**	9.5	**0.8**	**98.4**
20	**1**	**37.1**	**4.7**	9.9	**8.5**	3.4	2.4	2.1	**11.4**	**10.8**	**1.1**	**92.3**
30	1.6	**7.4**	6.3	**15.7**	**4.7**	4.6	**7**	6.3	**16.8**	**21.3**	**0.5**	**92**

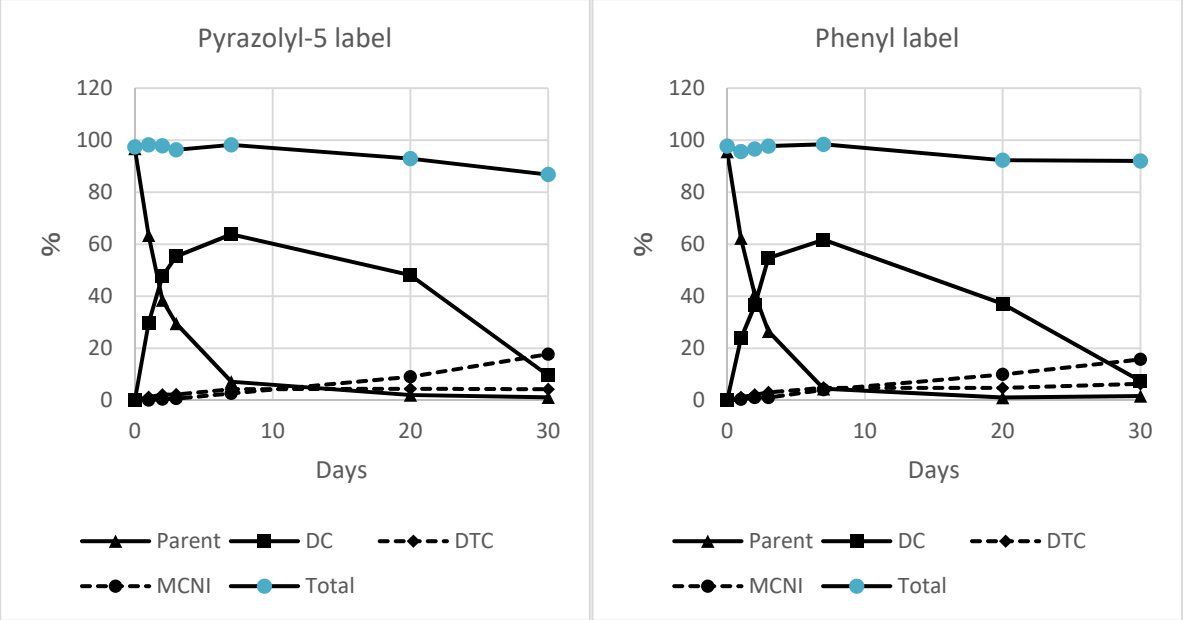


Figure 4 Photolysis of fenpyrazamine in pH 4 buffer

Under the UK/US summer sunlight, DT_{50} and DT_{90} of fenpyrazamine were calculated using the simple first-order kinetics as follows:

	DT_{50}, days	DT_{90}, days
Pyrazolyl label	1.7	5.5
Phenyl label	1.6	5.4

Fenpyrazamine is quite susceptible to photolysis in sterile buffer.

Photolysis on soil (C J Lewis K Troth, 2007, QNM-0020)

Photodegradation of fenpyrazamine was studied using radiolabelled fenpyrazamine on a surface of English soil (sand 5%, silt 29% and clay 20%; organic carbon 3.7%; pH 6.9) at 20±3 °C. Radiolabelled fenpyrazamine at 8.4 µg/g dry soil, equivalent to 0.84 kg/ha, assuming a penetration depth of 1 cm and a soil density of 1 g/cm^3, was added to about 5 g of soil spread on a metal plate to a depth of 3 to 4 mm. Soil layers were incubated at 20±3 °C and were adjusted to 75% of the maximum water holding capacity at 0.33 bar whenever samples were removed for analysis. Samples were continuously exposed to artificial sunlight or were incubated under equivalent conditions in the dark. Soil was irradiated with Xenon light, filtered to remove light below the wavelength of 290 nm, equivalent to 3.3-3.4 times the summer sunlight in Tokyo. The light intensity used was approximately equivalent to UK/US summer sunlight (ca 25 W/m^2). Moistened air was pulled over the surface of the soil samples and the units were connected to a series of traps that included an ethanediol trap, a 2% paraffin in xylene trap and two sodium hydroxide traps.

Duplicate samples were removed for analysis immediately following application and after 2, 7, 14, 21 and 30 days incubation. Each sample was sequentially extracted with methanol/water (5:1 v/v, 4 × 20 mL) (neutral extract) and methanol/0.5 M HCl (5:1 v/v, 3 × 20 mL) (acid extract). Neutral extracts were analysed separately from acid extracts. Concentrated extracts were analysed by HPLC and degradation products were confirmed in extracts by two-dimensional TLC. Radioactivity in the traps was quantified by LSC and carbon dioxide was confirmed by addition of barium chloride solution to the 2M sodium hydroxide traps. Radioactivity in the soil residues was quantified by combustion and LSC and bound residue fractionation was used to characterize the unextracted radioactivity at the terminal sampling interval.

There were no significant differences in the degradation of fenpyrazamine between irradiated sample and control (dark) sample. Carbon dioxide occurred under up to 2.9 and 8.4% of the applied radioactivity for phenyl- and pyrazolyl-labelled fenpyrazamine under irradiation, it occurred up to 1.6–2.4% of the applied radioactivity in the dark.

Methanol/water (5:1) extracted 96–97% of the applied radioactivity on day 0 gradually declining to 67–73% on day 30. In the extract, fenpyrazamine accounted for 96% of the applied radioactivity on day 0 and decreased to 67–70% on day 30. S-2188-OH was detected from day2 or 7, and at its highest on day 30 but still less than 1% of the applied radioactivity. S-2188-DC was detected only in the test with phenyl-labelled fenpyrazamine from day 21 but at less than 1% of the applied radioactivity. There were 3 unknown compounds but none exceeded 0.7% of the applied radioactivity. MCNI was not detected from either of the radiolabelled fenpyrazamine.

DT_{50} values were calculated using the simple first order kinetics to be 74–80 days. This indicates that fenpyrazamine is moderately stable to photolysis on the surface of soil than in aqueous solution.

		DT_{50}, days	DT_{90}, days
Light	Pyrazolyl label	74	246
	Phenyl label	80	265
Dark	Pyrazolyl label	50	167
	Phenyl label	60	199

RESIDUE ANALYTICAL METHODS

The Meeting received information on analytical methods together with validation data for the determination of residues of fenpyrazamine, S-2188-DC and/or S-2188-OH in plant commodities. In general, the methods involve extraction with aqueous acetone and a clean-up process with SPE or by gel permeation chromatography in case of the multi residue method. The determination of residues was performed by HPLC followed by tandem mass spectrometric detection.

Analytical methods for determination of fenpyrazamine residues

Analytical methods for plant matrices

Method SUM-0731V (HPLC-MS/MS) (Rzepka S., Jungklaus N., 2007: QNA-0008)

Matrix	Grapes, Oil Seed Rape (Seeds), Carrot, Green Pepper and Cereals (Grain and Straw)
Analyte:	Fenpyrazamine, S-2188-DC
LOQ:	0.01 mg/kg
Description:	Extraction: Following an initial addition of 1M sodium ascorbate solution, fenpyrazamine was extracted twice from samples of grapes (berries), oil seed rape (seeds), carrot (roots), green pepper (fruits) and cereal (straw and grain) using acetone/water (4:1, v/v) and was then made to volume using the same solvent. Clean-up: An aliquot was reduced in volume by evaporation and cleaned up on an Oasis HLB SPE cartridge. The cartridge was initially conditioned with acetonitrile, followed by water. The extract was loaded onto the cartridge and the flask was rinsed with water, followed by acetonitrile/water (1:10, v/v). The rinsing was added to the cartridge and the residues were eluted using acetonitrile/water (1:1, v/v). The extract was made to volume. Determination of residues: Residues were analysed by LC-MS/MS with monitoring of the ion transitions: Fenpyrazamine: m/z = 332 → 230 and m/z = 332 → 272 S-2188-DC: m/z = 232 → 190 and m/z = 232 → 145

Method RM-45C (HPLC-MS/MS) (Kowalsky J., 2008: Private company method)

Matrix	Grapes
Analyte:	Fenpyrazamine, S-2188-DC
LOQ:	0.02 mg/kg
Description:	Extraction and Clean-up: Identical to Method SUM-0731V above. Determination of residues: Residues were analysed by LC-MS/MS with monitoring of the ion transitions: Fenpyrazamine: m/z = 332 → 230 and m/z = 332 → 189 S-2188-DC: m/z = 232 → 190 and m/z = 232 → 145

Method RM-45C-1 ((HPLC-MS/MS) (Kowalsky J., 2010: Private company method)

Matrix	Grapes, almond hulls and nutmeat
Analyte:	Fenpyrazamine, S-2188-DC
LOQ:	0.02 mg/kg
Description:	The method is a modification of method RM-45C above for difficult matrices that require additional clean-up steps, i.e. purple-coloured grapes and almond hulls and nut meat. A smaller sample size was used, an additional dichloromethane partition step between the extraction and the Oasis HLB column was added, and there was no dilution after the clean-up column.

Method SUM-0706V (HPLC-MS/MS) (Rzepka S., Jungklaus N., 2007: QNA-0009)

Matrix	Grapes, Oil Seed Rape (Seeds), Carrot, Green Pepper and Cereals (Grain and Straw)
Analyte:	S-2188-OH
LOQ:	0.01 mg/kg
Description:	Extraction: S-2188-OH was extracted twice from samples of grapes (berries), oil seed rape (seeds), carrot (roots), green pepper (fruits) and cereal (straw and grain) using acetone/water (4:1, v/v) and was then made to volume using the same solvent. Clean-up: An aliquot was reduced in volume by evaporation and cleaned up on an Oasis HLB SPE cartridge. The cartridge was initially conditioned with acetonitrile, followed by water. The extract was loaded onto the cartridge and the flask was rinsed with water. The rinsing was added to the cartridge and the residues were eluted using acetonitrile/water (1:1, v/v). The extract was made to volume. Determination of residues: Residues were analysed by LC-MS/MS with monitoring of the ion transitions: S-2188-OH: m/z = 248 → 230 and m/z = 248 → 132

Cornell Analytical Laboratory (CAL) Method ver.1: Cornell Analytical Laboratory method: Residue Analysis of V-10135 on Ginseng by LC/MS/MS Detection; ver. 1

Matrix	Ginseng
Analyte:	Fenpyrazamine, S-2188-DC
LOQ:	0.02 mg/kg
Description:	Extraction: An aliquot of the homogenized sample (10 g) was weighed and stirred with 1M Sodium Ascorbate (5 g). Acetone/water (4:1, v/v; 60 mL) is added and the sample shaken for ~20 minutes. After filtration, the extract is evaporated to the aqueous reminder under reduced pressure. Clean-up: A sample aliquot is transferred to an Oasis HLB cartridge. After rinsing with water

and acetonitrile/water (1:10, v/v), residues were eluted with acetonitrile/water (1:1, v/v).

Determination of residues: Residues were analysed by LC-MS/MS with monitoring of the ion transitions:

Fenpyrazamine: m/z = 332 → 230
S-2188-DC: m/z = 232 → 190

Multi-residue Methods

Method L-00.00-34 (an Committee for Standardization) - formerly DFG S19: (HPLC-MS/MS) (Rzepka S., Jungklaus N., 2007: QNA-0006)

Matrix	Grapes, Rapeseed (Seeds), Carrot, Pepper and Cereals (Grain and Straw)
Analyte:	Fenpyrazamine
LOQ:	0.01 mg/kg
Description:	Extraction I (grape, carrot, pepper, cereal): Prior to extraction of grape the pH value of the specimens was adjusted to at least 8 by addition of sodium hydrogen carbonate. For all crops, water was added to adjust the total water present in the sample to 100 mL and mixed well by means of a glass rod. Thereafter, 200 mL of acetone were added and the specimen material was homogenized for 2 min using an Ultra-Turrax. Then 100 mL of ethyl acetate/cyclohexane (1:1, v/v) and 35 g of sodium chloride were added and homogenized again for 1 min. The phases were allowed to separate for 30 to 60 min. An aliquot of the organic phase was filtered through cotton wool covered with sodium sulphate into a round-bottom flask. The filter and the flask were rinsed four times with approx. 20 mL portions of ethyl acetate/cyclohexane (1:1, v/v). The filtrate was evaporated to an aqueous remainder (not to dryness). Exactly 7.5 mL of ethyl acetate were added to the evaporation residue. The residue was dissolved completely, immersing the flask in an ultrasonic bath. Approx. 5 g of a mixture of sodium sulphate/sodium chloride (1:1, w/w) were added and swirled. Then exactly 7.5 mL of cyclohexane were added to obtain a total volume of 15.0 mL and swirled vigorously again. The salt mixture was allowed to deposit. Extraction II (oilseed rape): The oil seed rape method involved blending the seeds with acetone, acetonitrile, Calflo E and Celite before gentle vacuum filtering. The filtrate was filtered again through a dry fluted filter covered with Calflo E and the volume of filtrate was measured. Isooctane was added to the filtrate and the volume was reduced by rotary evaporation. The last traces of solvent were removed by a gentle stream of nitrogen and the residues were reconstituted in ethyl acetate/cyclohexane (1:1, v/v). Clean-up: The extract from all matrices were then subjected to clean up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1:1, v/v) as eluent. The eluate was evaporated to dryness and reconstituted in ethyl acetate. Determination of residues: A further aliquot was evaporated to dryness, reconstituted in methanol, made to volume with water and analysed for residues of fenpyrazamine by LC-MS/MS with monitoring of the ion transitions m/z= 332→230 (quantitation) and m/z= 332→272 (confirmation), using a UPLC BEH C18 column.

Independent method validation of L-00.00-34 (DFG S19) (Bacher R. 2008, PTRL report no. P/B 1402 G)

Matrix	Grapes, Oil Seed Rape (Seeds), Carrot, Pepper and Cereals (Grain and Straw)
Analyte:	Fenpyrazamine
LOQ:	0.01 mg/kg
Description:	No significant deviations from the Method L-00.00-34 (DFG S19). The only deviation, the second mass transition for confirmation of fenpyrazamine was m/z=332→216 instead of m/z=332→272.

Method Validation for plant commodities

Validation data for the methods used for determining fenpyrazamine, S-2188-DC and S-2188OH in plant commodities are summarized below. Mean recoveries were within the acceptable range of 70–120% with RSD values ≤ 19%.

Table 29 Summary of method validation for plant commodities

Matrix	Analyte	Fortification (mg/kg)	n	Recovery (%)		RSD (%)	Method	Reference
				Range	Mean			
Grape (berries)	Fenpyrazamine m/z=332→230	0.01	5	96-110	102	5.2	SUM-0731V	SUM-0731V (QNA-0008)
		0.10	5	92-106	98	5.3		
	Fenpyrazamine m/z=332→272	0.01	5	91-110	97	8.2		
		0.10	5	86-109	94	9.7		
	S-2188-DC m/z=232→190	0.01	5	90-112	103	9.8		
		0.10	5	96-110	101	5.4		
	S-2188-DC m/z=232→145	0.01	5	94-110	105	6.0		
		0.10	5	91-107	99	5.8		
Oilseed Rape (seed)	Fenpyrazamine m/z=332→230	0.01	5	71-80	77	4.4	SUM-0731V	SUM-0731V (QNA-0008)
		0.10	5	72-78	75	3.1		
	Fenpyrazamine m/z=332→272	0.01	5	70-86	79	8.6		
		0.10	5	73-78	76	2.5		
	S-2188-DC m/z=232→190	0.01	5	86-103	93	6.6		
		0.10	5	84-93	89	3.8		
	S-2188-DC m/z=232→145	0.01	5	79-110	97	13		
		0.10	5	79-100	86	11		
Carrot (roots)	Fenpyrazamine m/z=332→230	0.01	5	79-87	84	3.7	SUM-0731V	SUM-0731V (QNA-0008)
		0.10	5	82-86	84	1.9		
	Fenpyrazamine m/z=332→272	0.01	5	79-91	85	5.9		
		0.10	5	81-84	83	1.6		
	S-2188-DC m/z=232→190	0.01	5	83-90	87	3.2		
		0.10	5	85-90	87	2.4		
	S-2188-DC m/z=232→145	0.01	5	86-94	90	3.9		
		0.10	5	89-93	91	1.6		
Pepper (fruit)	Fenpyrazamine m/z=332→230	0.01	5	97-105	100	3.1	SUM-0731V	SUM-0731V (QNA-0008)
		0.10	5	89-99	94	4.3		
	Fenpyrazamine m/z=332→272	0.01	5	92-108	100	6.7		
		0.10	5	92-97	94	2.0		
	S-2188-DC m/z=232→190	0.01	5	95-105	98	4.2		
		0.10	5	85-100	91	6.0		
	S-2188-DC m/z=232→145	0.01	5	84-109	92	11		
		0.10	5	85-96	90	4.3		
Cereal (straw)	Fenpyrazamine m/z=332→230	0.01	5	70-78	73	4.5	SUM-0731V	SUM-0731V (QNA-0008)
		0.10	5	79-85	82	2.7		
	Fenpyrazamine m/z=332→272	0.01	5	70-78	74	4.1		
		0.10	5	78-86	81	4.0		
	S-2188-DC m/z=232→190	0.01	5	76-87	83	5.3		
		0.10	5	82-84	83	1.3		
	S-2188-DC m/z=232→145	0.01	5	87-100	93	5.5		
		0.10	5	85-93	89	3.4		
Cereal (grain)	Fenpyrazamine m/z=332→230	0.01	5	77-83	79	2.9	SUM-0731V	SUM-0731V (QNA-0008)
		0.10	5	70-81	76	5.3		
	Fenpyrazamine m/z=332→272	0.01	5	74-92	84	8.9		
		0.10	5	72-80	76	4.2		
	S-2188-DC m/z=232→190	0.01	5	84-90	88	2.6		
		0.10	5	80-84	82	2.0		
	S-2188-DC m/z=232→145	0.01	5	70-88	79	10		
		0.10	5	81-86	84	2.3		
Grape (berries)	S-2188-OH m/z=248→230	0.01	5	93-103	100	4.6	SUM-0706V	SUM-0706V (QNA-0009)
		0.10	5	90-103	96	6.2		
	S-2188-OH m/z=248→132	0.01	5	77-101	85	12		
		0.10	5	79-106	96	12		

Matrix	Analyte	Fortification (mg/kg)	n	Recovery (%)		RSD (%)	Method	Reference
				Range	Mean			
Oilseed Rape (seed)	S-2188-OH m/z=248→230	0.01	5	71-101	80	17	SUM-0706V	SUM-0706V (QNA-0009)
		0.10	5	81-94	88	6.0		
	S-2188-OH m/z=248→132	0.01	5	72-106	91	14		
		0.10	5	81-98	87	7.5		
Carrot (roots)	S-2188-OH m/z=248→230	0.01	5	81-109	96	13	SUM-0706V	SUM-0706V (QNA-0009)
		0.10	5	76-91	86	7.5		
	S-2188-OH m/z=248→132	0.01	5	72-110	86	19		
		0.10	5	90-98	93	3.3		
Pepper (fruit)	S-2188-OH m/z=248→230	0.01	5	84-97	89	6.3	SUM-0706V	SUM-0706V (QNA-0009)
		0.10	5	79-88	82	4.5		
	S-2188-OH m/z=248→132	0.01	5	90-100	95	4.4		
		0.10	5	77-89	82	5.8		
Cereal (straw)	S-2188-OH m/z=248→230	0.01	5	97-125	108	11	SUM-0706V	SUM-0706V (QNA-0009)
		0.10	5	81-103	92	9.2		
	S-2188-OH m/z=248→132	0.01	5	74-104	92	14		
		0.10	5	78-96	87	8.2		
Cereal (grain)	S-2188-OH m/z=248→230	0.01	5	75-98	87	11	SUM-0706V	SUM-0706V (QNA-0009)
		0.10	5	74-82	78	3.8		
	S-2188-OH m/z=248→132	0.01	5	70-100	84	13		
		0.10	5	79-88	83	4.9		
Grapes (berries)	Fenpyrazamine m/z=332→230	0.01	5	96-110	102	5.2	SUM-0731V	GGU-06-1765 (QNR-0004)
		0.10	5	92-106	98	5.3		
	Fenpyrazamine m/z=332→272	0.01	5	91-110	97	8.2		
		0.10	5	86-109	94	9.7		
	S-2188-DC m/z=232→190	0.01	5	90-112	103	9.8		
		0.10	5	96-110	101	5.4		
	S-2188-DC m/z=232→145	0.01	5	94-110	105	6.0		
		0.10	5	91-107	99	5.8		
Grape (berries)	Fenpyrazamine m/z=332→230	0.02	3	89-95	92	3.4	RM-45C	VP-30097 (QNR-0088)
		0.10	6	86-99	91	5.5		
	S-2188-DC m/z=232→190	0.02	3	103-109	106	2.8		
		0.10	6	86-99	94	5.0		
Raspberries (berries)	Fenpyrazamine m/z=332→230	0.02	3	86-89	87	2.0	RM-45C	IR-4 PR No. 09444 (QNR-0123)
		0.2	3	96-98	97	1.0		
		2.0	3	100-101	101	0.6		
	S-2188-DC m/z=232→190	0.02	3	108-116	113	3.9		
		0.2	3	118-121	120	1.4		
		2.0	3	109-114	111	2.6		
Blueberries (berries)	Fenpyrazamine m/z=332→230	0.02	3	86-89	88	2.0	RM-45C	IR-4 PR No. 09445 (QNR-0122)
		0.2	3	95-97	96	1.0		
		2.0	3	88-94	90	3.6		
	S-2188-DC m/z=232→190	0.02	3	95-100	98	2.9		
		0.2	3	91-94	93	1.6		
		2.0	3	102-108	105	2.9		
Lettuce	Fenpyrazamine m/z=332→230	0.02	5	76-119	95	17	RM-45C-1	VP-30097 (QNR-0088)
		0.10	5	78-101	88	11		
	S-2188-DC m/z=232→190	0.02	5	78-94	84	7.6		
		0.10	5	74-87	79	6.3		
Ginseng (roots)	Fenpyrazamine m/z=332→230	0.02	3	70	70	0	CAL	IR-4 PR No. 09453 (QNR-0121)
		0.20	3	80-85	82	3.5		
		2.0	3	80	80	0		
	S-2188-DC m/z=232→190	0.02	3	100-110	107	5.4		
		0.20	3	100-110	107	5.4		
		2.0	3	105-110	108	2.7		
Grapes (berries)	Fenpyrazamine m/z=332→230	0.01	5	97-110	105	5.7	L-00.00-34	SUM 0701V (QNA-0006)
		0.10	5	100-110	107	3.6		
	Fenpyrazamine m/z=332→272	0.01	5	95-110	104	5.3		
		0.10	5	96-109	102	4.6		
Oilseed Rape (seeds)	Fenpyrazamine m/z=332→230	0.01	5	77-85	82	4.1	L-00.00-34	SUM 0701V (QNA-0006)
		0.10	5	84-88	87	2.0		

Matrix	Analyte	Fortification (mg/kg)	n	Recovery (%)		RSD (%)	Method	Reference
				Range	Mean			
	Fenpyrazamine m/z=332→272	0.01	5	81-89	83	4.1		
		0.10	5	78-91	85	6.6		
Carrot (roots)	Fenpyrazamine m/z=332→230	0.01	5	83-117	97	14	L-00.00-34	SUM 0701V (QNA-0006)
		0.10	5	94-104	100	4.0		
	Fenpyrazamine m/z=332→272	0.01	5	83-107	91	11		
		0.10	5	93-99	96	2.8		
Pepper (fruit)	Fenpyrazamine m/z=332→230	0.01	5	89-97	92	3.6	L-00.00-34	SUM 0701V (QNA-0006)
		0.10	5	81-91	86	4.2		
	Fenpyrazamine m/z=332→272	0.01	5	82-94	89	5.3		
		0.10	5	79-91	86	6.7		
Cereal (straw)	Fenpyrazamine m/z=332→230	0.01	5	80-95	90	6.7	L-00.00-34	SUM 0701V (QNA-0006)
		0.10	5	80-96	90	7.0		
	Fenpyrazamine m/z=332→272	0.01	5	79-89	85	5.1		
		0.10	5	79-93	88	7.0		
Cereal (grain)	Fenpyrazamine m/z=332→230	0.01	5	92-108	97	6.7	L-00.00-34	SUM 0701V (QNA-0006)
		0.10	5	80-101	95	9.2		
	Fenpyrazamine m/z=332→272	0.01	5	95-110	101	6.2		
		0.10	5	82-98	94	7.1		
Grapes (berries)	Fenpyrazamine m/z=332→230	0.01	5	80-88	83	4.0	L-00.00-34	P 1402 G (QNA-0014)
		0.10	5	97-114	106	6.6		
	Fenpyrazamine m/z=332→216	0.01	5	83-90	86	3.8		
		0.10	5	98-114	106	5.6		
Oilseed Rape (seeds)	Fenpyrazamine m/z=332→230	0.01	5	75-98	81	12	L-00.00-34	P 1402 G (QNA-0014)
		0.10	5	85-101	95	6.7		
	Fenpyrazamine m/z=332→216	0.01	5	77-96	84	8.7		
		0.10	5	88-100	94	5.3		
Tomato (fruit)	Fenpyrazamine m/z=332→230	0.01	5	93-108	102	5.9	L-00.00-34	P 1402 G (QNA-0014)
		0.10	5	70-110	94	19		
	Fenpyrazamine m/z=332→216	0.01	5	90-106	99	7.1		
		0.10	5	68-110	93	19		
Cereal (grain)	Fenpyrazamine m/z=332→230	0.01	5	68-80	74	7.5	L-00.00-34	P 1402 G (QNA-0014)
		0.10	5	75-86	82	5.4		
	Fenpyrazamine m/z=332→216	0.01	5	71-80	75	5.8		
		0.10	5	77-86	83	4.7		

Extraction Efficiency

The extraction efficiency of the residue analytical methods was investigated by comparison of the extraction procedures with the results previously obtained from the metabolism data. The compounds analysed were fenpyrazamine, S-2188-DC and S-2188-OH in grapes, lettuce, rapeseed (seeds), carrot, green pepper and cereals (grain and straw). All methods included an extraction twice with acetone/water (4:1, v/v) or, for the multi-residue method L-00.00-34, once with acetone/water (2:1, v/v). This extraction procedure is the same (or at least similar for method L-00.00-34) with the extraction performed in the plant metabolism studies. A sufficiently high amount of radioactivity in the range of 98–99% was recovered from grape (berries and foliage) and lettuce, 90–96% from rapeseed foliage and stalk when the crop was surface washed and extracted with acetone/water. The preceding surface wash is assumed to be also covered by the extraction step of the methods for the residues on the surface. In rapeseed (seeds) extraction with acetonitrile/water followed by extraction with water recovered 44–52% of the radioactivity.

Analytical methods for plant matrices

The Meeting received information on an analytical method for the determination of fenpyrazamine in animal commodities (Richter S., 2014; QNA 0038). The method was derived from the QuEChERS (EN 15662) multi-residue method.

Matrix	Meat, liver, fat, milk and eggs
Analyte:	Fenpyrazamine
LOQ:	0.005 mg/kg for milk and 0.01 mg/kg for other matrices
Description:	Extraction: Fenpyrazamine was extracted from samples by extraction by homogenization with a mixture of acetonitrile and water (10:1 for milk, and 10:6 for eggs and tissues). After addition of $MgSO_4$, NaCl and buffering citrate salts, the mixture is shaken intensively and centrifuged for phase separation. Clean-up: An aliquot was the organic extract is cleaned-up by dispersive SPE with PSA and $MgSO_4$. For animal fat the clean-up with dispersive SPE was done after freezing out and with addition of C_{18}. Determination of residues: Fenpyrazamine was analysed by LC-MS/MS in the positive ionization mode isolating the 332 m/z mother ion and monitoring two different fragment ions for quantitation (189 m/z) and confirmation (216 m/z; for animal fat 272 m/z was used)

Method Validation for animal commodities

Validation data for the methods used for determining fenpyrazamine in animal commodities are summarized below. Mean recoveries were within the acceptable range of 88–110% with RSD values ≤ 6%.

Table 30 Summary of method validation for animal commodities

Matrix	Fenpyrazamine	Fortification (mg/kg)	n	Mean Recovery (%)	RSD (%)
Milk	m/z=332→189	0.005	6	110	1
		0.05	6	99	3
	m/z=332→216	0.005	6	110	1
		0.05	6	106	2
Bovine meat	m/z=332→189	0.01	6	96	3
		0.10	6	97	4
	m/z=332→216	0.01	6	96	3
		0.10	6	96	1
Bovine liver	m/z=332→189	0.01	5	104	6
		0.10	5	109	4
	m/z=332→216	0.01	5	106	2
		0.10	5	109	4
Eggs	m/z=332→189	0.01	5	102	5
		0.10	5	95	5
	m/z=332→216	0.01	5	102	5
		0.10	5	95	3
Fat	m/z=332→189	0.01	5	98	5
		0.10	5	89	2
	m/z=332→216	0.01	5	96	6
		0.10	5	88	3

Storage Stability under Frozen Conditions

The stability of fenpyrazamine residues in samples stored frozen was investigated in various plant matrices (grape, grape juice, dried grape, strawberry, raspberry, blueberry, lettuce, ginseng root, cereal grains, rapeseed and almond hulls).

In most studies, homogenized samples were fortified by fenpyrazamine, S-2188-DC or S-2188-OH at 0.1 or 0.2 mg/kg and stored at -18 °C or below. At the time of initiation of storage and after certain interval of frozen storage, samples were analysed for fortified analytes. In some studies, 1M sodium ascorbate was added to samples to see the effect of ascorbate on stabilization. For the dried grapes, the concentration after the processing was used as the initial concentration and set for 100% and the samples were stored with 1M sodium ascorbate. The results are shown in Table 31.

Percent of fenpyrazamine and its metabolites remaining in the frozen samples was not corrected for procedural recoveries.

Procedural recovery data obtained concurrently with the field trials sample analyses for field trials on grapes, strawberries and almond performed in the USA were summarized in the residue trial section.

Table 31 Storage stability of fenpyrazamine, S-2188-DC and S-2188-OH in various plant matrices under frozen conditions

Analyte (analytical method)	Fortification, mg/kg	Temp., °C	Storage, days	Concentration, mg/kg	% Remaining (mean %)	Procedural recovery, %
Grape berry						
Fenpyrazamine	0.1	-18	0	0.094, 0.096, 0.097	100	97
(SUM-0731V) [a)]			28	0.085, 0.089	91	88
			85	0.085, 0.085	89	82
			182	0.079, 0.083	84	79
			364	0.092, 0.096	98	93
S-2188-DC	0.1	-18	0	0.087, 0.091, 0.092	100	91
(SUM-0731V) [a)]			28	0.087, 0.087	97	93
			85	0.105, 0.106	121	108
			182	0.082, 0.087	94	96
			364	0.081, 0.083	91	90
S-2188-OH	0.1	-18	0	0.078, 0.082, 0.078	100	79
(SUM-0706V) [b)]			28	0.100, 0.095	124	95
			84	0.087, 0.089	111	89
			180	0.083, 0.084	106	82
			272	0.089, 0.086	111	96
			366	0.074, 0.083	100	72
Fenpyrazamine	0.1	-18	0	0.0793, 0.0919, 0.0794	100	—
(RM-45C, 45C-1) [c)]			32	0.1001, 0.0881	113	115
			77	0.0764, 0.0864	98	87
			96	0.0844, 0.0830	102	99
			293	0.0677, 0.0737	86	77
			1065	0.0714, 0.0746	83	95
S-2188-DC	0.1	-18	0	0.0870, 0.0867, 0.0770	100	—
(RM-45C, 45C-1) [c)]			32	0.0857, 0.0786	99	98
			77	0.0937, 0.1108	123	115
			96	0.0937, 0.0962	113	112
			293	0.0875, 0.0793	100	88
			1065	0.0354, 0.0406	40	102
Fenpyrazamine	0.1	-18	0	0.0840, 0.0861, 0.0882	100	—
w/1M ascorbate			32	0.0958, 0.1127	122	115
(RM-45C, 45C-1) [c)]			77	0.0794, 0.0774	91	87
			96	0.0893, 0.0857	103	99
			293	0.0769, 0.0774	90	79
			1065	0.0727, 0.0657	76	91
S-2188-DC	0.1	-18	0	0.0926, 0.0845, 0.0898	100	—
w/1M ascorbate			32	0.0821, 0.0851	94	99
(RM-45C, 45C-1) [c)]			77	0.1048, 0.1046	118	110
			96	0.1027, 0.1034	116	111
			293	0.0857, 0.1184	115	98
			1065	0.0589, 0.0624	64	102
Grape Juice						
Fenpyrazamine	0.1	-18	0	0.0776, 0.0830, 0.0818	100	—
(RM-45C, 45C-1) [c)]			67	0.0816, 0.0810	101	91
			330	0.0646, 0.0617	74	75
S-2188-DC	0.1	-18	0	0.0774, 0.0839, 0.0811	100	—
(RM-45C, 45C-1)			67	0.0700, 0.0723	89	83

Analyte (analytical method)	Fortification, mg/kg	Temp., °C	Storage, days	Concentration, mg/kg	% Remaining (mean %)	Procedural recovery, %
[c]						
			330	0.0642, 0.0729	84	73
Dried grape (from processing study)						
Fenpyrazamine	0.1	-18	0	4.30, 4.16 (4.23)	100	83, 77, 73, 110
w/1M ascorbate			495	3.83, 3.68 (3.75)	89	72
(RM-45C, 45C-1) [c]						
S-2188-DC	0.1	-18	0	1.78, 1.67 (1.72)	100	71, 70, 70, 109
w/1M ascorbate			495	1.46, 1.40 (1.43)	83	77
(RM-45C, 45C-1) [c]						
Strawberry						
Fenpyrazamine	0.1	-18	0	0.0914, 0.0902, 0.0941	100	—
(RM-45C) [d]			30	0.0920, 0.0921	99	95
			76	0.0684, 0.0814	82	92
			114	0.0664, 0.0757	77	92
			337	0.0501, 0.0597	60	67
S-2188-DC	0.1	-18	0	0.0897, 0.0857, 0.0857	100	—
(RM-45C) [d]			30	0.0680, 0.0676	78	78
			76	0.0701, 0.0743	83	100
			114	0.0588, 0.0677	74	100
			337	0.0311, 0.0295	34	92
Fenpyrazamine	0.1	-18	0	0.1020, 0.1027, 0.0923	100	—
w/1M ascorbate			30	0.0832, 0.0766	80	91
(RM-45C) [d]			76	0.0942, 0.1010	99	111
			114	0.0764, 0.0852	82	99
			337	0.0598, 0.0562	59	70
S-2188-DC	0.1	-18	0	0.0995, 0.0997, 0.0904	100	—
w/1M ascorbate			30	0.0733, 0.0708	73	80
(RM-45C) [d]			76	0.0849, 0.0837	88	90
			114	0.0959, 0.0986	101	105
			337	0.1095, 0.0762	96	127
Raspberry						
Fenpyrazamine	0.2	-20	320	0.180, 0.181, 0.191	100	93
(RM-45C) [e]			384	0.187, 0.194, 0.188	103	94
S-2188-DC	0.2	-20	320	0.163, 0.157, 0.156	100	97
(RM-45C) [e]			384	0.163, 0.170, 0.166	105	96
Blueberry						
Fenpyrazamine	0.2	-20	258	0.181, 0.179, 0.178	100	97
(RM-45C) [f]			349	0.175, 0.171, 0.174	97	90
S-2188-DC	0.2	-20	258	0.193, 0.203, 0.196	100	95
(RM-45C) [f]			349	0.210, 0.207, 0.211	85	116
Lettuce						
Fenpyrazamine	0.1	-18	0	0.087, 0.089, 0.090	100	87
(SUM-0731V) [a]			28	0.085, 0.085	96	88
			89	0.080, 0.083	92	76
			182	0.073, 0.077	84	80
			364	0.084, 0.086	96	89
S-2188-DC	0.1	-18	0	0.089, 0.090, 0.092	100	90
(SUM-0731V) [a]			28	0.081, 0.082	91	90
			85	0.104, 0.112	120	103
			182	0.070, 0.078	82	85
			364	0.076, 0.083	89	102
S-2188-OH	0.1	-18	0	0.071, 0.075, 0.077	100	74
(SUM-0706V) [b]			28	0.075, 0.082	107	92
			84	0.050, 0.054	70	97
			180	0.060, 0.098	107	80
			272	0.043, 0.042	58	89
			366	0.064, 0.046	74	83

Analyte (analytical method)	Fortification, mg/kg	Temp., °C	Storage, days	Concentration, mg/kg	% Remaining (mean %)	Procedural recovery, %
Fenpyrazamine	0.1	-18	0	0.090, 0.095, 0.092	100	—
w/1M ascorbate			510	0.077, 0.075	83	82
(RM-45C-1) [g)]						
S-2188-DC	0.1	-18	0	0.080, 0.080, 0.080	100	—
w/1M ascorbate			510	0.073, 0.073	91	77
(RM-45C-1) [g)]						
Ginseng Root						
Fenpyrazamine	0.2	-18	0	0.16, 0.16, 0.16	100	80
w/1M ascorbate			350	0.16, 0.16, 0.16	100	80
(RM-45C-1) [g)]						
S-2188-DC	0.2	-18	0	0.24, 0.22, 0.21	100	100
w/1M ascorbate			350	0.15, 0.15, 0.16	69	130
(RM-45C-1) [g)]						
Cereal grain						
Fenpyrazamine	0.1	-18	0	0.073, 0.075, 0.079	100	79
(SUM-0731V) [a)]			28	0.067, 0.075	90	74
			89	0.051, 0.064	76	68
			182	0.064, 0.081	96	77
			364	0.073, 0.074	97	81
S-2188-DC	0.1	-18	0	0.072, 0.076, 0.082	100	72
(SUM-0731V) [a)]			28	0.066, 0.074	91	74
			85	0.076, 0.083	103	97
			182	0.057, 0.070	83	80
			364	0.066, 0.072	90	80
S-2188-OH	0.1	-18	0	0.079, 0.076, 0.075	100	77
(SUM-0706V) [b)]			28	0.085, 0.079	106	93
			84	0.083, 0.088	112	86
			180	0.080, 0.080	104	77
			272	0.090, 0.084	113	97
			366	0.100, 0.098	128	95
Rapeseed (seed)						
Fenpyrazamine	0.1	-18	0	0.066, 0.068, 0.076	100	66
(SUM-0731V) [a)]			28	0.072, 0.075	106	73
			89	0.065, 0.066	94	77
			182	0.065, 0.070	97	70
			364	0.053, 0.059	80	67
S-2188-DC	0.1	-18	0	0.075, 0.076, 0.076	100	75
(SUM-0731V) [a)]			28	0.066, 0.070	89	75
			85	0.072, 0.080	100	75
			182	0.061, 0.064	83	74
			364	0.065, 0.075	92	79
S-2188-OH	0.1	-18	0	0.072, 0.072, 0.071	100	72
(SUM-0706V) [b)]			28	0.075, 0.072	103	76
			84	0.080, 0.084	114	79
			180	0.078, 0.079	110	76
			272	0.081, 0.089	118	75
			366	0.085, 0.082	117	85
Almond hull						
Fenpyrazamine	0.1	-20	0	0.0685, 0.0697	100	66
(RM-45C-1) [h)]			440	0.0698, 0.0628	109	72

[a] Rzepka, S., 2008, and Amendment No. 1 by Daneva, E., 2009: QNR-0012

[b] Rzepka, S., 2009, and Amendment No. 1: Daneva, E., 2009: QNR-0034

[c] Kowalsky J., 2011; QNR-0088

[d] Kowalsky J., ,2010; QNR-0091

[e] Switek, T., 2011: QNR-0123

[f] Switek, T., 2011: QNR-0122

[g] Kowalsky J., 2011; QNR-0090

[h] Kowalsky J., 2011; QNR-0087

The results above showed that fenpyrazamine, S-2188-DC and S-2188-OH were stable up to about one year in the homogenized samples stored frozen at -18 °C or below, except strawberry when the percent remaining is calculated from the concentration of the day 0 as 100%. In some studies, the initial concentrations in homogenized samples were much lower (around 70%) than the planned fortification concentration. 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. In grape berries, fenpyrazamine and S-2188-DC were less than 70% of the initial concentration 1065 days of frozen storage with and without 1M ascorbate but was stable up to 293 days of storage.

USE PATTERN

Fenpyrazamine is intended for post-emergence use as a fungicide by foliar spray application for the control of *Botrytis cinerea* and various Monilia species on various crops: strawberries, cucumber, eggplant, pepper and tomato grown under glasshouse conditions and stone fruits, able and wine grapes, strawberries, bush and cane berries, lettuce, ginseng, almonds, and pistachio grown under field conditions. Fenpyrazamine is registered in many countries. Table 32 indicates registered uses in countries or regions where supervised trials were conducted or in countries with GAPs relevant to the supervised trials.

Table 32 Registered uses of fenpyrazamine for the crops for which supervised trials were conducted. All uses employ foliar spray method.

Crop	Country	Conc., g ai/kg, Form	Application					PHI days
			Max g ai/ha	Max g ai/hL	L/ha	Max No. (g ai/ha/ season)	Interval day	
Stone fruits								
Apricot, cherry, nectarine, peach, plum	Austria	500 WG	600		500/m	3 BBCH 75-87	7	1
Apricot, peach, nectarine	Italy	500 WG	600	40–60		3	7	3
	Spain	500 WG	600		500-1500	3 [e)]	7	1
Peach, nectarine	France	500 WG	600			3 BBCH 61-87	7	1
	Greece	500 WG	600	40–60	1000	3 BBCH 61-87	7	1
	Hungary	500 WG	600		500-1200	3 BBCH 61-87	7	1
	Poland	500 WG	600		500-1000	3 BBCH 61-87	7	1
Berries and other small fruits								
Cane berries	USA.	360 SC	560			3 [a)] (1680)	7–14	0
Blueberries	USA.	360 SC	560			3 [a)] (1680)	7–14	0
Table grapes	Austria	500 WG	600		1000	1 BBCH 61-85		14
	France	500 WG	600			1 BBCH 61-87		7
	Germany	500 WG	600		1600	1 BBCH 61-85		14
	Greece	500 WG	600	40–60	800-1000	1 BBCH 61-87		7
	Hungary	500 WG	600		800-1200	1 BBCH 61-85		14
	Italy	500 WG	600	50		1		7
	Spain	500 WG	600		100-1000	1	—	7
	USA	360 SC	560			3 [a)] (1680)	[b)]	3
Wine grapes	Austria	500 WG	600		1000	1 BBCH 61-85		21

Crop	Country	Conc., g ai/kg, Form	Application					PHI days
			Max g ai/ha	Max g ai/hL	L/ha	Max No. (g ai/ha/ season)	Interval day	
	France	500 WG	600			1 BBCH 61-87		14
	Germany	500 WG	600		1600	1 BBCH 61-85		21
	Greece	500 WG	600	40–60	800-1000	1 BBCH 61-87		14
	Hungary	500 WG	600		800-1200	1 BBCH 61-85		21
	Italy	500 WG	600	50		1	—	14
	Spain	500 WG	600		100-1000	1	—	14
	USA	360 SC	560			3 [a] (1680)	[b]	3
Strawberries (indoor & outdoor)	Austria	500 WG	600	40-60	2000	3 BBCH 61-87	7	1
	France	500 WG	600			3 BBCH 61-87	7	1
	Germany	500 WG	600		2000	3 BBCH 61-87	7–14	1
	Greece	500 WG	600	40–60	500-1200	3 BBCH 61-87	7	1
	Hungary	500 WG	600		500-2000	3 BBCH 61-87	7	1
	Italy	500 WG	600	40–60		3	7	3
	Netherlands	500 WG	600			3	7	1
	Poland	500 WG	600		500-1000	3 BBCH 61-69	7-14	1
	Spain	500 WG	600		500-1200	3	7	1
	USA	360 SC	560			4 [a] (2240)	7–14	0
Fruiting vegetables, Cucurbits								
Cucurbits with edible peel (indoor)	Austria	500 WG	600	60	600-1200	3 BBCH 61-87	10	1
	France	500 WG	600			3 BBCH 61-87	10	1
	Germany	500 WG	600		1500	3 BBCH 61-87	10–14	1
	Greece	500 WG	600	40–60	600-1200	3 BBCH 61-87	10	1
	Hungary	500 WG	600		600-1200	3 BBCH 61-87	10	1
	Italy	500 WG	600	40–60		3	10–12	3
	Netherlands	500 WG	600	40–60		3	10	1
	Poland	500 WG	600	60		3 BBCH 61-87	10	1
	Spain	500 WG	600		100-1500	3 (600)	10	1
Fruiting vegetables, other than Cucurbits								
Tomato, pepper, eggplant (indoor)	Austria	500 WG	600	60	600-1200	3 BBCH 61-87	10	1
	France	500 WG	600			3 BBCH 61-87	10	1
	Germany	500 WG	600		1500	3 BBCH 61-87	10–14	1
	Greece	500 WG	600	40–60	600-1200	3 BBCH 61-87	10	1
	Hungary	500 WG	600		600-1200	3 BBCH 61-87	10	1
	Italy	500 WG	600	40–60		3	10–12	3

Crop	Country	Conc., g ai/kg, Form	Application					PHI days
			Max g ai/ha	Max g ai/hL	L/ha	Max No. (g ai/ha/ season)	Interval day	
	Netherlands	500 WG	600	40–60		3	10	1
	Poland	500 WG	600	60	1000-1200	3 BBCH 61-87	10	1
	Spain	500 WG	600		100-1500	3 (600)	10	1
Leafy vegetables (including Brassica leafy vegetables)								
Lettuce (head and leaf)	USA	360 SC	560	—		3 [a)] (1680)	7–10	14
Root and tuber vegetables								
Ginseng	USA	360 SC	560	—		4 [a)] (2240)	7–14	2
Tree nuts								
Almond	USA	360 SC	420			3 [a)] (1270)	[c)]	21
Pistachio	USA	360 SC	420			3 [a)] (1270)	[d)]	21

[a] Do not make more than 2 sequential applications without alternating with an application of a fungicide of other group labelled for brown rot or green fruit rot control;

[b] First application at early bloom; second application at pre-bunch closure; third application at veraison;

[c] Begin applications at pink bud (approximately 5% bloom). If conditions are favourable for disease development, make additional applications at full bloom and at petal fall;

[d] Begin applications when conditions favour disease development (typically when the bloom/terminal shoot is 1/2 to 1 inch). Make a repeat application to young clusters if cool wet weather occurs;

[e] From the beginning of flowering (about 10% of the flowers open) to the end; or from the end of the flowering to maturity of fruits;

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

Supervised trials using foliar spray of fenpyrazamine have been conducted on the following crops: stone fruits, berries and other small fruits, cucurbits, fruiting vegetables other than cucurbits, leafy vegetables, root and tuber vegetables, and tree nuts. The results of these supervised trials are summarized in the following tables:

Crop Group	Commodity	Country/Region, year of trials	Table No.
Stone fruits	Cherries	Austria 2012, France 2011, 2012, Germany 2011, 2012, Hungary 2012, Italy 2012, Greece 2012, Poland 2012, Spain 2011,	33
	Plums	Austria 2011, France 2111, 2012, Germany 2011, 2012, Greece 2011, Italy 2011, Poland 2012, Spain 2012	34
	Apricot	Austria 2012, France 2012, Hungary 2012, Italy 2012, Spain 2012, Greece 2012	35
	Peach	Austria 2010, 2011, France 2010, 2011, Greece 2010, 2011, Hungary 2010, 2011, Italy 2010, 2011, Spain 2010, 2011	36
Berries and other small fruits	Cane berries		37
	Blackberry	USA 2009,	
	Raspberry	USA 2009	

Crop Group	Commodity	Country/Region, year of trials	Table No.
	Bush berries		38
	Blueberry	USA 2009	
	Table and wine grapes	Austria 2006, 2007, 2008, Germany 2006, 2007, 2008, Italy 2006, 2007, 2008, Spain 2006, 2007, 2008	39 and 40
		Canada 2008, USA 2006, 2007, 2008	
	Strawberries	France 2010, 2011, Germany 2011, Greece 2011, Hungary 2011, Italy 2010, Poland 2010, 2011, Spain 2010, 2011	41 and 42
		Canada 2008, USA 2006/7, 2007, 2008, 2009	
Fruiting vegetables, Cucurbits	Cucumber	Hungary 2007, 2008, Italy 2007, 2008, Netherlands 2007, 2008, Spain 2007, 2008	43
Fruiting vegetables, other than Cucurbits	Peppers	Hungary 2007, 2008, Italy 2007, 2008, Netherlands 2007, 2008, Spain 2007, 2008	44
	Tomato	Hungary 2007, 2008, Italy 2007, 2008, Netherlands 2007, 2008, Spain 2007, 2008	45
Leafy vegetables	Lettuce (head and leaf)	USA 2009, 2010	46
Root and tuber vegetables	Ginseng	USA 2008	47
Tree nuts	Almond nutmeat	USA 2008	48
	Almond hulls	USA 2008	49

In addition to the description and details of the field trials, each study report includes a summary of the analytical method(s), together with the corresponding procedural recoveries, LOQ(S) and information on storage of samples.

All appropriate trials are summarized and used. In the trials, where multiple analyses were conducted on a single sample, the mean value is reported. Where multiple samples were taken from a single plot, the mean residue value is reported. Where results from replicate plots are reported, the highest value was selected for estimating STMR, HR and MRL. Where results from separate plots with distinguishing characteristics such as different formulations, varieties or treatment schedules were reported, results are listed for each plot.

When residues were not quantifiable they are shown as below the LOQ of the relevant analytical method (e.g. < 0.01 mg/kg). Residues and application rates have generally been rounded to two significant figures or, for residues near the LOQ, to one significant figure.

Although control plots were included in the trials, control data are not reported in the following tables unless residues in control samples exceeded the LOQ. Results have not been corrected for concurrent method recoveries.

Residue values from the trials conducted according to the maximum GAP were used for the estimation of maximum residue levels. Those results are underlined in the tables for the estimation of STMR, HR and MRL. Where a higher residue value was obtained at a later DALA, the higher value was used.

Where a report contained trials or plots supporting the proposed GAP and others not supporting the GAP, only the relevant trials or plots are presented.

The proportionality principle has been applied where appropriate. The proposed MRLs were calculated using the OECD MRL calculator. The rationale for the proposed MRL is given for each commodity.

In all the trials reported below, fenpyrazamine was applied as foliar spray for the control of *Botrytis cinerea* and/or *Monilia* species. 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.

Summaries of the trial results are given in the following tables.

Stone fruits

Apricot, Cherry, Nectarine, Peach, Plum

A total of forty-six residue trials were conducted on stone fruits in Europe: 12 on cherries in 2011/12; 16 trials on plums in 2011/12; 12 trials on peaches in 2010/11; and six trials on apricot in 2012. In each trial, fenpyrazamine was applied three times at a nominal interval of seven days as a foliar spray of a 500 g/kg water dispersible granule (500 WG) formulation at a nominal rate of 600 g ai/ha.

Per crop, 50% of the trials were performed as harvest trials and 50% were performed as decline trials each with one untreated control and one treated plot. Samples were taken nominally 1 and 3 days after the last application (DALA) for harvest trials and at DALA of nominally 0, 1, 3 and 7 days. Samples from all trials/plots were harvested at commercial maturity.

Stone fruits were analysed for residues of fenpyrazamine and S-2188-DC using the validated analytical method SUM-0731V, with an LOQ of 0.01 mg/kg. Procedural recoveries were in the range of 72% to 109% for parent fenpyrazamine (0.01–4.0 mg/kg) and for S-2188-DC, in the range of 63% to 109% (0.01–1.0 mg/kg).

Samples were stored deep-frozen for a maximum of 5 months (163 days) for cherries, 5 months (162 days) for plums, 7 months (202 days) for peaches and 7 months (210 days) for apricot. This period is covered by storage stability data for lettuce as a high water containing crop over 12 months (364 days).

Table 33 Residues of fenpyrazamine in cherries from supervised trials conducted in Europe

Cherry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 7 days				
GGU-11-9201, FR01, St Nenis en Val, France, 2011 (Lapins)	500 WG	637 571 578	3	0 1 3 7	Whole fruit	0.55 0.35 <u>0.36</u> 0.21	0.12 0.12 0.16 0.14	0.72 0.52 0.59 0.41	QNR-0103
GGU-11-9201, FR02, Languedoc-Roussillon, France, 2011 (Summit)	500 WG	590 604 607	3	1 3	Whole fruit	<u>1.0</u> 0.76	0.16 0.09	1.23 0.89	QNR-0103
GGU-11-9201, DE03, Baden-Wurttemberg, Germany, 2011 (Morellenfeuer)	500 WG	601 603 599	3	1 3	Whole fruit	<u>0.60</u> 0.42	0.07 0.11	0.70 0.58	QNR-0103
GGU-11-9201, ES04, Alicante, Spain, 2011 (Burlar)	500 WG	586 616 628	3	0 1 3 7	Whole fruit	0.69 <u>0.34</u> 0.25 0.11	0.08 0.08 0.06 0.06	0.80 0.45 0.34 0.20	QNR-0103

Cherry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
12SGS018, FR01, Champagne-Ardenne, France, 2012 (Sweet Heart)	500 WG	598 608 609	3	0 1 3 7	Whole fruit[a)]	2.6 [b)] 1.8 [b)] 0.62 [b)] 0.45 [b)]	0.06 [b)] 0.10 [b)] 0.07 [b)] 0.08 [b)]	2.69 1.94 0.72 0.56	QNR- 0111
12SGS018, GE02, Hessen, Germany, 2012 (Schattenmorelle)	500 WG	646 632 611	3	0 1 3 7	Whole fruit[s)]	0.67 0.82 0.77 0.34	0.06 0.13 0.17 0.17	0.76 1.01 1.01 0.58	QNR- 0111
12SGS018, AU03, Upper Austria, Austria, 2012 (Regina)	500 WG	572 604 597	3	0 1 3 7	Whole fruit[s)]	0.93 0.54 0.44 0.25	0.07 0.05 0.08 0.08	1.03 0.61 0.55 0.36	QNR- 0111
12SGS018, FR04, St Denis en Val, France, 2012 (Lapins)	500 WG	601 605 617	3	1 3	Whole fruit[s)]	0.41 0.27	0.05 0.04	0.48 0.33	QNR- 0111
12SGS018, PL05, Mazowieckie, Poland, 2012 (Lutówka)	500 WG	604 609 606	3	1 3	Whole fruit[a)]	1.9 [b)] 1.8 [b)]	0.19 [b)] 0.27 [b)]	2.17 2.19	QNR- 0111
12SGS018, HU06, Hejer, Hungary, 2012 (Germers-dorfi óriás)	500 WG	627 601 603	3	1 3	Whole fruit[a)]	0.33 0.33	0.07 0.10	0.43 0.47	QNR- 0111
12SGS018, IT07, Lombardia, Italy, 2012 (Brooks)	500 WG	602 598 581	3	0 1 3 8	Whole fruit[a)]	0.84 0.61 0.27 0.16	0.08 0.12 0.11 0.07	0.95 0.78 0.43 0.26	QNR- 0111
12SGS018, GR08, Central Macedonia, Greece, 2012 (Lapins)	500 WG	602 601 602	3	1 3	Whole fruit[a)]	1.0 0.94	0.26 0.28	1.37 1.34	QNR- 0111

[a] Residues calculated from the flesh/stone ratio

[b] Mean of a multiple (2-3) determination

Table 34 Residues of fenpyrazamine in plums from supervised trials conducted in Europe

Plums Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 7 days				
GGU-11-9205, FR01, Pays de Loire, France, 2011 (TC Sun)	500 WG	630 595 585	3	0 1 3 7	Whole fruit	0.33 0.18 0.19 0.13	0.02 0.01 0.02 0.02	0.36 0.19 0.22 0.16	QNR- 0104
GGU-11-9205, AT02, Lower Austria, Austria, 2011 (President)	500 WG	596 603 598	3	1 3	Whole fruit	0.33 0.30	0.06 0.10	0.42 0.44	QNR- 0104
GGU-11-9205, GR03, Central Macedonia, Greece, 2011 (Antzelino)	500 WG	604 600 617	3	0 1 3 7	Whole fruit	0.36 0.20 0.23 0.11	0.08 0.09 0.10 0.09	0.47 0.33 0.37 0.24	QNR- 0104
GGU-11-9205, IT04, Lombardia, Italy, 2011 (AL 406)	500 WG	628 599 616	3	1 3	Whole fruit	0.40 0.16	0.03 0.03	0.44 0.20	QNR- 0104
GGU-11-9205, FR05, Lorraine, France, 2011 (Mirabelle de Nancy)	500 WG	592 594 587	3	1 3	Whole fruit	1.5 0.72	0.13 0.08	1.69 0.83	QNR- 0104

Plums Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
GGU-11-9205, DE06, Rheinland-Pfalz, Germany, 2011 (Mirabelle de Nancy)	500 WG	603 605 599	3	0 1 3 8	Whole fruit	0.71 <u>0.84</u> 0.56 0.50	0.02 0.06 0.07 0.06	0.74 0.93 0.66 0.59	QNR- 0104
12SGS017, FR01, Pays de Loire, France, 2012 (Reine Claude)	500 WG	572 624 603	3	0 1 3 6	Whole fruit[a)]	0.43 <u>0.40</u> 0.20 0.19	0.02 0.06 0.04 0.03	0.46 0.49 0.26 0.23	QNR- 0110
12SGS017, GE02, Hessen, Germany, 2012 (Hauszwetsche)	500 WG	613 606 613	3	0 1 3 7	Whole fruit[a)]	0.11 <u>0.12</u> 0.06 0.04	0.01 0.02 0.02 <0.01	0.12 0.15 0.09 0.05	QNR- 0110
12SGS017, GE03, Rheinland Pfalz, Germany, 2012 (Hauszwetsche)	500 WG	613 618 648	3	1 3	Whole fruit[s)]	0.16 <u>0.18</u>	0.02 0.02	0.19 0.21	QNR- 0110
12SGS017, PL04, Mazowieckie, Poland, 2012 (Amers)	500 WG	620 617 624	3	1 3	Whole fruit[a)]	<u>0.67</u> 0.30	0.07 0.03	0.77 0.34	QNR- 0110
12SGS017, FR05, Ste Livrade Sur Lot, Aquitaine, France, 2012 (D'Ente)	500 WG	604 615 595	3	0 1 3 8	Whole fruit[a)]	0.61 <u>0.56</u> 0.43 0.20	0.06 0.08 0.07 0.05	0.70 0.67 0.53 0.27	QNR- 0110
12SGS017, SP06, Valencia, Spain, 2012 (Golden Japan)	500 WG	602 604 607	3	0 1 3 6	Whole fruit[a)]	1.5 [b)] 0.75[b)] <u>0.87</u>[b)] 0.80[b)]	0.15[b)] 0.15[b)] 0.19[b)] 0.21[b)]	1.71 0.96 1.14 1.10	QNR- 0110
12SGS017, IT07, Piemonte, Italy, 2012 (Florentia)	500 WG	579 573 603	3	0 1 3 7	Whole fruit[a)]	0.45 <u>0.70</u> 0.24 0.27	0.07 0.09 0.13 0.16	0.55 0.83 0.43 0.50	QNR- 0110
12SGS017, FR08, Taillecavat, Aquitaine, France, 2012 (D'Ente)	500 WG	610 606 611	3	1 3	Whole fruit[a)]	<u>0.36</u> 0.10	0.05 0.02	0.43 0.13	QNR- 0110
12SGS017, SP09, Navarra, Spain, 2012 (Claudia)	500 WG	601 610 617	3	1 3	Whole fruit[a)]	<u>0.23</u> 0.05	0.02 0.02	0.26 0.08	QNR- 0110
12SGS017, GR10, Central Macedonia, Greece, 2012 (Angelino)	500 WG	602 595 598	3	1 3	Whole fruit[a)]	0.27 <u>0.30</u>	0.09 0.12	0.40 0.47	QNR- 0110

[a] Residues calculated from the flesh/stone ratio

[b] Mean of a duplicate determination

Table 35 Residues of fenpyrazamine in Apricots from supervised trials conducted in Europe

Apricots Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 7 days				
12SGS016, HU01, Moson-Sopron, Hungary, 2012 (Gonci Magyar)	500 WG	586 606 590	3	0 1 3 7	Whole fruit	0.61 0.42 <u>0.43</u> 0.24	0.21 0.29 0.17 0.13	0.91 0.83 0.67 0.43	QNR- 0109

Apricots Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
12SGS016, AU02, Upper Austria, Austria, 2012 (Golden Rich)	500 WG	587 586 589	3	1 3	Whole fruit	0.52 0.41	0.04 0.05	0.58 0.48	QNR-0109
12SGS016, FR03, Rhone-Alpes, France, 2012 (Farbaly)	500 WG	599 591 595	3	0 1 3 8	Whole fruit	1.1 0.89 0.45 0.30	0.20 0.18 0.18 0.20	1.39 1.15 0.71 0.59	QNR-0109
12SGS016, IT04, Piemonte, Italy, 2012 (Reale di Imola)	500 WG	601 601 634	3	0 1 3 7	Whole fruit	3.5 2.5 3.0 2.4	0.28 0.36 0.55 0.54	3.90 3.01 3.79 3.17	QNR-0109
12SGS016, SP05, Valencia, Spain, 2012 (Mitter de Castello)	500 WG	592 584 609	3	1 3	Whole fruit	1.1 0.95	0.33 0.37	1.57 1.48	QNR-0109
12SGS016, GR06, Central Macedonia, Greece, 2012 (Bebekou)	500 WG	598 608 593	3	1 3	Whole fruit	1.6 1.4	0.23 0.23	1.93 1.73	QNR-0109

Table 36 Residues of fenpyrazamine in Peaches from supervised trials conducted in Europe

Apricots Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 7 days				
GGU-10-6205, AU01, Styria, Austria, 2010 (Benedikte)	500 WG	626 588 588	3	1 3	Whole fruit	1.5 0.28	0.06 0.03	1.59 0.32	QNR-0100
GGU-10-6205, HU02, Komarom-Esztergom, Hungary, 2010 (Szegedi Arany)	500 WG	622 623 609	3	0 1 3 6	Whole fruit	1.5 0.44 0.76 0.36	0.25 0.10 0.24 0.02	1.86 0.58 1.10 0.39	QNR-0100
GGU-10-6205, FR02, Languedoc-Roussillon, France, 2010 (Opale)	500 WG	580 633 614	3	0 1 3 7	Whole fruit	1.6 0.53 0.85 0.40	0.27 0.11 0.28 0.17	1.99 0.69 1.25 0.64	QNR-0100
GGU-10-6205, GR01, Macedonia/Pella, Greece, 2010 (Katerina)	500 WG	600 607 609	3	1 3	Whole fruit	2.5 1.6	< 0.01 0.18	2.51 1.86	QNR-0100
GGU-10-6205, IT01, Piemonte, Italy, 2010 (Zee Lady)	500 WG	613 604 599	3	1 3	Whole fruit	0.94 0.65	< 0.01 < 0.01	0.95 0.66	QNR-0100
GGU-10-6205, SP01, Valencia, Spain, 2010 (Catherine)	500 WG	603 600 601	3	0 1 3 7	Whole fruit	1.0 1.1 0.46 0.69	0.10 0.11 0.09 0.14	1.14 1.26 0.59 0.89	QNR-0100
GGU-11-8897, AT01, Styria, Austria, 2011 (Redhaven)	500 WG	609 595 601	3	1 3	Whole fruit	0.36 0.13	0.26 0.07	0.73 0.23	QNR-0105
GGU-11-8897, HU02, Komarom-Esztergom, Hungary, 2011 (Champion)	500 WG	629 592 590	3	0 1 3 7	Whole fruit	0.46 0.61 0.42 0.30	0.10 0.11 0.13 0.15	0.60 0.77 0.61 0.51	QNR-0105
GGU-11-8897, FR03, Languedoc-Roussillon, France, 2011 (Melina)	500 WG	566 616 601	3	0 1 3 7	Whole fruit	1.1 0.43 0.44 0.28	0.21 0.22 0.31 0.13	1.40 0.75 0.88 0.47	QNR-0105

Apricots Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
GGU-11-8897, GR04, Central Macedonia, Greece, 2011 (Katerina)	500 WG	596 601 601	3	1 3	Whole fruit	<u>0.95</u> 0.89	0.13 0.17	1.14 1.13	QNR- 0105
GGU-11-8897, IT05, Lombardia, Italy, 2011 (Mirko)	500 WG	605 593 577	3	1 3	Whole fruit	<u>0.55</u> 0.05	0.04 0.02	0.61 0.08	QNR- 0105
GGU-11-8897, ES06, Valencia, Spain, 2011 (Red Cander)	500 WG	576 600 586	3	0 1 3 7	Whole fruit	0.90 <u>0.70</u> 0.57 0.30	0.12 0.17 0.21 0.09	1.07 0.94 0.87 0.43	QNR- 0105

Berries and other Small Fruits

Cane berries

Six residue trials (including one pair of replicate plots) were conducted on cane berries (3 on blackberries and 3 on raspberries) in the USA in 2009. In each trial, fenpyrazamine was applied three times as a foliar spray of a 479 g/L suspension concentrate (479 SC) formulation at a nominal rate of 560 g ai/ha with a nominal interval of seven days. Five trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken on the same day of the last application (DALA=0). Samples in all trials/plots were harvested at commercial maturity. One trial was performed as a decline trial with one untreated control and one treated plot. Samples were taken on the same day of the last application and at DALA of 1, 4, 7 and 9 days.

Cane berries were analysed for residues of fenpyrazamine and S-2188-DC using method RM-45C (Kowalsky J., 2009/10), with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 3.0 mg/kg were in the range of 87–107% for parent fenpyrazamine and 70–124% for S-2188-DC.

Samples of cane berries were stored deep-frozen for a maximum of 14 months (416 days), only slightly longer than the longest period tested for frozen storage stability, 13 months (384 days).

Table 37 Residues of fenpyrazamine in cane berries from supervised trials conducted in the USA

Cane berry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	556	3	PHI 0	Interval: 7 days Seasonal maximum: 1680 g ai/ha				
Blackberries IR-4 PR 09444, 09-OR24, Aurora, OR, USA, 2009 (Marion) [b]	479 SC	560 566 557	3	0 1 4 7 9	Berries	<u>2.81</u> 2.43 0.99 0.74 0.71	0.29 0.37 0.30 0.33 0.34	3.22 2.95 1.41 1.21 1.19	QNR- 0123
Raspberries IR-4 PR 09444, 09- OR25, Aurora, OR, USA, 2009 (Willamette) [b]	479 SC	571 566 562	3	0	Berries	1.20	0.19	1.47	QNR- 0123
Raspberries IR-4 PR 09444, 09- OR26, Aurora, OR, USA, 2009 (Willamette) [b]	479 SC	565 575 566	3	0	Berries	1.55	0.34	2.04	QNR- 0123

Cane berry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Blackberries IR-4 PR 09444, 09-NC09, Jackson Springs, NC, USA, 2009 (Kiowa)	479 SC	546 548 559	3	0	Berries	1.60	0.18	1.85	QNR-0123
Raspberries IR-4 PR 09444, 09-MI28, Clarksville, MI, USA, 2009 (Heritage)	479 SC	563 567 564	3	0	Berries	1.90	0.22	2.21	QNR-0123
Blackberries IR-4 PR 09444, 09-CA53, Kingsburg, CA, USA, 2009 (Apache)	479 SC	578 568 550	3	0	Berries	0.53	0.06	0.61	QNR-0123

[a] Duplicate samples analysed; residues represent a mean value

[b] Same location with the same soil and same variety. The applications were conducted with one week of time difference. Therefore, the highest concentration from the two plots was selected.

Bush berries

Eight residue trials were conducted on blueberries in the USA in 2009. In each trial, fenpyrazamine was applied three times as a foliar spray of a 479 SC formulation at a nominal rate of 560 g ai/ha with a nominal interval of seven days. Seven trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken on the same day of the last application (DALA=0). Samples from all trials/plots were harvested at commercial maturity. One trial was performed as a decline trial with one untreated control and one treated plot. Samples were taken on the same day of the last application and at DALA of 1, 3, 7 and 10 days.

Blueberries were analysed for residues of fenpyrazamine and S-2188-DC using analytical method RM-45C (Kowalsky J., 2009/10), with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 2.0 mg/kg were in the range of 91–106% for parent fenpyrazamine and 71–164% for S-2188-DC.

Samples of blueberries were stored deep-frozen for a maximum of 12.6 months (383 days), only slightly longer than the longest period tested for frozen storage stability, 11.5 months (349 days).

Table 38 Residues of fenpyrazamine in blueberries from supervised trials conducted in the USA

Blueberry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	556	3	PHI 0	Interval: 7 days Seasonal maximum: 1680 g ai/ha				
IR-4 PR 09445, 09- NJ06, Chatsworth, NJ, USA, 2009 (Bluecrop) [b]	479 SC	554 572 558	3	0	Berries	1.80	0.16	2.03	QNR-0122
IR-4 PR 09445, 09- NJ11, Chatsworth, NJ, USA, 2009 (Elliot) [b]	479 SC	558 556 571	3	0	Berries	2.31	0.34	2.80	QNR-0122
IR-4 PR 09445, 09- MI13, Fennville, MI, USA, 2009 (Rubel)	479 SC	560 559 557	3	0 1 3 7 10	Berries	0.74 0.41 0.31 0.15 0.09	0.08 0.15 0.19 0.15 0.12	0.85 0.62 0.59 0.37 0.26	QNR-0122

Blueberry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
IR-4 PR 09445, 09- MI14, Holt, MI, USA, 2009 (Jersey)	479 SC	539 560 565	3	0	Berries	0.15	0.05	0.22	QNR-0122
IR-4 PR 09445, 09- MI15, Benton Harbor, MI, USA, 2009 (Bluecrop)	479 SC	577 1139 555	3	0	Berries	0.38	0.09	0.51	QNR-0122
IR-4 PR 09445, 09- ME01, Jonesboro, ME, USA, 2009 (lowbush, wild types)	479 SC	527 530 535	3	0	Berries	1.04	0.16	1.27	QNR-0122
IR-4 PR 09445, 09- OR20, Aurora, OR, USA, 2009 (Bluecrop)	479 SC	595 591 560	3	0	Berries	0.35	0.10	0.49	QNR-0122
IR-4 PR 09445, 09- NC11, Castle Hayne, NC, USA, 2009 (Croatan)	479 SC	563 547 555	3	0	Berries	0.92	0.13	1.11	QNR-0122

[a] Duplicate samples analysed; residues represent a mean value

[b] Same location with the same soil but different varieties. The applications in each plot were conducted with 41 days of time difference. Therefore, both results were used.

Grape

Seventeen residue trials on grapes were conducted in Europe in 2006–2008 and in fourteen residue trials North America (13 from the USA and one from Canada) in 2006–2008, respectively.

In each European trial, fenpyrazamine was applied once as a foliar spray of a 500 WG formulation at a nominal rate of 600 g ai/ha.

Eight trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 7 and 14 days. Samples from all trials/plots were harvested at commercial maturity. Eight trials were performed as reversed decline trials with one untreated control and five plots treated nominally 0, 3, 7, 14 and 21 days prior to normal commercial harvest.

Grape berries were analysed for residues of fenpyrazamine and S-2188-DC using analytical method SUM-0731V, with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels in the range of 0.01 mg/kg to 0.50 mg/kg were in the range of 85% to 97% for parent fenpyrazamine and 75% to 102% for S-2188-DC.

Samples of grape berries were stored deep-frozen for a maximum of 10 months (294 days). This period is covered by storage stability data for grape over 12 months (364 days).

Table 39 Residues of fenpyrazamine in grapes from supervised trials conducted in Europe

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	1	PHI 7 PHI 14	PHI 7 for table grapes PHI 14 for wine grapes				
GGU-06-1765, AU01, Gottlesbrunn, Austria, 2006 (Grüner Veltliner)	500 WG	601	1	0	Berries	1.0	0.02	1.03	QNR-0004
		619	1	3	Berries	0.72	0.09	0.85	
		667	1	7	Berries	0.62	0.08	0.73	
		632	1	14	Berries	0.49	0.08	0.60	
		619	1	21	Berries	0.46	0.10	0.60	
GGU-06-1765, GE01,	500 WG	616	1	0	Berries	1.1	< 0.01	1.11	QNR-

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Jugenheim, Germany, 2006 (Spätburgunder)		616	1	3	Berries	0.63	0.02	0.66	0004
		573	1	8	Berries	0.49	0.04	0.55	
		608	1	14	Berries	0.74	0.08	0.85	
		608	1	21	Berries	0.15	0.02	0.18	
GGU-06-1765, GE02, Heuchelheim, Germany, 2006 (Riesling)	500 WG	608	1	8 13	Berries	0.45 0.29	0.06 0.03	0.54 0.33	QNR-0004
GGU-06-1765, IT02, Valledona, Italy, 2006 (Rossese)	500 WG	625	1	7 15	Berries	0.05 0.06	< 0.01 < 0.01	0.06 0.07	QNR-0004
GGU-06-1765, SP01, Valencia, Spain, 2006 (Tempranillo)	500 WG	601	1	0	Berries	1.5	0.02	1.53	QNR-0004
		601	1	3	Berries	1.8	0.32	2.26	
		601	1	7	Berries	1.2	0.39	1.76	
		612	1	14	Berries	0.62	0.20	0.91	
		601	1	21	Berries	0.25	0.07	0.35	
		612	1	14	Bunches	0.67	0.16	0.90	
GGU-07-2771 AU01, Langenlois, Austria, 2007 (Welschriesling)	500 WG	622	1	7 14	Berries	0.77 0.52	0.11 0.12	0.92 0.69	QNR-0011
GGU-07-2771 GE01, Wisenheim am Sand, Germany, 2007 (Spätburgunder)	500 WG	606	1	0	Berries	0.75	< 0.01	0.76	QNR-0011
		604	1	3	Berries	0.38	0.05	0.45	
		583	1	7	Berries	0.25	0.07	0.35	
		597	1	14	Berries	0.16	0.02	0.19	
		603	1	21	Berries	0.18	0.03	0.22	
GGU-07-2771 GE02, Partenheim, Germany, 2007 (Weissburgunder)	500 WG	624	1	7 13	Berries	0.37 0.23	0.05 0.04	0.44 0.29	QNR-0011
GGU-07-2771 IT01, Lombardia, Italy, 2007 (Riesling Italico)	500 WG	625	1	0	Berries	0.60	< 0.01	0.61	QNR-0011
		580	1	3	Berries	0.47	0.02	0.50	
		613	1	7	Berries	0.06	< 0.01	0.07	
		592	1	14	Berries	0.15	0.01	0.16	
		591	1	21	Berries	0.02	< 0.01	0.03	
		592	1	15	Bunches	0.09	< 0.01	0.10	
GGU-07-2771 IT02, Piemonte, Italy, 2007 (Cortese)	500 WG	577	1	7 13 13	Berries Berries Bunches	0.22 0.13 0.15	0.04 0.02 0.03	0.28 0.16 0.19	QNR-0011
GGU-07-2771 SP01, Casello de Gugat, Valencia, Spain, 2007 (Garnacha)	500 WG	600	1	0 3 7 14 21 14	Berries Berries Berries Berries Berries Bunches	0.35 0.40 0.12 0.37 0.07 0.15	< 0.01 0.08 < 0.01 0.05 < 0.01 < 0.01	0.36 0.51 0.13 0.44 0.08 0.16	QNR-0011
GGU-07-2771 SP02, Andilla, Valencia, Spain, 2007 (Meseguera)	500 WG	600	1	7 14	Berries	0.14 0.08	0.02 0.01	0.17 0.09	QNR-0011
GGU-08-4135, AU01, Gemeinlebrarn, Austria, 2008 (Zweigelt)	500 WG	600	1	6 13	Berries	0.53 0.54	0.04 0.06	0.59 0.63	QNR-0035
GGU-08-4135, GE01, Partenheim, Germany, 2008	500 WG	600	1	0	Berries	1.0	< 0.01	1.01	QNR-0035
		612	1	2	Berries	0.52	0.01	0.53	
		592	1	7	Berries	0.19	< 0.01	0.20	

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
(Müller-Thurgau)		592	1	14	Berries	< 0.01	< 0.01	0.02	
		615	1	21	Berries	0.23	0.02	0.26	
GGU-08-4135, IT01, Liguria, Italy, 2008 (Rossese)	500 WG	607	1	7 14 14	Berries Berries Bunches	0.9 1.2 0.42	0.09 0.27 0.08	1.03 1.59 0.53	QNR-0035
GGU-08-4135, IT03, Lombardia, Italy, 2008 (Chiavennasca)	500 WG	566	1	14	Bunches	0.32	0.06	0.41	QNR-0035
GGU-08-4135, SP01, Valencia, Spain, 2008 (Malvasa)	500 WG	600	1	0 3 7 14 22 14	Berries Berries Berries Berries Berries Bunches	1.2 0.65 0.31 1.0 0.11 0.31	< 0.01 0.03 0.03 0.08 0.01 0.02	1.21 0.69 0.35 1.11 0.12 0.34	QNR-0035

In each North American trial, fenpyrazamine was applied three times as a foliar spray of a 500 WG formulation (five trials) or 479 g/L SC formulation (five trials) at a nominal rate of 560 g ai/ha. In three trials both of these formulations were compared in side by side plots. In two trials a second plot was established, were the WG formulation was applied three times at an exaggerated (2×) rate of nominally 1120 g ai/ha. In one trial a second plot was established where the SC formulation was applied three times at an exaggerated (5×) rate of nominally 2800 g ai/ha to obtain samples for processing grapes to juice and raisin.

Twelve trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 3 days. Samples from all trials/plots were harvested at commercial maturity. Two trials were performed as decline trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 0, 1, 3, 7 and 10 days.

Grape berries were analysed for residues of fenpyrazamine and S-2188-DC using analytical methods RM-45C and RM-45C-1 (modification for purple-coloured grapes) (Kowalsky J., 2009/10), with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 5.0 mg/kg were in the range of 78–108% for parent fenpyrazamine and 72–114% for S-2188-DC.

Samples of grape berries were stored deep-frozen for a maximum of 10 months (371 days). This period is covered by storage stability data for grape over 12 months (364 days).

Table 40 Residues of fenpyrazamine in grapes from supervised trials conducted in Canada and the USA

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	560	3	PHI 3	Seasonal maximum: 1667 g ai/ha				
VP-30097 V-30097-A Kerman, CA, USA 2006 (Thompson Seedless)	500 WG	565 549 558	3	0 1 3 7 10	Berries	1.05 0.95 1.09 0.66 1.08	0.11 0.10 0.12 0.10 0.20	1.21 1.09 1.26 0.80 1.37	QNR-0088
VP-30097 V-30097-B Dundee, NY, USA 2007 (Vidal Blanc)	500 WG	559 562 562	3	3	Berries	1.01	0.19	1.28	QNR-0088
	500 WG	1107 1117 1122	3	3	Berries	2.22	0.35	2.72	QNR-0088

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	560	3	PHI 3	Seasonal maximum: 1667 g ai/ha				
VP-30097 V-30097-C Winters, CA, USA 2007 (Merlot)	500 WG	555 559 555	3	3	Berries	0.55	0.08	0.66	QNR-0088
	500 WG	1117 1117 1121	3	3	Berries	1.15	0.11	1.31	QNR-0088
VP-30097 V-30097-D Kerman, CA, USA 2007 (Thompson Seedless)	500 WG	559 562 566	3	3	Berries	2.08	0.76	3.17	QNR-0088
VP-30097 V-30097-E Granger, WA, USA 2007 (Chardonnay)	500 WG	575 554 572	3	3	Berries	0.80	0.16	1.03	QNR-0088
VP-30097 V-30097-F Dundee, NY, USA 2008 (DeChaunac)	479 SC	563 565 570	3	0 1 4 7 11	Berries	1.80 1.23 1.06 0.84 0.63	0.09 0.09 0.17 0.18 0.15	1.93 1.36 1.30 1.10 0.84	QNR-0088
VP-30097 V-30097-G Branchton, Ontario, Canada 2008 (Concordes)	479 SC	521 567 563	3	3	Berries	0.18	0.02	0.21	QNR-0088
	500 WG	520 571 552	3	3	Berries	0.33	0.03	0.37	QNR-0088
VP-30097 V-30097-H Artois, CA, USA 2008 (Ruby Red)	479 SC	580 580 574	3	3	Berries	0.74	0.35	1.24	QNR-0088
	500 WG	584 583 576	3	3	Berries	0.93	0.32	1.39	QNR-0088
VP-30097 V-30097-I Hickman, CA, USA 2008 (Chardonnay)	479 SC	559 560 569	3	3	Berries	0.42	0.14	0.62	QNR-0088
	500 WG	559 560 569	3	3	Berries	0.53	0.06	0.62	QNR-0088
VP-30097 V-30097-J Winters, CA, USA 2008 (Merlot)	479 SC	556 556 556	3	3	Berries	0.91	0.19	1.18	QNR-0088
VP-30097 V-30097-K Kerman, CA, USA 2008 (Thompson Seedless)	479 SC	555 563 555	3	3	Berries	1.10	0.36	1.62	QNR-0088
VP-30097 V-30097-L Corvallis, OR, USA 2008 (Pinot noir)	479 SC	564 569 561	3	2	Berries	1.24	0.13	1.43	QNR-0088
VP-30097 V-30097-M Granger, WA, USA 2008 (Riesling)	479 SC	589 572 566	3	3	Berries	0.88	0.13	1.07	QNR-0088
VP-30097 V-30097-N Madera, CA, USA 2008 (Thompson Seedless)	479 SC	563 557 550	3	3	Berries	0.71	0.17	0.95	QNR-0088
	479 SC	2838 2820 2827	3	3	Berries	4.00	1.07	5.52	QNR-0088

[a] Duplicate samples analysed; residues represent a mean value

Strawberries

Sixteen residue trials were conducted on strawberries in Europe in 2010/11 (eight indoor and eight outdoor). Another eight residue trials were conducted on strawberries in North America (seven in the USA and one in Canada) in 2006–2009.

In each European trial, fenpyrazamine was applied three times as a foliar spray of a 500 WG formulation at a nominal rate of 600 g ai/ha.

Half of the trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 1 and 3 days. Samples from all trials/plots were harvested at commercial maturity. Another Half of the trials were performed as decline trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 0, 1, 3 and 7 days.

Strawberry fruits were analysed for residues of fenpyrazamine and S-2188-DC using analytical method SUM-0731V, with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels in the range of 0.01 mg/kg to 4.0 mg/kg were in the range of 75% to 113% for parent fenpyrazamine and 75% to 112% for S-2188-DC.

Samples of strawberries berries were stored deep-frozen for a maximum of 140 days. This period is covered by demonstrated storage stability data for strawberries for 114 days.

Table 41 Residues of fenpyrazamine in strawberries from supervised trials conducted in Europe

Strawberry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 7 days for both indoor and outdoor				
Indoor									
GGU-10-6203, FR01, Rougeou, France, 2010 (Yamaska)	500 WG	587 592 579	3	1 3	Fruit	0.86 0.81	0.10 0.12	1.00 0.98	QNR-0086
GGU-10-6203, PL01, Lowitz, Poland, 2010 (Elsanta)	500 WG	602 607 605	3	0 1 3 7	Fruit	0.30 0.33 0.35 0.18	0.01 0.01 0.01 0.04	0.31 0.34 0.36 0.24	QNR-0086
GGU-10-6203, IT01, Piemonte, Italy, 2010 (Record)	500 WG	607 612 619	3	1 3	Fruit	0.24 0.10	0.03 0.03	0.28 0.14	QNR-0086
GGU-10-6203, SP01, Cartaya, Spain, 2010 (Camarosa)	500 WG	601 590 617	3	0 1 3 7	Fruit Storage: 140 days	0.82 0.76 0.43 0.13	0.17 0.22 0.19 0.08	1.06 1.08 0.70 0.24	QNR-0086
GGU-11-8895, FR01, Pays de Loire, France, 2011 (Sirène)	500 WG	588 608 628	3	1 3	Fruit	0.28 0.21	0.13 0.10	0.47 0.35	QNR-0101
GGU-11-8895, HU03, Kecskem, Hungary, 2011 (Clery)	500 WG	610 653 627	3	0 1 3 8	Fruit	0.70 0.45 0.27 0.09	0.01 0.06 0.03 0.04	0.71 0.54 0.31 0.15	QNR-0101
GGU-11-8895, GR02, Macedonia, Greece, 2011 (Kamaroza)	500 WG	603 599 602	3	1 3	Fruit	1.4 0.95	0.44 0.51	2.03 1.68	QNR-0101
GGU-11-8895, ES04, Huelva, Spain, 2011 (Camarosa)	500 WG	598 560 625	3	0 1 3 7	Fruit	1.4 0.92 0.61 0.38	0.15 0.16 0.10 0.07	1.61 1.15 0.75 0.48	QNR-0101
Outdoor									

Strawberry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 7 days for both indoor and outdoor				
GGU-10-6204, IT01, Lombardia, Italy, 2010 (Aromas)	500 WG	595 598 608	3	1 3	Fruit	0.28 0.18	0.09 0.09	0.41 0.31	QNR-0096
GGU-10-6204, SP01, Huelva, Spain, 2010 (Camarosa)	500 WG	601 614 612	3	0 1 3 7	Fruit Storage: 133-140 days	0.60 0.47 0.21 0.14	0.09 0.16 0.11 0.10	0.73 0.70 0.37 0.28	QNR-0096
GGU-11-8946, FR01, Nord-Pas de Calais, France, 2011 (Dar Select)	500 WG	608 599 590	3	0 1 3 7	Fruit	0.85 0.30 0.11 0.03	0.03 0.06 0.04 0.01	0.89 0.39 0.17 0.04	QNR-0102
GGU-11-8946, DE02, Untergruppenback, Baden-Wurtemberg, Germany, 2011 (Dar Select)	500 WG	602 604 593	3	0 1 3 7	Fruit	1.1 0.60 0.65 0.42	0.18 0.11 0.13 0.13	1.36 0.76 0.84 0.61	QNR-0102
GGU-11-8946, DE03, Onningen, Baden-Wurtemberg, Germany, 2011 (Dar Select)	500 WG	606 596 507	3	1 3	Fruit	0.64 0.58	0.13 0.18	0.83 0.84	QNR-0102
GGU-11-8946, PL04, Lodzkie, Poland, 2011 (Senga Sengana)	500 WG	607 603 596	3	1 3	Fruit	1.3 0.11	0.10 0.12	1.44 0.28	QNR-0102
GGU-11-8946, GR05, Macedonia, Greece, 2011 (Kamaroza)	500 WG	598 599 601	3	1 3	Fruit	1.4 0.03	0.18 0.10	1.66 0.17	QNR-0102
GGU-11-8946, ES06, Huelva, Spain, 2011 (Amiga)	500 WG	584 596 574	3	0 1 3 6	Fruit	0.56 0.54 0.29 0.23	0.09 0.19 0.12 0.09	0.69 0.81 0.46 0.36	QNR-0102

In each North American trial fenpyrazamine was applied four times as a foliar spray of a 500 WG formulation (three trials) or 479 SC formulation (two trials) at a nominal rate of 560 g ai/ha. In three trials, both of these formulations were compared in side by side plots. In two trials a second plot was established, where the WG formulation was applied four times at an exaggerated (2×) rate of nominally 1120 g ai/ha. Six trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken in duplicate at the day of last application (DALA = 0). Samples from all trials/plots were harvested at commercial maturity. Two trials were performed as decline trials with one untreated control and one treated plot. Samples were taken at DALA of 0, 1, 3, 5 and 7 days.

Strawberries berries were analysed for residues of fenpyrazamine and S-2188-DC using analytical methods RM-45C (Kowalsky J., 2009/10), with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 4.0 mg/kg were in the range of 74–110% for parent fenpyrazamine and 74–103% for S-2188-DC.

Samples of strawberries berries were stored deep-frozen for a maximum of 249 days. This period is longer than the demonstrated storage stability of 114 days.

Table 42 Residues of fenpyrazamine in strawberries from supervised trials conducted in the USA

Strawberry Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed [a]	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	560	4	PHI 0	Seasonal maximum: 2240 g ai/ha Interval: 7-14 days				
VP-30127 V-30127-A Plant City, FL, USA, 2006/7 (Festival)	500 WG	568 563 559 563	4	0 1 4 5 7	Fruit Storage: 242-249 days	1.7 1.4 0.57 0.49 0.28	0.07 0.06 0.18 0.16 0.11	1.80 1.49 0.83 0.72 0.44	QNR-0091
VP-30127 V-30127-B Penn Yan, NY, USA, 2007 (Honeyoye)	500 WG	537 571 551 549	4	0	Fruit	0.54	0.03	0.58	QNR-0091
	500 WG	1141 1108 1152 1141	4	0	Fruit	0.93	0.04	0.99	QNR-0091
VP-30127 V-30127-C Aromas, CA, USA, 2007 (Raritan)	500 WG	565 564 557 569	4	0	Fruit	1.3	0.13	1.43	QNR-0091
	500 WG	1119 1122 1138 1108	4	0	Fruit	2.4	0.29	2.82	QNR-0091
VP-30127 V-30127-D North Augusta, SC, USA, 2008 (Camarosa)	479 SC	566 567 564 556	4	0	Fruit	0.65	0.06	0.74	QNR-0091
	500 WG	558 560 558 550	4	0	Fruit	0.87	0.05	0.94	QNR-0091
VP-30127 V-30127-E Brantford, Ontario, Canada, 2008 (Mira)	479 SC	582 564 577 553	4	0	Fruit	0.32	0.06	0.41	QNR-0091
	500 WG	582 585 548 562	4	0	Fruit	0.41	0.06	0.50	QNR-0091
VP-30127 V-30127-F Fresno, CA, USA, 2008 (Chandler)	479 SC	567 560 564 563	4	0	Fruit	0.95	0.19	1.22	QNR-0091
	500 WG	565 563 570 565	4	0	Fruit	0.85	0.21	1.15	QNR-0091
VP-30127 V-30127-G Santa Maria, CA, USA, 2008 (Albion)	479 SC	561 564 562 561	4	0 1 3 5 7	Fruit	0.39 0.16 0.11 0.10 0.06	0.14 0.15 0.13 0.13 0.10	0.59 0.37 0.30 0.29 0.20	QNR-0091
VP-30127 V-30127-I Junction City, OR, USA, 2009 (Benton)	479 SC	542 561 561 561	4	0	Fruit	0.88	0.08	0.99	QNR-0091

[a] Duplicate samples analysed; residues represent a mean value

Fruiting Vegetables, Cucurbits

Cucumber

Eight residue trials were conducted on indoor cucumber in Europe in 2007/08. In each trial, fenpyrazamine was applied three times as a foliar spray of a 500 WG formulation at a nominal rate of 600 g ai/ha.

All trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 1 and 3 days. In four trials an additional sampling was done at 6–7 days after last application.

Cucumber fruits were analysed for residues of fenpyrazamine and S-2188-DC using analytical method SUM-0731V, with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels in the range of 0.01 mg/kg to 0.50 mg/kg were in the range of 79% to 110% for parent fenpyrazamine and 89% to 110% for S-2188-DC.

Samples of cucumber were stored deep-frozen for a maximum of 6 months (189 days). This period is covered by storage stability data for lettuce over 12 months (364 days).

Table 43 Residues of fenpyrazamine in cucumber from supervised trials conducted outdoor in Europe

Cucumber Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 10 days				
GGU-07-2774, HU01, Bacs-Kiskum, Hungary, 2007 (Darina)	500 WG	598 600 581	3	1 3	Whole fruit	0.34 0.26	0.03 0.05	0.38 0.33	QNR-0007
GGU-07-2774, NL01, Gelderland, Netherlands, 2007 (Sheila)	500 WG	592 591 589	3	1 3 7	Whole fruit	0.14 0.15 0.04	< 0.01 < 0.01 < 0.01	0.15 0.16 0.05	QNR-0007
GGU-07-2774, IT01, Lombardia, Italy, 2007 (Canan)	500 WG	580 584 604	3	1 3	Whole fruit	0.25 0.11	< 0.01 < 0.01	0.26 0.12	QNR-0007
GGU-07-2774, SP01, Castellon, Spain, 2007 (Darina)	500 WG	590 590 592	3	1 3 6	Whole fruit	0.12 0.11 0.03	< 0.01 < 0.01 < 0.01	0.13 0.12 0.04	QNR-0007
GGU-08-4138, HU01, Gyor-Moson-Sopron, Hungary, 2008 (Budaicsemege)	500 WG	581 578 607	3	1 3	Whole fruit	0.14 0.10	0.02 0.09	0.17 0.23	QNR-0016
GGU-08-4138, NL01, Gelderland, Netherlands. 2008 (Sheila)	500 WG	612 597 595	3	1 3 7	Whole fruit	0.22 0.13 0.13	< 0.01 < 0.01 < 0.01	0.23 0.14 0.14	QNR-0016
GGU-08-4138, IT01, Lombardia, Italy, 2008 (Caman)	500 WG	590 620 572	3	1 3	Whole fruit	0.16 0.08	< 0.01 < 0.01	0.17 0.09	QNR-0016
GGU-08-4138, SP01, Murcia, Spain, 2008 (Anico)	500 WG	597 610 576	3	1 3 6	Whole fruit	0.33 0.14 0.06	< 0.01 0.01 < 0.01	0.34 0.15 0.07	QNR-0016

Fruiting Vegetables, other than Cucurbits

Pepper

Eight residue trials were conducted on pepper indoor in Europe in 2007/08. In each trial, fenpyrazamine was applied three times as a foliar spray of a 500 WG formulation at a nominal rate of 600 g ai/h.

All trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 1 and 3 days. In four trials an additional sampling was done at 7 days after last application.

Pepper fruits were analysed for residues of fenpyrazamine and S-2188-DC using analytical method SUM-0731V, with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels in the range of 0.01 mg/kg to 0.50 mg/kg were in the range of 78% to 100% for parent fenpyrazamine and 76% to 103% for S-2188-DC.

Samples of pepper were stored deep-frozen for a maximum of 6 months (174 days). This period is covered by storage stability data for lettuce over 12 months (364 days).

Table 44 Residues of fenpyrazamine in peppers from supervised trials conducted indoor in Europe

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 10 days				
Bell pepper (sweet) GGU-07-2773, HU01, Bacs-Kiskum, Hungary, 2007 (Tezeki white)	500 WG	624 608 589	3	1 3	Whole fruit	1.3 1.1	0.10 0.10	1.44 1.24	QNR-0006
Bell pepper (sweet) GGU-07-2773, NL01, Gelderland, Netherlands, 2007 (Ferrari)	500 WG	583 577 577	3	1 3 7	Whole fruit	0.55 0.58 0.42	0.01 0.01 0.02	0.56 0.59 0.45	QNR-0006
Bell pepper (sweet) GGU-07-2773, IT01, Lombardia, Italy, 2007 (Pyrean)	500 WG	602 599 595	3	1 3	Whole fruit	1.4 1.0	0.04 0.02	1.46 1.03	QNR-0006
Bell pepper (sweet) GGU-07-2773, SP01, Castellon, Spain, 2007 (Almagro)	500 WG	585 625 592	3	1 3 7	Whole fruit	0.68 0.69 0.54	0.03 0.04 0.03	0.72 0.75 0.58	QNR-0006
Bell pepper (sweet) GGU-08-4137, HU01, Gyor-Moson-Sopron, Hungary, 2008 (Century)	500 WG	588 606 602	3	1 3	Whole fruit	0.60 0.48	0.14 0.16	0.80 0.71	QNR-0015
Bell pepper (sweet) GGU-08-4137, NL01, Gelderland, Netherlands. 2008 (Spider)	500 WG	608 600 595	3	1 3 7	Whole fruit	0.16 0.46 0.47	0.02 0.06 0.04	0.19 0.55 0.53	QNR-0015
Bell pepper (sweet) GGU-08-4137, IT01, Lombardia, Italy, 2008 (Quadrato)	500 WG	602 555 608	3	1 3	Whole fruit	0.74 0.94	0.04 0.04	0.80 1.0	QNR-0015
Bell pepper (sweet) GGU-08-4137, SP01, Murcia, Spain, 2008 (Gacela - California)	500 WG	585 584 574	3	1 3 7	Whole fruit	1.2 0.63 0.47	0.24 0.18 0.19	1.54 0.89 0.74	QNR-0015

Tomato

Eight residue trials were conducted on indoor cherry tomato in Europe in 2007/08. In each trial, fenpyrazamine was applied three times as a foliar spray of a 500 WG formulation at a nominal rate of 600 g ai/ha.

All trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 1 and 3 days. In four trials an additional sampling was done at 6–8 days after last application.

Tomato fruits were analysed for residues of fenpyrazamine and S-2188-DC using analytical method SUM-0731V, with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels in the range of 0.01 mg/kg to 0.50 mg/kg were in the range of 79% to 97% for parent fenpyrazamine and 75% to 98% for S-2188-DC.

Samples of tomatoes were stored deep-frozen for a maximum of 5 months (165 days). This period is covered by storage stability data for lettuce over 12 months (364 days).

Table 45 Residues of fenpyrazamine in cherry tomatoes from supervised trials conducted indoor in Europe

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in Europe	500 WG	600	3	PHI 1	Interval: 10 days				
GGU-07-2772, HU01, Bacs-Kiskum, Hungary, 2007 (Cheramy)	500 WG	600 588 611	3	1 3	Whole fruit	0.56 0.28	0.06 0.06	0.65 0.37	QNR-0005
GGU-07-2772, NL01, Brabant, Netherlands. 2007 (Claree)	500 WG	585 587 593	3	1 3 6	Whole fruit	1.0 1.5 0.88	0.03 0.02 0.03	1.04 1.53 0.92	QNR-0005
GGU-07-2772, IT01, Lombardia, Italy, 2007 (Datterino Dasher)	500 WG	595 603 606	3	1 3	Whole fruit	0.71 0.28	0.02 < 0.01	0.74 0.29	QNR-0005
GGU-07-2772, SP01, Castellon, Spain, 2007 (Lucinda)	500 WG	587 587 593	3	1 3 7	Whole fruit	1.8 1.65 1.2	0.02 0.02 0.04	1.83 1.68 1.26	QNR-0005
Tomato (cherry), GGU-08-4136, HU01, Bacs-Kiskum, Hungary, 2008 (Caramella)	500 WG	604 585 584	3	1 3	Whole fruit	0.66 0.46	0.01 < 0.01	0.67 0.47	QNR-0014
Tomato (cherry), GGU-08-4136, NL01, Zuid-Holland, Netherlands. 2008 (Claree)	500 WG	585 579 600	3	1 3 7	Whole fruit	0.85 0.67 0.66	0.02 0.02 0.02	0.88 0.70 0.69	QNR-0014
Tomato (cherry), GGU-08-4136, IT01, Lombardia, Italy, 2008 (Birillino)	500 WG	630 620 605	3	1 3	Whole fruit	0.43 0.65	0.06 0.04	0.52 0.71	QNR-0014
Tomato (cherry), GGU-08-4136, SP01, Granada, Spain, 2008 (Catalina)	500 WG	595 607 600	3	1 3 8	Whole fruit	1.2 1.4 1.1	< 0.01 0.02 0.02	1.21 1.43 1.13	QNR-0014

Leafy vegetables

Lettuce

Sixteen residue trials were conducted on lettuce in the USA (eight each in head lettuce and leaf lettuce) in 2009/10. In each trial, fenpyrazamine was applied three times as a foliar spray of a 479 SC formulation at a nominal rate of 840 g ai/ha. In four trials (two in head varieties and two in leaf varieties) a second plot was established, were fenpyrazamine was applied three times at an exaggerated (2×) rate of nominally 1680 g ai/ha. In all trials, application rates were outside of ±25%

of the critical rate but other conditions were within the GAP conditions. Scaling of residues according to the proportionality principle was necessary for the results to be used for estimating an MRL.

Six trials per each crop type (head and leaf lettuce) were performed as harvest trials with one untreated control and one treated plot. Samples were taken in duplicate at the day of harvest (DALA = 13–14). Samples from all trials/plots were harvested at commercial maturity.

Two trials per each crop type were performed as decline trials with one untreated control and one treated plot. Samples were taken at DALA of nominally 3, 7, 14 and 21 days.

Lettuce samples were analysed for residues of fenpyrazamine and S-2188-DC using RM-45C-1 (Kowalsky J., 2010), with an LOQ of 0.01 mg/kg. Head lettuce samples were analysed with and without wrapper leaves. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 5.0 mg/kg were in the range of 76–126% for parent fenpyrazamine and 69–119% for S-2188-DC.

The maximum interval of frozen storage as whole lettuce was 296 days. Except for samples from trials V-32906-G and -I, the samples were stabilized within 36 days of harvest with ascorbate. The maximum frozen storage interval for thus stabilized lettuce was 519 days. Both, fenpyrazamine and S-2188-DC residues were found to be stable during frozen storage in macerated lettuce for 364 days and in macerated lettuce stabilized with 1 M sodium ascorbate during frozen storage for 510 days.

Table 46 Residues of fenpyrazamine in lettuce from supervised trials conducted in the USA

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg) [a]			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	560	3	PHI 14	Seasonal maximum: 1680 g ai/ha Interval: 7-10 days				
Head Lettuce									
VP-32906, V-32906-A North Rose, NY, USA, 2009 (Mighty Joe)	479 SC	843 861 842 (2546)	3	14	Head with wrapper leaves	0.01	< 0.01	0.02	QNR-0089
				14	Head without wrapper leaves	< 0.01	< 0.01	< 0.02	
VP-32906, V-32906-B Oviedo, Fl, USA, 2009 (Great Lakes Head Lettuce)	479 SC	834 818 834 (2486)	3	14	Head with wrapper leaves	< 0.01	< 0.01	< 0.02	QNR-0089
				14	Head without wrapper leaves	< 0.01	< 0.01	< 0.02	
VP-32906, V-32906-C Porterville, CA, USA, 2009 (Vandenberg)	479 SC	850 832 839 (2521)	3	3 6 14 21	Head with wrapper leaves	0.81 0.13 0.02 0.02	1.46 0.20 0.03 0.01	2.90 0.42 0.06 0.03	QNR-0089
				3 6 14 21	Head without wrapper leaves	0.07 0.01 < 0.01 < 0.01	0.02 < 0.01 < 0.01 < 0.01	0.10 0.02 < 0.02 < 0.02	
VP-32906, V-32906-D Aromas, CA, USA, 2009 (Legacy)	479 SC	836 859 857 (2552)	3	3 7 14 20	Head with wrapper leaves	0.63 0.17 0.05 0.03	1.72 0.39 0.14 0.09	3.09 0.73 0.25 0.16	QNR-0089
				3 7 14 20	Head without wrapper leaves	0.04 < 0.01 < 0.01 < 0.01	0.07 < 0.01 < 0.01 < 0.01	0.14 < 0.02 < 0.02 < 0.02	
VP-32906 V-32906-E Madera, CA, USA, 2009 (Canary Row)	479 SC	844 832 838 (2514)	3	14 14	Head with wrapper leaves	0.05	0.03	0.09	QNR-0089
					Head without wrapper leaves	< 0.01	< 0.01	< 0.02	
		1678 1667 1669 (5014)	3	14	Head with wrapper leaves	0.27	0.23	0.60	QNR-0089
				14	Head without wrapper leaves	< 0.01	< 0.01	< 0.02	

Grape Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)[a]			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
VP-32906 V-32906-F Hickman, CA, USA, 2009 (Vandenberg)	479 SC	839 840 836 (2515)	3	13	Head with wrapper leaves	0.10	0.01	0.11	QNR-0089
				13	Head without wrapper leaves	0.05	0.01	0.06	
		1685 1669 1681 (5035)	3	13	Head with wrapper leaves	0.21	0.04	0.27	QNR-0089
				13	Head without wrapper leaves	0.08	0.01	0.09	
VP-32906 V-32906-G Yuma, AZ, USA, 2009 (Desert Storm)	479 SC	833 846 817 (2496)	3	14	Head with wrapper leaves	0.33	0.56	1.13	QNR-0089
				14	Head without wrapper leaves	< 0.01	< 0.01	< 0.02	
VP-32906 V-32906-I Santa Maria, CA, USA, 2010 (Vandenberg)	479 SC	845 846 852 (2543)	3	13	Head with wrapper leaves	0.91	0.29	1.33	QNR-0089
				13	Head without wrapper leaves	0.01	< 0.01	0.02	
Leaf Lettuce									
VP-32904, V-32904-A North Rose, NY, USA, 2009 (Lasting Green)	479 SC	856 842 863 (2561)	3	14	Leaves	1.07	0.13	1.26	QNR-0090
VP-32904, V-32904-B Oviedo, FL, USA, 2009 (Buttercrunch Bibb Lettuce)	479 SC	831 826 817 (2474)	3	14	Leaves	< 0.01	< 0.01	< 0.02	QNR-0090
VP-32904, V-32904-C Madera, CA, USA, 2009 (Red Tide)	479 SC	813 829 819 (2461)	3	3 7 13 21	Leaves	1.19 0.47 0.33 0.07	3.09 1.14 0.29 0.09	5.62 2.10 0.75 0.20	QNR-0090
VP-32904, V-32904-D Paso Robles, CA, USA, 2009 (Outback)	479 SC	831 834 838 (2503)	3	3 7 14 20	Leaves	1.37 0.40 0.14 0.02	3.31 1.05 0.24 0.03	6.11 1.90 0.48 0.06	QNR-0090
VP-32904, V-32904-E Porterville, CA, USA, 2009 (Green Star)	479 SC	835 832 837 (2504)	3	14	Leaves	0.13	0.11	0.29	QNR-0090
		1726 1679 1680 (5085)	3	14	Leaves	1.58	3.66	6.82	
VP-32904, V-32904-F Aromas, CA, USA, 2009 (Green Towers)	479 SC	839 844 833 (2516)	3	13	Leaves	0.01	< 0.01	0.02	QNR-0090
		1680 1697 1692 (5069)	3	13	Leaves	0.01	< 0.01	0.02	
VP-32904, V-32904-G Fresno, CA, USA, 2009 (Valley Heart)	479 SC	836 840 844 (2520)	3	14	Leaves	0.12	0.12	0.29	QNR-0090
VP-32904, V-32904-H Yuma, AZ, USA, 2009 (Winterhaven)	479 SC	845 849 838 (2532)	3	14	Leaves	0.44	0.73	1.49	QNR-0090

[a] Duplicate samples analysed; residues represent a mean value

Root and tuber vegetables

Ginseng

Three residue trials were conducted on ginseng in the USA in 2008. In each trial, fenpyrazamine was applied four times as a foliar spray of a 479 SC formulation at a nominal rate of 560 g ai/ha.

Two trials were performed as harvest trials with one untreated control and one treated plot. Samples of ginseng roots were taken in duplicate at a DALA of 2 days. Samples from all trials/plots were harvested at commercial maturity.

One trial was performed as a decline trial with one untreated control and one treated plot. Samples were taken at DALA of 0, 2, 7 and 13 days.

All ginseng roots were dried in a commercial drying facility immediately after sampling, simulating commercial drying practices.

Dried ginseng roots were analysed for residues of fenpyrazamine and S-2188-DC using the Cornell Analytical Laboratory (CAL) method, with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 0.20 mg/kg were in the range of 55–85% for parent fenpyrazamine and 100–150% for S-2188-DC. For the metabolite S-2188-DC the generally high procedural recoveries show that the residues were overestimated. However, residues of S-2188-DC were below the LOQ in all the trials.

Samples of ginseng roots were stored deep-frozen for a maximum of 8 months (253 days). This period is covered by storage stability data for ginseng roots over 12 months (350 days).

Table 47 Residues of fenpyrazamine in ginseng from supervised trials conducted in the USA

Ginseng Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg)[a]			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	560	4	PHI 2	Seasonal maximum: 2240 g ai/ha Interval: 7-14 days				
IR-4 Project no. 09453, 09453.08-MI15 Wausau, WI, USA, 2008 (American)	479 SC	583 560 595 597	4	2	dried roots	0.17	< 0.02	0.20	QNR-0121
IR-4 Project no. 09453, 09453.08-MI16 Athens, WI, USA, 2008 (American)	479 SC	575 554 555 576	4	2	dried roots	0.32	< 0.02	0.35	QNR-0121
IR-4 Project no. 09453, 09453.08-MI17 Poniatowski, WI, USA, 2008 (American)	479 SC	566 560 554 569	4	0 2 7 13	dried roots	0.15 0.15 0.098 0.11	< 0.02 < 0.02 < 0.02 < 0.02	0.18 0.18 0.13 0.14	QNR-0121

[a] Duplicate samples analysed; residues represent a mean value

Tree Nuts

Almond

Five residue trials were conducted on almond in the USA in 2008. In each trial, fenpyrazamine was applied three times as a foliar spray of 479 SC at a nominal rate of 447 g ai/ha. In two trials a second plot was established, where fenpyrazamine was applied three times at an exaggerated (2×) rate of nominally 894 g ai/ha.

Four trials were performed as harvest trials with one untreated control and one treated plot. Samples were taken in duplicate at DALA of 20–21 days. Samples from all trials/plots were harvested at commercial maturity.

One trial was performed as a decline trial with one untreated control and one treated plot. Samples were taken at DALA of 16, 21, 26 and 31 days.

Almond nutmeat samples were analysed for residues of fenpyrazamine and S-2188-DC using analytical method RM-45C-1, with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels of 0.02 mg/kg and 0.10 mg/kg were in the range of 70–106% for parent fenpyrazamine and 71–97% for S-2188-DC.

Samples of almond nutmeat were stored deep-frozen for a maximum of 827 days (27 months), thereof 818 days (27 months) stabilized with sodium ascorbate. Storage stability data is available for fenpyrazamine and S-2188-DC in rapeseed, as oily crop, demonstrating stability over a period of 12 months (364 days) without addition of ascorbate. This period is not covering the whole storage duration for almond nutmeat over 827 days for field trial samples.

Table 48 Residues of fenpyrazamine in almond nutmeat from supervised trials conducted in the USA

Ginseng Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg) [a]			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	420	3	PHI 21	Seasonal maximum: 1270 g ai/ha				
VP-30088 V-30088-B Sutter, CA, USA, 2008 (Nonpareil)	479 SC	448 447 448	3	21	Nutmeat Storage >364 days	< 0.01	< 0.01	< 0.02	QNR-0087
	479 SC	903 901 899	3	21	Nutmeat Storage >364 days	< 0.01	< 0.01	< 0.02	
VP-30088 V-30088-C Kerman, CA, USA, 2008 (Carmel)	479 SC	446 454 451	3	21	Nutmeat	< 0.01	< 0.01	< 0.02	QNR-0087
	479 SC	893 870 897	3	21	Nutmeat	< 0.01	< 0.01	< 0.02	
VP-30088 V-30088-D Orland, CA, USA, 2008 (Nonpareil)	479 SC	450 449 449	3	16 21 26 31	Nutmeat Storage for ca 800 days	< 0.01 < 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01 < 0.01	< 0.02 < 0.02 < 0.02 < 0.02	QNR-0087
VP-30088 V-30088-E Madera, CA, USA, 2008 (Nonpareil)	479 SC	451 459 455	3	20	Nutmeat	< 0.01	< 0.01	< 0.02	QNR-0087
VP-30088 V-30088-F Terra Bella, CA, USA, 2008 (Nonpareil)	479 SC	457 456 462	3	21	Nutmeat	< 0.01	< 0.01	< 0.02	QNR-0087

[a] Duplicate samples analysed; residues represent a mean value

Animal feeds

Almond hulls

Almond hulls were analysed for residues of fenpyrazamine and S-2188-DC using analytical method RM-45C-1, with an LOQ of 0.02 mg/kg. Procedural recoveries at fortification levels in the range of 0.02 mg/kg to 5.0 mg/kg were 71–96% for parent fenpyrazamine and 65–120% for S-2188-DC.

Samples of almond hulls were stored deep-frozen for a maximum of 830 days (27 months), thereof 812 days (27 months) stabilized with sodium ascorbate. In almond hulls stability is only

confirmed for fenpyrazamine over a storage period of 14 months (440 days) also in ascorbate stabilized samples. S-2188-DC was analysed in all the samples after storage periods of longer than 800 days.

Table 49 Residues of fenpyrazamine in almond hulls from supervised trials conducted in the USA

Ginseng Study, Trial, Location, Year (Variety)	Application			DALA (days)	Portion analysed	Residues (mg/kg) [a]			Ref
	Form. (g ai/kg)	Rate g ai/ha	No.			Fenpyrazamine	S-2188-DC	Sum	
Critical GAP in the USA	479 SC	420	3	PHI 21	Seasonal maximum: 1270 g ai/ha				
VP-30088 V-30088-B Sutter, CA, USA, 2008 (Nonpareil)	479 SC	448 447 448	3	21	Hull	0.29	0.06	0.38	QNR-0087
	479 SC	903 901 899	3	21	Hull	1.07	0.26	1.44	
VP-30088 V-30088-C Kerman, CA, USA, 2008 (Carmel)	479 SC	446 454 451	3	21	Hull	0.48	0.07	0.58	QNR-0087
	479 SC	893 870 897	3	21	Hull	1.74	0.24	2.08	
VP-30088 V-30088-D Orland, CA, USA, 2008 (Nonpareil)	479 SC	450 449 449	3	16 21 26 31	Hull	1.37 0.90 1.37 1.07	0.17 0.09 0.25 0.14	1.61 1.03 1.73 1.27	QNR-0087
VP-30088 V-30088-E Madera, CA, USA, 2008 (Nonpareil)	479 SC	451 459 455	3	20	Hull	0.50	0.06	0.59	QNR-0087
VP-30088 V-30088-F Terra Bella, CA, USA, 2008 (Nonpareil)	479 SC	457 456 462	3	21	Hull	0.31	0.03	0.35	QNR-0087

[a] Duplicate samples analysed; residues represent a mean value

FATE OF RESIDUES IN STORAGE AND IN PROCESSING

Information and Data from Residues in Processed Commodities

The Meeting received information on processing of grapes to processed commodities.

Hydrolysis

The hydrolysis of [Pyrazolyl-5-^{14}C] fenpyrazamine was investigated by incubation in sterile buffered aqueous solution under the conditions simulating pasteurization, baking/brewing/boiling and sterilization (Lewis C.J., Troth K., 2007; QNM-0030).

[Pyrazolyl-5-^{14}C] fenpyrazamine was dissolved in the aqueous buffer solutions to give a final concentration of *ca.* 1.0 µg/ml and incubated at 90 °C (pH 4) for 20 minutes, 100 °C (pH 5) for 60 minutes or 120 °C (pH 6) for 20 minutes. For each set of conditions, the total radioactivity was determined at the beginning and the end of the incubation period after cooling. The total radioactivity in each sample was determined by LSC, both directly and after dilution with a small volume of acetonitrile. The residue levels reported were determined from the diluted samples. Radioactivity in the diluted samples was analysed by HPLC. The identities of fenpyrazamine and S-2188-DC were confirmed by 2D-TLC of the undiluted samples.

The mean recovery of applied radioactivity was 99–101% before incubation and 97–102% after incubation. At the end of the incubation periods, the major component in each case was unchanged fenpyrazamine. The only degradation product of any significance was S-2188-DC,

comprising 0.5%, 1.0% and 8.6% of the applied radioactivity after incubation under pasteurisation, baking/brewing/boiling and sterilization conditions, respectively.

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

Table 50 Effect of hydrolysis on fenpyrazamine in buffers at pH 4, 5 and 6 simulating food processing

Process simulated	Test condition			% Applied radioactivity			
	pH	Temp, °C	Time, min	Before incubation		After incubation	
				Fenpyrazamine	S-2188-DC	Fenpyrazamine	S-2188-DC
Pasteurization	4	90	20	100	0.3	95.1	0.5
Baking/brewing/boiling	5	100	60	98.1	0.1	99.8	1.0
Sterilization	6	120	20	101	0.1	89.1	8.6

Grapes

A number of studies were conducted on the processing of grapes in conjunction with supervised residue trials.

General processing procedures are briefly described in Figure 5 below.

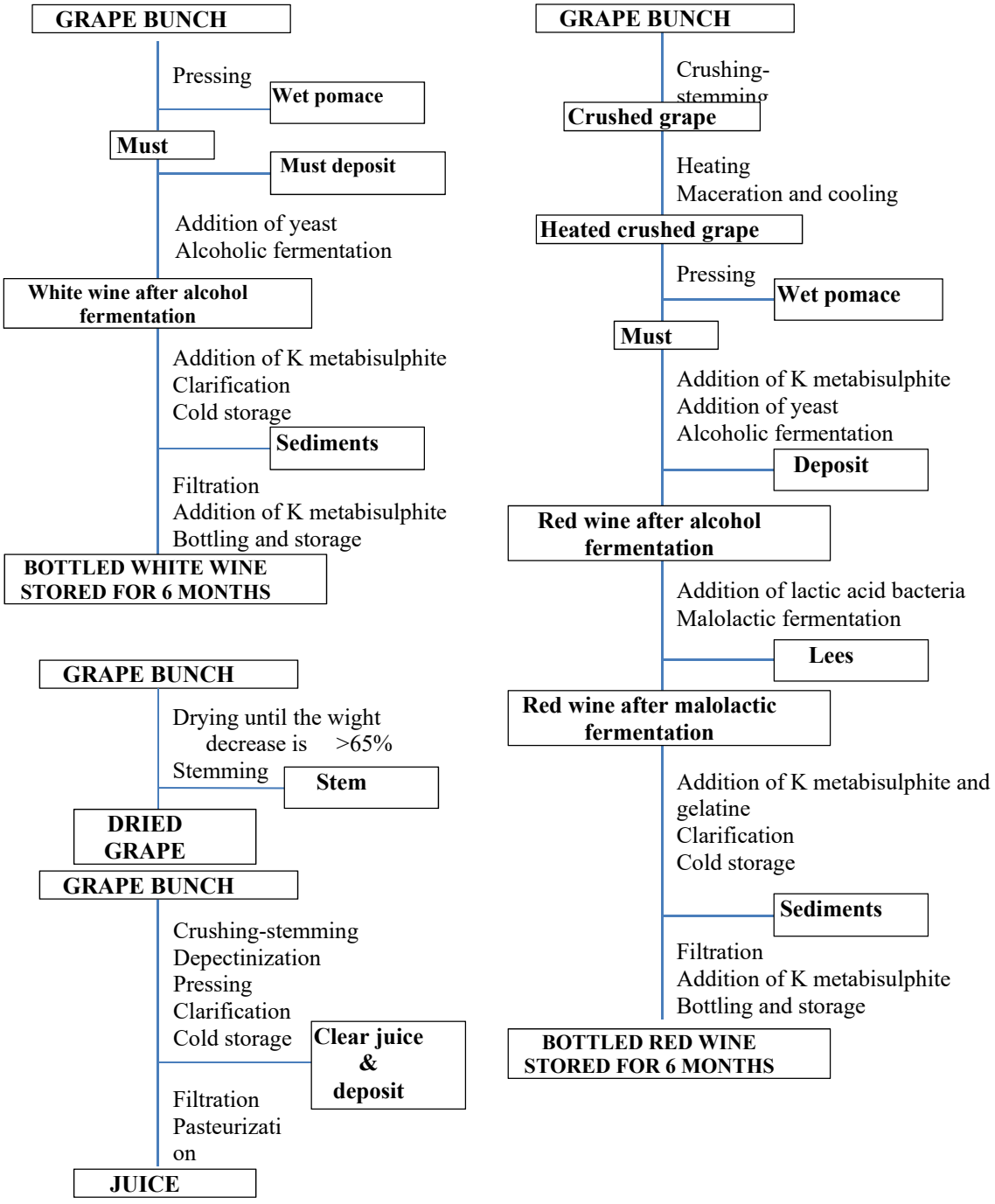


Figure 5 Processing of grapes into white and red wines and dried grapes

First study (Grolleau, G., 2008; QNR-0004)

In supervised trial SP01 in study GGU-06-1765 conducted in Spain, a red variety of grapevine was treated once according to critical GAP in Europe (dose rate of 600 g ai/ha 14 days before harvest). Grape bunches for processing were collected and transported to the processing laboratory within one day. Bunches were processed to must and red wine simulating industry procedure in a small volume. Samples were collected at various stages of wine production and residues of fenpyrazamine and S-2188-DC were determined using analytical method SUM-0731V within 275 days from sampling.

Results and calculated processing factors are shown in Table 51. Higher concentrations of residues were found in crushed grapes and wet pomace with the processing factors for both fenpyrazamine and the total residues of about 2–3, and 5–6, respectively.

Table 51 Processing of grape from Study GGU-06-1765, Trial SP01 to red wine

Commodity/ Processed Fraction	Residue (mg/kg)		Processing Factor	
	Fenpyrazamine	Fenpyrazamine + S-2188-DC	Fenpyrazamine	Fenpyrazamine + S-2188-DC
Bunches used for processing	0.67	0.90	—	—
Crushed grapes	2.0	2.79	3.0	3.1
Heated crushed grapes	1.2	1.76	1.8	2.0
Wet pomace	3.9	4.49	5.8	5.0
Must deposit	0.82	1.51	1.2	1.7
Red wine after alcoholic fermentation stage	0.32	0.95	0.5	1.1
Lees	0.50	1.26	0.7	1.4
Red wine after malolactic fermentation stage	0.30	1.00	0.4	1.1
Sediments	0.34	1.10	0.5	1.2
Red wine at bottling	0.29	1.01	0.4	1.1
Red wine after 6-months storage	0.30	1.03	0.4	1.1

Second study (Grolleau, G., 2008; QNR-0011)

In supervised trials IT01, IT02 and SP01 in study GGU-07-2771 conducted in Italy and Spain, white and red variety of grapevine were treated once according to critical GAP in Europe (dose rate of 600 g ai/ha 13-15 days before harvest). Grape bunches for processing were collected and transported to the processing laboratory within one day. Bunches were processed to dried grape, juice, wine and by-products. Residues in all processed fractions were determined by analytical method SUM-0731V within 184 days from sampling.

Results and calculated processing factors are shown in Table 52. Residues were concentrated in dried grape and wet pomace with processing factors for both fenpyrazamine and the total residues of around 1-5 and 2, respectively.

Table 52 Processing of grape from Study GGU-07-2771, Trial IT01, IT02 and SP01 to processed products

Commodity/ Processed Fraction	Residue (mg/kg)		Processing Factor	
	Fenpyrazamine	Fenpyrazamine + S-2188-DC	Fenpyrazamine	Fenpyrazamine + S-2188-DC
IT01				
Bunches	0.09	0.10	—	—
Stems (dried)	0.92	0.96	10.2	9.6
Dried grape	0.42	0.45	4.7	4.5
Must	0.14	0.15	1.6	1.5
Wet pomace	0.21	0.22	2.3	2.2
Must deposit	0.50	0.53	5.6	5.3
White wine after alcoholic fermentation	0.10	0.13	1.1	1.3
Lees	0.21	0.24	2.3	2.4
Sediment	0.10	0.11	1.1	1.1
White wine at bottling	0.10	0.13	1.1	1.3
White wine after 6-months storage	0.09	0.12	1.0	1.2
IT02				
Bunches	0.15	0.19	—	—
Stems (dried)	1.4	1.51	9.3	7.9
Dried grape	0.21	0.22	1.4	1.2
Stems	0.52	0.56	3.5	2.9

Commodity/ Processed Fraction	Residue (mg/kg)		Processing Factor	
	Fenpyrazamine	Fenpyrazamine + S-2188-DC	Fenpyrazamine	Fenpyrazamine + S-2188-DC
Raw juice	0.05	0.06	0.3	0.3
Wet pomace	0.29	0.30	1.9	1.6
Clear juice	0.04	0.05	0.3	0.3
Deposit	0.06	0.07	0.4	0.4
Juice	0.04	0.07	0.3	0.4
White wine at bottling	0.22	0.29	1.5	1.5
White wine after 6-months storage	0.18	0.25	1.2	1.3
SP01				
Bunches	0.15	0.16	—	—
Stems	0.59	0.65	3.9	4.1
Raw juice	0.06	0.09	0.4	0.6
Wet pomace	0.24	0.25	1.6	1.6
Clear juice	0.06	0.09	0.4	0.6
Deposit	0.07	0.10	0.5	0.6
Juice	0.06	0.09	0.4	0.6
Red wine at bottling	0.06	0.10	0.4	0.6
Red wine after 6-months storage	0.07	0.13	0.5	0.8

Third study (Grolleau, G., 2009; QNR-0035)

In trials GGU-08-4135-IT01, IT03 and SP01 conducted in Italy and Spain, red and white varieties of grapevine were treated once according to critical GAP in Europe (dose rate of 600 g ai/ha, 14 days before harvest). Grape bunches for processing were collected and transported to the processing laboratory within one day. Bunches were processed to dried grape, grape juice and wine. Residues were determined in all processed fractions by analytical method SUM731V within 84 days from sampling.

Results and calculated processing factors are shown in Table 53. Residues were concentrated in dried grape and white wine with processing factors for both fenpyrazamine and the total residues of around 4–5 and 2–3, respectively.

Table 53 Processing of grape from trials GGU-08-4135-IT01, IT03 and SP01 to processed products

Commodity/ Processed Fraction	Residue (mg/kg)		Processing Factor	
	Fenpyrazamine	Fenpyrazamine + S-2188-DC	Fenpyrazamine	Fenpyrazamine + S-2188-DC
IT01				
Bunches	0.42	0.53	—	—
Dried grape	2.0	2.19	4.8	4.1
Juice	0.07	0.17	0.2	0.3
IT03				
Bunches	0.32	0.41	—	—
Red wine at bottling	0.08	0.31	0.3	0.8
Red wine after 6-months storage	0.09	0.35	0.3	0.9
SP01				
Bunches	0.31	0.34	—	—
Juice	0.09	0.13	0.3	0.4
White wine at bottling	0.75	0.94	2.4	2.8
White wine after 6-months storage	0.78	0.97	2.5	2.9

Fourth study (Kowalsky J., 2011; QNR-0088)

In the trial V-30097-N conducted in California, USA in 2008, table grapes were treated at an exaggerated rate of 2800 g ai/ha (5× the US label rate). Samples of grape berries were harvested at a DALA of three days. Grape berries were processed to dried grape and grape juice. Residues were determined in all processed fractions by analytical method RM-45C within 274 days from sampling.

Results and calculated processing factors are shown in Table 54. In this study, concentration of residues was observed in both dried grape and juice with processing factors for both fenpyrazamine and the total residues of slightly above 1 and around 2, respectively.

Table 54 Processing of grape from trial V-30097-N to dried grape and juice

Commodity/ Processed Fraction	Residue (mg/kg)		Processing Factor	
	Fenpyrazamine	Fenpyrazamine + S-2188-DC	Fenpyrazamine	Fenpyrazamine + S-2188-DC
Berries	4.00	5.52	—	—
Dried grape	4.23	6.70	1.1	1.2
Juice	8.05	10.5	2.0	1.9

Processing studies were conducted on grapes to grape juice, dried grape, and white and red wine. Individual and best estimates of processing factors for fenpyrazamine and the sum of fenpyrazamine and S-2188-DC expressed as fenpyrazamine from these processing studies are summarized in Table 55.

Table 55 Summary of processing factors for processed grape products

Processed Commodity	Fenpyrazamine		Fenpyrazamine + S-2188-DC	
	Individual processing factor	Best estimate	Individual Processing Factors	Best estimate
Dried grape	1.1, 1.4, 4.7, 4.8	3.1	1.2, 1.2, 4.1, 4.5	2.7
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
Wet pomace	1.6, 1.9, 2.3, 5.8,	2.1	1.6, 1.6, 2.2, 5.0	1.9
White wine (at bottling)	1.1, 1.5, 2.4	1.5	1.3, 1.5, 2.8	1.5
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
Red wine (after 6 months storage)	0.3, 0.4, 0.5	0.4	0.8, 0.9, 1.1	0.9

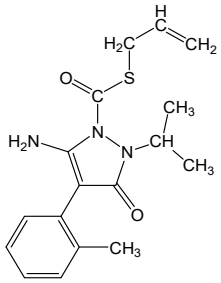
RESIDUES ON ANIMAL PRODUCTS

Livestock Feeding Studies

No feeding studies were received by the current Meeting.

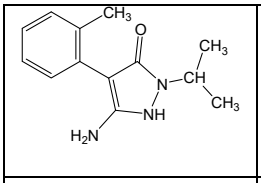
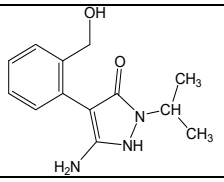
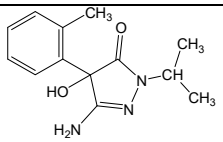
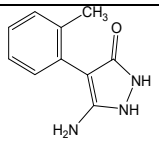
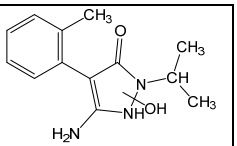
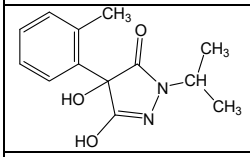
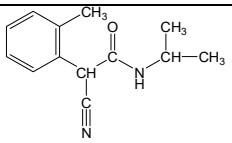
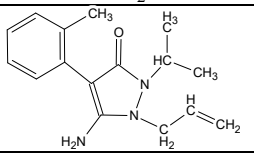
APPRAISAL

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 deoxygenated 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

Almonds

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 2× 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 below 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.

Commodity		Recommended MRL, mg/kg		STMR/STMR-P/mean residue mg/kg	HR/HR-P/Highest residue mg/kg
CCN	Name	New	Previous		
FS 0013	Subgroup of Cherries	3		0.74	2.2

Commodity		Recommended MRL, mg/kg		STMR/STMR-P/mean residue mg/kg	HR/HR-P/Highest residue mg/kg
CCN	Name	New	Previous		
FS 0014	Subgroup of Plums	2		0.455	1.7
FS 2001	Subgroup of Peaches	4		1.1	3.8
FB 2005	Subgroup of Cane berries	5		2.05	3.3
FB 2006	Subgroup of Bush berries	4		0.985	2.9
FB 0269	Grapes	4		1.25	3.4
DF 0269	Dried grapes	12		3.38	9.2
FB 0275	Strawberry	3		0.94	2.0
VC 0424	Cucumber	0.7		0.23	0.38
VO 0445	Peppers, sweet	3		0.90	1.5
VO 0448	Tomato	3		0.81	1.8
VO 2700	Cherry tomato	3		0.81	1.8
VO 2046	Subgroup of eggplants	3		0.81	1.8
VL 0482	Lettuce, head	1.5		0.195	2.4
VL 0483	Lettuce, leaf	1.5		0.195	2.4
VR 0604	Ginseng	0.7		0.20	0.38
TN 0660	Almond	0.01*		0.02	
MF 0100	Mammalian fats (except milk fats)	0.02*	-	0	0
MM 0095	Meat (from mammals other than marine mammals)	0.02*	-	0	0
ML 0106	Milks	0.01*	-	0	-
MO 0105	Edible offal (mammalian)	0.05	-	0.018 Kidney 0.038 Liver	0.018 Kidney 0.038 Liver

For calculating dietary intake

Commodity		STMR-P mg/kg	HR-P mg/kg
CCN	Name		
	Grape juice	0.5	-
	White wine	1.88	-
	Red wine	1.12	

For calculating animal dietary burdens

Commodity		Median residue mg/kg	Highest residue mg/kg
CCN	Name		
	Wet pomace of grape	2.4	-

Expressed on an “as received” basis

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.

The International Estimated Short-Term Intakes (IESTI) of fenpyrazamine were calculated for the commodities using HRs/HR-Ps and STMRs/STMR-Ps estimated by the current Meeting (see Annex 4 of the 2017 Report). The calculated IESTIs were 0–40% of the ARfD for the general population and 0–30% of the ARfD for children. The Meeting concluded that the short-term dietary exposure to residues of fenpyrazamine, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.

REFERENCES

Reference No	Author(s)	Year	Study Title
QNA-0006	Rzepka S., Jungklaus N.	2007	Validation according to DFG Method S19 (extended and revised version) for the Determination of Residues of S-2188 in Grapes, Oil Seed Rape (seeds), Carrots, Pepper and Cereals (straw and grain); Eurofins Analytik GmbH report no.: SUM-0701V
QNA-0008	Rzepka S., Jungklaus N.	2007	Validation of a Method for the Determination of Residues of S-2188 and its Metabolite, S-2188-DC, in Grapes, Oil Seed Rape (Seeds), Carrot, Green Pepper and Cereals (Grain and Straw), Eurofins Analytik GmbH report no.: SUM-0731V
QNA-0009	Rzepka S., Jungklaus N.	2007	Validation of a Method for the Determination of Residues of S-2188-OH, one of the metabolites of S-2188, in Grapes, Oil Seed Rape (Seeds), Carrot, Green Pepper and Cereals (Grain and Straw), Eurofins Analytik GmbH report no.: SUM-0706V
QNA-0014	Bacher R.	2008	Independent Laboratory Validation of the Multi-Residue Enforcement Method DFG S19 (Extended Revision, 35 LMBG) for the Determination of S-2188 in Plant; PTRL Europe report no. P 1402 G
QNA-0038	Richter S.	2014	Validation of a Residue Method for the Determination of Fenpyrazamine (S-2188) in Foodstuff of Animal Origin; PTRL Europe ID: P 3261 G
QNM-0013	Lewis C.J., Cooke C.	2006	[^{14}C]S-2188: Metabolism in Grapevine Covance Laboratories Ltd. Report no. 0333/248-D2149
QNM-0014	Lewis C.J., Cooke C.	2007	[^{14}C]S-2188: Metabolism in Lettuce Covance Laboratories Ltd. Report no. 0333/250-D2149
QNM-0017	Lewis, C.J.	2007	[^{14}C]S-2188: Hydrolytic Stability Covance Laboratories Ltd, Report no. 0333/257-D2149
QNM-0019	Lewis C.J., Troth K.	2007	[^{14}C]S-2188: Metabolism in Oilseed Rape Covance Laboratories Ltd. Report no. 0333/249-D2149
QNM-0020	Lewis C.J., Troth K.	2007	[^{14}C]S-2188: Photodegradation on a Soil Surface Covance Report no. 0333/259-D2 149
QNM-0024	Panthani A.M., Herczog K.J.S., Savides M.C.	2007	Metabolism of [^{14}C]S-2188 by Lactating Goats Ricerca Biosciences LLC Report no. 019493-1
QNM-0025	Panthani A.M., Herczog K.J.S., Savides M.C.	2007	Metabolism of [^{14}C]S-2188 by Laying Hens Ricerca Biosciences LLC Report no. 019494-1
QNM-0029	Lewis, C.J., Troth, K.	2007	[^{14}C]S-2188: Photodegradation and Quantum Yield in Sterile, Aqueous Solution Covance Laboratories Ltd, Report no. 0333/258-D2149
QNM-0030	Lewis C.J., Troth K.	2007	Nature of the Residue (High Temperature Hydrolysis) study; Covance Laboratories Ltd. Report no. 0333/279-D2149
QNM-0039	Jalal M.A.F.	2009	A Confined Accumulation in Rotational Crops Study on [Pyrazolyl-5-^{14}C]-S-2188; Valent USA Corporation Report no. VP-30829
QNP-0001	Beckwith, R.C., DiFrancesco, D.	2005	Determination of Dissociation Constant (pKa) - S-2188 Ricerca Biosciences LLC, Report no. 018410-1
QNP-0003	Lentz, N.R.	2005	Determination of Water Solubility - S-2188 Ricerca Biosciences LLC, Report no. 018315-1

Reference No	Author(s)	Year	Study Title
QNP-0004	DiFrancesco, D.	2006	Determination of Vapour Pressure - S-2188 Ricerca Biosciences LLC, Report no. 018435-1
QNP-0006	Sweetapple, G.G., Lentz, N.R.	2006	Determination of Physical-Chemical Properties of S-2188PAI (amended report) Ricerca Biosciences LLC, Report no. 19388-1-1
QNP-0007	Sweetapple, G.G., Lentz, N.R.	2006	Determination of Physical-Chemical Properties of S-2188TGAI (amended report) Ricerca Biosciences LLC, Report no. 19387-1-1
QNP-0010	Tani, N.	2009b	Storage Stability of S-2188 Technical Grade at Normal and Elevated Temperatures, Metals and Metal ions, Study no. 0027
QNP-0011	Tani, N.	2009a	Storage Stability of S-2188 Technical Grade, Study no. 0024
QNR-0004	Grolleau, G.	2008	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in grapevine raw agricultural commodity and processed fractions - Northern and Southern Europe - 2006; Staphyt Report no. GGU-06-1765
QNR-0005	Grolleau, G.	2008	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in protected cherry tomato raw agricultural commodity - Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-2772
QNR-0006	Grolleau, G.	2008	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in protected sweet pepper raw agricultural commodity - Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-2773
QNR-0007	Grolleau, G.	2008	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in protected cucumber raw agricultural commodity – Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-2774
QNR-0008	Grolleau, G.	2008	Magnitude of the residue of S-2188-OH in protected cherry tomato raw agricultural commodity - Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-3194
QNR-0009	Grolleau, G.	2008	Magnitude of the residue of S-2188-OH in protected sweet pepper raw agricultural commodity - Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-3195
QNR-0010	Grolleau, G.	2008	Magnitude of the residue of S-2188-OH in protected cucumber raw agricultural commodity – Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-3196
QNR-0011	Grolleau, G.	2008	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in grapevine raw agricultural commodity and processed fractions - Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-2771
QNR-0012	Rzepka S. and Daneva, E.	2009	Freezer Storage Stability Study of S-2188 and its Metabolite S-2188-DC, in Grapes, Oil Seed Rape (seeds), Lettuce and Cereal (Grain), Eurofins Analytik GmbH Report no. SUM-0711; (Amendment No. 1)
QNR-0013	Grolleau, G.	2008	Magnitude of the residue of S-2188-OH in grapevine raw agricultural commodity and processed fractions – Northern and Southern Europe - 2007; Staphyt Report no. GGU-07-3193
QNR-0014	Grolleau, G.	2009	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in protected cherry tomato raw agricultural commodity - Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4136
QNR-0015	Grolleau, G.	2009	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in protected sweet pepper raw agricultural commodity - Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4137
QNR-0016	Grolleau, G.	2009	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in protected cucumber raw agricultural commodity – Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4138
QNR-0017	Grolleau, G.	2009	Magnitude of the residue of S-2188-OH in protected cherry tomato raw agricultural commodity - Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4286
QNR-0018	Grolleau, G.	2009	Magnitude of the residue of S-2188-OH in protected sweet pepper raw agricultural commodity - Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4287; Sumitomo ref: QNR-0018
QNR-0019	Grolleau, G.	2009	Magnitude of the residue of S-2188-OH in protected cucumber raw agricultural commodity – Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4288
QNR-0034	Rzepka S. and Daneva, E.	2009	Freezer Storage Stability Study of S-2188-OH, one of the metabolites of S-2188, in Grapes, Oil Seed Rape (seeds), Lettuce and Cereal (Grain), Eurofins Analytik GmbH Report no. SUM-0712; (Amendment No. 1)
QNR-0035	Grolleau, G.	2009	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in grapevine raw agricultural commodity and processed fractions - Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4135

Reference No	Author(s)	Year	Study Title
QNR-0058	Grolleau, G.	2009	Rotational field crops residue study after applications of S-2188 50WG to open-field tomato, Southern Europe - 2007; Staphyt Report no. GGU-07-3192
QNR-0068	Grolleau, G.	2009	Magnitude of the residue of S-2188-OH in grapevine raw agricultural commodity and processed fractions – Northern and Southern Europe - 2008; Staphyt Report no. GGU-08-4285
QNR-0086	Grolleau, G.	2011	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in greenhouse strawberry raw agricultural commodity. Northern and Southern Europe – 2010; Staphyt Report no. GGU-10-6203
QNR-0087	Kowalsky J.	2011	Magnitude of the Residue of V-10135 on Almonds; Valent Report no. VP-30088
QNR-0088	Kowalsky J.	2011	Magnitude of the Residue of V-10135 on Grapes and it's Processed Products; Valent Report no. VP-30097
QNR-0089	Kowalsky J.	2011	Magnitude of the Residue of V-10135 on Head Lettuce; Valent Report no. VP-32906
QNR-0090	Kowalsky J.	2011	Magnitude of the Residue of V-10135 on Leaf Lettuce; Valent Report no. VP-32904
QNR-0091	Kowalsky J.	2010	Magnitude of the Residue of V-10135 on Strawberries; Valent Report no. VP-30127
QNR-0096	Grolleau, G.	2011	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in open-field strawberry raw agricultural commodity. Southern Europe – 2010; Staphyt Report no. GGU-10-6204
QNR-0097	Grolleau, G.	2011	Magnitude of the residue of S-2188-OH in greenhouse strawberry raw agricultural commodity. Northern and Southern Europe – 2010; Staphyt Report no. GGU-10-6226
QNR-0098	Grolleau, G.	2011	Magnitude of the residue of S-2188-OH in open-field strawberry raw agricultural commodity. Southern Europe – 2010; Staphyt Report no. GGU-10-6227
QNR-0099	Grolleau, G.	2011	Magnitude of the residue of S-2188-OH in peach raw agricultural commodity, Northern and Southern Europe - 2010; Staphyt Report no. GGU-10-6228
QNR-0100	Grolleau, G.	2011	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in peach raw agricultural commodity, Northern and Southern Europe - 2010; Staphyt Report no. GGU-10-6205
QNR-0101	Grolleau, G.	2012	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in greenhouse strawberry raw agricultural commodity. Northern and Southern Europe – 2011; Staphyt Report no. GGU-11-8895
QNR-0102	Grolleau, G.	2012	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in open-field strawberry raw agricultural commodity. Northern and Southern Europe – 2011; Staphyt Report no. GGU-11-8946
QNR-0103	Grolleau, G.	2012	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in cherry raw agricultural commodity, Northern and Southern Europe - 2011; Staphyt Report no. GGU-11-9201
QNR-0104	Grolleau, G.	2012	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in plum raw agricultural commodity, Northern and Southern Europe - 2011; Staphyt Report no. GGU-11-9205
QNR-0105	Grolleau, G.	2012	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in peach raw agricultural commodity, Northern and Southern Europe – 2011; Staphyt Report no. GGU-11-8897
QNR-0109	Bousquet C.	2013	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in apricot raw agricultural commodity, Northern and Southern Europe - 2012; SGS AGRI MIN Report no. 12SGS016
QNR-0110	Bousquet C.	2013	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in plum raw agricultural commodity, Northern and Southern Europe - 2012; SGS AGRI MIN Report no. 12SGS017
QNR-0111	Bousquet C.	2013	Magnitude of the residue of S-2188 and its metabolite S-2188-DC in cherry raw agricultural commodity, Northern and Southern Europe - 2012; SGS AGRI MIN Report no. 12SGS018
QNR-0121	Corley J.	2010	V-10135: Magnitude of the Residue on Ginseng; IR-4 Project no. 09453
QNR-0122	Switek, T.	2011	V-10135: Magnitude of the Residue on Blueberry; Rutgers, The State University of New Jersey ,USA, IR-4 PR No.09445
QNR-0123	Switek, T.	2011	V-10135: Magnitude of the Residue on Caneberry; Rutgers, The State University of New Jersey ,USA, IR-4 PR No.09444